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Synthesis of novel secosteroids

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SYNTHESIS OF NOVEL SECOSTEROIDS

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The importance of anaesthesia.

Dedicated to my parents and grandma

for their constant love and support

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To all of you, cheers and all the best for the future.

ABSTRACT

It was hoped that secosteroids of the type **107**, and to a lesser degree secosteroids such as **60** and **65**, and *abeo*steroids **117** and **128** would probe conformational requirements for inducing and maintaining general anaesthesia after intravenous administration.

The 3-acetoxy-5α-hydroxy steroid **38** underwent fragmentation of the C(5)-C(10) bond after treatment with ceric ammonium nitrate in refluxing acetonitrile, with the formation of 10-membered ring containing 5,10-secosteroidal compounds with a $\Delta^{1,10}$ double bond. The synthesis afforded exclusively the (E)-form **39**, the configuration of the double bond being assigned by means of NMR spectrometry. Epoxidation of the $\Delta^{1,10}$ double bond occurred stereoselectively to give the 1β,10α-epoxide, which served as a protecting group for the olefin and allowed deoxygenation of the 5-carbonyl. Selective hydrolysis of the 3-acetate in the presence of the 20-acetate allowed manipulation of both the *head* and *tail* ends of the seco- and *abeo*steroids to afford the 3α-hydroxyl and 20-keto moieties, required for optimum anaesthetic potency (detailed in the *review of steroidal anaesthetics*, see Appendix). It was hoped that the 3α-hydroxyl and 20-keto moieties would be less constrained and thus more free to adopt a suitable conformation for $GABA_A$ receptor binding.

Cyclization of the (E)-5,10-secosteroids of the type **39** under thermal conditions afforded compounds of the 5(10→1βH)*abeo* type **117**, exclusively the *trans* 1β,5α-configuration. Also incorporation of a good leaving group at position C(5) facilitated the facile transannular reaction to afford 5(10→1βH)*abeo*steroids of the type **117**. The ease of the transannular reaction plus results of conformational studies indicates that the transannular interaction of the double bond with the

carbonyl group may strongly influence the thermodynamic stability of the system.

Conformational studies on the (E)-5,10-seco-pregnene **39** showed the cyclodecenone ring to adopt an extended *crown* conformation, and analysis of the ^{1}H-NMR data of the 3α-silyl ether revealed that in solution the 10-membered ring exists in at least 2 distinct forms.

X-ray analysis of the 5(10→1βH)*abeo* diol **117** revealed the unusual configuration of the 19α-methyl group plus a solid state molecular packing dominated by intermolecular hydrogen bonds through the corresponding hydroxyl groups. The consequence of these hydrogen bonds is an array of infinite herringbone polymers, unique to this type of structure.

ABBREVIATIONS

Å	-	angström
Ac	-	acetyl
AFU	-	anaesthetic fish unit
AIBN	-	azobisisobutyronitrile
Ar	-	aryl
ATP	-	adenosine triphosphate
BI	-	benz[e]indene
Bu	-	butyl
Bz	-	benzyl
CAN	-	ceric ammonium nitrate
C.I.	-	chemical ionisation
CNS	-	central nervous system
CTMS	-	chlorotrimethylsilane
2D COSY	-	two dimensional correlated spectroscopy
DCA	-	desoxycorticosterone acetate
DDQ	-	2,3-dichloro-5,6-dicyano-1,4-benzoquionone
DEAD	-	diethyl azodicarboxylate
DEG	-	diethylene glycol
DHP	-	dehydropregnenolone
DMAP	-	4-dimethylaminopyridine
DMF	-	dimethylformamide
DMPC	-	dimyristoylphosphatidylcholine
DMSO	-	dimethyl sulphoxide
DPPC	-	dipalmitoylphosphatidylcholine
ED_{50} (AD_{50})	-	50 percent effective dose

E.I.	-	electron ionisation
Et	-	ethyl
FAB	-	Fast Atom Bombardment
GABA	-	gamma-amino-*n*-butyric acid
GAD	-	glutamic acid decarboxylase
h	-	hour
HCG	-	human chorionic gonadotrophin
HMPA	-	hexamethyl phosphoric acid
Hz	-	Hertz
Im	-	Imidazole
IR	-	infrared
J	-	coupling constant
Kd	-	dissociation constant for a given drug
KJ	-	kilo joule
LD_{50}	-	50 percent lethal dose
LH	-	luteinizing hormone
MAC	-	minimum alveolar concentration
Me	-	methyl
min	-	minute
m.p.	-	melting point
NMDA	-	N-methyl-D-aspartate
NMO	-	4-methylmorpholine N-oxide
NMR	-	nuclear magnetic resonance
PCC	-	pyridinium chlorochromate
PCN	-	3β-hydroxy-20-oxo-5α-pregnan-16α-carbonitrile
Ph	-	phenyl
PMS	-	pregnant mare serum
ppm	-	parts per million
p-TSA	-	*para*-toluenesulphonic acid

rbf	-	round bottomed flask
REM	-	rapid eye movement
Rf	-	retention factor
rt	-	room temperature
SAR	-	structure activity relationship
t	-	*tert-*
TBAF	-	tetrabutylammonium fluoride
tBDMS	-	*t*-butyldimethylsilyl
TBPS	-	*t*-butyl bicyclophosphorothionate
Tf	-	triflate
THF	-	tetrahydrofuran
T.I.	-	therapeutic index
tlc	-	thin layer chromatography
TPAP	-	tetrapropylammonium perruthenate
Ts	-	tosyl
U.V.	-	ultraviolet
W	-	Watt

NOMENCLATURE

General

The chemical name of a compound specifies its stereochemistry but not the conformation. Though all steroids can be assigned a unique systematic name based on IUPAC nomenclature[1], trivial and non-systematic names are used persistantly. For example, the trivial name for pregn-4-ene-3,20-dione is progesterone.

The systematic names are based on certain fundamental stem names and the stereochemistry implied in them. Substituents are indicated by systematic application of the rules of general organic chemical nomenclature. According to the IUPAC rules hydroxyl and keto groups appear after the name of the parent compound, characterized by the ending "-ol" or "-one" respectively, added to the number of the carbon atom to which it is attached, while all other substituents are listed before the parent name in order of their position on the steroid skeleton. Prefixes and suffixes are added to the parent name, their positions being indicated by the number of the carbon atoms to which they are attached. The prefix Δ is used as an alternative to denote a double bond. The standard atom numbering scheme and the lettering of the rings of the steroid skeleton are shown in Figure 1. The carbon atoms are numbered around the rings, starting from the top of ring A.

Additional to these rules are the application of the methods of skeleton modification, such as the use of *nor* for group elimination.

When the rings of a steroid are denoted as projections onto the plane of the paper,

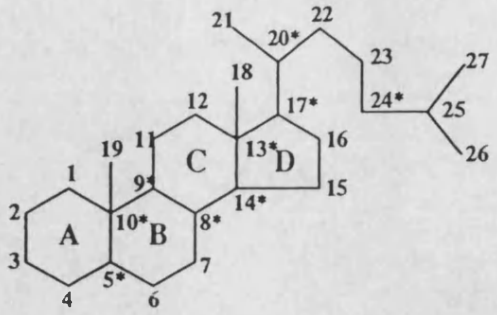


Figure 1. Numbering scheme of steroid skeleton and ring lettering. Asterisks denote asymmetric C - atoms.

the standard formula should be oriented as used here. This convention corresponds to the absolute configuration of the nucleus of natural steroid molecules. In structural formulae, the configuration of groups attached to the nucleus which are above the plane of the ring system is termed β, and for groups below the plane it is termed α. For a steroid name, the configuration at an asymmetric centre is indicated by these Greek letters prefixed by the appropriate numeral. Bonds to atoms or groups lying below the plane of the paper are shown as broken lines, and bonds to atoms or groups lying above the plane of the paper are shown as solid lines preferably thickened. Substituents lying in the plane of the nucleus are equatorial whereas those extending away are axial substituents (Figure 2).

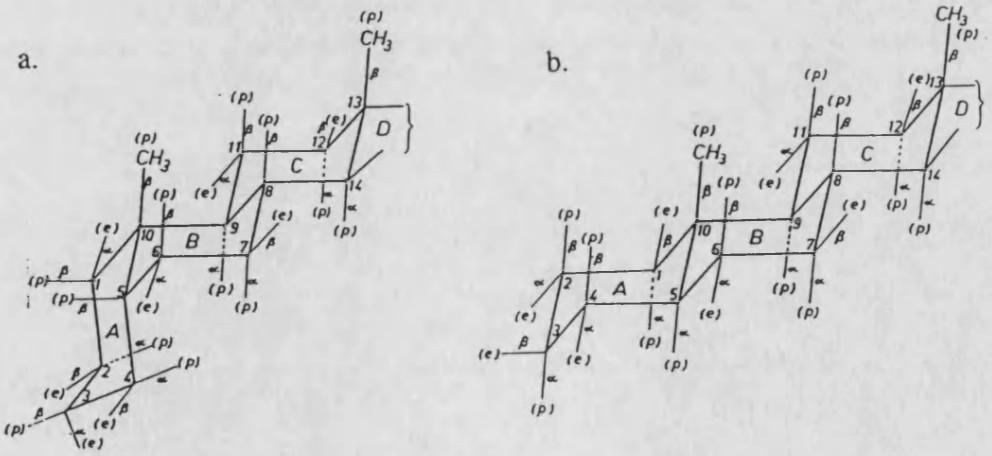


Figure 2. A/B-ring junction and configuration of substituents

a) A/B *cis*, b) A/B *trans*

There are two A/B-ring fusions which occur naturally. These are the *cis* fusion and the *trans* fusion (Figure 2). Common steroids have all rings *trans* fused.

Anaesthetic steroids

The structures of the parent skeletons that are relevant to anaesthetic steroids are depicted in Figure 3.

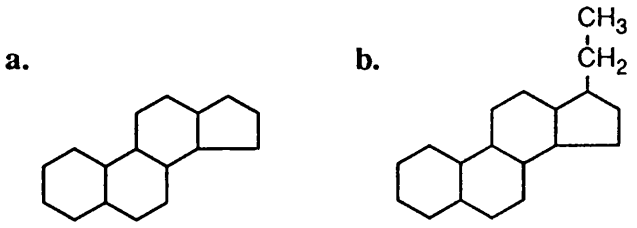


Figure 3. Basic chemical structures of pregnanes, from which steroid anaesthetics are derived. a)Steran, b)Pregnane.

The basic skeleton of all known steroid anaesthetics is the pregnane ring system, which is derived from the basic, cyclopentanophenanthrene (steran) ring (Figure 3). Pregnanes contain 21 carbon atoms, as distinguished from the estranes, androstanes, and ethiochoianes, which contain 19 carbon atoms. The pregnane skeleton is common to progesterone and many synthetic steroids with progestational activity.

Pregnanes occur in two stereoisomeric forms ; 5α-pregnane (allopregnane) and 5β-pregnane (epipregnane). These differ only with respect to the steric positions of the H and methyl substituents at the C-5 and C-10 carbon atoms of the A ring, respectively. In 5α-pregnane the relative position of the two groups is *trans* (opposite), while in 5β-pregnane it is *cis* (parallel).

Substitutions, usually by "-OH" (hydroxyl) or "=O" (keto) groups, may occur at almost any of the 21 carbon atoms, but most frequently exist at the 3, 11, 17, 20

and 21 carbons. The freely rotating hydroxyl groups may point "upward" from the plane of the ring system (β-position) or "downward" from the plane (α-position).

The structure of progesterone differs from that of most metabolites because the C(4)-C(5) double bond eliminates a C(5)-C(10) steric constellation. Furthermore, its two keto groups, in contrast to hydroxyl groups, are sterically fixed (Figure 4).

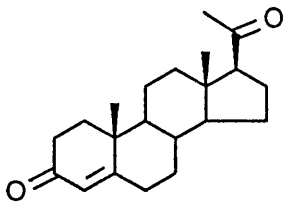


Figure 4. Progesterone (Pregn-4-ene-3,20-dione).

CONTENTS

INTRODUCTION

INTRODUCTION

1.1 What is anaesthesia?

"....it has an agreeable taste, so that even chickens take it gladly, and thereafter fall asleep for a long time, awaking unharmed...., its use may be recommended for painful illness, and it will mitigate the disagreeable complications of them"

-Paracelsus, sixteenth century alchemist on diethyl ether.

Anaesthesia is the art or science of rendering the patient unaware, thus producing partial or total loss of the sense of pain, providing an indispensable foundation for surgery. Since the introduction of anaesthesia into medical practice in the first half of the nineteenth century, considerable progress has been achieved in the form of safer and more effective anaesthetic agents and vastly improved surgical techniques. Thus anaesthesia has played an indirect, yet vital part in the major advance in the quality of human life.

There are two types of anaesthesia, general and local anaesthesia. Local or regional anaesthesia is brought about by pharmacological agents capable of producing a loss of sensation in a circumscribed area of the body ranging from a wart to the entire abdomen and lower limbs. Local anaesthetic is a clinical term and should be used only to refer to blocking agents that produce reversible clinical local anaesthesia.

In contrast, general anaesthesia is described as a condition wherein the patient has cerebral cortical depression, loss of the sensation of pain, loss of motor responses,

and is generally unconscious. As evidenced by alterations in physiological responses, general anaesthetics act by depressing nervous function. As higher concentrations of anaesthetic agents in nervous tissue are obtained, depth of anaesthesia increases.

Anaesthesia was divided into four separate stages, which were subsequently subdivided by Gillespie[2];

Stage I. *Analgesia.* A mild depression of higher cortical centres, suitable for surgical procedures not requiring extensive muscular relaxation.

Stage II. *Delirium.* Excitement as a result of depression of cortical motor centres resulting in possible extensive involuntary activity.

Stage III. *Surgical anaesthesia.* Subdivided into four planes representing progressive increases in depth of anaethesia as characterised by; first, loss of spinal reflexes; second, decreased muscle reflexes; third, paralysis of intercostal muscles; and fourth, disapppearance of muscle tone.

Stave IV. *Respiratory paralysis.* More correctly referred to as toxic or overdose stage involving respiratory and vasomotor cessation.

All anaesthetics do not bring about analgesia. They do produce insensitivity to pain, but this is related to loss of consciousness rather than to specific analgesic response. Anaesthetic-induced analgesia is related to an induced release of endorphin[3].

Stage II extends from the loss of consciousness to the beginning of surgical anaethesia and may be accompanied by uncoordinated muscular movements

associated with delirium, urinary incontinence and related responses. Depression of cerebral cortex and reticular formation may cause excitement, which in turn increases heart rate, blood pressure, and respiration. The first two stages are termed the "induction period", which is ideally short and free of excitement, involuntary activity, and irritation.

Most surgical procedures are carried out during stage III, which is characterised by regular, involuntary breathing, roving eyeball movements, and the absence of certain reflexes. As surgical anaesthesia deepens these physical signs progressively change, differentiating the four planes of surgical anaesthesia. Effects on respiration as patients enter stage III vary with different anaesthetic agents.

Cessation of respiration generally signifies stage IV, and breathing must be assisted until anaesthetic effect decreases.

The best estimate of anaesthetic potency is MAC - the minimum alveolar concentration (at 1 atmosphere) of an agent that produces immobility in 50 percent of those subjects exposed to a noxious stimulus. In humans the stimulus is a surgical skin incision, whereas in animals it is produced by clamping the tail or by passing an electrical current through subcutaneous electrodes.

The anaesthetic concentration that abolishes the righting reflex in 50 percent of the animals is often used to measure anaesthetic potencies in smaller animals. That is, it is an anaesthetic 50 percent effective dose (ED_{50}). It must be noted that the tail-clamp ED_{50} (MAC) and the righting-reflex ED_{50} are not identical. The tail-clamp ED_{50} is higher than the righting-reflex ED_{50}. This implies that the righting-reflex is depressed, at least in part, by a different mechanism from that which depresses the response to the application of a tail clamp. Thus the relative

potencies of anaesthetics may depend on the end point measured.

1.2 History of anaesthesia

1.21 History of anaesthetic practice

Operations have been performed for centuries but always for superficial diseases - a fracture, amputation, cataract extraction, or removal of bladder calculus. To these ends, the anaesthetic properties of hypnosis and trance, pressure over peripheral nerves and blood vessels, application of cold, alcohol intoxication, ingestion of herbal concoctions, or even physical restraint or shock were used.

With techniques of anaesthesia administration more or less divided into schools, the choice now lies among inhalation, intravenous, regional techniques, or combinations thereof. The seeds of all three methodologies were implanted during the middle ages.

Inhalational anaesthesia

Around 1540, Paracelsus, a swiss physician and alchemist, sweetened the feed of fowl with sweet oil of vitriol, a substance earlier prepared by Valerius Cordus, then named Aether by Frobenius - the familiar diethyl ether that would later be inhaled by most surgical patients over a span of 100 years or more. Paracelsus claimed that the diethyl ether was taken unwittingly by chickens, which then fell asleep and later awakened without harm.

Local anaesthesia

In the 16th century slaves were paid off in coca leaves, an effective method of

increasing and prolonging their productivity. Customarily, coca leaves bound into a ball (cocada) with guano and cornstarch were chewed with lime or alkaline ash to release the active alkaloid. Documentation of that era indicates that trephination was successful, as the operator permitted cocaine-drenched saliva to drip from the mouth onto the wound, thereby providing creditable local anaesthesia.

Intravenous anaesthesia

Following Harvey's studies of the circulation Percival Christopher Wren, in 1665 wrote that he could "...easily contrive a way to convey a liquid thing immediately into the circulating mass of blood; thus, in pretty big and lean dogs, by making ligatures on the veins and then opening them on the side of the ligature towards the heart; and by putting into them slender syringes or quills, fastened to bladders containing the matter to be infacted....whereof the success was that opium, being soon circulated into the brain did within a short time stupefy, though not kill the dog; but a large dose of the crocus metallorum, made another dog vomit up life and all".

The dried crocus or saffron was at the time employed as a stimulant, or antispasmodic.

1.22 A brief history of steroidal anaesthetics

The anaesthetic properties of some steroids, notably progesterone and desoxycorticosterone acetate were first reported[4] by Hans Selye in 1941. It was later claimed, also by Selye[5] that 5β-pregnane-3,20-dione exhibited enhanced activity. It took another 13 years before Pfizer introduced the first water-soluble steroidal intravenous anaesthetic agent, *viz.* hydroxydione[6] - a hemisuccinate salt. It possessed a good therapeutic index but several disadvantages, namely slow induction time, low potency and a high incidence of thrombophlebitis. However it

was marketed for many years before being withdrawn due to its side effects.

Glaxo[7] and Syntex made the next significant development towards a better steroidal i.v. anaesthetic by introducing a range of 3α-hydroxy steroids as potent hypnotic agents. Glaxo went on to develop 3α-hydroxy-5β-pregnan-11,20-dione 3-phosphate disodium which was water soluble, stable, did not cause thrombophlebitis and induced anaesthesia although not instantly. Although the agent was anaesthetic in man it also caused paraesthesia.[8]

In 1971 the Glaxo group introduced Althesin, a 3:1 mixture of alphaxalone and alphadalone acetate in cremaphor EL-water. Althesin produced a moderate duration of surgical anaesthesia, had a high therapeutic index and was compatable with common pre- and post-anaesthetic agents and inhalation anaesthetics. Unfortunately it had to be withdrawn from the market as it was found to cause a sometimes fatal allergic reaction due to the cremaphor EL. Althesin is discussed in more detail later, in a review of steroidal anaesthetics (see Appendix).

Glaxo's follow-up was the water-soluble i.v. steroidal anaesthetic minaxolone (Figure 5), in 1979. However this drug was also withdrawn due to excitation during induction and a slow recovery time.[9]

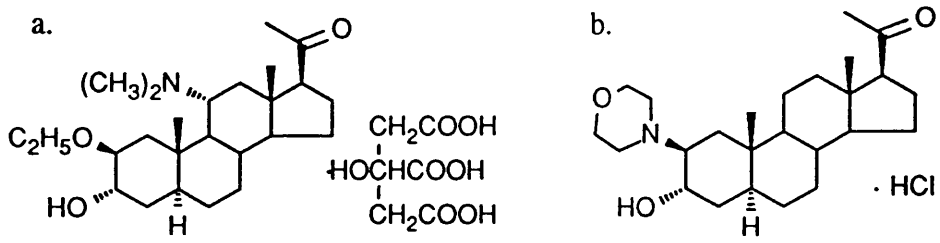


Figure 5. Two anaesthetically potent steroids. a)Minaxolone, b)ORG. 9843

A review by Witzel[10] and subsequent reports by Glaxo[11] and Syntex[12] groups suggested that water-soluble salts of anaesthetic steroidal conjugates are usually

less active and slower acting than the alcohol from which they are derived. Organon Teknika discovered that this was also the case with salts of aminated pregnanolones. For instance 3α-hydroxy-5α-pregnan-20-one is more active than the 2β-morpholinyl derivative when adminstered in aqueous solution as a salt, eg. the hydrochloride - Org. 9843 (Figure 5). But the steroid, Org. 9843, had an interesting level of anaesthetic activity and for this reason Organon Teknika is involved in the synthesis and biological evaluation of water soluble analogues related in Org. 9843[13].

1.3 Local anaesthesia

Local anaesthetic agents belong to that special class of pharmacological agents that are purposely administered as close to the site of action as possible. A local effect is, in fact specifically desired, and absorption in the general circulation and subsequent redistribution in the body can lead to undesirable systemic effects and toxicity. The desired local effect is a selective analgesia or anaesthesia of some part of the body. This localised effect is due to inhibition at nerve endings or to a conduction in a peripheral nerve.

Types of local anaesthesia consist of epidural, spinal and topical anaesthesia. All are types of central neural blocks. Epidural anaesthesia is used in caesarian pregancies and allows the patient to be awake, but feel no pain. Topical anaesthesia refers to a diversity of sites of application that includes for example, the skin, the cornea, the tracheobronchial tree and the gastrointestinal tract.[14] Depending on the characteristics of the site of application, specific agents and even specifically formulated vehicles may be required in order to effect local anaesthesia of acceptable intensity and duration.

1.31 Local anaesthetic agents

Cocaine, a naturally occurring alkaloid, was isolated from "Erythroxylon coca" Lam. (1860) and was also prepared from a semisynthetic procedure, from ecgonine, Figure 6, obtained from natural sources.

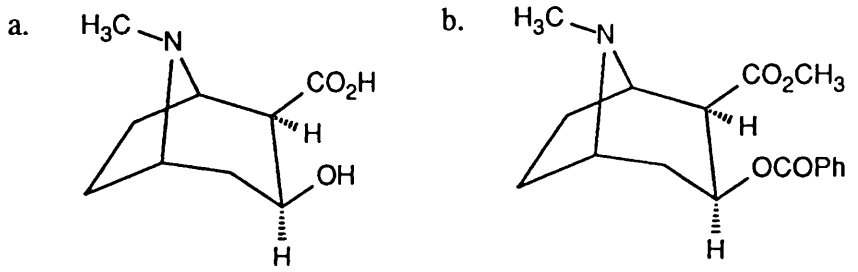


Figure 6. a)Ecgonine, b)Cocaine, [3(S)-benzoxy-2(R)-methoxycarbony-l-1(R)-tropane].

Although today cocaine is of greater historic than practical importance (allergic reactions, tissue irritation, poor stability in aqueous solution, and serious addiction liability), few developments in the chemistry of local anaesthetics can disclaim relationship to cocaine.

In 1903, Braun observed that by combining cocaine and other available agents with the natural endogenous hormone epinephrine, the duration of action of anaesthesia was considerably prolonged.[15] When a local anaesthetic is administered *in vivo*, its duration is primarily influenced by its removal from the site of administration in the local blood supply. Local anaesthetic drugs tend to have a vasodilator activity and therefore tend to potentiate their own removal from the site of administration by increasing the local blood supply. Because epinephrine causes vasoconstriction it has been hypothesized that it reduces the local blood supply and thereby allows the blocking agent to remain at the nerve for a longer period of time.

Shortly after Braun's observation there followed the introduction of the first

injectable local anaesthetic agent (1905) into the clinic - Procaine, synthesised by Einhorn.[16]

The intrinsic potency of this compound was low and the duration of action was relatively short, but both were considerably enhanced when it was combined with epinephrine.

Following the clinical introduction of procaine hundreds of compounds were synthesised, most of which were structurally related to procaine and cocaine. The major advancement during this period was the introduction of tetracaine by Eisleb in 1931.

This agent was found to be 8 to 10 times as potent as procaine, gave a long duration of anaesthesia and could be used safely even though it was toxic. Clinical use also gave the first indication that blockade of nerve conduction for several hours did not lead to irreversible nerve damage. Tetracaine and chloroprocaine are procaine-like agents that remain in current use.

The next major development in local anaesthetic agents was the synthesis of lidocaine (Figure 7) by Löfgren[17] in 1946. Lidocaine was the first generally acceptable local anaesthetic that did not contain the ester linkage present in cocaine, procaine and tetracaine, and therefore its introduction led to chemical synthesis on a much broader base. In place of the ester linkage lidocaine possessed an amide linkage. Lidocaine was found to be more potent than procaine and this combined with its short onset of action and reasonable degree of toxicity, made it a superior and more reliable agent. The incidence of allergic reactions with lidocaine was low, but was observed fairly often with ester type anaesthetics. A further practical advantage of lidocaine is its stability in aqueous solution, which allows heat sterilization and guarantees pharmacological activity after prolonged

storage. Other amide substances include mepivacine, prilocaine, bupivacaine, and etidocaine.

Since 1948 there have been many developments in the field of local anaesthesia, but in the fields of neurophysiology and neurochemistry rather than synthetic medicinal chemistry. In the near future, this research may lead to more specific and more potent nerve blocking agents.

1.32 Structure-activity relationships

The clinically useful drugs fall essentially into two chemical categories; agents with an ester link between the aromatic end of the molecule and the intermediate chain (procaine-like) and agents with an amide link between the aromatic portion and the intermediate group (lidocaine-like).

Chemical compounds that demonstrate clinically useful local anaesthetic activity possess the following chemical arrangement : aromatic portion - intermediate chain - amino portion or alternatively, lipophilic portion - intermediate chain - hydrophilic portion (Figure 7). Alteration in any portion or sequence will modify their anaesthetic activity. Lengthening of the intermediate chain tends to increase anaesthetic potency until a certain length beyond which potency decreases.

Chemical modification of either end of the molecule will affect the lipid solubility and protein-binding characteristics. For example, within the ester series tetracaine, which is formed by the addition of a butyl group to the aromatic end of the procaine molecule, is an agent of increased lipid solubility and protein binding, greater intrinsic anaesthetic potency, and longer duration of anaesthetic activity (Figure 7).

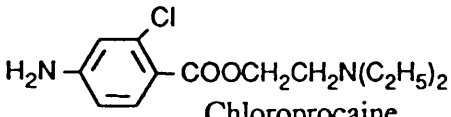
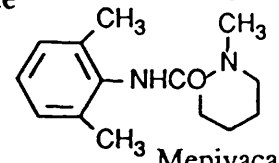
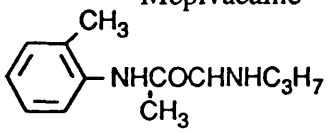
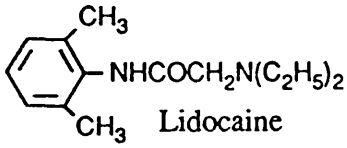
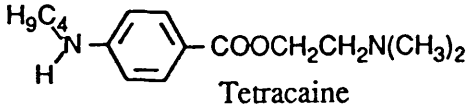
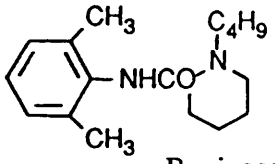
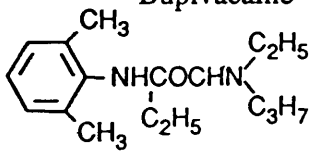
AGENT	RELATIVE POTENCY	DURATION (MIN)
Low Potency		
 Procaine	1	60 - 90
 Chloroprocaine	1	30 - 60
Intermediate Potency		
 Mepivacaine	2	120 - 240
 Prilocaine	2	120 - 240
 Lidocaine	2	90 - 200
High Potency		
 Tetracaine	8	180 - 600
 Bupivacaine	8	180 - 600
 Etidocaine	6	180 - 600

Figure 7. Local anaesthetic agents according to *in vivo* potency and duration of action.

In the amide series the addition of the same butyl group to the amine end of mepivacaine transforms this agent into a compound, bupivacaine, which is also more lipid soluble, more highly protein bound, more potent and longer acting (Figure 7). Alterations in the chemical structure of homologous compounds may also affect their rate of degradation and therefore, their intrinsic toxicity. Thus tetracaine, which is hydrolysed at a considerable slower rate than is procaine, has a considerably greater toxicity.

The basic difference between heterologous types of local anaesthetic agents, i.e. the ester and amide compounds, residues in; the rate and manner in which they are metabolised , their allergic potential, and their physiochemical properties such as lipid solubility and protein-binding, which alter their anaesthetic properties. This last point is more so for chemical alterations within a homologous group, i.e. within the ester group or the amide group.

1.4 General anaesthesia

General anaesthesia is brought about by a wide range of substances which are administered at high concentrations and gain access to all parts of the body. They exert their actions on peripheral organs, such as the neuromuscular function and the heart, as well as their primary target(s) in the central nervous system. The central nervous system is extremely complex and our rudimentory understanding of its physiology has prevented elucidation of how general anaesthetics reversibly depress consciousness.

The phenomenon of general anaesthesia can be brought about by a remarkable range of substances - from the inert gas xenon to the steroid althesin (Figure 8). This suggests that no single, structurally specific receptor for general

anaesthetics exists. Given this, there are two possible overall classes of mechanism which might give rise to general anaesthesia. The *first* mechanism assumes that a non-specific locus of action is involved, with which general anaesthetics of all molecular classes can interact. For example, the lipid bilayer of neuronal membranes may provide such a site. This is consistant with there being no known chemical antagonist of general anaesthesia and leads to the so-called *unitary hypothesis*, which states that a single molecular mechanism underlies general anaesthesia. The *second* mechanism assumes that there are a number of different ways of causing general anaesthesia. For example, one structural class of anaesthetic might act on a binding site on a protein in one region of the brain and another structural class on a differently shaped protein site elsewhere in the brain. Alternatively, one protein might possess a number of different binding sites, each capable of interacting with one structural class of general anaesthetic. This has been called the *degenerate perturbation hypothesis* of general anaesthetic action[22], see chapter on nerve impulse and receptor pharmacology.

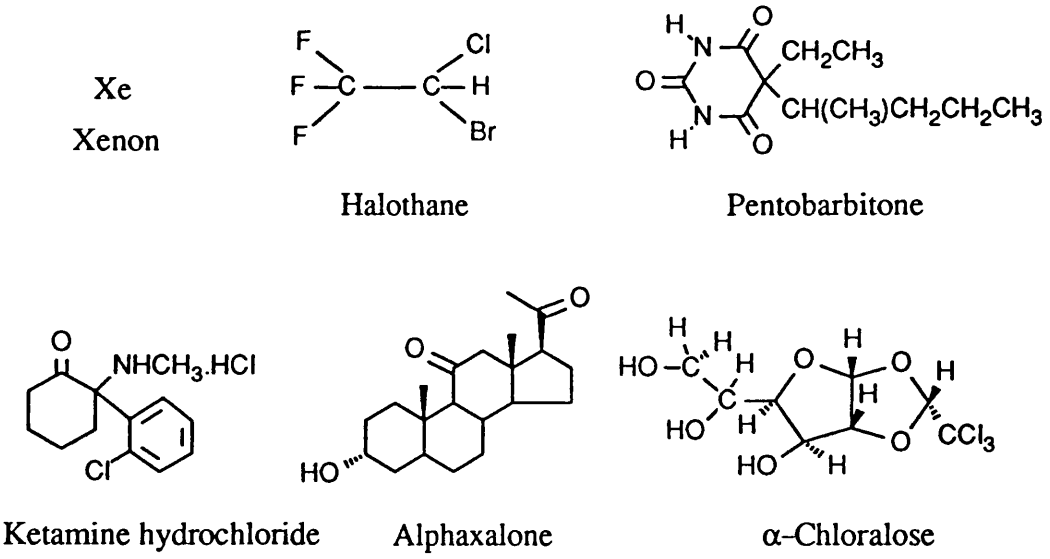


Figure 8. The structures of some general anaesthetics.

It is not unthinkable that general anaesthetics can exert both types of action, when one considers the high concentrations at which general anaesthetics are

administered and the number of side effects they exert.

Thus there is no one theory to account for all drugs which can be anaesthetic. Briefly, gases of one atom (xenon), simple gases (nitrous oxide) and vapours (hydrocarbons and complex ethers) or the large molecular structures of steroids (althesin) can all cause reversible depression of the central nervous system.

General anaesthetic agents may be divided into three classes; gases, volatile liquids and intravenous or fixed anaesthetics. The last class are called the *ultrashort-acting babiturates.*

The gases and volatile liquids are grouped together as inhaled anaesthetics. During administration of a volatile anaesthetic its concentration in the blood rapidly increases toward that in the inspired gas. As the anaesthetic enters tissues, this concentration approaches that of the arterial blood supply. Organs transfused by a large amount of blood, such as the brain, rapidly acquire high concentrations of anaesthetic. Depth of anaesthesia is a function of the amount of anaesthetic in the brain, and the time of induction and recovery are determined by the rate of change of concentration in the brain. Thus induction and recovery are determined by the solubility of the agent in blood. Relatively soluble agents, such as diethyl ether, have slow induction and recovery times, whereas relatively insoluble agents, such as cyclopropane, have short induction and recovery periods. Before appreciable amounts of anaesthetic diffuse into brain tissue, the blood reservoir must be nearly saturated.

In contrast to the inhalation agents, intravenous anaesthetics are extremely easy to administer, in fact, fatally so. Delayed onset of anaesthesia may tempt the unwary to continue injection, thereby adding to the dose that is already making its way to the patients brain. Unlike inhalation anaesthetics rapid elimination by controlled

ventilation is impossible.

The ultrashort-acting barbiturates are used to produce both surgical and basal anaesthesia. When injected intravenously they quickly produce unconsciousness, and emergence is rapid. Ultrashort-acting barbiturates are frequently used in combination with a volatile anaesthetic, such as nitrous oxide. The barbiturate produces rapid, pleasant anaesthesia, which is then maintained with the volatile anaesthetic.

The barbiturates were the first group of drugs to be introduced for intravenous administration, followed by eugenols, of which propanidid is currently available, and is notable for its rapid recovery.

A somewhat different approach has been the development of steroids with anaesthetic potency. The first usable compound was hydroxydione, but this was not a satisfactory compound due to its slow onset of action, slow recovery and a high incidence of venous thrombosis. Many other steroids have been investigated. Althesin has been the most promising to date.

To date many drugs have been used, and many more tested. The ideal induction agent must meet a formidable list of requirements, including, e.g., smooth induction, predictable dosage, high safety (therapeutic index), minimal cardiovascular and respiratory disturbances, rapid and complete recovery, absence of pain or injury from injection, and stability in solution. The barbiturate thiopental, although it does not meet every requirement, has been used for 30 years and is still the standard against which any new drug is compared.

1.41 Nonbarbiturate intravenous anaesthetics

Intravenous anaesthetic agents may be used for the induction of anaesthesia, as the sole agent for short operations, to supplement general anaesthesia or regional analgesia, or for sedation.

The introduction of thiopental into clinical practice in 1934 was the advent of intravenous anaesthesia. Thiopental and other barbiturates are not ideal intravenous anaesthetics primarily because they provide only hypnosis. The ideal intravenous anaesthetic would provide hypnosis, analgesia, and amnesia. The physical properties of an ideal intravenous anaesthetic include stability in solution, a long shelf-life, and water solubility. The drug should be a non-irritant and possess a low incidence of venous thrombosis. Excitatory effects on induction are undesirable, as are coughing, hiccup, and laryngospasm. An ideal agent should also have a very low incidence of hypersensitivity reaction and histamine release even in patients who show a sensitivity to other drugs. Exposure to the drug should not sensitise the patient to subsequent administration.

Many drugs have been used that offer some of these properties, but none has truly replaced thiopental as the most widely used intravenous anaesthetic drug. A great variety of intravenous drugs are available for use in the care of patients requiring general anaesthesia. The selection of a particular drug must be based on the individual patients need for hypnosis, amnesia and analgesia. Drug selection must match the physiology and/or the pathophysiology of the individual patient with the pharmacology of the particular drug. There is no single perfect drug for any particular patient, but rather it is the informed practitioner who wisely employs the appropriate drug or drugs in the practice of good anaesthesia care. The future of anaesthetic management probably involves the use of several drugs used together, including inhaled anaesthetics with intravenous drugs.

Intravenous agents differ from inhalational anaesthetics in that, once injected, there is nothing that can be done to facilitate the removal of the drug, hence the adage "thiopental is fatally easy to give". The most important properties of existing agents rests on their ability to penetrate the tissues of the body, particularly the central nervous system, without delay. Their rapid course of action is explained by their high lipid solubility coupled with a rich cerebral blood flow which ensures rapid penetration into the brain. This being so, the intensity and duration of this action is critically influenced, not only by the dose, but also by the speed at which that dose in injected. This is the *bolus effect.* The drug should produce its action in one arm-brain circulation time.

Once admixture with the circulating blood volume has occurred, the drug effect declines predominantly in most instances with redistribution of the drug into less richly perfused areas of the body, muscle, skin and finally fat depots. This decline reflects a rapid fall in plasma drug concentration defined pharmocokinetically as the α-*distributional phase*, which is contrasted with the slower β-*elimination phase.* Only after exceptionally large or repeated doses can elimination, mainly by metabolic degradation in the liver, exert a critical influence. The pharmacokinetics of intravenous anaesthetics are discussed in a paper by Ghonesin and Korltila.[20] The ideal anaesthetic agent should produce a rapid recovery with little hangover effect. Redistribution to non-nervous tissue and rapid detoxification to inert metabolites is ideal.

Intravenous anaesthetics can of course, like all drugs, induce adverse reactions. The subject has been extensively reviewed by Whitwam.[28] Adverse reactions can usefully be described under four headings, namely:

1. Reactions associated with induction - muscle movements, cough,

hiccup, respiratory and cardiovascular depression.

2. Tissue complications - local tissue damage, thrombophlebitis at the site of injection.
3. Reactions associated with recovery - psychic problems, motor disturbances, pain, nausea, prolonged somnolence.
4. Hypersensitivity - anaphylaxis, anaphylactoid reactions, skin rashes, bronchospasm.

The adverse reactions to the barbiturates thiopental and methohexitone are for the most part dose-related and they can therefore be avoided by care in the anaesthetic techniques.

Intravenous hypnotics

Propanidid, althesin, minaxolone, ketamine hydrochloride, etomidate, propofol, and gamma-aminobutyric acid (GABA) have all been used to induce anaesthesia. Propanidid is a eugenol derivative that is highly water insoluble. Cremophor EL is added to bring about solubility. Onset of anaesthesia and recovery is rapid. The rapid recovery is due to both redistribution and metabolism. The drug is metabolised by pseudocholinesterase in the liver and plasma, resulting in an elimination half-life of 10 minutes. The major disadvantage of propanidid is a high incidence of severe allergic reaction and occasional case of thrombophlebitis, which resulted in its withdrawal.

Propofol, 2,6-diisopropyl phenol, is formulated in lipid emulsion. It is a rapidly acting hypnotic agent with a short duration of action. Pain is experienced by some patients during intravenous injection and the blood pressure usually falls. Recovery is rapid.

Etomidate, *R*-1-ethyl-1(α-methylbenzyl) imidazole-5-carboxylate, is a

water-soluble induction agent, supplied as a solution containing propylene glycol, which gives a rapid onset of anaesthesia. On repeated administration there is little tendency to cummulation.[23] Pain at the site of injection is common and there is a high incidence of involuntary movement[24] which can be decreased with diazopam premedication. The drug is without analgesic action. Etomidate is rapidly metabolised and thus recovery is rapid, thus the drug is ideal for outpatient anaesthesia. However, the immediate post-anaesthetic period is frequently complicated by nausea and vomiting so that adjuvant anti-emetic medication is needed. Altogether the drug has no great advantage over other agents, it was recommended only if established agents are contraindicated.

Ketamine hydrochloride (ketalar), 2-(O-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride is a white crystalline solid, soluble in water with a pH between 3.5 and 5.5. It is stable at room temperature and benzethonium chloride is added as a preservative. Ketamine has unusual anaesthetic properties in that it produces unconsciousness with profound analgesia. It does not potentiate GABA (see mechanism of anaesthesia), but selectively blocks the ion channel activated by the selective excitatory amino acid N-methyl-D-aspartate (NMDA). Selective inhibition of the excitatory NMDA receptor in the central nervous system by ketamine also leads to CNS depression. The duration of anaesthesia brought about by ketamine is 5-10 minutes with a rise in blood pressure and heart rate. The drug also produces a high incidence of hallucinations, which are reduced by premedication with lorazepam.

GABA is a naturally occurring neuroinhibitor and is capable of producing anaesthesia. However it results in significant side effects, including prolonged recovery, emergence delirium, extra pyramidal muscle movement, and nervous irritation. Thus the drug was not approved for clinical practice.

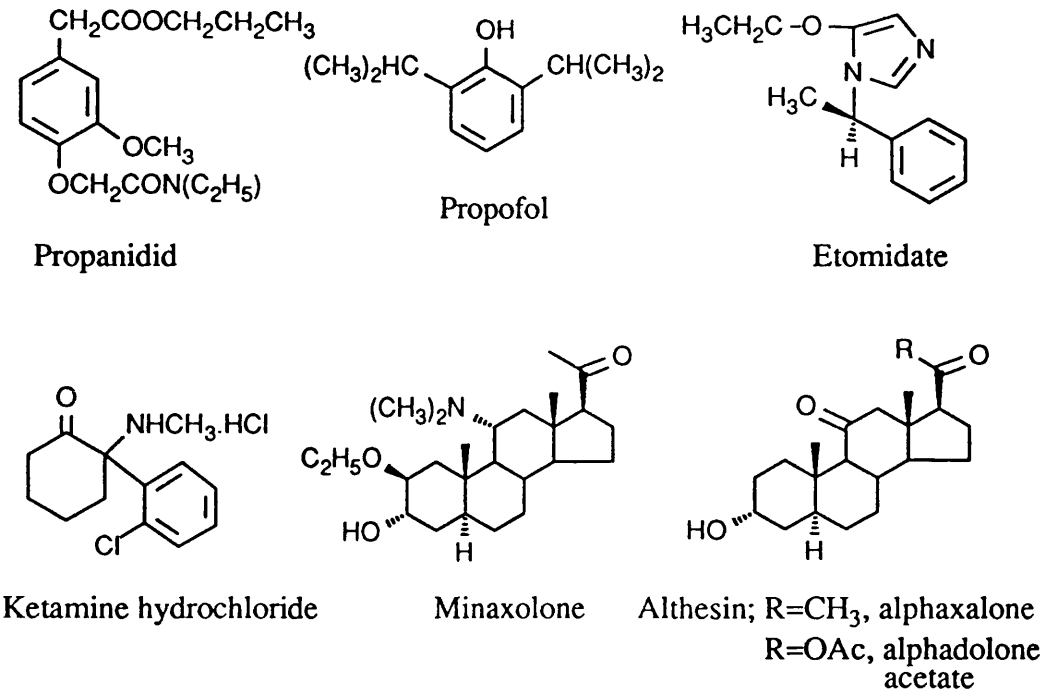
Althesin is a combination of two steroids, alphaxalone (primary active steroid) and alphadolone acetate (to increase solubility). It is presented as a clear colourless isotonic solution of neutral pH containing 9 mg alphaxalone and 3 mg alphadolone acetate in each ml, which also contains 20 percent cremophor EL. It is a brief acting intravenous anaesthetic agent which is usually non-irritant at the site of injection. Onset of action after administration is rapid, as is recovery. Termination of its action is determined largely by rapid metabolism in the liver[25] rather than redistribution. It should therefore not be given to patients with liver disease. Althesin is also useful for the maintenance of anaesthesia and sedation when given by infusion. Like propanidid, a high incidence of hypersensitivity reactions has resulted in the total withdrawal of this intravenous anaesthetic. Althesin will be discussed further in the 'review of steroidal anaesthetics' (see Appendix).

Minaxolone is an attempt to provide a water-soluble steroid i.v. anaesthetic. It is 11α-dimethylamino-2β-ethoxy-3α-hydroxypregnan-20-one, a water-soluble derivative of althesin. In limited clinical trials it has been shown not to cause venous irritation and to have a rapid onset of action, though accompanied by a high incidence of muscle movements.[26] By comparison with althesin, recovery from anaesthesia is slow[27], presumably because of the high water solubility. Though initially promising in some ways, this agent has now been withdrawn because of adverse effects on long-term toxicity testing in animals.

1.5 Mechanism of anaesthesia

To understand the medicochemical aspects of anaesthetics it is necessary to have a working knowledge of neuroanatomy and neurophysiology.

The central nervous system (CNS) is made up of a brain and a spinal cord. This system is connected to all parts of the body by nerves, which are made up of



Propanidid | Propofol | Etomidate

Ketamine hydrochloride | Minaxolone | Althesin; R=CH_3, alphaxalone; R=OAc, alphadolone acetate

Figure 9. Intravenous induction agents.

thousands of long thin nerve fibres. Like all other organ systems the nervous system consists of different types of cells. The cells which conduct the nerve impulses are called *neurones*.

The cell body has cytoplasm which unlike other cells extends, from the cell body outwards forming long thin threads as thin as 0.005nm in diameter and up to 1 metre in length. These threads are the nerve fibres along which travel the 'messages' made up of nerve impulses to and from all parts of the body.

Nerve impulses pass along the nerve fibres in only one direction. They pass into the CNS along the fibres of sensory neurones, and out of the CNS along fibres called *motor neurones*.

1.51 Neuroanatomy[28]

The sensory, or input fibres course together in bundles with the motor, or output, fibres from the periphery to the spinal cord.

Each nerve axon has its own membranous covering, often just called the *nerve membrane*, tightly surrounded by a myelin sheath called a *Schwann cell covering*. The myelin is not continuous along the fibre. The interruptions are the *nodes of Ranvier*, which are of great importance for nerve functioning.

The nerve fibres are collected in bundles similar to a telephone exchange. Conduction in these fibres would be slow if they were not insulated with the myelin coat. The interruptions in the myelin coat by the nodes of Ranvier, are where current enters and exits. Ionic fluxes occur at these nodes. The nerve impulse jumps along the fibre from node to node faster than in unmyelinated fibres.[29]

1.52 Neurochemistry[30]

The main inorganic constituents of nerve axons and cell bodies are sodium, potassium, calcium, magnesium, and chloride. The predominant intracellular cation is potassium and the predominant extracellular ions are sodium and chloride. Because there is not enough chloride within the cell for electroneutrality, at least half of the negative charges must be made up of fixed-charge lipid and protein complexes. Lipids and proteins are found free or in association with each other, forming lipoproteins, and also together with phosphorus, forming phospholipoproteins.

1.53 Neurophysiology[31]

What is a nerve impulse?

Some nerve impulses originate inside the CNS, others come from the sense organs.

The sole function of a sense organ is to change various forms of stimulus, such as light or sound, into nerve impulses which pass along sensory neurone fibres to the brain. Figure 10 illustrates a simple way of explaining what happens during the conduction of an impulse.

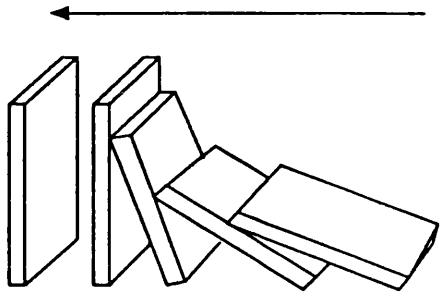


Figure 10. A simple analogy of a nerve impulse.

Imagine blocks of wood, such as dominoes, arranged in a row. The first domino is knocked over and falls against the next, which falls against the next,and so on to the end of the line. Note two important facts. Nothing, except a certain amount of energy, has moved along the line of dominoes, and this cannot happen again until the dominoes have been restored to their original positions. These events can be compared with a nerve impulse in the following way.

An impulse begins as a change in the arrangement of chemicals in a small area at one end of a nerve fibre, i.e. changes in permeability lead to depolarisation. Like one domino falling against the next and knocking it over, this changed area of nerve fibre excites an identical change in the area adjacent to it, which excites the next area, and so on to the end of the fibre. No material object has moved along the fibre, only a wave of chemical rearrangement. Just as the dominoes must be stood on end before they can be knocked down again, so a nerve fibre must recover before it can conduct another impulse. But this recovery period is only a few thousandths of a second.

There is another similarity between falling dominoes and nerve impulses. If the first domino in the line is touched very lightly it may rock back and forth, but will not knock over the next and trigger off the impulse. The first domino must be pushed with a specific amount of force before it falls against the next. Similarly, there are stimuli that are so weak that they do not stimulate the nerve enough to trigger off impulses to the brain. Stimulation of a nerve fibre must reach what is called the *threshold level* before the fibre sends a nerve impulse to the brain.

Another feature of nerve impulses is that there is no such thing as a weak or strong impulse. They either occur or do not, all are exactly alike no matter where they originate. This is called the *all-or-none* principle. The only feature of an impulse which ever varies is the number of them which pass along a nerve fibre per second. The frequency of the impulse depends on the strength of the stimulus. Thus a strong stimulus results in hundreds of impulses per second being sent to the brain, whereas a weak stimulus produces only a few impulses per second and a stimulus below the threshold level produces none at all.

Neurones, are not continuous with one another. Nerve impulses must cross a synapse where the axon of one neurone meets the dendrites, or cell body, of another. The dendrites and cell body of a motor neurone have synapses with thousands of other neurones.

Synapses also have a threshold level. The threshold of a synapse is the number of impulses per second at which the synaptic gap is 'bridged' and impulses begin to flow in the next neurone. The threshold level of a sense organ, plus the threshold of every synapse in the chain of neurones connected to it, form a barrier to the movement of impulses between that sense organ and the brain. This barrier is only penetrated by the high frequency impulses which result from strong stimuli. The

low frequency impulses resulting from weaker stimuli are unable to cross the synapse, and so do not reach the brain. The velocity at which an impulse is conducted along the nerve is known as the *conduction velocity* and is proportional to the diameter of the fibre.

In summary, the nervous system is a mass of interconnected nerve fibres which conduct impulses from receptors to effectors throughout the whole body.

Nerve transmission

A nerve impulse is a physico-chemical process in a nerve fibre. In a fibre in the resting state, there is a potential difference across the membrane of approximately 60 to 90 mV, inside negative to outside.[32] This is known as the *resting membrane potential.*

The origin of the resting potential is of great interest. The nerve cell contains a high concentration of potassium, which flows in and out of the cell with ease. The cell was believed to be impermeable to sodium because of the low sodium concentration in the excitable cell. Because the excitable cell appeared to be highly permeable to potassium and impermeable to sodium, it was treated by neurophysiologists as if it were an electrochemical, or Nernst, cell. Thus the resting potential was determined using the Nernst equation. However it was later shown that in the resting state the cell is slightly permeable to sodium but somehow able to extrude this ion. The mechanism for extrusion has been called a *sodium pump*. Thus the resting potential is intimately related to the ratio of potassium ions between the inside (Ki) and outside (Ko) of the cell membrane. At rest, a Ki:Ko ratio of approximately 30 to 1 exists.

During nervous activity, *an action potential*, the transmembrane potential goes from about -70 to about +40mV and promptly returns to the resting potential; the

event lasts about 1 millisecond.

When a nerve is stimulated the cell becomes transiently highly permeable to sodium. The sodium pump is inactivated, sodium permeability increases, and sodium ions pass from the exterior to the interior of the nerve cell through the so-called sodium channels. This process is called *depolarization.* A relatively slow phase of depolarization occurs during which the electrical potential within the cell becomes progressively less negative, until it reaches the threshold potential, about -50mV. At this stage a massive change in sodium permeability occurs and a rapid phase of depolarization follows resulting in a reversal in the membrane potential to about +30mV. The total amplitude of the action potential is approximately 100mV (Figure 11A). As the cell approaches its peak action potential the sodium permeability is rapidly reduced (sodium inactivation) and potassium permeability increases until the normal resting membrane potential is re-established. Thus, after an action potential the cell is left with a very small sodium increase and potassium decrease.

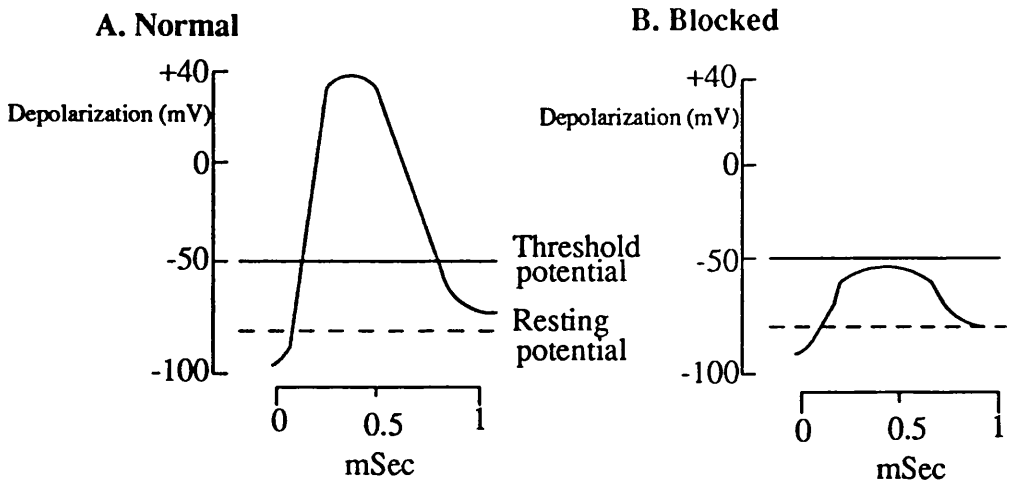


Figure 11. Intracellular nerve action potential prior to and following exposure to an anaesthetic.

This subsequent process is known as *repolarization.* The electrical status of the cell is restored by potassium diffusing out of the cell. The movement of potassium

speeds up the repolarization process which would be slower if it depended on the sodium permeability changes above.

The initial membrane electrical state has now been restored; however, the cell has gained sodium and lost potassium. With the reactivation of the sodium pump the normal intra- and extracellular cation ratios are restored. It has been suggested that by using the energy derived from splitting adenosine triphosphate (ATP), an ATPase system could serve this function and act as a sodium pump.[33]

The nerve impulse passes from the active area of depolarization to the adjacent inactive region, which is thereby depolarized. The area of activation are the nodes of Ranvier. No ions flow through the myelinated sheath, thus the spacing of the areas of activation increases the impulse transmission velocity.

Immediately after an impulse has been propagated, the area is *absolutely refractory* or completely inexcitable, and so stimulus, no matter how strong or long, cannot excite it. Shortly thereafter, the axon becomes *relatively refractory*; it responds with a propagated impulse only to stimulation that is greater than the normal threshold. The length of the refractory period is affected by the frequency of stimulation and by many drugs.

Anaesthetically active drugs, in someway, act on, or bring about changes to the nerve cell whereby depolarization is interfered with and the action potential fails to reach the threshold potential (Figure 11B). Therefore, the stimulus is unable to propagate any nerve impulses. This could be brought about by interaction of the anaesthetic agent with a *receptor*.

1.54 Receptor theory

Living cells possess special sites of drug action, the properties of which are to react with drugs of a specific nature and to initiate a chain of events leading to the pharmacological effect. Such sites of action are *receptors.*

Receptor structure

The overall physical structure of a receptor determines the three-dimensional configuration of the specific area of receptor protein that will bind a drug. The pharmacological structure of a drug must match the three dimensional configuration of the binding area of a receptor. Hence, subtle changes in drug structure may dramatically alter its ability to bind to a specific receptor population. In addition, two drugs with seemingly unrelated two-dimensional chemical structures may bind to the same receptor if their three-dimensional structures and charged areas are similar.

Recent advances in molecular biology have enabled researchers to deduce the primary structure of many receptors. Common features of all membrane receptors appear to be hydrophobic transmembrane regions as well as intracellular and extracellular regions. The exact arrangements of the three regions varies according to receptor type.

Classic receptor theory

To mediate effects drugs must bind to receptors. Such drug-receptor interactions provide the basis for the *classic receptor theory.* Class receptor theory assumes the compound or drug binds reversibly with the specific receptor site to produce a drug-receptor complex. The generation of this drug-receptor complex represents an intermediate step in producing a specific effect. The effect may cease when there is dissociation of the drug-receptor complex. This process is analogous to the

classic model of Michaelis-Menton enzyme kinetics. Mathematically, this may be expressed as

$$[D] + [R] \rightleftharpoons [DR]$$

where [D] = the concentration of drug, [R] = the concentration of receptor, and [DR] represents the concentration of drug-receptor complex. At equilibrium, one can define a K_d as the dissociation constant for that given drug. Thus, mathematically

$$K_d = \frac{[D][R]}{[DR]}$$

It is assumed that the drug-receptor complex represents an intermediate step in the production of a specific effect. An effect can be any biochemical compound, an enzyme level, heart rate, or blood pressure.

The delivery of a drug to a given receptor will be time and dose dependent. If the receptor is located within the central compartment, where there may be instantaneous equilibrium after a intravenous injection, the peak effect may occur immediately. If the drug-receptor complex occurs in a peripheral compartment, pharmacokinetic alterations in drug absorption and distribution may further alter the time course of a given drug effect.

The binding of a hormone or drug to its receptor does not instantly produce clinical effects. Instead a series of rapid biochemical events couple receptor binding to the ultimate clinical effect. These biochemical events are called *second messengers.* Alterations in second messenger coupling can alter the effectiveness of a drug.

Drug-receptor interactions

The attachment of drug to receptor involves physical bonding by a number of forces including ionic, van der Waals, and hydrogen bonding. The extent to which each of these contributes to the drug-receptor attraction varies from one drug to another and from one receptor to another. The number of bonds involved is usually multiple and their steric arrangement critical. Thus it is usual in the case of stereoisomers, for one isomer to be much more potent than the other. The relationship between receptor structure and bonding is the subject of a review.[34]

Drugs which stimulate receptors and induce a pharmacological effect are *agonists*; those which block them are *antagonists*. Agonist drugs induce an effect that mimics endogenous hormones or neurotransmitters when bound to a receptor. This effect may be stimulatory or inhibitory. The term *affinity* as related to a given agonist is a measure of the attraction between the given drug and the receptor.

Antagonist drugs inhibit or prevent receptor-mediated agonist effects by competing for receptor occupancy. A *competitive antagonist* can generally be displaced from the receptor complex by the administration of a receptor agonist if given in a large enough concentration, thus permitting the agonist to produce the expected effect. It is so called because the agonist and antagonist compete for the same receptor. A *non-competitive antagonist*, when bound to the receptor complex, will produce a loss of the expected effect that cannot be reproduced by the concurrent administration of a receptor agonist.

Drugs which possess both agonist and antagonist actions are referred to as *partial agonists*.

Clarke[35] proposed that tissue response was proportional to the number of receptor sites occupied. Once occupied, the receptor is unavailable for further stimulation.

On this theory the critical difference between agonists and antagonists lies in the rates of dissociation of the drug-receptor complexes. An agonist should dissociate rapidly, thus freeing the receptor for a fresh act of combination. An antagonist, on the other hand, should dissociate slowly, thus reducing the availability of receptors to agonist impacts. A partial agonist should lie between these extremes.

Drugs exert their effects on receptors according to their *efficacy*. Agonists are assumed to have variable degrees of efficacy and to induce changes in their receptors which, in turn, initiate pharmacological responses. By contrast, antagonists have zero efficacy and induce no changes in their receptors, but they prevent access of agonists and therefore inhibit the pharmacological responses. For a more detailed study of drug receptors, refer to a review by Rang.[36]

In addition to receptors, pharmacological agents act on other excitable cell membrane proteins such as ion channels. It has been shown that these channels mediate neural signalling by modulating ion permeability of electrically excitable membranes. Both the nicotinic acetylcholine receptor and the $GABA_A$ receptor, described in detail later, are receptor-ion channel complexes. The combination of a classic receptor protein plus an ion channel gives ligand-gated ion channels the unique ability to have drugs directly alter membrane permeability to cations.

Finally, drug effects are evaluated using dose-response curves (correlation between drug dose and measured effect), efficacy, potency, 50 percent effective dose (ED_{50}), 50 percent lethal dose (LD_{50}) and therapeutic index after stimulating excitable cell membrane proteins.

1.6 Biology of steroidal anaesthetics

There is now considerable evidence to suggest that the anaesthetic steroids elicit

their effect by binding allosterically and probably extracellularly to the cell membrane-bound $GABA_A$ receptor[37-43]

1.61 Gamma-amino-n-butyric acid (GABA)[44]

Gamma-amino-n-butyric acid (GABA) is a major inhibitory neurotransmitter in the vertebrate central nervous system and in invertebrate central and peripheral nervous systems. Inhibitory neurotransmitters cause increased chloride conductance of postsynaptic membranes and decreased neuronal firing rate.

GABA is formed in the CNS of vertebrate organisms by the decarboxylation of L-glutamic acid by the enzyme glutamic acid decarboxylase (GAD).

GABA is liberated from the terminals of specific inhibitory nerves in vertebrate and invertebrate species. GABA typically produces an increase in membrane permeability to chloride ions that can be measured as an increase in membrane conductance. It is in this way that this naturally occurring transmitter can counteract the depolarizing action of excitatory processes to maintain the polarization of a cell at an equilibrium level near that of its resting value, acting essentially as a chemical voltage clamp. GABA has been shown to exert a hyperpolarizing or inhibitory effect by this mechanism. However, if intracellular chloride concentrations should occur, GABA can produce a decrease in membrane potential or depolarization. The impression is that of looking at a highly restrained nervous system, the inhibitory neurons acting like reins that serve to keep the neuronal horses from running away.

Potentiation of the actions of GABA would, therefore, be expected to depress the CNS to produce anaesthesia. Organon Teknika Laboratories have shown that clinically relevant concentrations of the general anaesthetics alphaxalone,

propofol, etomidate, propanidid, and pentobarbitone potentiate the actions of GABA on $GABA_A$ receptors of tissue cultured cells.

1.62 Gamma-aminobutyric acid (GABA) receptors[45]

The sites to which the inhibitory neurotransmitter, GABA, binds to exert its inhibitory effect are the functional GABA receptors.[46]

In the vertebrate species, GABA receptors are found only in nerve cell membranes and are sufficiently widespread that most neurones in the CNS are believed to possess them. There are two major types of vertebrate GABA receptors. The $GABA_A$ receptor is distributed more widely than the $GABA_B$ receptor. The physiological importance of these receptors depends upon the availability of endogenous GABA to activate them. Activated $GABA_A$ receptors exert their most profound influence, (1) at cell bodies and dendrites to alternate excitatory postsynaptic potentials, thereby preventing initiation of an action potential, and (2) at nerve terminals to reduce transmitter release by an incoming action potential. At some nerve terminals, activated $GABA_B$ receptors also reduce transmitter release.

The primary classification of the two types of GABA receptors is made according to the sensitivity of the receptor to drugs. Characteristically $GABA_A$ receptors are activated most potently by muscimol (GABA receptor agonist), and rather less potently by GABA. $GABA_B$ receptors are activated most potentently by GABA and (-) baclofen, the latter being inactive on $GABA_A$ receptors. Muscimol is much less potent on $GABA_B$ receptors.

Because of the limited range of drugs available to act specifically at the $GABA_B$ receptor, knowledge of its physiological importance is derived solely from the use

of baclofen as a centrally acting muscle relaxant. The role of the $GABA_A$ receptor complex is much clearer. The $GABA_A$ receptors are hetero-oligomeric ligand-gated ion channel proteins,[47] which also serve as the receptor target of a cation for several categories of important drugs and perhaps endogenous modulators; the picrotoxin-like convulsants, the benzodiazepines, barbiturates and steroid anaesthetics; inhalational anaesthetics and alcohols may also act, at least in part, on some $GABA_A$ receptors.[48] It plays an essential part in the local control of neuronal excitability throughout the CNS.

At concentrations in the anaesthetic range alphaxalone, propofol, etomidate, propanidid, and pentobarbitone directly activate the $GABA_A$ receptor in addition to potentiating GABA. This secondary action may be important in contributing to anaesthesia. Demonstration of general anaesthesia being induced by $GABA_A$ agonists supports the notion that the $GABA_A$ receptor is a specific target for intravenous anaesthetics.

1.63 Mechanism of action

The enhancement of GABA-mediated inhibition of barbiturates involves prolongation of the GABA-activated chloride channel lifetime[49], direct opening of the chloride channel by barbiturates is also observed.[50] This cellular action of barbiturates on GABA chloride channels is a good candidate for a molecular mechanism of anaesthesia,[51] numerous nonbarbiturate anaesthetics share this action on GABA, including volatile anaesthetics,[52] ethanol[53] and alphaxalone and naturally occurring steroid analogs.[54] The GABA mechanism is the most likely to explain the action of steroid anaesthetics, and possibly the other anaesthetics as well.[55]

Modulation of chloride channel kinetics by steroids probably involves binding to the receptor-channel protein.[56] Nevertheless, an interaction with the lipid membrane has not been ruled out, and a chemically specific interaction with lipids that increases membrane fluidity has been observed for all anaesthetics, including steroids.[57] Fesik and Makriyannis[58] proposed from their NMR studies with hypnotically active and inactive steroids that perturbation of membrane lipid structure by the steroids may be transmitted to $GABA_A$ receptors. However, membrane fluidisation has not been proven to be associated with alteration of any biological function that could result in anaesthesia. The observed biological activity of the anaesthetic steroids at nanomolar concentrations[59] and their stringent SARs including stereospecificity favours the modulation of $GABA_A$ receptors via a specific binding site. Thus proteins, e.g., the GABA chloride channel, are excellent candidates for being receptors for anaesthetics. These actions seem to be selective (occurring at concentrations at which other receptor/channels are unaffected) and show a high affinity and stereoselectivity.

Majewska *et al*[54] reported that metabolites of progesterone and desoxycorticosterone (anaesthetically active steroids) alter radioligand binding to the GABA receptor-chloride ionophore complex in rat brain in a manner that closely resembles the action of hypnotic barbiturates. These steroids share the ability of barbiturates to enhance chloride-dependent responses to GABA or the GABA agonist muscimol.[60]

Harrison *et al*[38] illustrated several similarities between the actions of the active steroids and those of the hypnotic barbiturates in parallel radioligand binding assays. First, barbiturates and steroids that enhance $GABA_A$ receptor function in the CNS displace or inhibit the binding of the convulsant chloride channel ligand [^{35}S]-*t*-butyl bicyclophosphorothionate ([^{35}S]-TBPS) with similar coefficients. Second, both barbiturates and steroids stimulate the binding of the benzodiazepine,

flunitrazepam, as well as that of the GABA agonist muscimol.[61] Furthermore, like barbiturates and benzodiazepines the steroids potentiate inhibitory synaptic transmission mediated by GABA.[62] All of the findings suggested a common site or mechanism of interaction of steroids and barbiturates with the GABA receptor complex. The active steroids may act as an endogenous 'barbiturate-like substances' at the barbiturate receptor site on the $GABA_A$ receptor complex.[54]

SAR for activity and the nature of the "steroid site"

There are exacting steric requirements for steroid activity at the $GABA_A$ receptor complex. All of the active compounds found to potentiate GABA-activated chloride conductance responses in the present experiments have a 3α-hydroxyl, a ketone moiety at C(20) and a reduced pregnane skeleton.[38] In addition, all of these active compounds are active in displacing TBPS binding. Conversely, steroid structures inactive in modulating GABA-activated chloride conductance are inactive in TBPS binding assays. These observations suggested a high degree of structural discrimination at the steroid "binding sites".

Structure-activity studies showed a high degree of structural and stereochemical specificity for the interactions between steroids and the GABA receptor complex in neuronal membranes, such as might be expected for a membrane protein binding site.[38] However, in the case of these highly lipophilic steroids other interpretations are also plausible.

It has also been demonstrated that anaesthetic steroids of the 3α-hydroxy type produce profound membrane disordering, whereas 3β-hydroxy compounds produced no such effect. Thus, there may be no "specific" binding sites for steroids as such binding is normally imagined, and the interaction between steroids and the GABA receptor complex may arise as a consequence of the interaction between the steroid molecules and the lipid membrane.

Alphaxalone was found to be effective in potentiating responses to GABA,[63] whereas the β-hydroxy isomer of alphaxalone (betaxalone) was ineffective in potentiating responses to GABA[64] and at relatively high concentrations acted as an antagonist.[63] The 5α- and 5β-pregnane steroid nuclei of active parent steroids are both active. The 5α-analog of hydroxydihydroprogesterone was found to be quite active in enhancing GABA function and inhibiting binding of the chloride channel ligand, [^{35}S]TBPS.[34,54,65]

Fesik and Makriyannis proposed[58] that anaesthetic steroids are accomodated lengthwise between two fatty acyl chains of membrane phospholipids, like cholesterol. The presence of a polar 17-substituent (in addition to a 3α-hydroxyl) in anaesthetic steroids however, makes the cholesterol-like interaction with phospholipids very unfavourable. Rather the two polar groups of hypnotic steroids may interact with polar head groups of phospholipids or polypeptides, while their hydrophobic backbone interacts with the methylene residues of the fatty acyl chains near the membrane surface. On the strength of these considerations, it is reasonable to propose that anaesthetic steroids may have two points of contact (probably through hydrogen bonding) with $GABA_A$ receptors through the two polar functional groups and their hydrophobic moieties remaining in contact with the fatty acyl chains of phospholipids surrounding $GABA_A$ receptors. Such interactions could demand stringent structural requirements for the ligands, because their two polar groups have to be positioned in a particular spacial orientation. One may then observe the disappearance or reduction of anaesthetic actions with some chemical modifications of the steroids that lead to distortion of the relative spacial configurations of the two polar groups. Such modifications may include not only the residues near the functional groups but also introduction of hydrophilic groups in the hydrophobic backbone of the steroid, which cause destabilisation of their hydrophobic interactions and eventually lead to profound

changes in overall configuration of the compounds.

Despite the aforementioned similarities, studies evaluating the anaesthetic effect of binary mixtures of the synthetic steroid alphaxalone and hyponotic barbiturates suggest they do not act through a common site or mechanism.[66] On [^{3}H] muscimol binding[40] the effects of pentobarbital and alphaxalone were similar and clearly additive, but they potentiated each others inhibitory effect on TBPS binding.[39] In contrast the stimulatory effects of combinations of the barbiturates, pentobarbitone and secobarbitone or the hypnotic steroids, alphaxalone and 5β-pregnan-3α-ol-20-one were not additive.[40] On the chloride flux assay the barbiturate and steroid direct activations were clearly synergistic, giving very large responses together in the absence of muscimol, despite small direct effects on their own,[39] i.e., alphaxalone potentiated barbiturates and/or *vice versa.* It has previously been demonstrated that the direct agonist action of alphaxalone, 5β-pregnan-3α-ol-20-one or 5α-pregnan-3α-ol-20-one is potentiated by the barbiturate, phenobarbitone,[63,67] and 5β-pregnan-3α-ol-20-one was found to enhance greatly the amplitude of membrane currents elicited by pentobarbitone.[40] This is consistent with different sites or mechanisms of action and suggests the existence of a unique binding site for the steroid in $GABA_A$ receptors.

Evidence supporting separate sites of action for the barbiturates and steroids was obtained by Kirkness and Turner,[68] who showed that the enhancement of benzodiazepine binding *in vitro* by the two types of compounds was differentially sensitive to inhibition by phenobarbital, picrotoxin, and avermectin, as well as additive.

Whatever the nature of the barbiturate/steroid binding site(s), the demonstration of the potent modulation of $GABA_A$ receptors by endogenous steroids offers the future prospect of the development of new steroidal general anaesthetics, and a

better understanding of the influence of the endocrine system on central nervous system function.

1.7 Structural requirements of steroidal anaesthetics

It must be noted that when more than one group is introduced into a parent structure, the overall effect of the groups on activity is not necessarily additive and may show a substantial reduction or increase in the "expected" activity. It was clear from the work of Pfizer,[69] Glaxo[7,10] and Syntex[12] groups that for high anaesthetic activity within the steroidal series the 3α-hydroxyl and 17β-acetyl groups are essential moieties, with both the 5α- and 5β-compounds affording potent hypnotics.

Organon Teknika Laboratories backed this up by showing that in both the 5α- and 5β-series, 3α-hydroxypregnan-20-ones exhibit very good hypnotic activity, whereas the 3β-hydroxy analogues do not.[7] If the shape of ring A, and more importantly the relative position of the 3-hydroxyl, is indeed so important, it is surprising that compounds of the 5α- and 5β-series show similar activity. The great difference in shape can be seen by comparing the 5α-compound with the 5β all-chair conformation (Figure 12). The 3α-hydroxyl group in both the 5α- and 5β-series is on the underside of the steroid nucleus, although the 3α-hydroxyl group in the 5β-series is carried much lower than in the 5α-series.

However, a similar position of the 3α-hydroxyls in the 5α- and 5β-analogs is required for each isomer to possess similar activity. The only conformation in which the 5β-isomer carries the 3α-hydroxyl in roughly the same position as in the

5α-series, is the unfavoured one with both the A and B rings as boats (Figure 13),

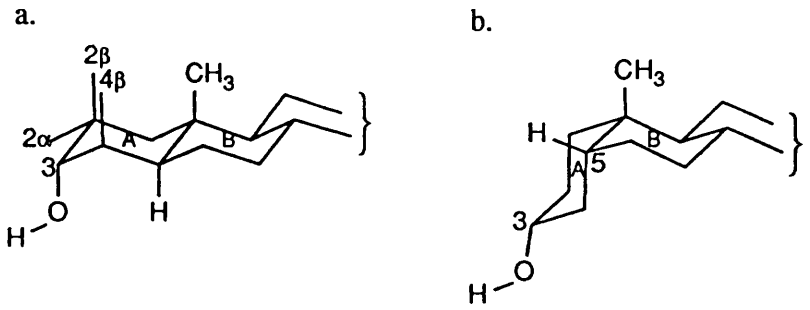


Figure 12. a) The 5α-conformation, A\B *trans*, b) the 5β all-chair conformation, A\B *cis*.

christened the *catamaran* by Phillips.[70] To account for similar activity of the 5α- and 5β-series it is proposed that this is the likely conformation when it binds to the $GABA_A$ receptor, or a conformation very close to it.

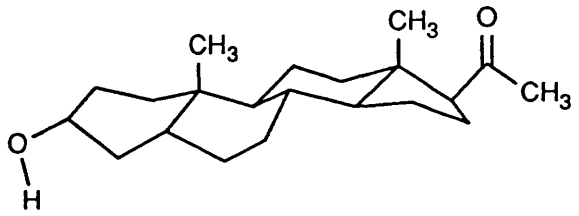
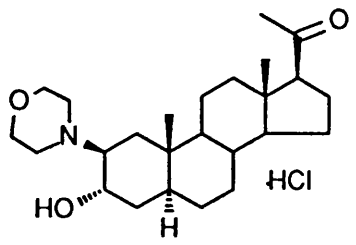
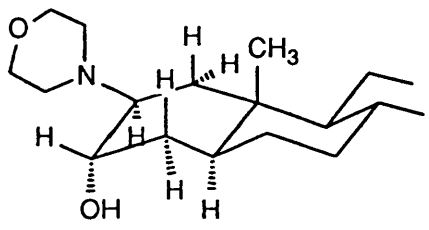


Figure 13. The A and B ring dispositions when both assume the boat conformation in the 5β-series. The "catamaran".

Similar to the *catamaran* conformation of the 5β-series, Organon Teknika suggested that the likely conformation of ring A in Org. 9843 (anaesthetically potent steroid) when it binds to the $GABA_A$ receptors, is the α-boat form, or a conformation very close to it (Figure 14).



ORG. 9843



Ring A; α - boat

Figure 14. Possible conformation of ORG. 9843 at the $GABA_A$ receptor.

X-ray analysis of crystalline ORG. 9843 reveals that ring A is in the chair conformation, in which the 2β-morpholinyl and the 3α-hydroxyl groups are *trans* diaxial, whereas the infrared spectrum of the compounds in methylene chloride shows that ring A is mainly in the β-boat form (in which there is strong hydrogen bonding between the hydroxyl group and the nitrogen atom) - with the hydroxyl group nearly co-planar with the plane of the steroid (Figure 15). Neither of these conformations are likely to be present when Org. 9843 binds to the $GABA_A$ receptor, when it is probably in the α-boat form with the hydroxyl group below the plane of the steroid and nearly orthogonal to it.

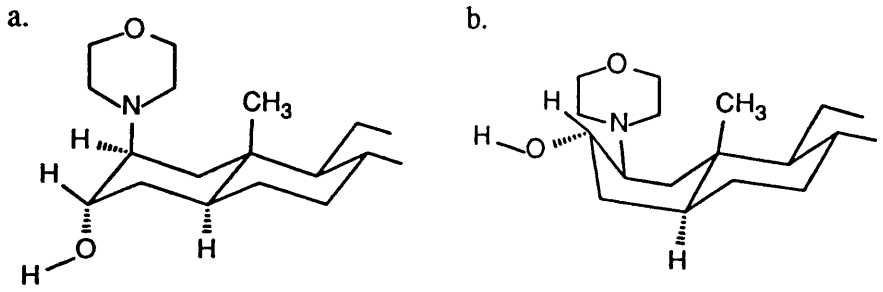


Figure 15. a) X-ray of ORG. 9843, with ring A in chair conformation,
b) ORG. 9843 in solution, with ring A in the β-boat form.

On the basis of the structure-activity data available the following modifications in structure were suggested.

Synthesis of novel secosteroids

Two essential features for steroid actions at the $GABA_A$ receptor appear to be the presence of a 5α- or 5β-reduced tetracyclic steroid skeleton and a hydroxy group in the 3α-position.[71] Although a number of studies have examined steroid structure-activity issues,[38,41,71] there is little information regarding the necessity of the tetracyclic steroid nucleus.

In an effort to examine the importance of the tetracyclic structure, as well as to create potential steroid site ligands with greater molecular flexibility,

Rodgers-Neame *et al*[72] synthesized a series of substituted benz[e]indenes (BIs). The BIs are tricyclic molecules that can be envisioned as steroid-like but that contain only a portion of the steroid A-ring. Rodgers-Neame *et al* examined the most effective of these compounds synthesized, BI-1 (Figure 16) and compared its action on GABA-gated chloride currents with responses produced by 3α-hydroxy-5α-pregnan-20-one, 3α-OH-DHP (Figure 16).

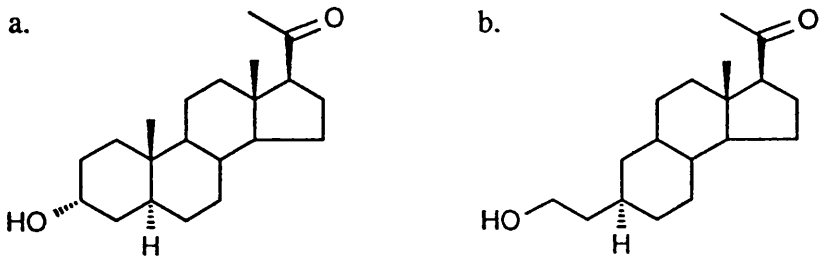


Figure 16. a) 3α-OH-DHP, b) the benz[e]indene, BI-1.

Both compounds were found to be effective potentiators of GABA responses, however, unlike 3α-OH-DHP, BI-1 is much less effective at directly gating chloride channels in the absence of GABA. Similar actions were found of other BIs, suggesting that these effects are characteristic of this class of drugs.

The failure of benzodiazepine and picrotoxin site antagonists to block the BI-1 effects, coupled with the inability of BI-1 to potentiate GABA currents in the presence of high concentrations of 3α-OH-DHP and *vice versa*, suggests that the BI acts at the steroid site on $GABA_A$ receptors. Clearly, removal of the steroid A ring does not eliminate GABA-potentiating ability but markedly alters the ability to gate chloride channels directly. It is possible that the GABA-potentiating and direct chloride channel-gating effects may be mediated by separate sites.

Org.9843 is a relatively rigid structure, diaxially substituted at C(2) and C(3). Subtle conformational changes in ring-A (or any of the other rings for that matter)

may be reflected in changes in (a) potentiation of GABA, or (b) activation of the $GABA_A$ receptor, and hence anaesthetic activity. Thus the synthesis of three families of structurally mobile variants of Org. 9843 is of potential interest (Figure 17).

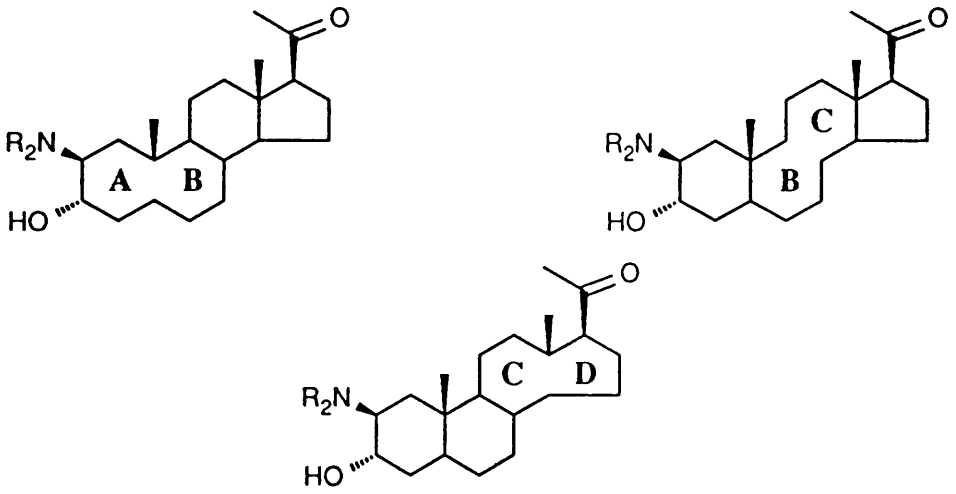


Figure 17. Structures of proposed secosteroids.

This thesis involves the synthesis of the first of these families, the A,B-secosteroids (or 5,10-secosteroids). The initial target was the 3α-hydroxy A,B-seco system. If anaesthetic properties were detected, the prime target in a further project would be a 2β-morphilino-3α-hydroxy variant to mimic Org. 9843 and to further probe the conformational requirements at the receptor. Chapter 2 will therefore describe approaches to the initial target.

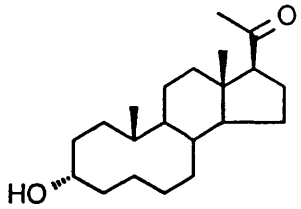
RESULTS AND DISCUSSION

DISCUSSION

2.1 Aims and objectives

The objective of this project was the synthesis of new families of secosteroids designed to probe conformational requirements for inducing and maintaining general anaesthesia after intravenous administration.

The primary target was the ring A,B-secosteroid (**107**).



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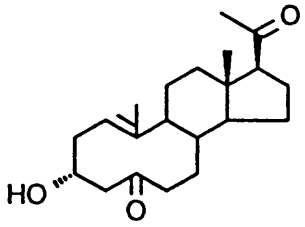
Intermediate targets included:

a. the preparation of 3α-hydroxy-5,10-secopregnen-20-ones, **60** and **65**,



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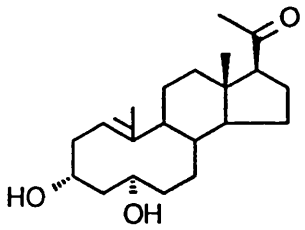
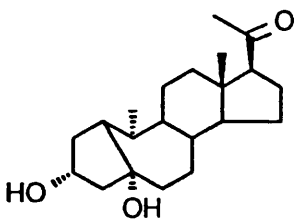


65

and b. the synthesis of 3α-hydroxy-5(10→1βH)*abeo*pregnan-20-ones, **117** and **128**.



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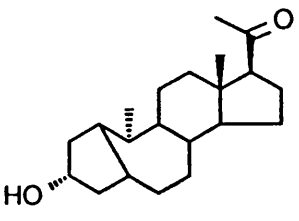

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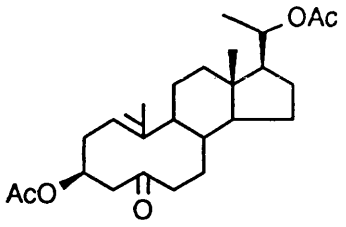
A key intermediate in the preparation of the final secosteroids, **60** and **65**, and the *abeo*steroids, **117** and **128** would be the 5,10-secopregn-1(10)-en-5-one (**39**).



39

The strategy employed in the preparation of the 5,10-secopregn-1(10)-en-5-one (**39**) would involve the oxidative β-fragmentation of 5α-hydroxy pregnanes using ceric ammonium nitrate as the oxidising agent, or irradiating with a 500W tungsten lamp in the presence of mercury oxide-iodine.

In order to reduce both the $\Delta^{1,10}$ double bond and the 5-carbonyl moiety from the 10-membered ring to give the C(3)-hydroxy-saturated macrocycle, as in **107**, the double bond would have to be 'protected' as the corresponding epoxide.

Structure-activity relationships reported in the literature (see Appendix, review of steroidal anaesthetics) have shown that the 3α-hydroxyl and 20-carbonyl moieties are necessary for anaesthetic potency. These stereospecific functional groups would be incorporated into the final synthetic products. Since all starting steroids possessed a 3β-hydroxyl group they would at some point, usually after ring opening, have to undergo a Mitsonubu reaction to invert the stereochemistry at C(3). The 20-carbonyl moiety would be achieved via a series of selective deprotections and protections at both ends of the steroid molecule.

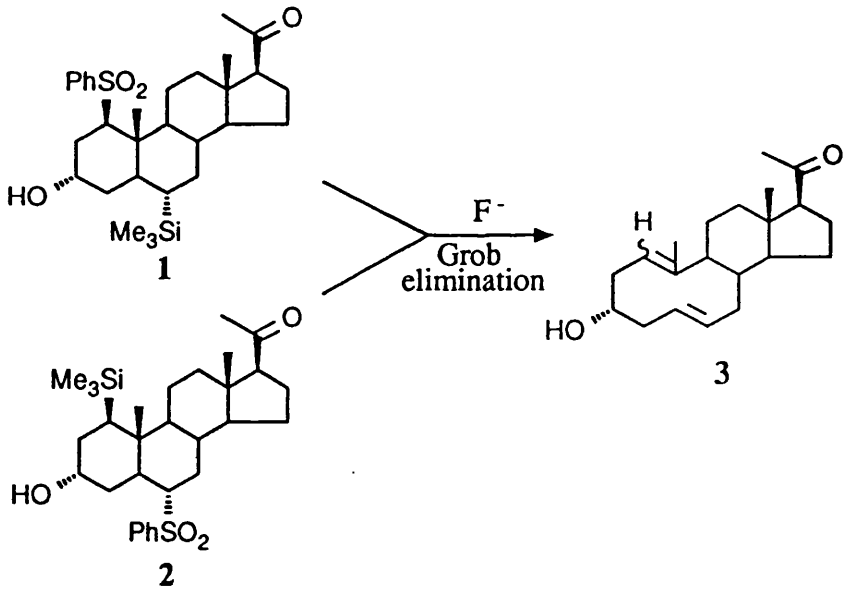
It must also be noted that throughout this report all of the chemistry performed after AB-ring opening, to give steroids of the type **39**, was carried out on the $\Delta^{1,10}$

trans-isomer of the 5,10-secosteroid, and at no point was the *cis*-isomer used. Hydrogenation of the $\Delta^{1,10}$ double bond would be carried out at a later stage in the synthesis of **107**.

2.2 Attempted preparation of 5,10-secopregnan-3α-ol-20-one (107)

Via Grob fragmentation

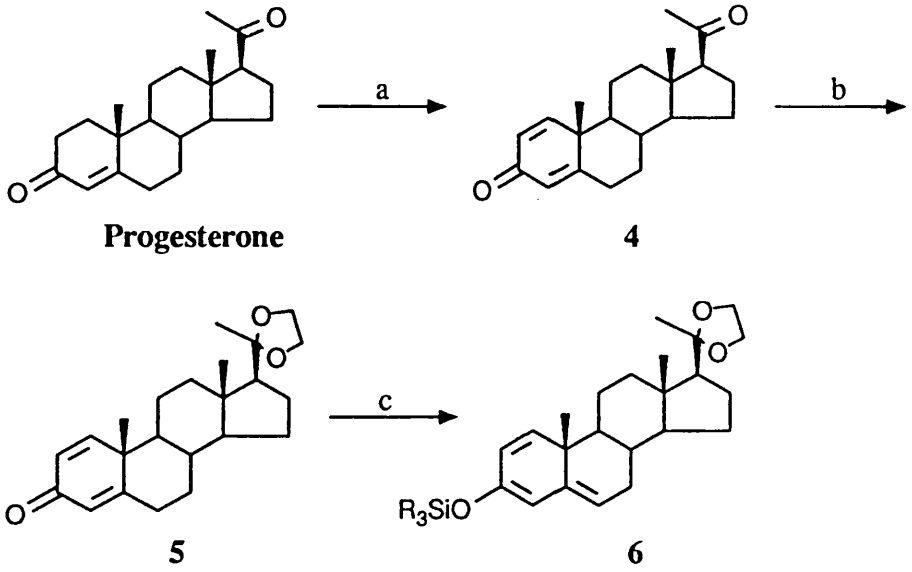
Initial attempts focussed on the Grob rearragement[73], whereby the bonds of the substituents in the C(1) and C(6) positions were perfectly aligned geometrically for a fluoride-mediated ring-opening to give the macrocyclic diene (3) as the first member of the target series (Scheme 1).



Scheme 1

It was proposed that the dienone (**5**) be converted to silyloxytrienolates (**6**). Formation of the conjugated dienone (**4**) via DDQ oxidation[74] of progesterone allowed the selective protection of the 20-carbonyl to give 20-ethylenedioxypregna-1(2),4(5)-dien-3-one (**5**). However attempts to form the silyloxytrienolate (**6**) (R = Me_3, $^tBu(Me)_2$), failed, presumably due to the stability of the conjugated

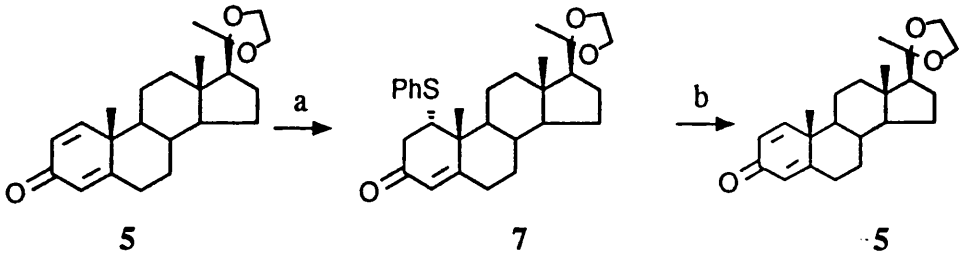
dienone, as only starting material was recovered (Scheme 2).



Reagents and conditions: a. DDQ, $PhCH_3$, Δ, 24 hr, b. $HOCH_2CH_2OH$, $HC(OEt)_3$, p-TSA; c. R_3SiCl, base.

Scheme 2

The conjugation was disrupted by the preferential addition of thiophenol at C(1) to give 1α-phenylthio-20-ethylenedioxypregn-4(5)-en-3-one (**7**)[75] (Scheme 3). Unfortunately reaction of the derivative (**7**) with base and silyl chloride (both trimethyl and *tert*butyldimethylsilyl chloride) gave only the elimination product (**5**), and not the desired phenylthiosilyloxy dienolate.

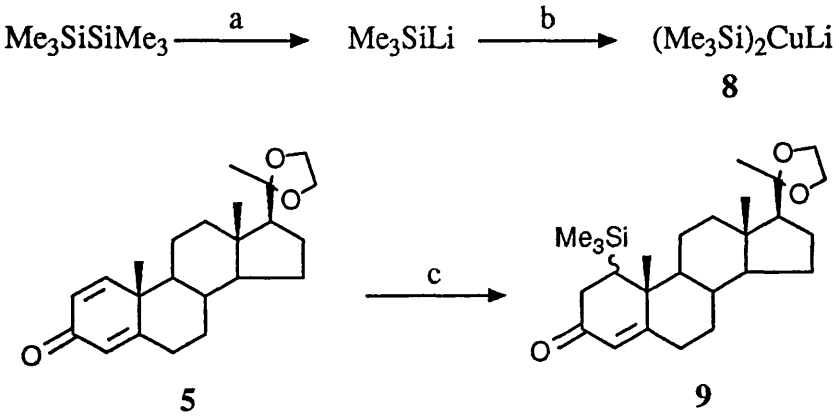


Reagents and conditions: a. PhSNa, Et_3N, THF; b. R_3SiCl, base.

Scheme 3

The 1-trimethylsilyl steroid (**9**) was prepared according to the method of Fleming

et al[76] by the conjugate addition of lithium bis(trimethylsilyl)cuprate (**8**) to the dienone (**5**) (Scheme 4). The analogous reaction with lithium bis(*tert*butyldimethylsilyl)cuprate failed due to the steric bulk of the silyl cuprate, and in this case only starting material was recovered.



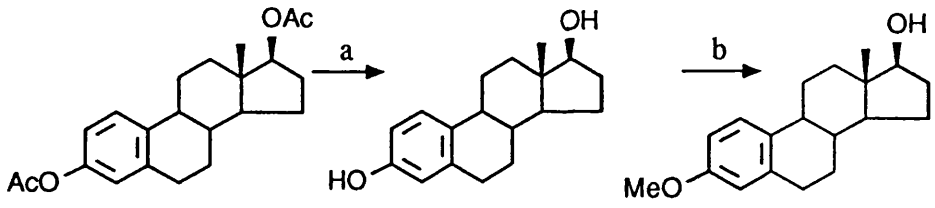
Reagents and conditions: a. MeLi, HMPA; b. CuCN, THF; c. **8**, THF.

Scheme 4

However, 1-trimethylsilyl-20-ethylenedioxypregn-4(5)-en-3-one (**9**), a mixture of 1α- and 1β-isomers, was formed in a disappointing yield of 25%. This, coupled with the promising look of other routes led to the discontinuation of this approach.

Via ozonolysis of 19-nor-3-ethylenedioxypregn-5(10)-en-20β-ol (**20**)

Deprotection of β-oestradiol diacetate (**10**) using potassium carbonate in methanol gave a quantitative yield of β-oestradiol (**11**), which was selectively methylated at the 3-hydroxyl, in a yield of 73%, using methyl iodide and sodium hydride (Scheme 5), to give **12**.

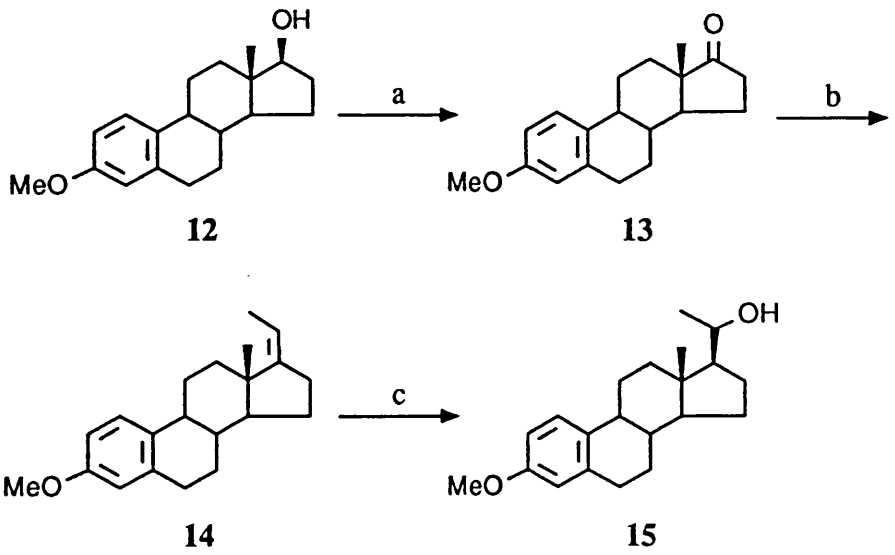


10 **11** **12**

Reagents and conditions: a. K_2CO_3, MeOH; b. MeI, NaH, THF.

Scheme 5

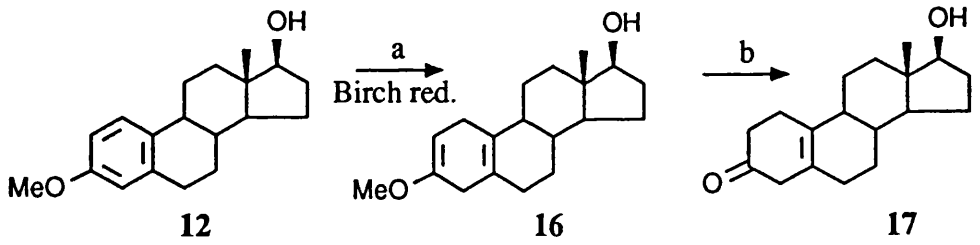
Selective protection of the 3-hydroxyl of **11** would allow the appropriate side chain to be attached at the 17-position. This would be achieved by a 'Wittig' type reaction. Before this could be achieved the 17-hydroxyl was oxidized. Carrying out the oxidation with PCC[77] (pyridinium chlorochromate) or Jones' reagent,[78] CrO_3/H_2SO_4, gave poor yields of 40% and 20% respectively. This low yield could possibly be attributed to the formation of aryl-chromium complexes, which are quite common. However, this was not investigated any further as carrying out the reaction with 5 mol% tetrapropylammonium perruthenate (TPAP)[79] and 1.5 equiv 4-methylmorpholine N-oxide (NMO) in methylene chloride gave a good yield of **13**, 77%, and the reaction offered a convenient work-up (Scheme 6). The alkene (**14**) was formed via an efficient and very convenient Wittig reaction[80] using (ethyl)triphenylphosphonium bromide, dimethyl sulphoxide (DMSO) and sodium hydride. The methylsulphinyl carbanion is first formed from dimethyl sulphoxide-sodium hydride at 75-80°C. The solution was cooled and treated with the phosphonium salt to give the alkylidene phosphorane, or Wittig reagent, almost instantaneously, as a dark red solution. Subsequent addition of the 17-ketone (**13**), and appropriate reaction time and temperature completed the process. Hydroboration of the alkene (**14**) yielded the 17-hydroxyl steroid (**15**).



Reagents and conditions: a. TPAP, NMO, CH_2Cl_2; b. $CH_3CH_2PPh_3Br$, DMSO, NaH, Δ; c. i. B_2H_6, THF; ii. NaOH, H_2O_2.

Scheme 6

Modification of the aromatic ring-A in 3-methoxyoestra-17β-ol (**12**) gave the $\Delta^{5,10}$ 3-keto-steroid (**17**) (Scheme 7).

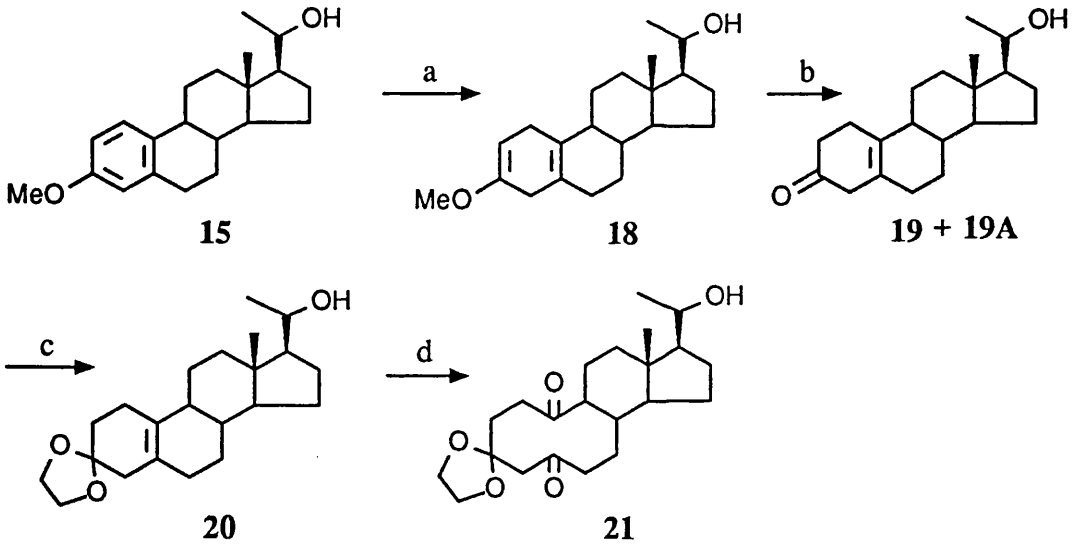


Reagents and conditions: a. NH_3, Li, EtOH, Et_2O; b. $(COOH)_2$, MeOH.

Scheme 7

Similar chemistry was applied to 3-methoxy-17β-(1-hydroxyethane)-oestrane (**15**), with similar results. Birch reduction[78,81] of the aromatic ring-A of **15** gave the diene (**18**), which underwent oxalic acid promoted deprotection[78] to give the $\Delta^{5,10}$ 3-keto steroid (**19**). A small amount, 10%, of the conjugated product, the $\Delta^{4,5}$ 3-ketone (**19A**), was also isolated. Conventional ethylene acetal derivatization of

the 3-carbonyl using ethylene glycol and catallytic p-TSA gave **20**. Care was used in this reaction as over-refluxing resulted in the double bond shifting to give the $\Delta^{4,5}$ steroid. Hence the $\Delta^{5,10}$ steroid (**20**) was only isolated in a 50% yield (Scheme 8). The next step was the ozonolysis[82] of the $\Delta^{5,10}$ bond of **20** to give the dione (**21**). This was carried out with disappearance of starting material giving a streaky product by tlc, suggesting the formation of an ozonide had been achieved. However, the attempted decomposition of the ozonide using powdered zinc and acetic acid failed. It may be the case that the zinc/acetic acid mixture was acidic enough to deprotect the 3-acetal, and thus give a complex reaction mixture. It was at this point that this approach was discontinued.



15 **18** **19 + 19A**

20 **21**

Reagents and conditions: a. NH_3, Li, EtOH, Et_2O; b. $(COOH)_2$, MeOH, $HO(CH_2)_2OH$, p-TSA, $PhCH_3$, Δ; d. i. O_3, EtOAc, ii. Zn, HOAc.

Scheme 8

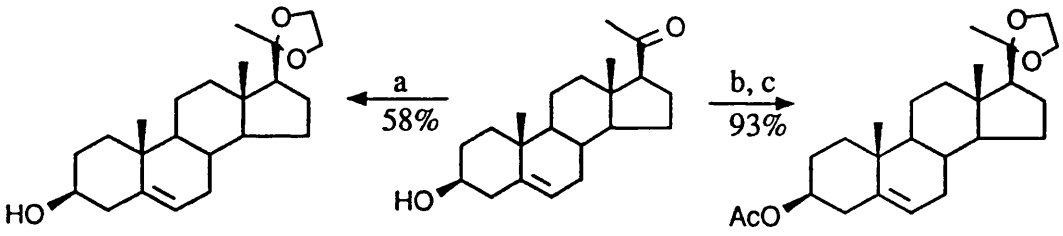
2.3 Preparation of 3α-hydroxy-5,10-secopregnen-20-ones

Synthesis of 5,10-secopregnenes

It has been reported that 5α-(or5β)hydroxy pregnanes undergo oxidations resulting

in the fragmentation of the C(5)-C(10) bond to give the A,B-secosteroid.[83] This oxidation may be brought about by oxidising agents such as lead tetraacetate,[84] ceric ammonium nitrate[85] or alternatively using photochemical methods.[86]

Protection of the 20-carbonyl of pregnenolone was best achieved in a 58% yield by co-distilling ethylene glycol and water from the reaction mixture.[87] The poor yield of this 'protection' was due to the relative insolubility of pregnenolone in the reaction solvent. Protection of the 3-hydroxyl as the acetate, to give the readily soluble 3β-acetoxypregn-4(5)-en-20-one (**23**), prior to ketalisation of the 20-carbonyl afforded an overall yield of 93% for **24** (Scheme 9).



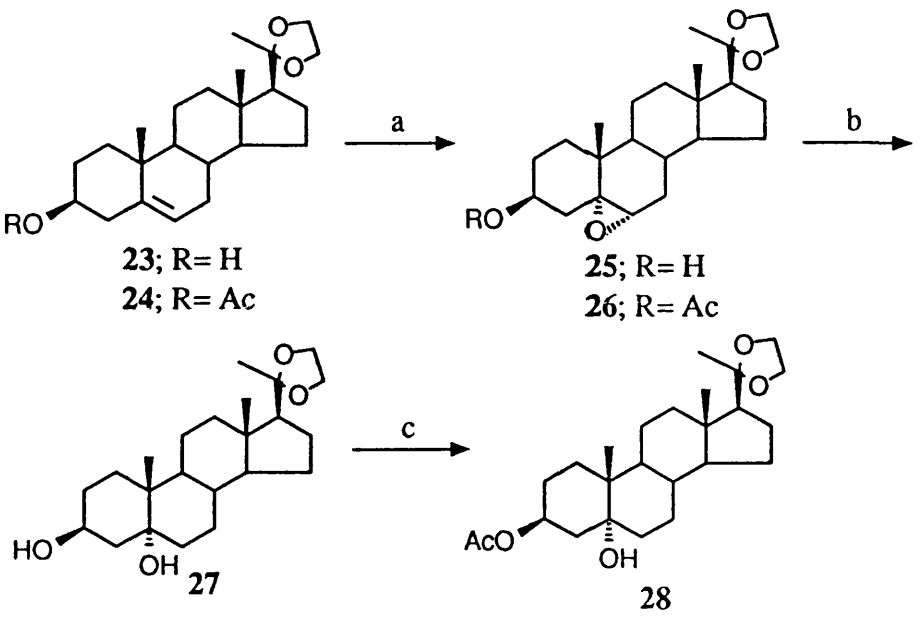
23 **Pregnenolone** **24**

Reagents and conditions: a.$HOCH_2CH_2OH$, p-TSA, Δ; b. Ac_2O, DMAP, Et_3N, CH_2Cl_2; c. $HOCH_2CH_2OH$, $HC(OEt)_3$, p-TSA, Δ.

Scheme 9

Epoxidation of the $\Delta^{5,6}$ double bond[86] of **23** gave the 5α,6α-epoxide (**25**), which upon treatment with lithium aluminium hydride was regioselectively opened to give the 5α-hydroxy steroid (**27**)[86] in a quantitative yield. Similar epoxidation of the $\Delta^{5,6}$ double bond of **24**, followed by regioselective ring-opening, and simultaneous deprotection of the 3-acetate of **26** gave the 5α-hydroxy steroid (**27**). Regiospecific acylation of the 3-hydroxyl of **27** gave the monoacetate **28**, the tertiary 5α-hydroxyl being too hindered to react (Scheme 10).

Ring-A,B-opening of **28** was investigated. However, the 20-acetal proved to be



23; R= H
24; R= Ac

25; R= H
26; R= Ac

27

28

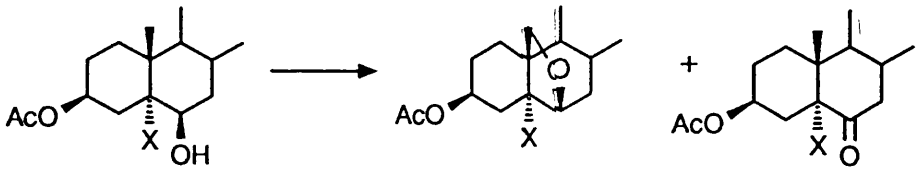
Reagents and conditions: a. m-CPBA, CH_2Cl_2; b. $LiAlH_4$, THF; c. Ac_2O, py.

Scheme 10

too labile thus resulting in, in most cases, a 5,20-dione. This was disappointing as selective deoxygenation of the 5-carbonyl in the presence of the 20-carbonyl would be virtually impossible as the carbonyls would be chemically indistinguishable. Also selective protection of either carbonyl in the presence of the other would hold out similar problems.

Tetravalent cerium (ceric ion) is a powerful oxidant, and has been shown to possess the ability to oxidize a wide variety of organic compounds.[88] It has been reported that 6β-hydroxy steroids undergo smooth oxidative cyclization to give the corresponding C(6β,19)-oxido compounds in fair to good yields, in addition to some ketone formation (Scheme 11), after reaction with ceric ammonium nitrate in aqueous acetonitrile or aqueous acetic acid at 80°C.

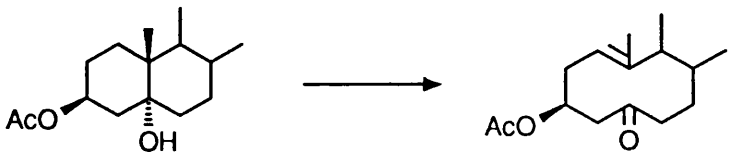
Under the same experimental conditions the tertiary, 5α-hydroxy steroid underwent smooth fragmentation to give the 5,10-secosteroid in high yield (Scheme 12). The reaction was almost instantaneous, occurring within 3 minutes,



X = H, Cl, or Br

Scheme 11

and was accompanied by a colour change from dark red to colourless.

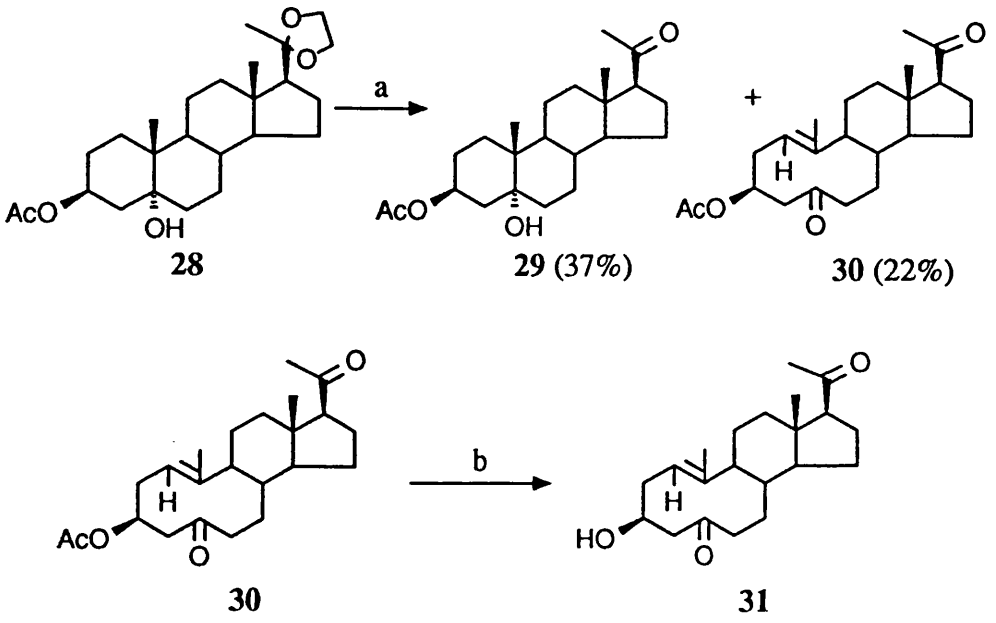


Scheme 12

The initial step in the ceric ion-induced reactions is probably an inner sphere process.[89] The dark red alcohol-cerium complex formed initially may dissociate with transfer of one electron from the alcohol to the metal ion, and the resulting electron deficient oxygen may then abstract hydrogen from an adjacent δ-carbon atom. Water is required for a successful reaction, in the absence of water complex reaction mixtures result.

The reaction conditions were applied to **28** to give both the deprotected 20-ketone (**29**) and the E-isomer of the deprotected 20-keto secosteroid (**30**) in the yields shown (Scheme 13). Treatment of **30** with potassium carbonate in methanol gave the 3β-hydroxy secosteroid (**31**), in a yield of 87%, after just 10 minutes at room temperature. Hydrolysis of the 20-ketal of **28** may also be achieved using an aqueous solution of sulphuric acid in acetone, to give the 20-keto steroid (**29**).

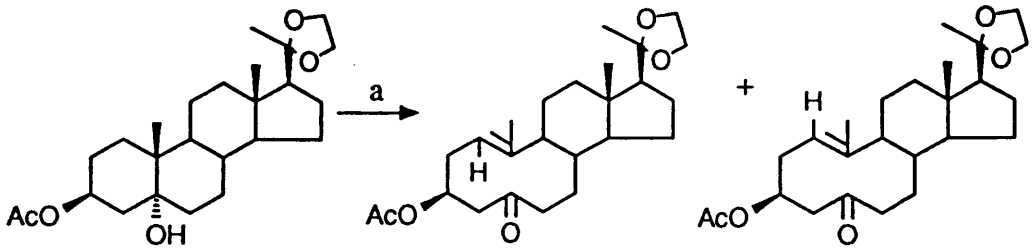
Mihailović *et al.* reported[83] that irradiating the 5α-hydroxy steroid with a 500W tungsten lamp in the presence of mercury oxide and iodine gave rise to a mixture



Reagents and conditions: a. $(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O, 80°C; b. K_2CO_3, MeOH.

Scheme 13

of (E)- and (Z)-A,B-secosteroids.[90] In the case of the 5α-hydroxy steroid (**28**) a mixture of the (E)-secosteroid (**32**) and the (Z)-secosteroid (**33**) was formed in yields of 14% and 8% respectively. Together with this, 8% of starting material (**28**) was isolated as well as 26% of the C(20) deprotected (E)-secosteroid (**30**) (Scheme 14).

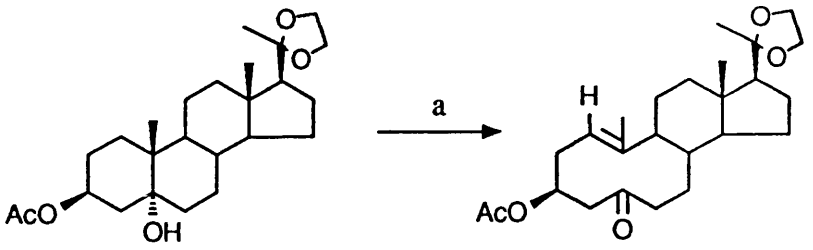


Reagents and conditions: a. HgO, I_2, CCl_4, *hυ*.

Scheme 14

Lead tetraacetate as oxidant,[84] in the presence of calcium carbonate, gave a disappointingly low yield of 10% of the (Z)-secosteroid (**33**). No (E)-secosteroid

was detected (Scheme 15).



28 **33** (10%)

Reagents and conditions: $Pb(OAc)_4$, $CaCO_3$, $PhCH_3$, Δ, 48hr.

Scheme 15

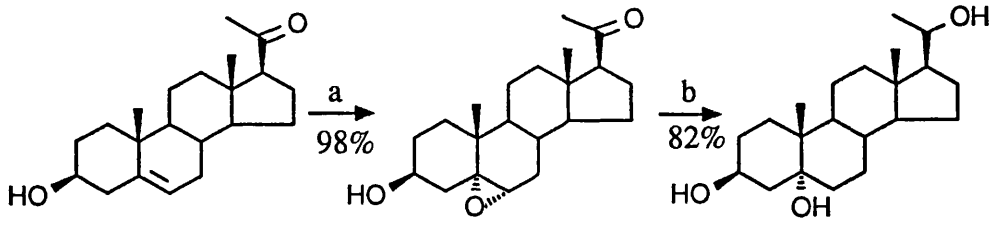
In summary, employing ceric ammonium nitrate as oxidant gave rise to the E-isomer, irradiating in the presence of mercury (II) oxide and iodine gave a mixture of E- and Z-isomers, whereas lead tetraacetate as oxidant gave only the Z-isomer. Whilst reaction with lead tetraacetate gave the desired product it gave a poor yield. The conditions employed in the oxidation with ceric ammonium nitrate and the ring-opening with irradiation proved to be too acidic for the acid labile 20-acetal. Hence, a more acid stable 20-hydroxyl protecting group was required.

The activity of ceric ammonium nitrate will be discussed further in chapter 2.5, which describes preparation of 3α-hydroxy-5(10→1βH)*abeo*pregnan-20-ones.

Providing it would be possible to selectively deprotect at C(3), the synthesis of a 3,20-diacetoxy-secosteroid could provide an answer to the problem of the acid labile 20-acetal. The acetate protecting group is stable to acid and is hydrolysed readily by treatment with a base,[91] such as potassium carbonate.

Epoxidation, as before, of the $\Delta^{5,6}$ double bond of pregnenolone gave the 5α,6α-epoxide (**34**). It is known that 20-ketones may be reduced with sodium borohydride in methanol to give the 20β- and 20α-hydroxyderivatives in the high

ratio of *ca* 92:8.[92] Lithium aluminium hydridle has been shown to be much less stereoselective.[93] However, reduction of simple pregnan-20-ones with sodium borohydride gives only *ca* 80% of 20β-alcohol[94], whereas reduction with lithium aluminium hydride gave quantitative yields of the 20-alcohol. It was not important if a mixture of 20α- and 20β-alcohols was obtained, as both hydroxyls would be oxidized to the 20-carbonyl at a later stage. However, the 20β-hydroxy steroid could be isolated in an excellent yield, *ca* 97%, after recrystallisation. The 20α-isomer could be obtained as the major component by reduction of the 20-carbonyl with lithium in liquid ammonia-THF.[92] Simultaneous reduction of the 20-carbonyl and regiospecific ring opening of the epoxide **34** gave the 3β,5α,20β-triol (**35**)[92] after refluxing in the presence of lithium aluminium hydride (Scheme 16). The overall yield of the triol (**35**) from pregnenolone was 80%.

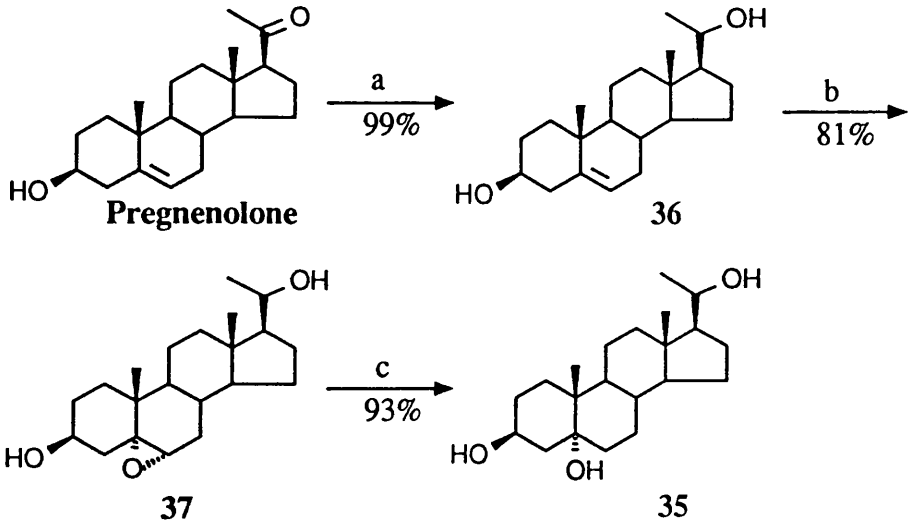


Pregnenolone **34** **35**

Reagents and conditions: a. m-CPBA, CH_2Cl_2; b. $LiAlH_4$, THF, Δ.

Scheme 16

To avoid using large amounts of lithium aluminium hydride an alternative synthesis was used to prepare the triol (**35**) in quantities in excess of 100g. The 20-carbonyl of pregnenolone was reduced to give the diol (**36**), prior to epoxidation. Epoxidation of the $\Delta^{5,6}$ double bond, followed by regiospecific epoxide-opening with lithium aluminium hydride was carried out as previously described to give the triol (**35**) (Scheme 17). The overall yield of **35** from pregnenolone was 75%.

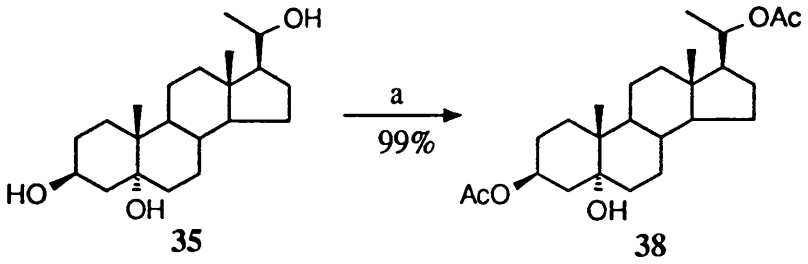


Pregnenolone 36 37 35

Reagents and conditions: a. $LiAlH_4$, THF; b. m-CPBA, CH_2Cl_2; c. $LiAlH_4$, THF, Δ.

Scheme 17

Selective protection of the 3- and 20-hydroxyls as acetates was affected by stirring the triol (**35**), with acetic anhydride, in pyridine overnight. The tertiary 5α-hydroxyl is too hindered to react[83] (Scheme 18).



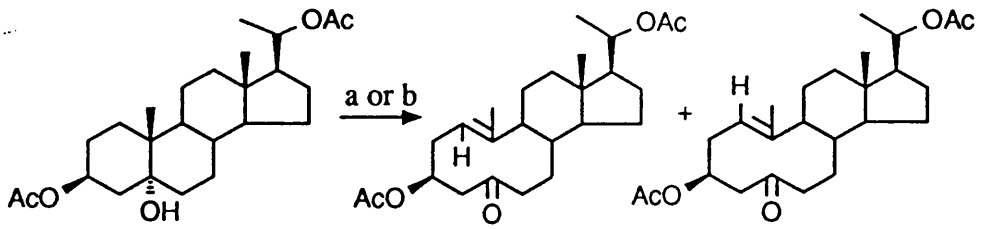
35 **38**

Reagents and conditions: a. Ac_2O, py., rt, 24 hr.

Scheme 18

Oxidation of the 5α-hydroxyl, again, brought about fragmentation of the C(5)-C(10) bond to give the secosteroid. Refluxing **38** in acetonitrile, with 2.5 equivalents of ceric ammonium nitrate[85] in water, for 3 minutes gave exclusively the (**E**)-A,B-secosteroid (**39**) in a yield of 82%. No **Z**-isomer was formed. Ring-opening of **38** by irradiating with a 500W tungsten lamp in the presence of

mercury oxide and iodine[83] gave a mixture of (E)- and (Z)-A,B-secosteroids, **39** and **40** respectively, in respective yields of 48% and 11% (Scheme 19). Both isomers were easily seperable by chromatography and were both isolated as white solids.

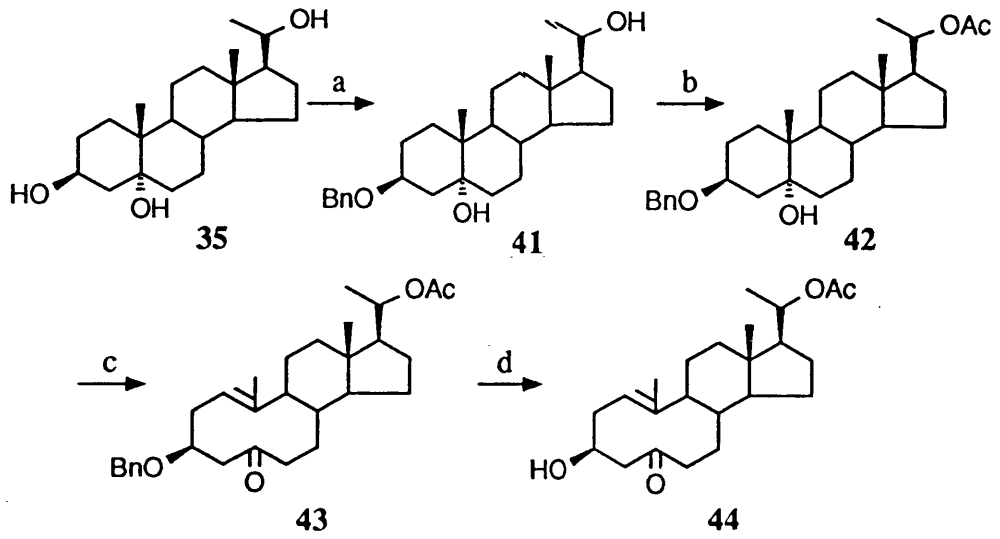


38 **39** (a; 82%, b; 48%) **40** (a; 0%, b; 11%)

Reagents and conditions: a. $(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O, 80°C; b. HgO, I_2, CCl_4, *hυ*.

Scheme 19

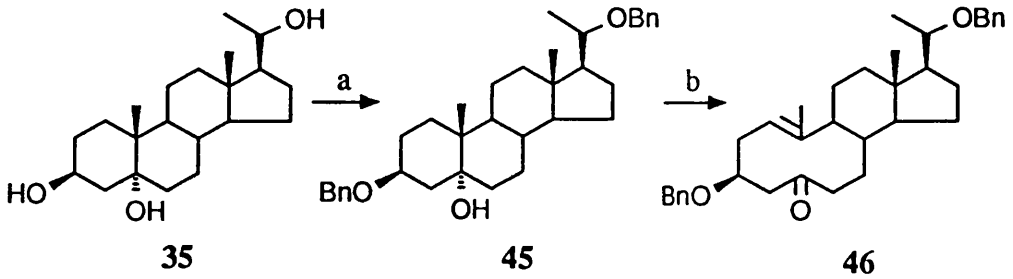
Further investigation of the 3- and 20-protecting groups revealed that it was possible to either mono-protect or di-protect the 3- and 20-hydroxyls as benzyl ethers. Reaction of the triol (**35**) with 1.1 equivalents of benzyl bromide in the presence of sodium hydride[95] affected mono-benzylation at the 3-position, to give **41** in a yield of 73%. Selective protection, as previously described, of the 20-hydroxyl as an acetate gave the 3-benzyloxy-20-acetoxy steroid (**42**), which underwent A,B-ring opening to give (E)-3β-benzyloxy-20β-acetoxy-5,10-secopregn-1(10)-en-5-one (**43**), in a yield of 46%, as a white solid. This allowed selective deprotection of the 3-hydroxyl to give the alcohol (**44**) in a quantitative yield (Scheme 20).



Reagents and conditions: a. 1.1 eq., $PhCH_2Br$, NaH, THF; b. Ac_2O, py.; c.$(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O, 80°C; d. H_2, Pd/C, EtOH.

Scheme 20

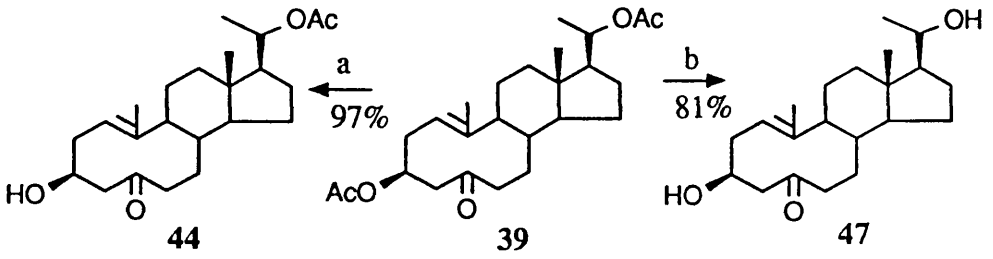
Dibenzylation of the 3- and 20-hydroxyls (the 5-hydroxyl being virtually inert to the reaction conditions) of **35** was achieved by using 2 equivalents of benzyl bromide in similar conditions to the mono-benzylation. A,B-ring opening of the dibenzyl protected steroid (**45**) was affected again using the ceric ammonium nitrate conditions to give exclusively the **E**-isomer of the dibenzyl protected secosteroid (**46**) in a good yield of 70% (Scheme 21).



Reagents and conditions: a. 2 eq. $PhCH_2Br$, NaH, THF; b. $(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O, 80°C.

Scheme 21

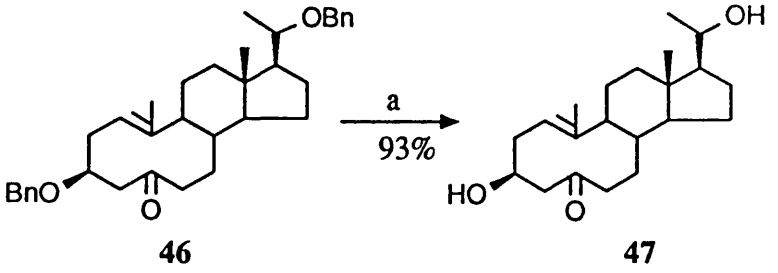
An insight into the conformation of the 10-membered ring of the E-isomer of the A,B-secosteroid could be gained from the relative rates of deprotection of the 3- and 20-protecting groups. The 3β-acetate of **39** was hydrolysed to the free alcohol after stirring a methanolic solution of **39** in the presence of potassium carbonate at room temperature for only 5 minutes. The 3β-hydroxy secosteroid (**44**) was formed in a quantitative yield. However deprotection of the 20-acetate required refluxing a methanolic solution of **39** for 24 hours, using potassium carbonate as base. The 3,20-diol (**47**) was formed in a yield of 81% (Scheme 22). This will be discussed further in chapter 2.6.



Reagents and conditions: a. K_2CO_3, MeOH, rt, 5min; b. K_2CO_3, MeOH, Δ, 24hr.

Scheme 22

Selective deprotection of the dibenzyl secosteroid (**46**) proved unsuccessful, with hydrogenation giving the 3,20-diol (**47**) in a yield of 93% (Scheme 23).



Reagents and conditions: a. H_2, Pd/C, EtOH.

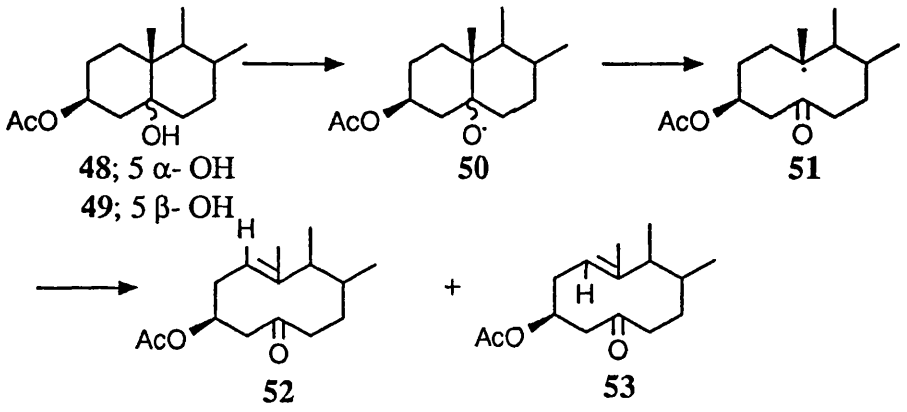
Scheme 23

Oxidative β-fragmentation to form 5,10-secosteroids

On treating 5-hydroxy steroids with the mercury oxide-iodine reagent and

irradiating with a 500W tungsten lamp, they undergo fragmentation of the C(5)-C(10) bond with formation of a new type of 10-membered ring containing 5,10-secosteroidal compounds.

The mechanism for this conversion is unclear, although the Mihailović group are close to understanding it.[83,84,86] It is thought that alkoxy radicals (**50**) obtained from 5-hydroxy steroids, such as **48** and **49**, readily undergo β-fragmentation involving scission of the C(5)-C(10) bond to give as final products (via the carbon radical intermediate (**51**)) the diastereomeric **Z**- and **E**-1(10)-unsaturated-5,10-secosteroid-5-ketones, **52** and **53**, in high yield (Scheme 24).



Scheme 24

Subsequent investigations have shown that, (1) oxidative C(5)-C(10) β-fragmentation is a general process which could be applied to various steroidal 5α- and 5β-hydroxy derivatives independent of the substituent at the 17-position[96] (although it has been shown in this thesis that the reaction conditions are sufficiently acidic to hydrolyse a 20-acetal, to a certain extent) or that the presence and orientation[97] of the 3-acetoxy group (although 5-hydroxy substrates with modified[98] or substituted[99] ring B can give products other than 5,10-seco ketones); and (2) this type of fragmentation can be affected by various oxidative agents or methods (for example, with lead tetraacetate under thermal[83,84] or U.V. photolytic

conditions,[96,97] with mercury oxide-iodine[86] or lead tetraacetate-iodine[83,100], or with ceric ammonium nitrate[85]).

Since, in general, the direction of β-fragmentation is unsymmetrical, alkoxy radicals can be rationalised in terms of the relative stability of the resulting C-centred free radical moiety and C-O moiety, as well as of steric and/or stereo-electronic factors.[101] It appeared that in the case of the 5-hydroxy steroids, such as **48** and **49**, the main factor controlling C(5)-C(10) bond cleavage is the stability of the tertiary C-10 radical intermediate (**51**), bearing the angular 19-Me group at the C-10 radical centre. Thus it may be assumed that in the 5-hydroxy steroid compounds the ease and direction of the fragmentation process should be strongly influenced by the presence, or absence, of a methyl substituent at position 10.

It has since been found that this is the case.[102] Oxidations were carried out on 19-*nor*-5-hydroxy steroids by methods previously used to effect fragmentation of the C(5)-C(10) bond in the analogous 19-methyl-5-hydroxy steroids of type **48** and **49**, i.e. lead tetraacetate under thermal conditions (28h) or U.V. photolytic conditions (3h), with lead tetraacetate in the presence of iodine (6h), and with the mercury oxide-iodine reagent (3h), the last two being irradiated with a 500W tungsten lamp. It was found that, in contrast to the 19-methyl containing steroid, the three procedures using lead tetraacetate were unsuccessful in inducing β-fragmentation of the 19-*nor*-5α-hydroxy steroid. In all these cases only unchanged starting material was isolated. However in the reaction with the mercury oxide-iodine reagent the C(5)-C(10) bond of the 19-*nor*-5-hydroxy steroid was readily broken, forming both the Z- and E-isomeric 5,10-secosteroids, although not as selectively or in as high a yield as in the 19-methyl series.

Hence it was deduced that the presence of the 19-methyl group in steroidal

5-alcohols enhanced cleavage of the C(5)-C(10) bond in β-fragmentation of the corresponding 5-alkoxy radicals, when compared to the analogous 19-*nor* compounds. Also, that various oxidative reagents, or methods, which are equally efficient (but not necessarily stereochemically equivalent[96]) when applied to 19-methyl-5-hydroxy derivatives, become selective in inducing β-fragmentation in 19-*nor* steroidal systems. The difference in reactivity between mercury oxide-iodine and lead tetraacetate (under various conditions) in the 19-*nor* steroids was probably due to factors dependent on the nature and mode of action of the oxidizing agents investigated. But there was no precise rationalistion for the results available at the moment.

The conformations of the 10-membered ring in the Z-isomers of the 19-methyl and 19-*nor* 5,10-secosteroids in solution, and the solid state, were very similar and correspond to A[97] (Figure 18). The conformation of the 10-membered ring in the E-isomeric 19-*nor* product in solution, and the solid state, also closely resembles the main conformation in solution, and in the solid state, of the 19-methyl-5,10-secosteroid, both corresponding to B[97] (Figure 18).

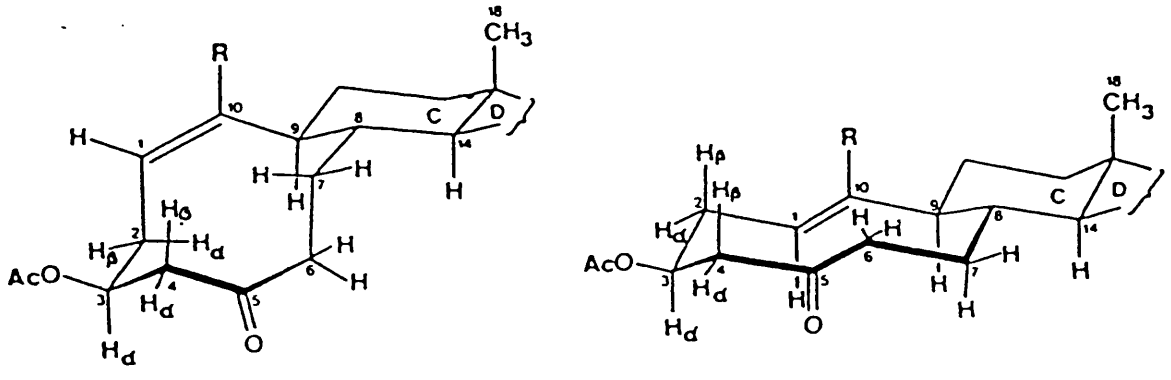


Figure 18

The similarity in the spatial arrangements of carbon atoms in both the

Z-secosteroid and their **E**-isomers was substantiated by comparison of their corresponding similar ^{13}C NMR spectra. It must be noted that the peak corresponding to the C(5) carbon for the (**Z**)-19-methyl isomer is absent, but is present in the corresponding (**Z**)-19-*nor* secosteroid and the **E**-isomers. The stereochemistry of **39** was determined by NMR studies.

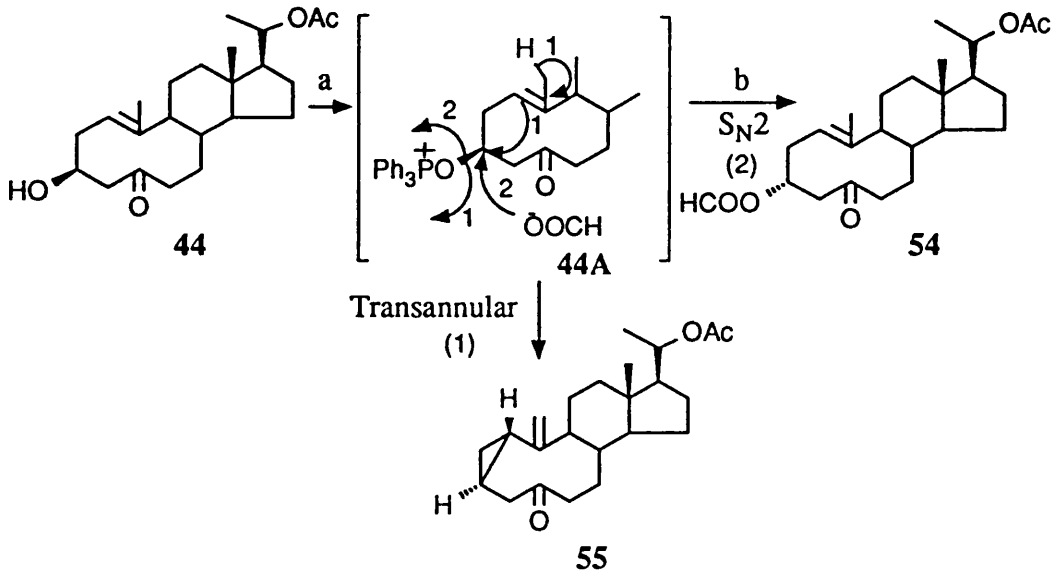
The **E**-isomer of the 5,10-secosteroid has a characteristic short wavelength adsorption in the U.V. spectrum, (293 nm) which is absent in the U.V. spectrum of the **Z**-isomer. There is also a difference in the proton NMR spectrum of the two isomers. The signal for the proton H-C(1) appears as an unresolvable multiplet for the **Z**-isomer at *ca.* 5.25 ppm, and those of the 4α-proton and the 4β-proton appear as a double doublet at *ca.* 2.4 and 3.1 ppm respectively. On the other hand, the resonance of **H**-C(1) for the **E**-isomer is, due to shielding by the 5-keto group, displaced upfield to *ca.* 4.8 ppm with a double doublet pattern. Also, in the spectrum of the **E**-isomer, there are no signals observed between 2.5 and 4.5 ppm (assuming no substituents on rings C and D possess a resonance in this range).

The **E**-isomer undergoes, more readily, transannular reactions[83] with participation of the $\Delta^{1,10}$ double bond, because of the favourable position of the carbonyl group with respect to the olefinic bond. However in the **Z**-isomer the $\Delta^{1,10}$ double bond and 5-ketone are on opposite sides of the ring (Figure 18), and would need to change to a less stable conformation to favour intramolecular cyclisation.

In summary, the unsaturated **Z**- and **E**-isomeric secosteroids behave differently towards reagents which might effect or participate in reactions involving bond formation across the 10-membered ring, and that the **E**-isomer is more reactive in these transannular processes than the **Z**-isomer. But local reactions on substituents of the 10-membered ring occur as predicted, in a similar manner for both the **E**- and **Z**- isomers.

Synthesis of (E)-3α-hydroxy-5,10-secopregn-1(10)-ene-5,20-dione (**60**)

Selective deprotection of the 3β-acetate of **39** has been discussed previously, and gave the 3β-hydroxy secosteroid (**44**) in a quantitative yield. Inversion of the 3β-hydroxyl of **44** was carried out according to the procedure described by Mitsonubu.[103] Conventional Mitsonubu reaction of the 3β-hydroxyl gave the 3α-formate ester (**54**) in a yield of 67%, via an S_N2 mechanism giving an inversion of configuration at C(3). Also formed was the cyclopropyl secosteroid (**55**), in a yield of 14%, via a transannular route (Scheme 25).



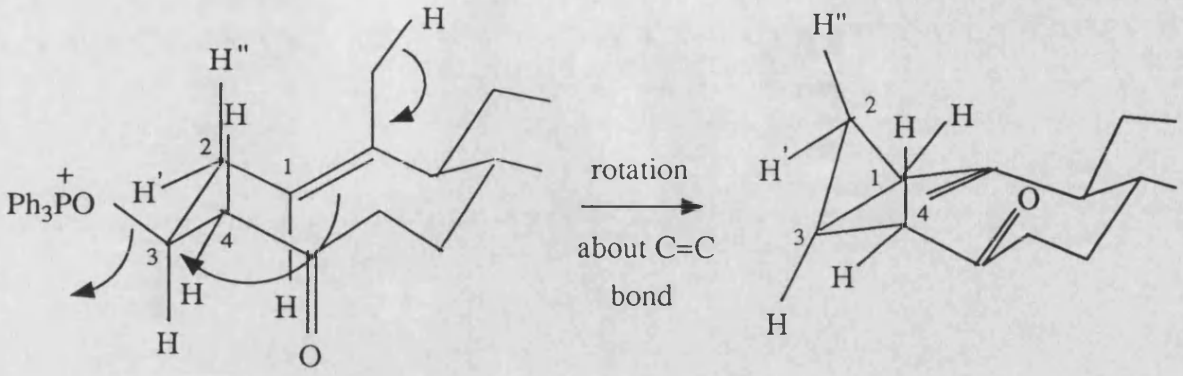
Reagents and conditions: a. DEAD, PPh_3, THF, b. HCO_2H, THF.

Scheme 25

The formation of such cyclopropane ring containing compounds has previously been reported,[104] from the solvolysis of 3-tosylates of (E)- and (Z)-5,10-sec-osteroids. Compound **44A** contains a homoallylic system, which participates in an *i*-steroid-type rearrangement.

The absolute stereochemistry of **55** was determined by NMR studies. The ^1H NMR spectra indicated an ABX_2 system for the cyclopropyl protons [δ 2.78 (1H,

dddd, $J_{3,4'}$, 2.1, $J_{3,2'}$ 6.3, $J_{3,2''}$ 12.4, $J_{3,4''}$ 19.1 Hz, 3-H), 3.05 (1H, dd, $J_{1,2'}$ 5.7, $J_{1,2''}$ 15.4 Hz, 1-H)] (Figure 19).



44A **55**

Figure 19

Recrystallisation of **55** from ethyl acetate provided crystals suitable for an X-ray crystallographic determination, which duly confirmed that the proposed structure was indeed correct [(Figure 20) full details are in Appendix].

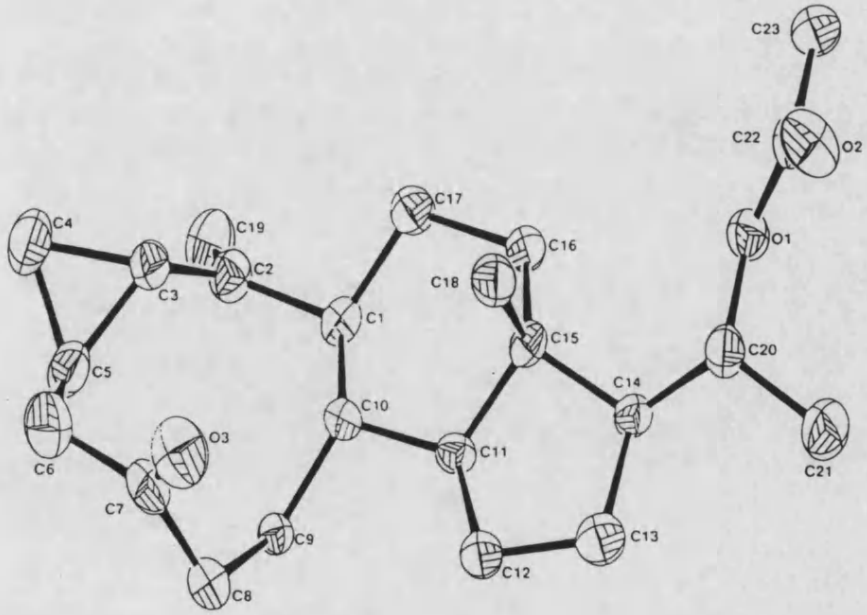
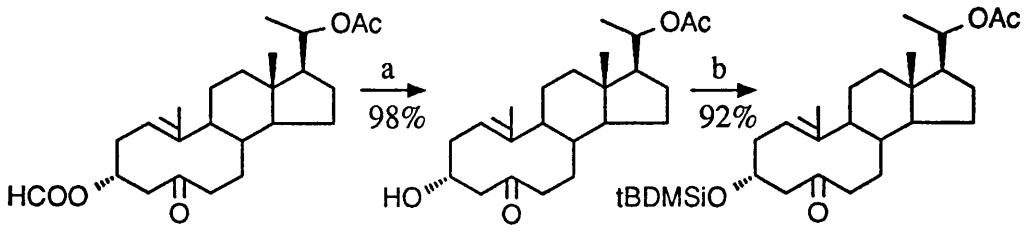


Figure 20. X-ray plot of 20β-acetoxy-1,3(10→1βH,5→3αH)*abeo*pregn-10(19)-e-n-5-one (**55**), (ORTEP plot).

The results of the X-ray analysis established the nature of the fusion of the three- and nine-membered rings in the bicyclo [7.1.0] decanone system. The transannular reaction of the 3β-phosphate (**44A**) occurs in an S_Ni fashion. Displacement of the 3β-phosphate afforded the cyclopropane ring β to the plane of the cyclononanone ring (Figure 19).

The 3-formyl ester was chosen ahead of, for example, the benzoate ester due to its relative ease of hydrolysis. Treatment of the 3α-formate ester (**54**) with potassium carbonate gave the 3α-hydroxy steroid (**56**) in a quantitative yield. The yield of the 3α-hydroxy steroid from the 3β-hydroxy steroid was 66%. The choice of 3α-hydroxyl protecting group was the silyl ether due to stability to base, and its ease of attachment and hydrolysis.[105] Also hydrolysis, with acid, would not be vigorous enough to affect transannular reaction of the $\Delta^{1,10}$ double bond with the 5-ketone, as demonstrated in chapter 2.5. Preparation of 3α-hydroxy-5(10→1βH)-*abeo*pregnan-20-ones. Protection of the 3α-hydroxy group as the *tert*butyldimethylsilyl ether[106] proceeded in 30 minutes at room temperature in the presence of imidazole to give the 3α-silyl ether (**57**) in a yield of 92% (Scheme 26).



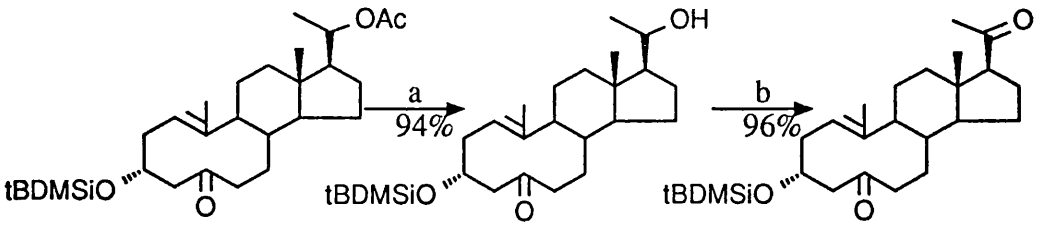
54 **56** **57**

Reagents and conditions: a. K_2CO_3, MeOH, rt, 30 min; b. tBDMSiCl, Im, DMF.

Scheme 26

Manipulation of the 20-substituent would give the desired 20-carbonyl group.

Relatively vigorous conditions were required to hydrolyse the 20-acetate group. A methanolic solution of **57** was refluxed in the presence of potassium carbonate for 24 hours to give the 20-alcohol (**58**). Oxidation of the 20-hydroxyl using pyridinium chlorochromate (PCC)[107] gave the desired 20-keto functionality (**59**) in high yield (Scheme 27).

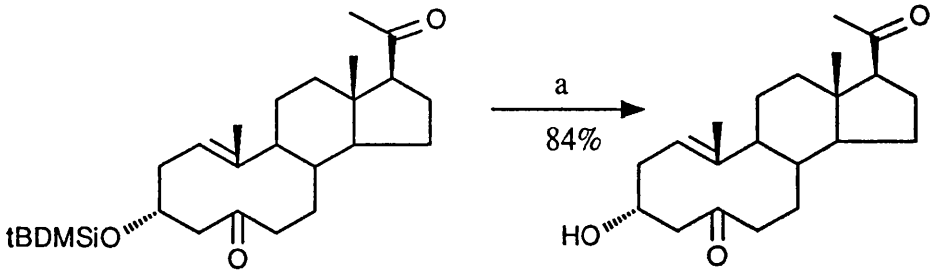


57 **58** **59**

Reagents and conditions: a. K_2CO_3, MeOH, Δ, 24 hr; b. PCC, CH_2Cl_2, rt, 3 hr.

Scheme 27

Conventional deprotection of the 3α-silyl ether[105] gave the target secosteroid, (**E**)-3α-hydroxy-5,10-secopregn-1(10)-ene-5,20-dione (**60**) (Scheme 28), in an overall yield of 29% from pregnenolone (over 11 steps).



59 **60**

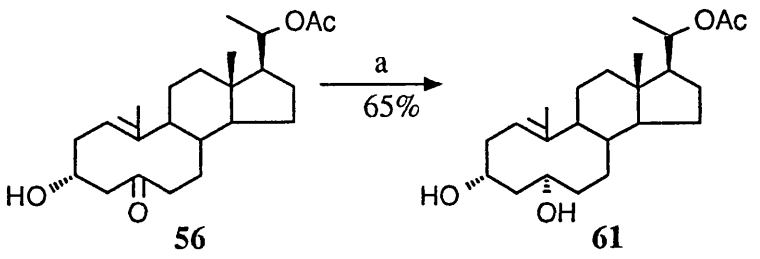
Reagents and conditions: a. $(CH_3)_4CN^+F^-$, THF, rt.

Scheme 28

Synthesis of (E)-3α,5α-dihydroxy-5,10-secopregn-1(10)-en-20-one (**65**)

Stereoselective reduction of the 5-carbonyl of **56** with sodium borohydride in

ethanol (the rate of reduction being dramatically reduced in *iso*-propanol) gave a 65% yield of the 3α,5α-diol (**61**) (Scheme 29). No 5β-alcohol was formed, hence the 5-carbonyl was reduced from the β-plane of the steroid. This can be explained by the steric environment of the 5-ketone, clearly visualised in Figure 18B, and explained in chapter 2.6.

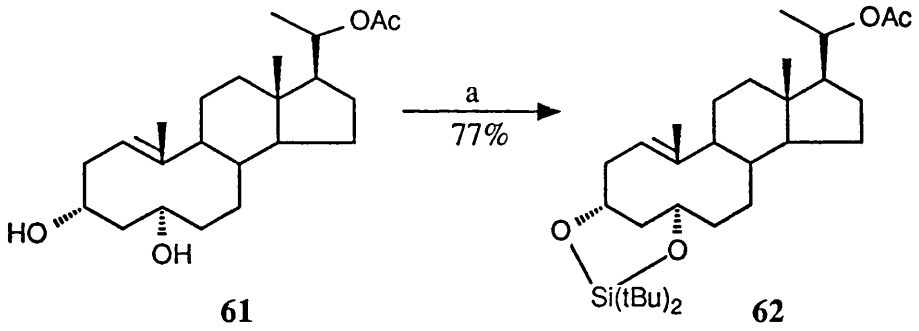


56 **61**

Reagents and conditions: a. $NaBH_4$, EtOH, 0 - 10°C.

Scheme 29

Trialkylsilyl triflates have been used for the silylation of hindered alcohols under mild conditions.[108] Also di-*tert*-butylsilyl ditriflate has been shown to react with 1,2-, 1,3- and 1,4-diols in the presence of 2,6-lutidine to provide the corresponding dialkylsilylene derivatives in high yield.[109] Di-*tert*-butylsilyl ditriflate may be prepared as described by Corey and Hopkins,[109] or purchased from Aldrich.[110] The ease of formation, stability towards hydrolysis, and mild conditions for deprotection meant that the dialkylsilylene derivatives were ideal for the protection of the 1,3-diol (**61**). Reaction of **61** with di-*tert*-butylsilyl ditriflate in the presence of 2,6-lutidine (3 equiv) at rt for 10 minutes, after work-up, gave the di-*tert*-butylsilylene derivative (**62**) (Scheme 30). The six-membered ring derivative of the 1,3-diol, **61**, was stable to chromatography on silica gel.

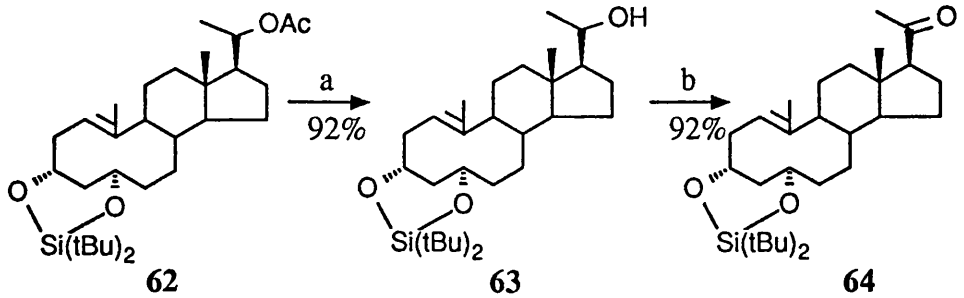


61 **62**

Reagents and conditions: a. $((CH_3)_3C)_2Si(OTf)_2$, 2,6-lutidine, CH_2Cl_2, rt.

Scheme 30

Conversion of the 20-acetate to the 20-carbonyl was carried out as previously described. Hydrolysis of the acetate gave the alcohol (**63**), which upon oxidation with PCC gave the 20-keto steroid (**64**) (Scheme 31).



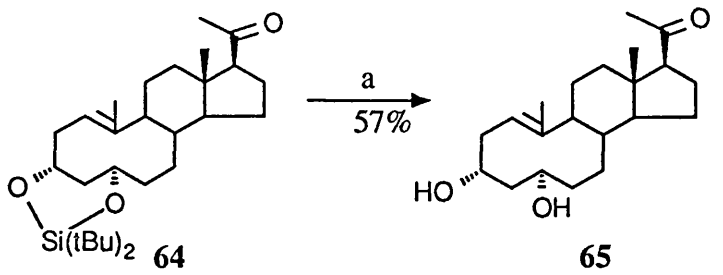
62 **63** **64**

Reagents and conditions: a. K_2CO_3, MeOH, Δ, 24 hr; b. PCC, CH_2Cl_2.

Scheme 31

Deprotection of the dialkylsilylene derivatives can be accomplished satisfactorily with aqueous hydrofluoric acid in acetonitrile[111] or with pyridinium hydrofluoride.[112] Deprotection of **64** with HF-pyridine, for 1 hour at room temperature, afforded (E)-3α,5α-dihydroxy-5,10-secopregn-1(10)-en-20-one (**65**)

as a white solid, in an overall yield of 10% from pregnenolone, over 12 steps (Scheme 32).



64 **65**

Reagents and conditions: a. HF - py, THF, rt.

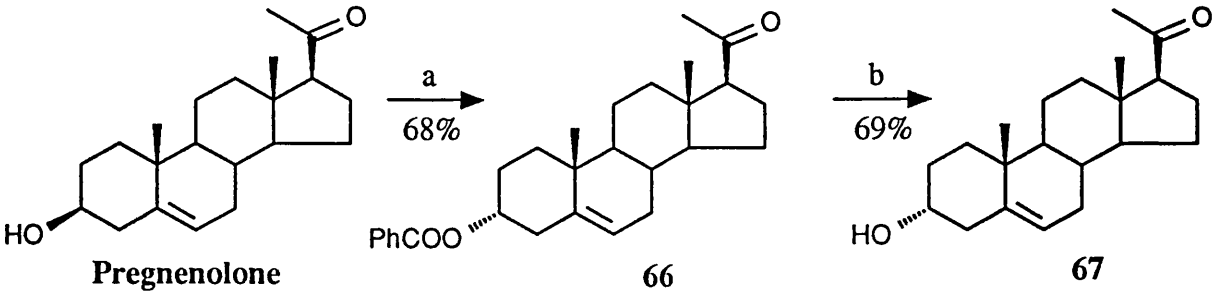
Scheme 32

Synthesis of (E)-3α,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one (**72**)

As has been discussed earlier, the 3α-hydroxyl group is necessary for anaesthetic activity. All the starting materials used possessed a 3β-hydroxyl group, thus in the previous preparations the 3β-hydroxyl group was inverted after A,B-ring opening. However, inversion of the 3β-hydroxyl group was also investigated prior to ring opening.

Pregnenolone underwent the Mitsonubu reaction[103] *via* two separate esters. Esterification of the 3β-hydroxyl as the benzoate, coupled with simultaneous inversion in configuration, gave the 3α-benzoate (**66**) in a yield of 68%. Relatively vigorous conditions, of refluxing in the presence of a strong base,[113] were required for hydrolysis of the 3α-benzoate ester to give 3α-pregnenolone (**67**), in a yield of 47% from 3β-pregnenolone (Scheme 33).

However, the preferred route of inversion of the 3β-hydroxyl group was *via* the formyl ester due to its ease of esterification and hydrolysis, and increased yields compared to the 3α-benzoate ester (**66**). The 3α-formyl ester[114] (**68**) was formed

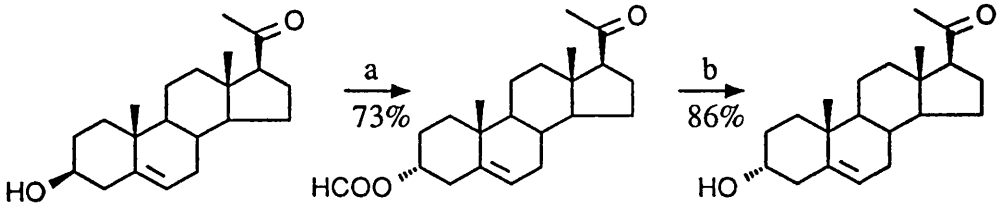


Pregnenolone **66** **67**

Reagents and conditions: a. DEAD, PPh_3, $PhCO_2H$, THF; b. NaOH, MeOH, 80°C, 1 hr.

Scheme 33

as *per* the benzoate ester (**66**) in an increased yield of 73%. Hydrolysis of the facile formyl ester occurred in 30 minutes at room temperature using potassium carbonate as base. 3α-Pregnenolone (**67**) was prepared in an overall yield of 63% from 3β-pregnenolone (Scheme 34).



Pregnenolone **68** **67**

Reagents and conditions: a. DEAD, PPh_3, HCO_2H, THF; b. K_2CO_3, MeOH, rt, 30 min.

Scheme 34

Both 3α- and 3β-pregnenolone were isolated as white solids, but were inseparable by chromatography. 3α-Pregnenolone had a lower melting point than 3β-pregnenolone (see experimental section) and a greatly increased solubility in corresponding organic solvents.

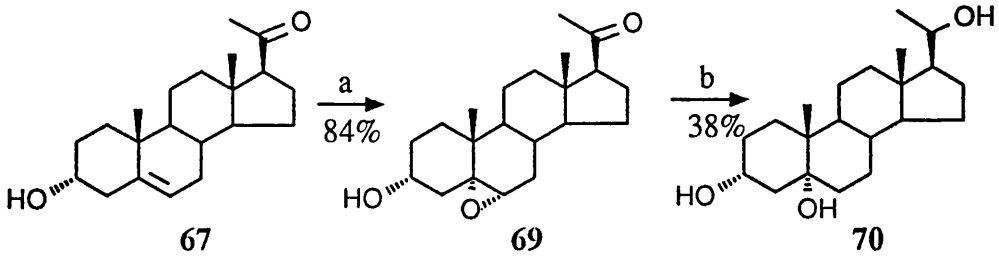
The ^1H NMR signal for the 3-proton is shifted considerably downfield in 3α-pregnenolone with respect to 3β-pregnenolone. The δ values are given in

Table 1. Although the signals for both the steroids are multiplets, the couplings to adjacent protons for the 3-proton of 3α-pregnenolone are smaller than those for 3β-pregnenolone, thus giving a narrower signal.

	δ_H (ppm) **H** - C(3)	multiplicity
3α - pregnenolone	4.05 - 3.98	m
3β - pregnenolone	3.58 - 3.48	m

Table 1

Epoxidation of the $\Delta^{5,6}$ double bond gave the 5α,6α-epoxide (**69**) in a yield of 84%. Simultaneous epoxide ring-opening and 20-carbonyl reduction, as previous, gave the triol (**70**) in a low yield of 38% (Scheme 35). The reason for the low yield is not fully understood, as a complex reaction mixture was obtained.

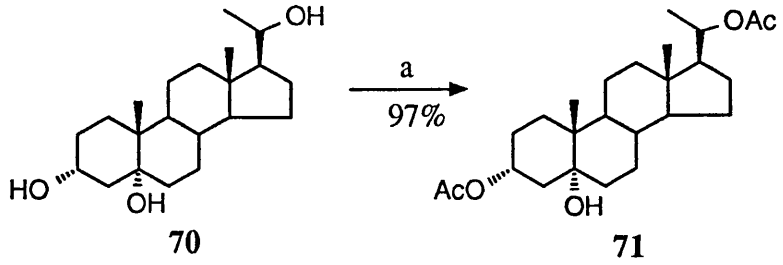


67 **69** **70**

Reagents and conditions: a. m-CPBA, CH_2Cl_2; b. $LiAlH_4$, THF, Δ.

Scheme 35

Selective protection of the 3α- and 20β-hydroxyls was carried out, as previous, to give the diacetate (**71**) in a quantitative yield (Scheme 36).

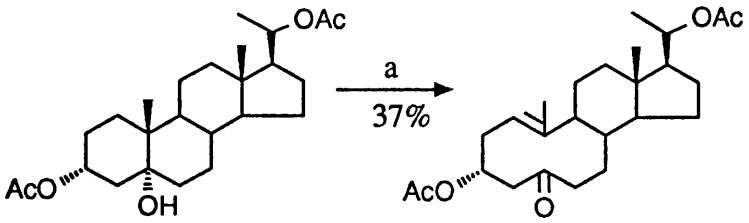


70 **71**

Reagents and conditions: a. Ac_2O, py., rt, 12 hr.

Scheme 36

A,B-Ring opening of the tertiary 5α-alcohol was affected using ceric ammonium nitrate in refluxing acetonitrile to give the 3α,20β-diacetoxy secosteroid (**72**) in a relatively low yield of 37% (Scheme 37).



71 **72**

Reagents and conditions: a. $(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O, 80°C.

Scheme 37

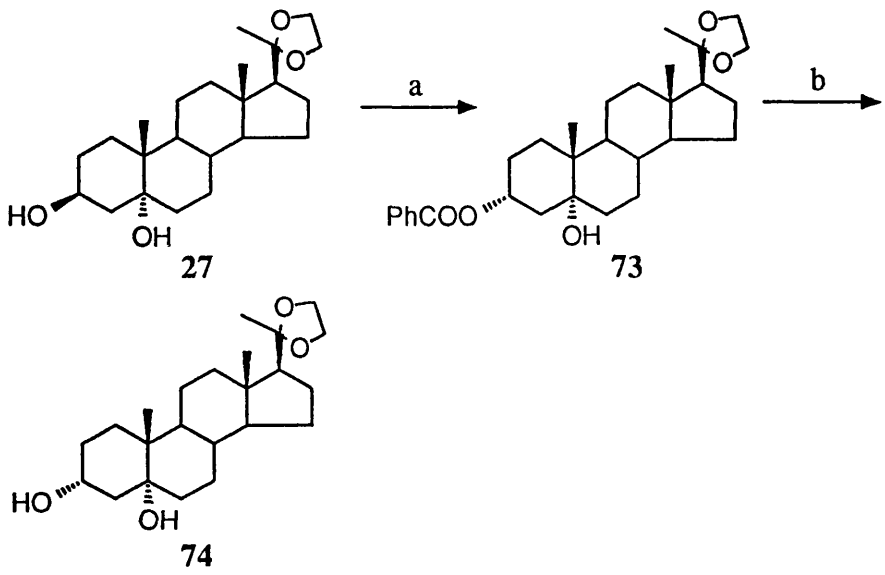
The overall yield of the 5,10-secosteroid from 3α-pregnenolone was 12% compared to 65% from 3β-pregnenolone. Thus it was concluded that it would be more viable to invert the stereochemistry at C(3) to give the desired 3α-hydroxyl moiety, after A,B-ring opening.

Synthesis of 3α,5α-dihydroxypregnan-20-one (**75**)

It was of interest to not only submit novel 3α-hydroxy-20-keto secosteroids for biological evaluation, but also any untested 3α-hydroxy-20-keto pregnanes.

Mitsonubu reaction[103] of **27** gave selective inversion of the 3β-hydroxyl to give the

3α,5α-diol (**74**) *via* the 3α-benzoate ester (**73**) (Scheme 38). The 5α-hydroxyl group was too sterically hindered to react.

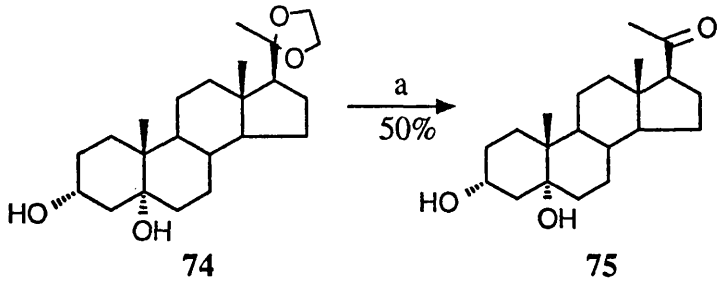


27 **73** **74**

Reagents and conditions: a. DEAD, Ph_3P, $PhCO_2H$, THF; b. NaOH, MeOH.

Scheme 38

Deprotection of the 20-acetal was achieved, using sulphuric acid in refluxing methanol, to give the desired product, 3α,5α-dihydroxypregnan-20-one (**75**) (Scheme 39).



74 **75**

Reagents and conditions: a. H_2SO_4, MeOH, Δ, 30 min.

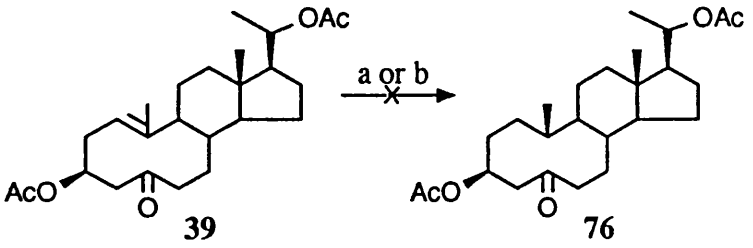
Scheme 39

2.4 Preparation of 5,10-secopregnan-3α-ol-20-one (107)

In order to access the saturated 10-membered ring analogues it was necessary to deoxygenate the 5-carbonyl and reduce the $\Delta^{1,10}$ double bond of 39.

Attempted reduction of $\Delta^{1,10}$ double bond

Unfortunately reduction of the $\Delta^{1,10}$ double bond prior to deoxygenation of the 5-carbonyl failed. Hydrogenation was attempted using hydrogen gas with a palladium catalyst at atmospheric pressure and a pressure of 150 atmospheres, and also with a platinum catalyst at atmospheric pressure (Scheme 40). The attempted hydrogenations were carried out in an ethanolic solution, and only starting material was isolated.

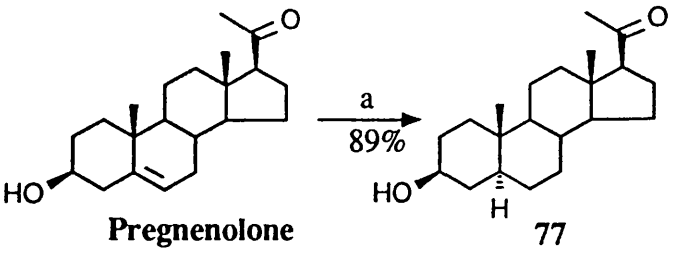


Reagents and conditions: a. H_2, Pd/C, EtOH; b. H_2, Pt/C, EtOH.

Scheme 40

The procedure and equipment were checked when a sample of pregnenolone was hydrogenated successfully at atmospheric pressure using a palladium catalyst (25% w/w) in a yield of 89% (Scheme 41).

Further attempts were made to reduce the $\Delta^{1,10}$ double bond using diimide.[115] It has been shown that the *trans* double bond was much easier to reduce than the *cis* form in the diimide reduction of 12-membered cyclic olefins.[115] The attempted reduction of **39** was carried out under three different modes of generating diimide.



Pregnenolone **77**

Reagents and conditions: a. H_2, Pd/C, EtOH, 2.5 hr.

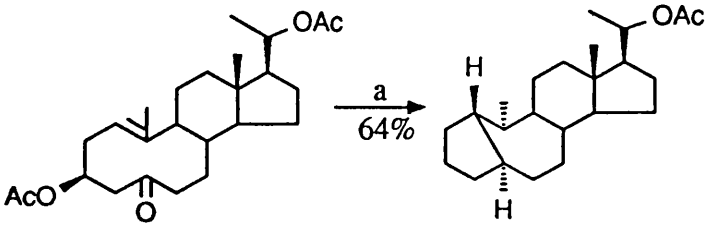
Scheme 41

These methods were; (1) oxidation of hydrazine hydrate with 30% hydrogen peroxide in the presence of cupric salts,[116] (2) oxidation of hydrazine hydrate in the presence of selenium,[117] and (3) decomposition of potassium azodicarboxylate in the presence of base.[118] However, as with the attempted hydrogenations, the diimide reductions failed and only starting material was isolated.

Also, in a further attempt to reduce the $\Delta^{1,10}$ double bond of **39**, transfer hydrogenations[119] were used. Transfer hydrogenation is the process whereby hydrogen is formed *in situ* by, for example, the oxidation of cyclohexene to give cyclohexadiene or even benzene. Three different methods of transfer hydrogenation were used; (1) oxidation of cyclohexene with palladium (II) hydroxide as catalyst,[120] (2) oxidation of cyclohexene in the presence of Wilkinsons' catalyst[121] and, (3) oxidation of ammonium formate in the presence of palladium catalyst.[122] All of the attempted 'hydrogenations' were carried out in refluxing ethanol or methanol for up to 1 week. Unfortunately the transfer hydrogenations also failed and only starting material was recovered.

From the failure of the attempted reductions of the $\Delta^{1,10}$ double bond of **39**, together with the fact that no reduction occurred and only starting material was recovered, it was surmised that the $\Delta^{1,10}$ double bond of **39** was too sterically hindered by the 5-carbonyl moiety and the 19-methyl group to undergo hydrogenation. Thus it was decided that deoxygenation of the 5-carbonyl would be necessary prior to reduction of the $\Delta^{1,10}$ double bond.

It was also found that carrying out the aforementioned hydrogenation in methylene chloride in place of ethanol gave a reaction, although not the desired one. The 5,10-secosteroid diacetate (**39**) underwent a rearrangement, together with elimination of the resulting 5-hydroxyl and the 3-acetate groups, and *in situ* reduction of the resulting $\Delta^{10,19}$ exocyclic double bond, when stirred in an atmosphere of hydrogen in methylene chloride in the presence of palladium on activated carbon for 72h to give the 5(10→1βH)*abeo*steroid (**78**) (Scheme 42). Steroids of this type will be discussed in detail in chapter 2.5, *Preparation of 3α-hydroxy-5(10→1βH)abeopregnan-20-ones.*

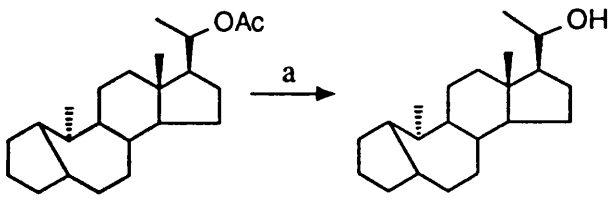


39 **78**

Reagents and conditions: a. H_2, Pd/C, CH_2Cl_2, 72 hr.

Scheme 42

Treatment of the acetate (**78**) with potassium carbonate and refluxing for 96 hours gave the 20-hydroxy-5(10→1βH)*abeo*steroid (**79**) in a quantitative yield (Scheme 43).



78 **79**

Reagents and conditions: a. K_2CO_3, MeOH, Δ, 96 hr.

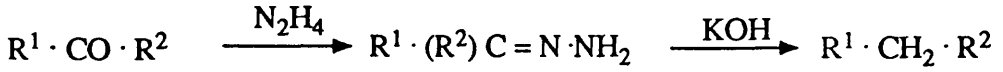
Scheme 43

Attempted deoxygenation of the C(5) carbonyl moiety

Three methods were attempted for the deoxygenation of the 5-carbonyl; (1) Wolff-Kischner reduction, (2) formation of a C(5) dithiane followed by cleavage by Raney nickel, and (3) dehydration of the C(5) hydroxyl group by various methods following reduction the of C(5) carbonyl.

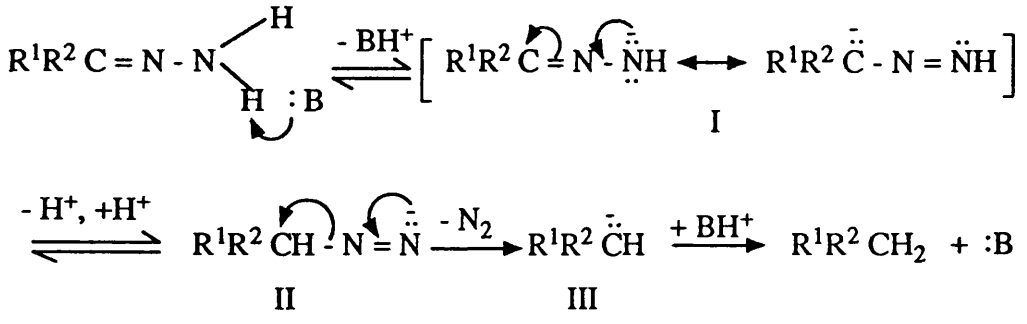
Wolff-Kischner reduction

Aldehydes and ketones may be reduced to the corresponding hydrocarbon by the Wolff-Kischner method[123] which involves heating the corresponding hydrazone with base, usually potassium hydroxide (Scheme 44).



Scheme 44

The mechanism of the Wolff-Kischner reduction may involve the initial formation of the mesomeric anion (I) by removal of a proton by the base from the hydrazone. A prototropic shift gives the anion (II) which loses nitrogen to form the carbanion (III) which accepts a proton to yield the hydrocarbon (Scheme 45).



Scheme 45

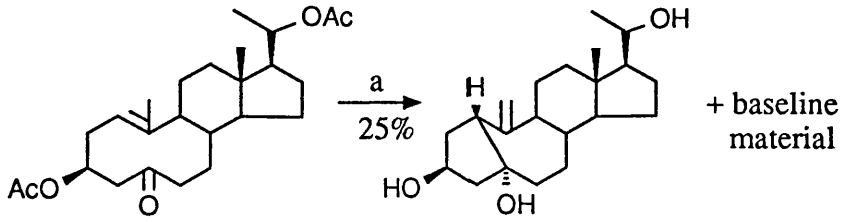
Huang-Minlon demonstrated that a modified Wolff-Kischner reduction proceeded

normally on keto groups at the steroidal positions 3,7,12,17 and 20 to give the normal methylenic compounds.[124] The keto group at position 11 remained unattacked due to steric hinderance. Moffett and Hunter[125] have reported an alternative Wolff-Kischner procedure for the reduction of 11-oxo steroids, as have Djerassi and Thomas.[126]

The Huang-Minlon modification has the following advantages: (1) the isolation of the hydrazone is unnecessary, (2) the reaction time is considerably reduced, (3) the reaction can be carried out at atmospheric pressure , and (4) the yields are usually higher. The hydrazone is first formed *in situ* by refluxing a solution of the carbonyl compound in diethylene glycol or triethylene glycol with the commercial hydrazine hydrate and about 3 equivalents of potassium hydroxide[127] (or potassium carbonate may be used[128]) for 1 hour; the water and excess hydrazine are removed by distillation until a favourable temperature for the decomposition of the hydrazone is attained (170-190°C) and the solution refluxed for 3-5 hours longer.

Attempted reduction of the 5-carbonyl of **39** using the Huang-Minlon modification, with either potassium hydroxide or potassium carbonate as base, failed to give the desired product. In both cases a complex reaction mixture resulted. One problem could be that the 5,10-secosteroid is highly base sensitive. As has been previously shown in this report, treatment with potassium carbonate is sufficient to hydrolyse the 3β-acetate and, to some extent, the 20-acetate. Refluxing the 5,10-secosteroid (**39**) in diethylene glycol with potassium hydroxide gave the triol (**80**) and baseline material (Scheme 46).

A further problem was that of the previously mentioned transannular reaction. Mihailović *et al* have reported on the transannular reaction of hydroxylamine

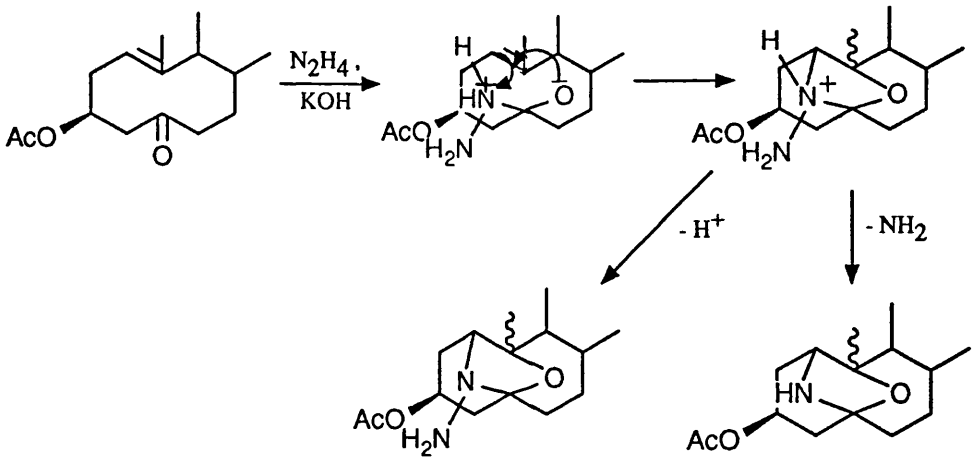


39 **80**

Reagents and conditions: aKOH, diethylene glycol, Δ, 2 hr.

Scheme 46

across the 10-membered ring.[83] Since hydrazine was used to effect the reduction of the 5-carbonyl this could be relevant. Proton NMR of the crude reaction mixture of the attempted reduction of the 5-carbonyl of **39** showed no signal for the olefinic proton, and the IR showed no peak corresponding to a carbonyl. The mass spectra showed a mass ion in excess of the desired product. It may be that a transannular type reaction has occurred, and a possible explanation is illustrated in Scheme 47.



Scheme 47

Chromatography showed the attempted reduction of the 5-carbonyl moiety *via* the Huang-Minlon modification of the Wolff-Kischner reaction to be very complex, and for this reason it was not purified any further.

Preparation of C(5) dithioketal

An important method for accomplishing complete reduction of a carbonyl group involves dithioketal formation followed by hydrogenolysis of the carbon-sulphur bonds. The mild and relatively neutral conditions employed in this procedure help make it a useful companion to the Wolff-Kischner reduction.

Many studies of the reactions of steroid ketones have demonstrated that ethanedithiol will condense with carbonyl functions at various positions.[129] Several efficient procedures for preparing ethylene thioketals have been devised by Fieser *et al.*[130] In the simpliest and often most effective of these methods boron trifluoride etherate is added, with stirring, to a solution (or suspension) of the carbonyl compound in ethanedithiol. Selective thioacetal formation of diones was also reported. For example, it was found that the addition of acetic acid to the ethanedithiol and acid catalyst caused a milder reaction medium allowing the synthesis of the 3-dithioacetal of cholestane-3,6-dione. In the absence of acetic acid the 3,6-bis-dithioacetal was obtained.

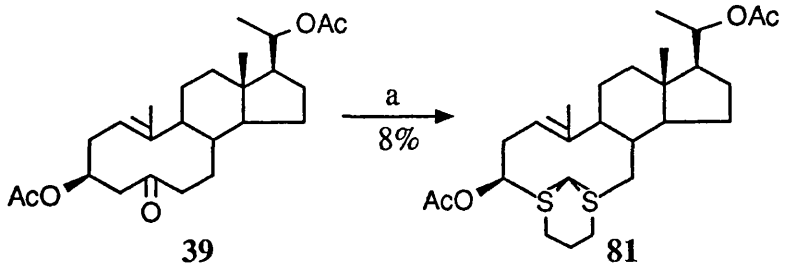
It has also been reported that when acetic acid and p-toluenesulphonic acid, as catalyst, were used the selectivity of the 3-position over that of the 17 was greatly reduced.[131] It was also found that using methanol as solvent gave greater selectivity than acetic acid and also avoided the problem of acetate formation.[131] Thus when methanol was used as the solvent, selective 3-thioacetal formation could be achieved in high yield in the presence of other groups such as the 17-ketone and the 19-alcohol.

Desulphurisation of thioketals may be accomplished by refluxing an ethanol or dioxane solution of the sulphur compound with Raney nickel (W-2, W-4, or W-7).[132] For best results, a large excess of the catalyst is required (i.e., weight ratio

of catalyst to substrate greater than 6). Freshly prepared catalyst may reduce other functional groups,[133] but this can be at least partly avoided if the Raney nickel is deactivated before use by refluxing for several hours in acetone.

The attempted condensation of the ketone (**39**) with ethanedithiol failed. The reaction was attempted for both the diacetate (**39**) and the diol (**47**) using ethanedithiol. It was attempted in neat dithiol, 1.5 equivalents of dithiol and methanol, and also 1.5 equivalents of dithiol and acetic acid. In all the above cases boron trifluoride etherate was used as catalyst. The reaction mixture, in all the cases, was very complex and for this reason was not purified in any way.

However, carrying out the reaction in acetic acid with propane-1,3-dithiol, using p-toluenesulphonic acid as catalyst, gave the corresponding dithioketal (**81**) of the ketone (**39**) (Scheme 48). Unfortunately the dithioketal was isolated in a low yield of 8%, and the reaction could not be repeated using boron trifluoride etherate as catalyst, or methanol as solvent, or using ethanedithiol.



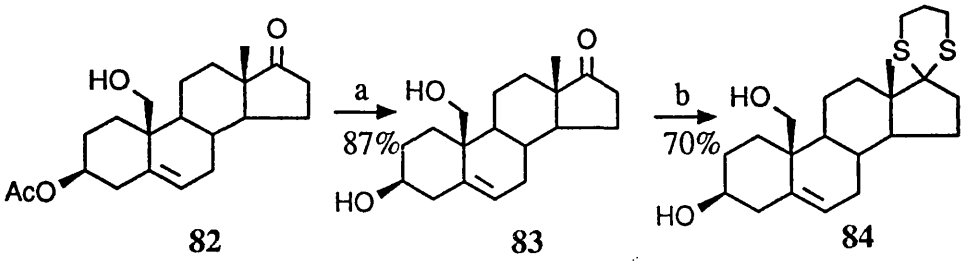
39 **81**

Reagents and conditions: a. $HS(CH_2)_3SH$, p - TSA, AcOH, rt.

Scheme 48

As a trial reaction the ketone (**83**) gave the propane dithioketal (**84**), after condensation with propane-1,3-dithiol in methanol using boron trifluoride etherate as catalyst, in a good yield of 70%. Also as a comparison of the rate of hydrolysis of 3-acetates, the 3β-acetate of **82** was hydrolysed. Hydrolysis of **82** was affected by stirring a methanolic solution of **82** for 3 hours in the presence of potassium

carbonate (Scheme 49) compared to 5 minutes under the same conditions for the hydrolysis of the 3-acetate of **39** (Scheme 22).

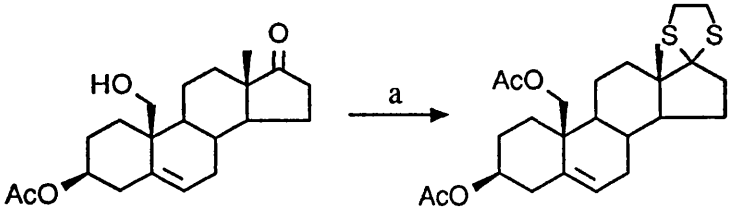


82 **83** **84**

Reagents and conditions: a. K_2CO_3, MeOH, rt, 3 hr; b. $HS(CH_2)_3SH$, $BF_3.OEt_2$, MeOH, rt, 30 min.

Scheme 49

Also condensation of the ketone (**82**) with ethane dithiol in acetic acid with boron trifluoride etherate as catalyst gave the ethane dithioketal (**85**), with simultaneous acetylation of the 19-hydroxyl (Scheme 50).

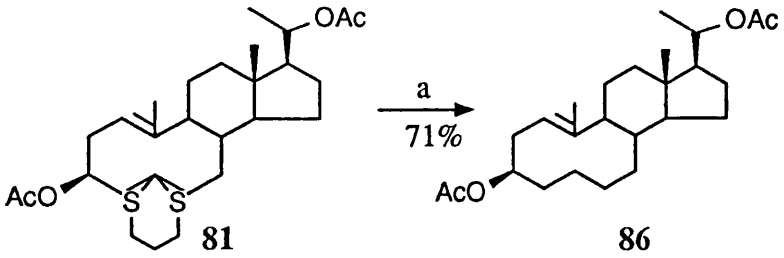


82 **85**

Reagents and conditions: a. $HS(CH_2)_2SH$, BF_3 .OEt_2, AcOH, rt, 2 hr.

Scheme 50

Desulphurisation of the thioketal (**81**) was affected by refluxing an ethanolic solution of **81** with a twenty-fold excess of W-2 Raney nickel,[134] prepared as described in the literature.[135] The C(5) methylene steroid (**86**) was formed in a good yield of 71% (Scheme 51). An overall yield of 6% from the ketone (**38**).



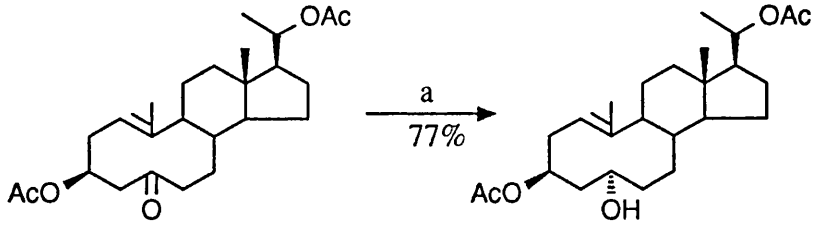
81 86

Reagents and conditions: a. Raney Ni, EtOH, Δ.

Scheme 51

Dehydration/elimination of the 5-hydroxyl moiety

The third attempted route to the deoxygenation of the 5-carbonyl was *via* the elimination of the 5-hydroxyl. Careful reduction of the 5-carbonyl of **39**, with cooling and premature quenching to avoid hydrolysis of the 3-acetate, using sodium borohydride gave the stereospecific 5α-hydroxy steroid (**87**) (Scheme 52). The conformation at C(5) was verified by ^{1}H NMR spectroscopy methods (see experimental section).



39 87

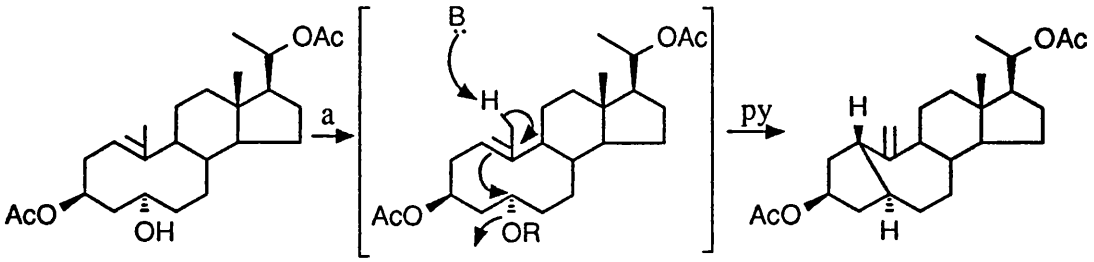
Reagents and conditions: a. $NaBH_4$, EtOH, 0°C - rt.

Scheme 52

Elimination of 5-tosylate

It was proposed that on formation of the 5-tosylate of **87**, this tosylate group could undergo elimination using collidine[136] to give the corresponding olefin,[137] or reaction with lithium aluminium hydride to give the C(5) methylene group.[138] Attempted synthesis of the 5-tosylate involved reaction of the alcohol (**87**) with tosyl chloride in pyridine, which acts as solvent and base. Unfortunately though,

even at reduced temperatures, the 5-tosylate could not be isolated as transannular elimination of the tosylate occurred instantaneously to give the 5(10→1βH)*ab-eo*steroid (**88**) as a white solid (Scheme 53). Although the reaction almost certainly proceeds *via* the 5-tosylate, it was not detected *via* chromatography. The transannular elimination was repeated when the reaction proceeded *via* the 5-mesylate, i.e., mesyl chloride was used in place of tosyl chloride (Scheme 53).



87 **88**

R = Ts, Ms

Reagents and conditions: a. TsCl (or MsCl), py., -78 - 25°C.

Scheme 53

Reductive removal of the 5-hydroxyl with chlorotrimethylsilane-sodium iodide

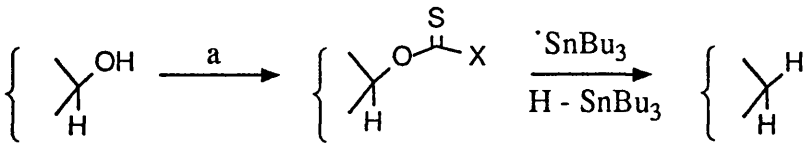
It has been shown that chlorotrimethylsilane-sodium iodide (CTMS-NaI) effects reductive removal of hindered tertiary hydroxyls to give saturation.[139] Hence it was expected that treatment of the alcohol (**87**) with CTMS-NaI would give the C(5) methylene steroid (**86**). Unfortunately the reaction seems to proceed as in Scheme 53, as the only product was the 5(10→1βH)*abeo*steroid (**88**), see experimental section.

Attempted radical deoxygenation of 5-alcohol

In the last decade there has been a marked increase in the use of radical reactions in organic synthesis.[140] The radical deoxygenation of secondary alcohols is one of the reactions of this kind.[141] The radical deoxygenation of secondary alcohols by tin hydride reduction of appropriate thiocarbonyl derivatives is a preparatively useful reaction, especially for complex molecules.

Barton *et al* suggested that a thioformate derivative would have a relative thermal stability and ease of reductive elimination.[142] They reported that the thioformates of tertiary alcohols were smoothly reduced by tributyltin hydride to the corresponding hydrocarbon in a radical chain reaction.[142]

The reaction of Barton and McCombie,[141] the radical deoxygenation of secondary alcohols, was improved by the use of the reagents 2,4,6-trichlorophenoxythiocarbonyl chloride and its congener pentafluorophenoxythiocarbonyl chloride. Deoxygenations with these derivatives were found to be fast and quantitative.[143] Acylations were carried out in benzene or toluene. The deoxygenation reaction could then be performed in the same solvent with tributyltin hydride, this being a significant advantage over any previous procedure. Also when X was 2,4,6-trichlorophenoxy, or especially pentafluorophenoxy, the reaction with tributyltin hydride was rapid (minutes rather than hours) and the yields were excellent (Scheme 54).

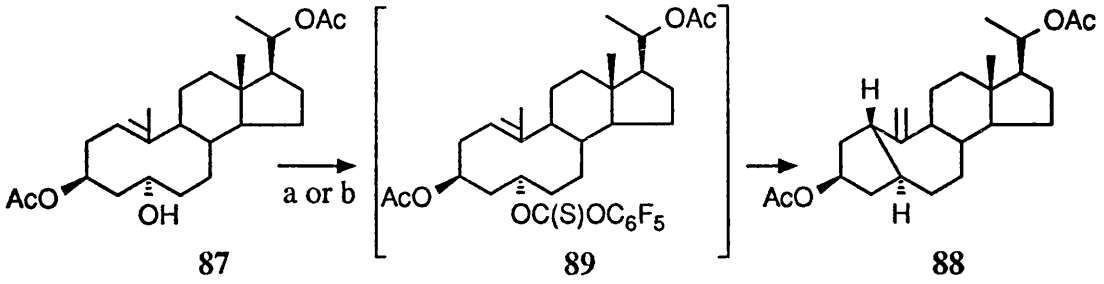


Reagents and conditions: a. $C_6F_5OC(S)Cl$ (or 2,4,6 - $C_6H_2Cl_3OC(S)Cl$), py, HOSu, $PhCH_3$, 80°C.

Scheme 54

Attempted acylation of the 5-hydroxyl group of **87** using pentafluorophenoxythiocarbonyl chloride in refluxing toluene unfortunately failed. The only product isolated was that of the 5(10→1βH)*abeo*steroid (**88**). Thus it was probable that on forming the 5-pentafluorophenoxythioformate (**89**), again a rapid transannular cyclization occurred (Scheme 55), as in the case of the 5-tosylate. The reaction was repeated at a lower temperature, ~78 - ~40°C, in an attempt to isolate the

thioformate (**89**). However, even at such low temperatures **89** was not detected by chromatography, and after appropriate work-up the only product was the *abeo*steroid (**88**) (Scheme 55).



87 **89** **88**

Reagents and conditions: a. $C_6F_5OC(S)Cl$, py., HOSu, $PhCH_3$, 80°C; b. $C_6F_5OC(S)Cl$, py., HOSu, CH_2Cl_2, -78°C - -40°C.

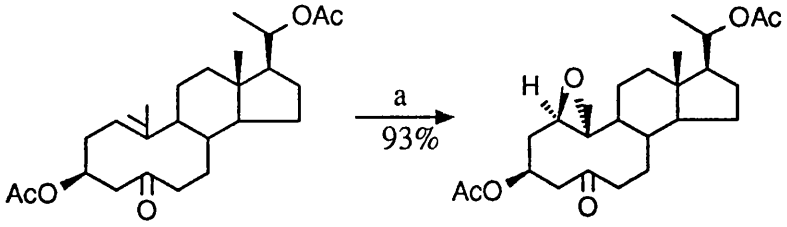
Scheme 55

In summary, it was not possible to reduce the $\Delta^{1,10}$ double bond of the 5,10-secosteroid whilst both the bulky 19-methyl and 5-carbonyl surrounded it. Unfortunately, attempts to deoxygenate the 5-carbonyl moiety also failed (with the exception of a low yield of the C(5) methylene steroid via the dithioketal) due to problems from transannular reactions brought about by the $\Delta^{1,10}$ double bond. It was not possible to eliminate the 5-hydroxyl moiety via 'a good leaving group' approach also, due to the aforementioned transannular reactions. Hence the $\Delta^{1,10}$ double bond could not be reduced with the 5-carbonyl present, but the 5-carbonyl could not be deoxygenated whilst the $\Delta^{1,10}$ double bond was present. A possible way round this could be by protection of the olefinic moiety. Protection of the carbonyl moiety would have no advantage, as it would not lessen the steric bulk.

Synthesis of 5,10-secopregnan-3α-ol-20-one (**107**)

Epoxidation of the $\Delta^{1,10}$ double bond of **39** proceeded uneventfully[144] to give the 'olefin protected' epoxide (**90**) as a white solid in a yield of 93% (Scheme 56). The conformation of the epoxide was proved by ^1H NMR spectroscopy. The

splitting pattern corresponding to the H-C(1) proton appeared as a doublet with a vicinal coupling constant of 9.2Hz. This suggested that the dihedral angle between this proton and one of the C(2) protons was very close to, if not, 90°, i.e. a coupling constant of 0Hz. The other C(1)-C(2) dihedral angle would thus be in the order of 150°, to give the said coupling constant. This suggestion was verified by models, and may only be brought about by the stereochemistry of the C(1) proton being α to the plane of the steroid backbone.[145] The 19-methyl signal was unchanged and thus was assumed to be β to the plane of the steroid, as in the starting material.



39 **90**

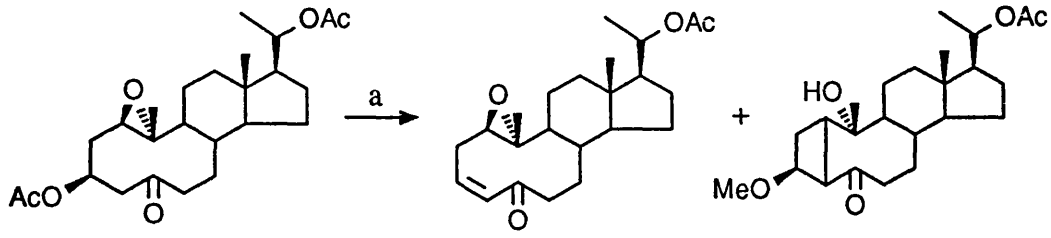
Reagents and conditions: a. m-CPBA, CH_2Cl_2, rt, 2 hr.

Scheme 56

Protection of the $\Delta^{1,10}$ double bond, as an epoxide, dramatically changed the character of the 5-carbonyl, suggesting that some intramolecular 'bonding' existed between the $\Delta^{1,10}$ double bond and the 5-carbonyl. This assumption explains the observed ease of transannular reaction, as was seen by the attempted isolation of derivatives of the 5-alcohol (**87**).

The 5-carbonyl of **90** behaved much more like a carbonyl is expected to, compared to that of **39**. Also the adjacent protons became a great deal more acidic, than those of **39**, thus adding to the suggestion of a "partial transannular bond" existing between the $\Delta^{1,10}$ olefinic bond and the 5-carbonyl. The acidity of the C(4) protons of **90** was demonstrated by treatment of **90** with potassium carbonate in methanol. It was expected that potassium carbonate would hydrolyse the 3-acetate, as was the case with **39** (Scheme 22). But this was not the case. One of

the C(4) protons was extracted by the base leading to elimination of the 3-acetate to give the $\Delta^{3,4}$ olefin (**91**). Conjugate addition of the solvent methanol, to the double bond led to a transannular ring opening of the epoxide of **91** to give the 3-methoxy alcohol (**92**) (Scheme 57).

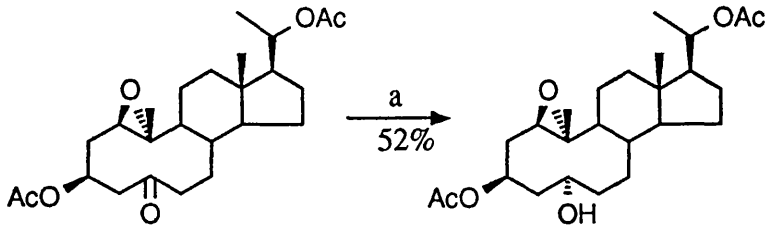


90 **91** (21%) **92** (76%)

Reagents and conditions: a. K_2CO_3, MeOH, rt, 25 min.

Scheme 57

Another example of the 'new found freedom' of the 5-carbonyl was the much increased speed of reduction using sodium borohydride in ethanol. The 5-carbonyl of **90** underwent reduction in less than a tenth of the time taken to reduce the 5-carbonyl of **38**, to give the 5-hydroxy epoxide (**93**) as a white solid (Scheme 58).



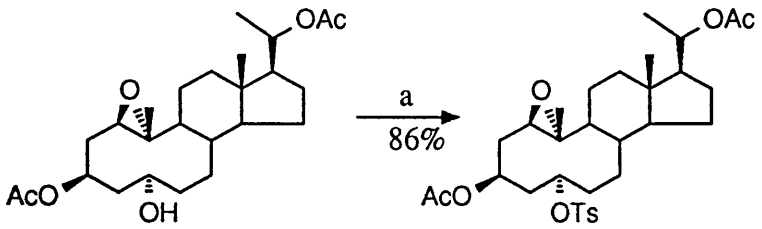
90 **93**

Reagents and conditions: a. $NaBH_4$, EtOH, rt, 45 min.

Scheme 58

Now, with the $\Delta^{1,10}$ double bond protected it was possible to derivatize the 5-hydroxyl moiety. The 5-tosylate (**94**) was isolated in a yield of 86% after the reaction of **93** with tosyl chloride and pyridine (Scheme 59). The reaction was stirred for 72 hours, thus the 10-membered ring was now stable to transannular

reactions.



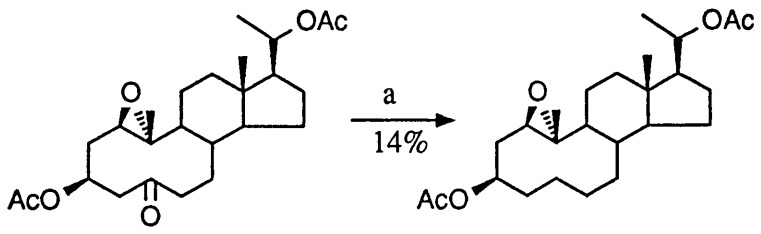
93 **94**

Reagents and conditions: a. $CH_3C_6H_4SO_2Cl$, py., CH_2Cl_2, rt, 72 hr.

Scheme 59

Hence, it should now be possible to either deoxygenate the 5-carbonyl of **90** or dehydrate/eliminate the 5-hydroxyl to give the corresponding C(5) hydrocarbon. It has been reported that the reduction of ketone tosylhydrazones with the mild reducing agent, sodium cyanoborohydride, in acidic 1:1 DMF-sulfolane provides a mild, convenient, and high yielding method for deoxygenation by either a direct hydride attack or a tautomerisation-then-reduction route.[146]

In situ reduction of the hydrazone of **90** with sodium cyanoborohydride gave the C(5) methylenic steroid (**95**) in a yield of 14% (Scheme 60).



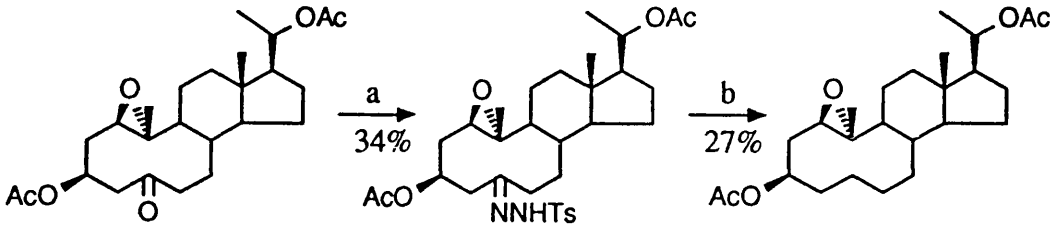
90 **95**

Reagents and conditions: a. $TsNHNH_2$, $NaCNBH_3$, p-TSA, DMF, sulfolane, Δ.

Scheme 60

Alternatively the hydrazone (**96**) of **90** could be isolated by stirring a solution of the ketone (**90**) with tosyl hydrazine for 5 days. The hydrazone was isolated in a yield of 34%, as a white solid. Reduction of the hydrazone (**96**) with sodium

cyanoborohydride gave the corresponding hydrocarbon (**95**) in an overall yield of 9% (Scheme 61).

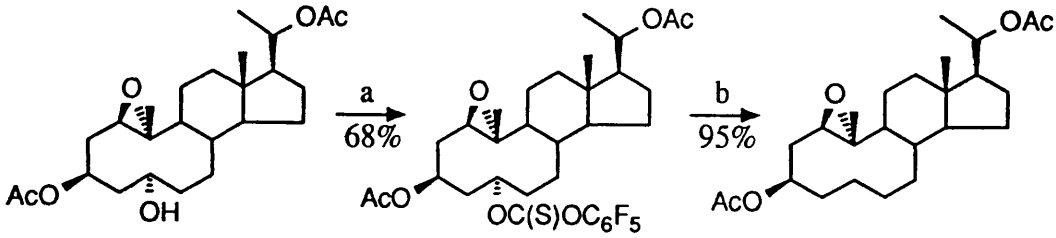


90 **96** **95**

Reagents and conditions: a. $TsNHNH_2$, CH_2Cl_2, 5 day; b. $NaCNBH_3$, p-TSA, DMF, sulfolane, Δ.

Scheme 61

Following the procedure of Barton and Jaszberenyi, for the radical deoxygenation of secondary alcohols,[143] the 5-hydroxyl group of **93** was deoxygenated in a yield of 65% (34% from the ketone (**90**)). The pentafluorophenoxythioformate derivative (**97**) of **93** was prepared as described earlier and isolated in a yield of 68%. Treatment of the xanthate (**97**) with tributyltin hydride[147] in the presence of azobisisobutryonitrile (AIBN) gave the C(5) methylenic steroid (**95**) after 15 minutes at 110°C (Scheme 62).



93 **97** **95**

Reagents and conditions: a. $C_6F_5OC(S)Cl$, py., HOSu, $PhCH_3$, 80°C; b. Bu_3SnH, AIBN, $PhCH_3$, 110°C.

Scheme 62

Deoxygenation of the epoxide would give the $\Delta^{1,10}$ olefin,[148] which it would now, hopefully, be able to reduce as the bulky carbonyl was no longer present.

Deoxygenation of the epoxide of **95** was initially attempted using samarium (II) iodide in THF.[149] The most stable oxidation state of the lanthanide elements is +3. Thus, divalent lanthanide salts[150] should behave as strong reducing agents.[151] Samarium metal reacts smoothly with 1,2-diiodoethane in THF to give samarium diiodide (Scheme 63). Ytterbium may be used in place of samarium.[149]

$$Sm + ICH_2CH_2I \longrightarrow SmI_2 + CH_2 = CH_2$$

Scheme 63

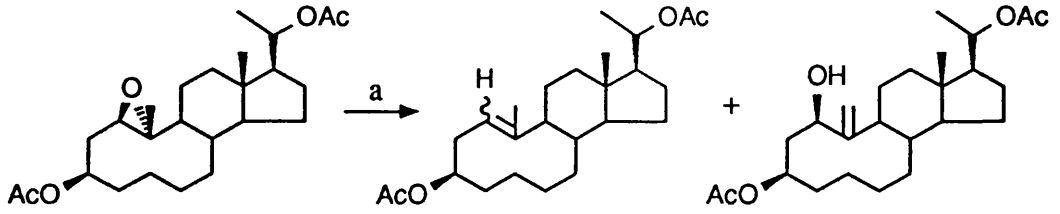
The reaction should be carried out at room temperature, under an inert atmosphere and anhydrous conditions, and yields are reported to be high. The resulting samarium diiodide-THF solution is coloured blue-green. Epoxides are deoxygenated to olefins by samarium diiodide, and to a lesser extent by ytterbium diiodide. Use of an excess of samarium diiodide in the presence of *tert*-butyl alcohol resulted in virtually quantitative conversion into olefin without by-products resulting from epoxide rearrangement[152] or reduction into alcohol.

Unfortunately the deoxygenation of the epoxide of **95** with samarium diiodide proceeded very slowly; by tlc only approx. 10% of a mixture of *trans*- and *cis*-olefins was formed after 5 days at 35-40°C.

It has long been known that bromohydrins give olefins on treatment with zinc.[153] Reduction of an iodohydrin to an olefin has also been reported.[154] When an epoxide was added to sodium iodide in acetic acid, an iodohydrin was formed rapidly, and subsequent addition of zinc powder gave an olefin.[155] The yield was improved upon when the mixture of acetic acid, sodium iodide, and zinc was buffered by sodium acetate. In the process, protonation of the epoxide was presumably followed by nucleophilic addition of the iodide ion, and then reduction

of the iodohydrin before solvolytic processes can destroy much of it. Iodide ion consumed in the second stage was regenerated in the third, so that a catalytic amount of sodium iodide should suffice to promote olefin formation.[156] Reduction of the epoxide was shown to give a mixture of roughly equal parts of *trans*- and *cis*-olefins.[154]

On treatment of **95** with sodium iodide, zinc, sodium acetate, and acetic acid the $\Delta^{1,10}$ olefin (**86**), a mixture of **E**- and **Z**- isomers, was formed in a yield of 73%, corrected for recovered starting material. Also isolated was the 1α-hydroxysteroid (**98**), resulting from epoxide rearrangement, in a yield of 13% (Scheme 64).



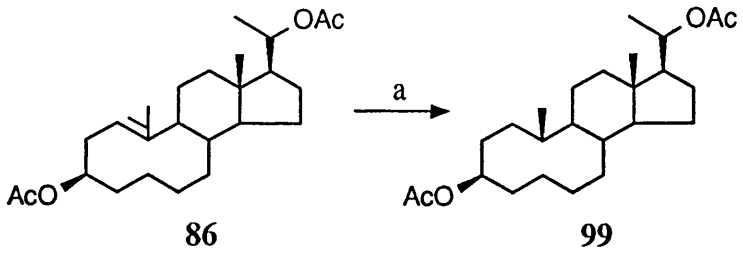
95 E/Z; **86** (73%) **98** (13%)

Reagents and conditions: a. Zn, NaI, NaOAc, AcOH, rt, 5 day.

Scheme 64

The fact that an isomeric mixture of olefin (**86**) was formed was immaterial, as the next step was the hydrogenation of the $\Delta^{1,10}$ double bond. Hydrogenation of the $\Delta^{1,10}$ double bond of **86** was very slow, 50% conversion in 2 weeks, but gave the fully saturated steroid (**99**) (Scheme 65). Hydrogenation of the $\Delta^{1,10}$ double bond was carried out prior to inversion of configuration at C(3) in order to eliminate the problem of side-products, of the type (**55**), formed in the Mitsonubu reaction, as a result of transannular reactions.

Selective hydrolysis of the 3β-acetate of **99** was less rapid than that of **38**. The 3β-hydroxy steroid (**100**) was formed by treatment of the 3β-acetate (**99**) with base

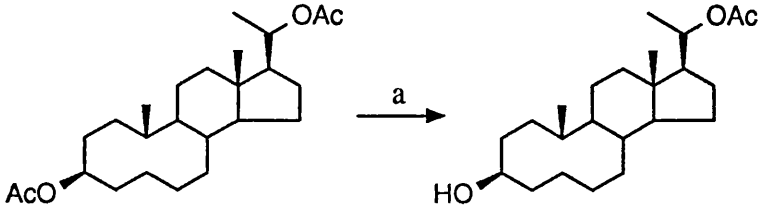


86 **99**

Reagents and conditions: a. H_2, Pd/C, EtOH, 14 day.

Scheme 65

for 24 hours (Scheme 66).



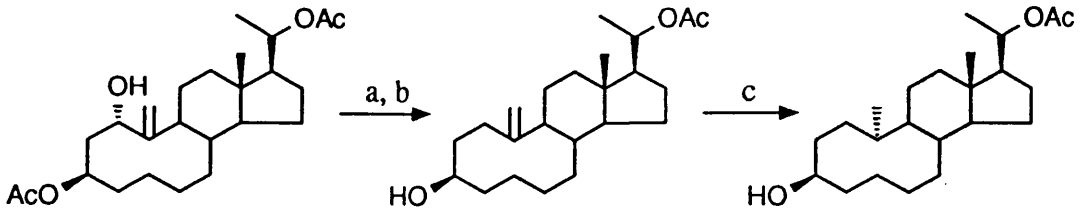
99 **100**

Reagents and conditions: a. K_2CO_3, MeOH, rt, 24 hr.

Scheme 66

The by-product of the deoxygenation of the epoxide (**95**), **98** may also be converted to the 19α-methyl isomer of the 3β-hydroxy saturated steroid (**100**). Radical deoxygenation of the 1α-hydroxyl group of **98** was carried out, as described previously, using the method of Barton and Jaszberenji,[143] in one step to give the olefinic diacetate (**101**). Selective hydrolysis of the 3β-acetate, over 24 hours, followed by hydrogenation of the $\Delta^{10,19}$ double bond gave the 3β-hydroxy steroid (**100A**) (Scheme 67).

Inversion of the configuration at C(3) using the Mitsonubu reaction[103] was performed on **100** to give the 3α-hydroxy steroid (**103**) via the 3α-formyl ester (**102**). Hydrolysis of the 3α-formyl group took 24 hours at room temperature (Scheme 68), compared to 1 hour for the more facile 3α-formyl ester of the 5-keto secosteroids. The ^1H NMR signal for the 3β-proton of **103** was shifted

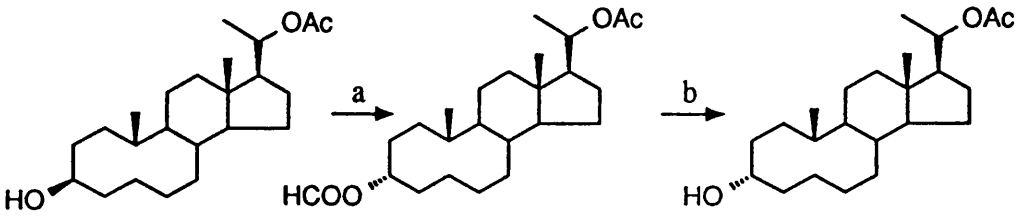


98 **101** **100A**

Reagents and conditions: a. i. $C_6F_5OC(S)Cl$, py., HOSu, $PhCH_3$, 80°C, ii. Bu_3SnH, AIBN, $PhCH_3$, 110°C; b. K_2CO_3, MeOH, rt, 24 hr; c. H_2, Pd/C, EtOH.

Scheme 67

considerably upfield with respect to that of the 3α-proton of **100**, proving an inversion in stereochemistry at this position had indeed occurred, giving rise to the desired axial 3α-hydroxyl group.



100 **102** **103**

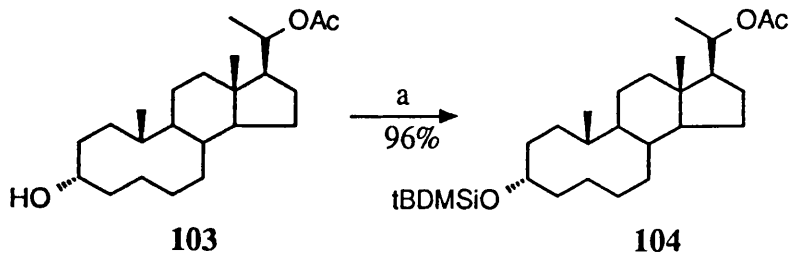
Reagents and conditions: a. DEAD, PPh_3, HCO_2H, THF; b. K_2CO_3, MeOH, rt, 24 hr.

Scheme 68

The following preparative steps were all carried out on 15 milligrammes of the 3α-hydroxysteroid (**103**), and therefore limited spectral data is available (see experimental section).

Protection of the 3α-hydroxyl group was effected, as before, using *tert*-butyldimethylsilyl chloride and imidazole to give the 3α-silyl ether (**104**) in a quantitative yield (Scheme 69).

Manipulation of the C(20) substituent was then carried out to give the desired 20-ketone. Hydrolysis of the 20-acetate, with potassium carbonate, took 24 hours

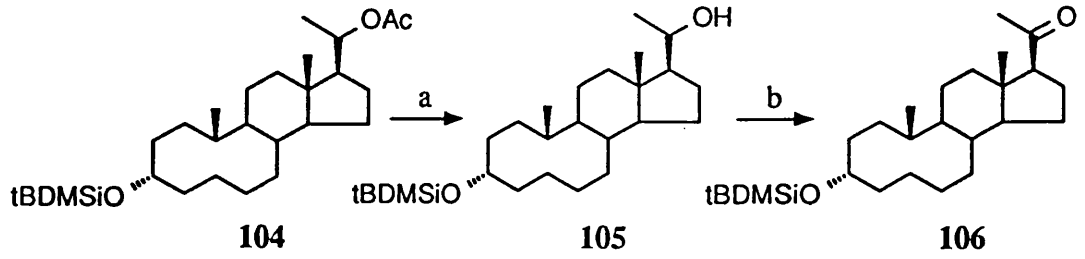


103 **104**

Reagents and conditions: a. tBDMSiCl, Im, DMF, rt, 24 hr.

Scheme 69

at reflux to give the 20-hydroxy steroid (**105**) which, upon treatment with PCC gave the 20-keto steroid (**106**). Only 3 mg of the 20-keto-steroid was isolated (Scheme 70).

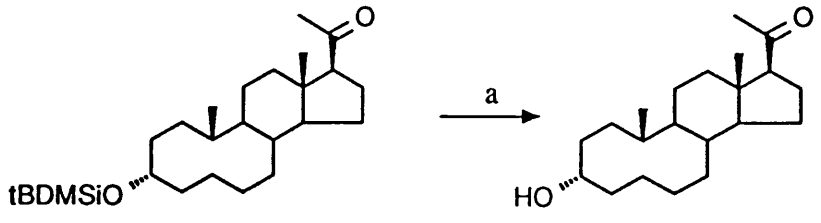


104 **105** **106**

Reagents and conditions: a. K_2CO_3, MeOH, Δ, 24 hr; b. PCC, CH_2Cl_2, rt, 3 hr.

Scheme 70

Deprotection of the 3α-silyl ether of **106** was carried out using a THF solution of TBAF[105] to give 0.5 mg. of the target compound, 5,10-secopregna-3α-ol-20-one (**107**) (Scheme 71).



106 **107**

Reagents and conditions: a. TBAF, THF, rt, 24 hr.

Scheme 71

2.5 Preparation of 3α-hydroxy-5(10→1βH)*abeo*pregnan-20-ones

The Baldwin rules for ring closure

Baldwin has provided a specific set of rules for certain closings of 3- to 7-membered rings.[157] These rules distinguish two types of ring closure, called *exo* and *endo*, and three kinds of atoms at the starred positions: *Tet* for sp^3, *Trig* for sp^2, and *Dig* for *sp* (Figure 21).

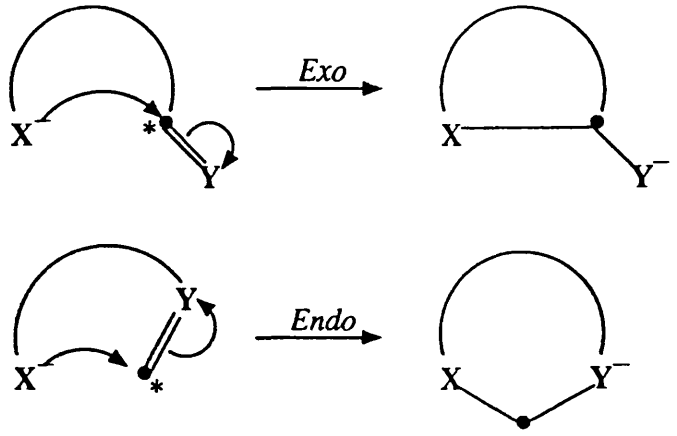


Figure 21. Baldwin's rules for ring closure.

The following are Baldwin's rules for closing rings of 3 to 7 members.

Rule 1. Tetrahedral systems

a. 3- to 7-*Exo-Tet* are all favoured processes,

b. 5- to 6-*Endo-Tet* are all disfavoured.

Rule 2. Trigonal systems

a. 3- to 7-*Exo-Trig* are favoured,

b. 3- to 5-*Endo-Trig* are disfavoured,

c. 6- to 7-*Endo-Trig* are favoured.

Rule 3. Digonal systems

a. 3- to 4-*Exo-Dig* are disfavoured,

b. 5- to 7-*Exo-Dig* are favoured,

c. 3- to 7-*Endo-Dig* are favoured.

"Disfavoured" does not mean it cannot be done, only that it is more difficult than the favoured cases, due greatly to the disfavoured processes requiring severe distortion of bond angles and distances in the linking chain for reaction. These rules are empirical and have a stereochemical basis.

Synthesis of 3α,5α-dihydroxy-5(10→1βH)abeopregnan-20-one (**116**)

Evidence exists for a transannular interaction between the carbon-carbon double bond and the carbonyl group, when these groups are located opposite each other in a ring of medium size.[158] Kosower *et al*[159] reported the use of solvent effects to identify a transannular electronic transition involving the carbon-carbon double bond and the carbonyl group of 5-cyclodecenone. It was suggested that such a transition, which led to a bond in the excited state between atoms not bonded in the ground state, be called *photodesmotic* (Figure 22).

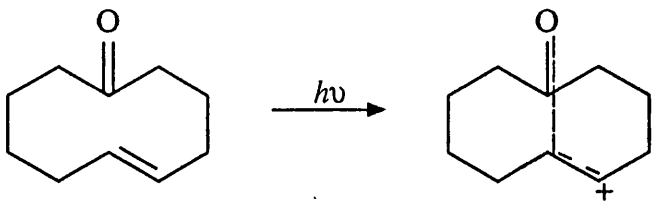


Figure 22. The photodesmotic transition of 5-cyclodecenone.

Leonard and Owens[158] showed that the transannular interaction between the carbon-carbon double bond and the carbonyl group in 5-cyclodecenone was apparent in the excited state from the ultraviolet spectrum, but not detected in the

ground state by infrared, indicating that the transannular interaction was an excited state phenomenon. They suggested that, within a solvent cage, in the 10-membered ring the aforementioned groups were held close together in conformations possessing little strain.

Lange and Bosch[160] have also reported on the ease of transannular reaction between the carbon-carbon double bond and the carbonyl group of *trans*-5-cyclodecenone. An example of a transannular Paterno-Buchi reaction, Figure 23, was demonstrated by the photochemistry of *trans*-5-cyclodecenone.

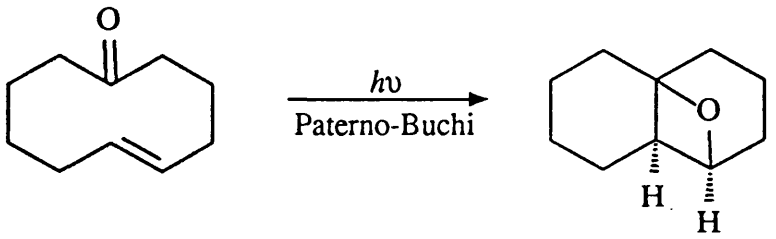
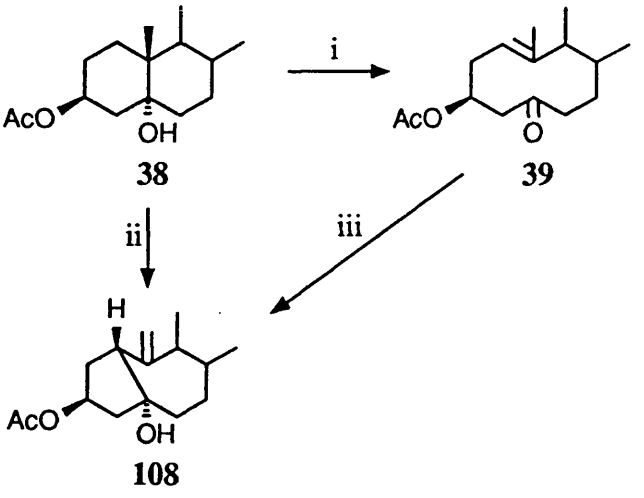


Figure 23. The transannular Paterno-Buchi reaction of *trans*-5-cyclodecenone.

Irradiation of *cis*-5-cyclodecenone resulted in complete isomerisation to the *trans*-isomer,[160] which would then undergo the transannular Paterno-Buchi reaction.

It is known that (E)-cyclodecenones of similar type to the 5,10-secosteroid **(39)** easily undergo thermal and acid-catalysed cyclisations.[161] Acid-catalysed cyclisations of **39** afforded steroid compounds of the 5(10→1)*abeo* type, involving intramolecular C(1)-C(5) bond formation to give A- nor B-homo derivatives with the *trans*-1β,5α-configuration (i.e., 5(10→1βH)*abeo*-5α-steroids),[97,162] more details in chapter 2.6. Both the five(5-*Exo-Trig*)- and the seven(7-*Exo-Trig*)-membered ring formations are favoured by Baldwin's rules for ring closure.[157]

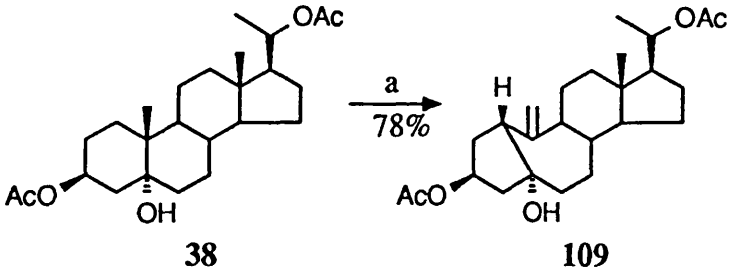
The 5,10-secosteroid (**39**) was formed by oxidation of the 5α-hydroxy steroid (**38**) using ceric ammonium nitrate in refluxing acetonitrile, the reaction mixture being quenched after 3 minutes. However if the reaction was allowed to proceed for a further 2 minutes, the 5(10→1βH)*abeo*steroid (**108**) is isolated (Scheme 72).



Reagents and conditions: i. ceric ammonium nitrate, CH_3CN, 80°C, 3 min; ii. ceric ammonium nitrate, CH_3CN, 80°C, 5 min; iii. 2N HCl, 0°C, 4 hr.

Scheme 72

Reaction of the 5α-hydroxy pregnane (**38**) with ceric ammonium nitrate at 80°C for 5 minutes yielded the 5(10→1βH)*abeo*steroid (**109**) as a white solid (Scheme 73).

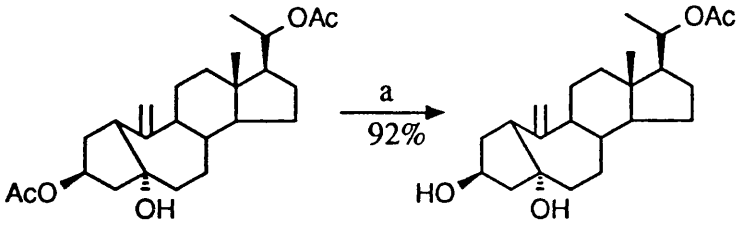


Reagents and conditions: a. CAN, CH_3CN, H_2O, 80°C, 5 min.

Scheme 73

Selective hydrolysis of the 3β-acetate again occurred rapidly at room temperature

using potassium carbonate to give the diol (**110**) (Scheme 74).

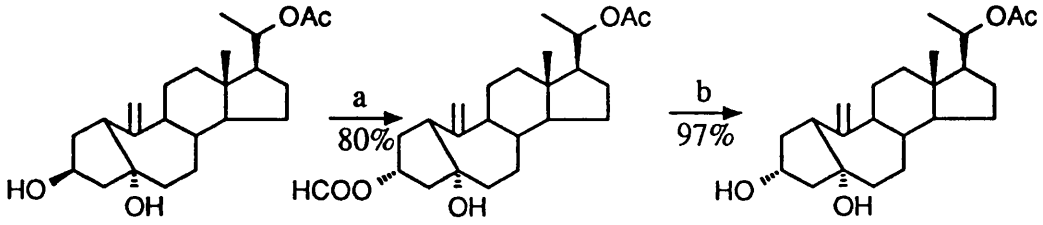


109 **110**

Reagents and conditions: a. K_2CO_3, MeOH, rt, 30 min.

Scheme 74

Inversion of the 3β-hydroxyl of **110** occurred regioselectively, with the 5α-hydroxyl group being too hindered to undergo any reaction. Thus the 3α-formyl ester (**111**) was formed[114] in a good yield of 80% (Scheme 75), and underwent hydrolysis to give the 3α-alcohol (**112**) in a quantitative yield.



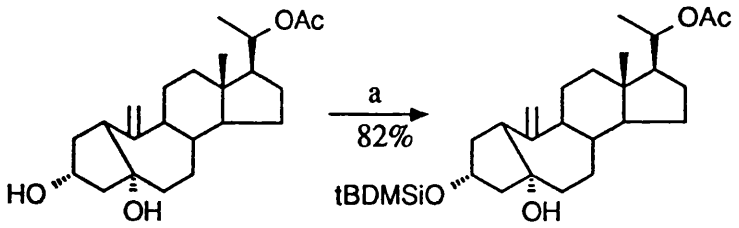
110 **111** **112**

Reagents and conditions: a. DEAD, PPh_3, HCO_2H, THF; b. K_2CO_3, MeOH, rt, 30 min.

Scheme 75

It was possible to chemically distinguish between the 3α-hydroxyl and the 5α-hydroxyl groups of **112** by using a sufficiently bulky protecting group, although at this stage in the synthesis di-protection would not be a problem. The 3α-silyl ether (**113**) was formed in the conventional manner (Scheme 76).

Hydrolysis of the 20-acetate of **113** gave the 20-alcohol (**114**) by refluxing in the presence of potassium carbonate for 24 hours. Oxidation of **114** using PCC was regioselective in giving the 20-keto steroid (**115**), i.e., the 5α-hydroxyl was inert to

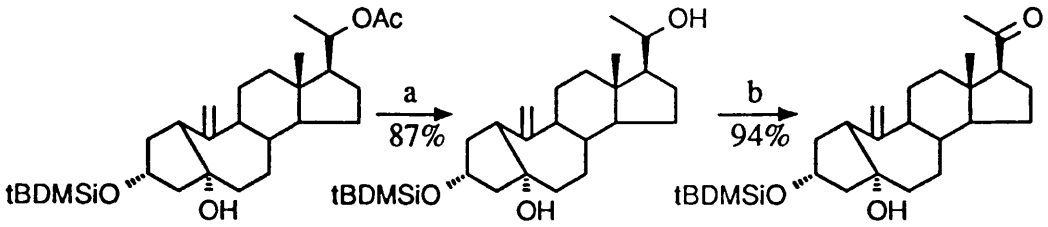


112 **113**

Reagents and conditions: a. tBDMSiCl, Im, DMF, rt.

Scheme 76

the reaction conditions (Scheme 77).

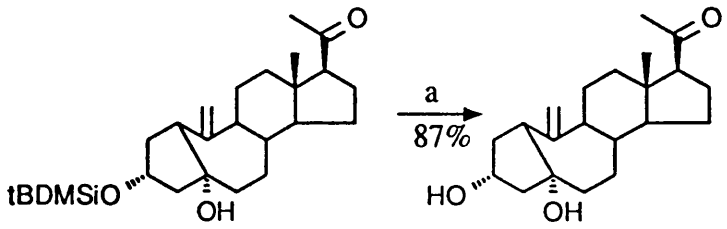


113 **114** **115**

Reagents and conditions: a. K_2CO_3, MeOH, Δ, 24 hr; b. PCC, CH_2Cl_2, rt, 3hr.

Scheme 77

Deprotection of the 3α-silyl ether was carried out using a THF solution of tetrabutylammonium fluoride to give the 3α-hydroxy steroid (**116**) as a white solid (Scheme 78).



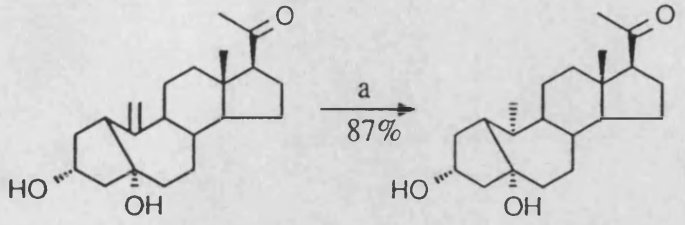
115 **116**

Reagents and conditions: a. TBAF, THF, rt.

Scheme 78

Finally, hydrogenation of the exocyclic $\Delta^{10,19}$ double bond of **116** occurred smoothly at atmospheric pressure, using palladium catalyst, to give the desired

compound, 3α,5α-dihydroxy-5(10→1βH)*abeo*pregnan-20-one (**117**) (Scheme 79) in an overall yield of 22% from pregnenolone, or 36% from the 5(10→1βH)*abeo*-steroid (**109**) over 12 and 8 steps respectively.



116 **117**

Reagents and conditions: a. H_2, Pd\C, EtOH, 15 hr.

Scheme 79

Recrystallisation of **117** from ethyl acetate provided crystals suitable for an X-ray crystallographic determination, which duly confirmed the structure as 3α,5α-dihydroxy-5(10→1βH)*abeo*pregnan-20-one [(Figure 24) for full details see Appendix].

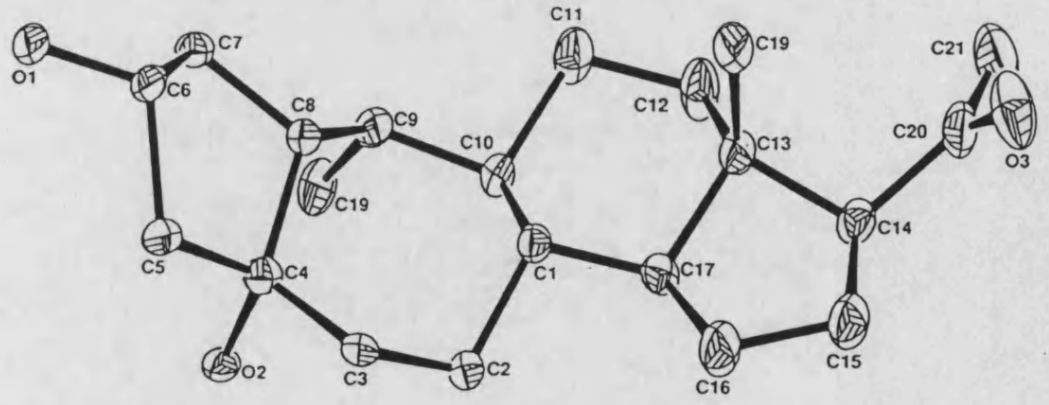


Figure 24. X-ray plot of 3α,5α-dihydroxy-5(10→1βH)*abeo*pregnan-20-one (**117**), (ORTEP plot).

As may be seen from the X-ray structure, hydrogenation of the $\Delta^{10,19}$ double bond of **116** gave rise to the α-methyl substituent at C(10). Also, examination of the solid state molecular packing revealed that the lattice was dominated by intermolecular hydrogen bonds through the hydroxyl groups. Throughout the lattice hydrogen bonds occur between the 3α-hydroxyl group of one steroid and the 5α-hydroxyl of another steroid, and *vice versa*. The ultimate consequence of these intermolecular contacts is an array of infinite herringbone polymers parallel to the a axis. The backbone of each herringbone is composed of two one-dimensional polymers. These polymers are cross-linked by the hydrogen bonds [(Figure 25), full details are in Appendix]. This is, to the best of our knowledge, a new supermolecular structural type (for a relevant review see Bishop *et al* [163]).

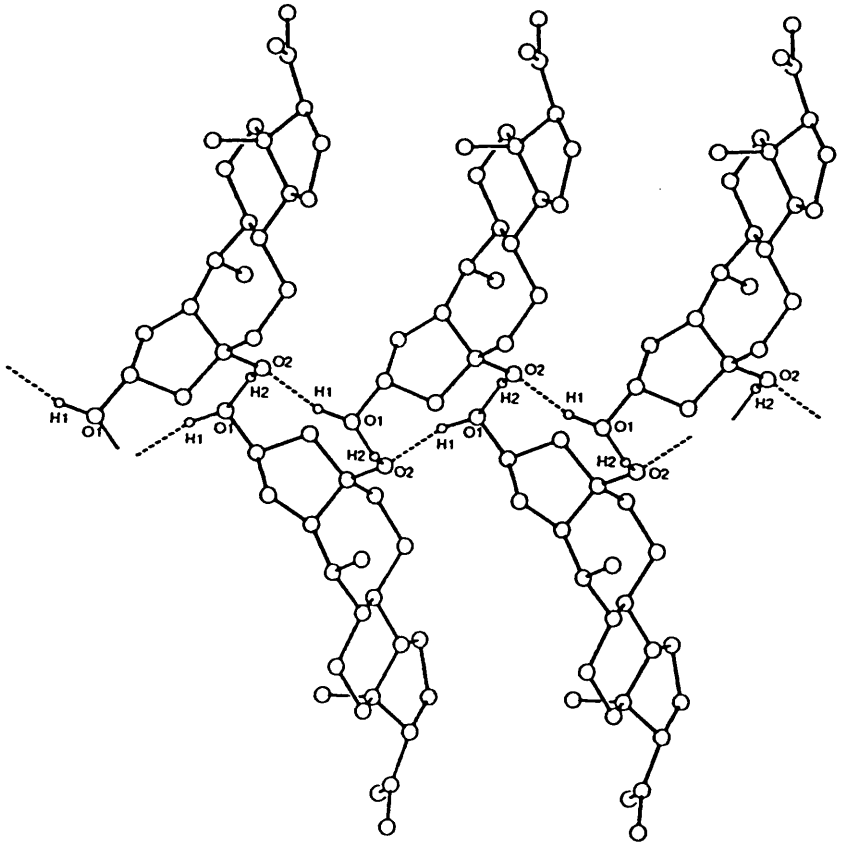
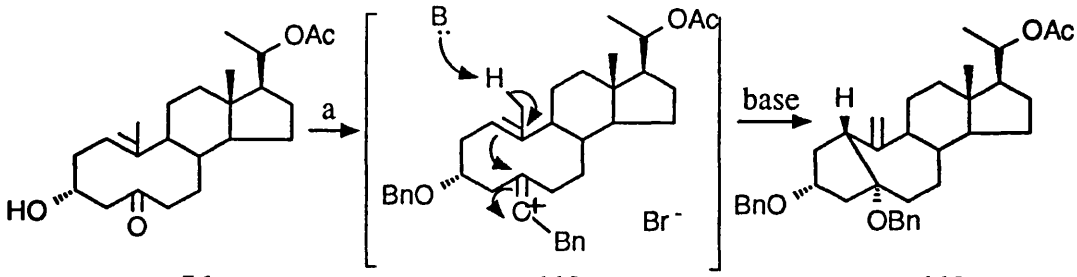


Figure 25. Solid state molecular packing of **117.**

The target compound (**117**) may be synthesized by an alternative route. Attempted benzylation of the 3α-hydroxyl of **56** resulted in protection of the 3-hydroxyl together with transannular ring closure across the 10-membered ring *via* coordination of the benzyl group to the 5-carbonyl (**118**). Thus the dibenzyl ether (**119**) was formed as a white solid in a 68% yield (Scheme 80). From the ^{1}H NMR of **119**, one of the exocyclic vinylic protons exists above the plane of the rearranged A,B rings, and one below, β- and α-protons respectively. The α-proton interacts, in some way, with the π system of the 5α-benzyl group. The ^{1}H NMR signal for this proton is thus shifted considerably downfield from approximately 5 ppm in steroids such as **117** to 9.5 ppm in the π-coordinated 5α-benzyl steroids such as **119**.

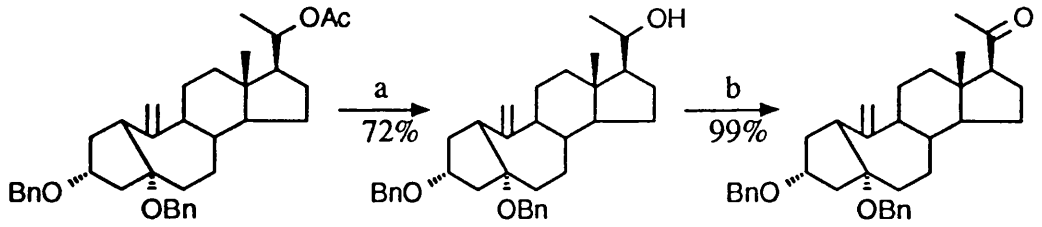


56 **118** **119**

Reagents and conditions: a. $PhCH_2Br$, NaH, THF, rt, 24 hr.

Scheme 80

Base hydrolysis of the 20-acetate of **119**, followed by PCC oxidation of the resulting 20-hydroxyl (**120**) gave the desired 20-ketone substituent (**121**) (Scheme 81).

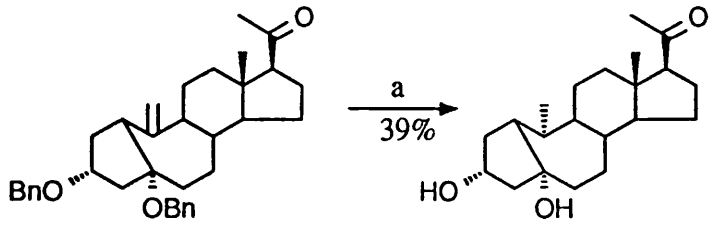


119 **120** **121**

Reagents and conditions: a. K_2CO_3, MeOH, Δ, 24 hr; b. PCC, CH_2Cl_2, rt, 3 hr.

Scheme 81

Hydrogenation of **121** achieved both deprotection of the benzyl ethers together with reduction of the $\Delta^{10,19}$ double bond to give the target compound **117**, 3α,5α-dihydroxy-5(10→1βH)*abeo*pregnan-20-one (Scheme 82), in an overall yield of 8% from pregnenolone over 11 steps.



121 **117**

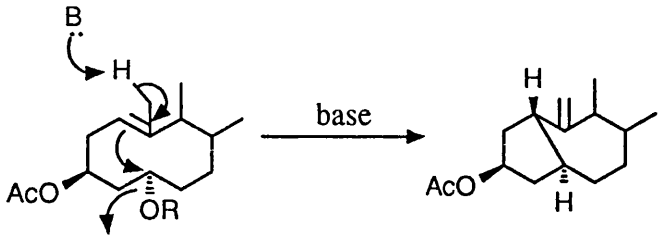
Reagents and conditions: a. H_2, Pd\C, EtOH, 24 hr.

Scheme 82

Synthesis of 5(10→1βH)abeopregnan-3α-ol-20-one (**128**)

It has also been shown, in Chapter 2.4, that transannular ring closure across the 10-membered ring of the 5,10-secosteroid may be promoted by base, as well as acid, via extraction of a 19-methyl proton (Scheme 83). Ring closure between C(1) and C(5) occurred stereoselectively, via olefination of the C(10)-C(19) bond to give the exocyclic $\Delta^{10,19}$ double bond (characterised by two singlets in the proton NMR for the olefinic protons, around 5 ppm), and the 1β-proton. It was usually not possible to isolate the 5-substituted secosteroid (**122**). Again, both the

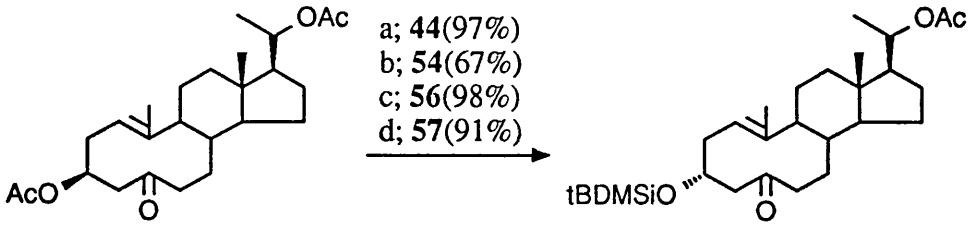
five(5-*Exo-Tet*)- and the seven(7-*Exo-Tet*)-membered ring formations are favoured according to Baldwin's rules for ring closure.[157]



122; R = Ts, Ms, or Xanthate **88**

Scheme 83

The preparation of the 3α-silyloxy secosteroid (**57**) has been described previously in Chapter 2.3. Its synthesis from the 5,10-secosteroid (**39**) is summarized in Scheme 84.



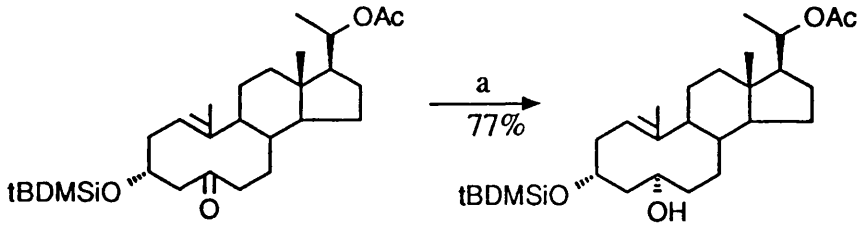
39 **57**

Reagents and conditions: a. K_2CO_3, MeOH, rt; b. DEAD, PPh_3, HCO_2H, THF; c. K_2CO_3, MeOH, rt; d. tBDMSiCl, Im, DMF, rt.

Scheme 84

Stereoselective reduction of the 5-carbonyl of **57**, using sodium borohydride gave the 5α-hydroxy secosteroid (**123**) as the only product (Scheme 85).

Advantage was taken of the fact that attempted elimination of the 5α-hydroxyl group of the 5,10-secosteroid resulted in transannular ring closure, as discussed previously. Reaction of the 5α-hydroxy steroid (**123**) with tosyl chloride and

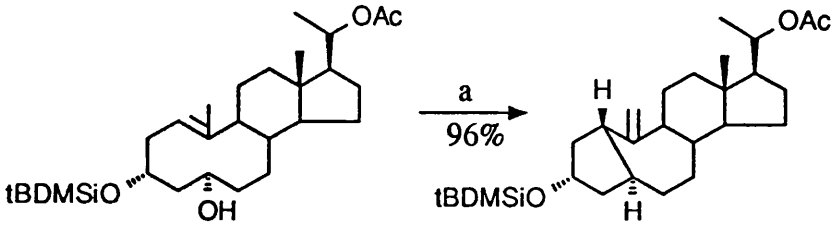


57 **123**

Reagents and conditions: a. $NaBH_4$, EtOH, rt, 48 hr.

Scheme 85

pyridine resulted in the *in situ* elimination of the resulting tosyl group to give to 5(10→1βH)*abeo*steroid (**124**) in a yield of 96% (Scheme 86).

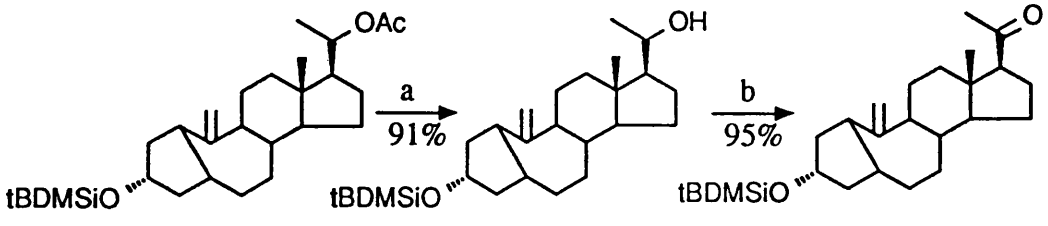


123 **124**

Reagents and conditions: a. TsCl, py., CH_2Cl_2.

Scheme 86

As before (Chapter 2.3), the 20-acetate (**124**) was hydrolyzed to give the 20-hydroxy steroid (**125**), which on reaction with PCC gave the desired 20-carbonyl (**126**) (Scheme 87).



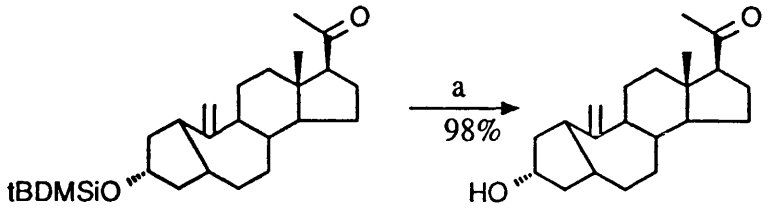
124 **125** **126**

Reagents and conditions: a. K_2CO_3, MeOH, Δ, 24 hr; b. PCC, CH_2Cl_2, rt,3 hr.

Scheme 87

Deprotection of the 3α-silyl ether of **126** was achieved using tetrabutylammonium

fluoride to give a quantitative yield of the 3α-hydroxy steroid (**127**) as a colourless oil (Scheme 88).

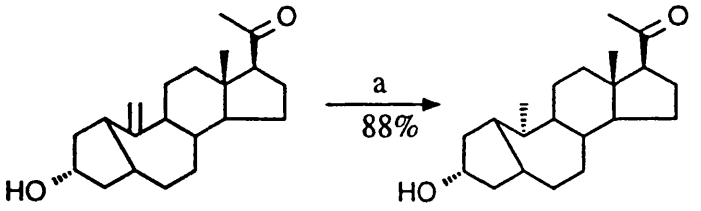


126 **127**

Reagents and conditions: a. TBAF, THF, rt.

Scheme 88

The target compound, 5(10→1βH)*abeo*pregnan-3α-ol-20-one (**128**) was formed by hydrogenation of the $\Delta^{10,19}$ double bond of **127** at atmospheric pressure, using a palladium catalyst (Scheme 89). The 5(10→1βH)*abeo*steroid was isolated as a white solid, and in an overall yield of 21% from pregnenolone, over 14 steps (or 32% from the 5,10-secosteroid (**39**)).



127 **128**

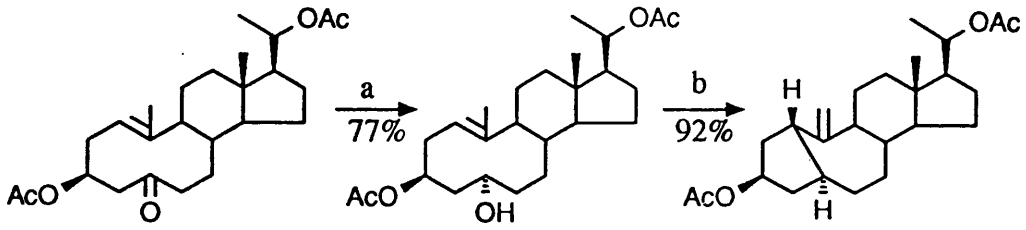
Reagents and conditions: a. H_2, Pd\C, EtOH, 12 hr.

Scheme 89

The 3α-silyloxy-5(10→1βH)*abeo*steroid (**124**) may be synthesized by an alternative route.

Reduction of the 5-ketone of **39** occurred stereoselectively, from the β face of the carbonyl, to give the 5α-alcohol (**87**). *In situ* elimination of the intermediate 5α-tosylate, formed from the 5α-alcohol (**87**), via transannular reaction gave the

5(10→1βH)*abeo*steroid (**88**) in a yield of 92% (Scheme 90).

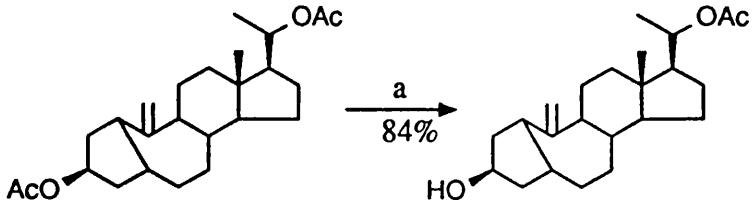


39 **87** **88**

Reagents and conditions: a. $NaBH_4$, EtOH, 0°C - rt; b. TsCl, py.

Scheme 90

Regioselective hydrolysis of the 3β-acetate of **88** was possible, although at a slower rate than that of **39**. The 3β-alcohol (**129**) was formed after treatment of **88** with potassium carbonate for 3.5 hours (Scheme 91).

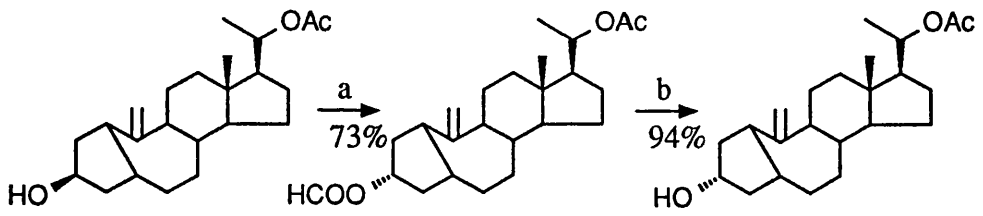


88 **129**

Reagents and conditions: a. K_2CO_3, MeOH, rt, 3.5 hr.

Scheme 91

Inversion of the configuration of the 3β-hydroxyl of **129** was carried out following the procedure of Mitsonubu,[103] generating the 3α-hydroxy steroid (**131**) via the 3α-formyl ester (**130**) in an overall yield of 69% (Scheme 92).

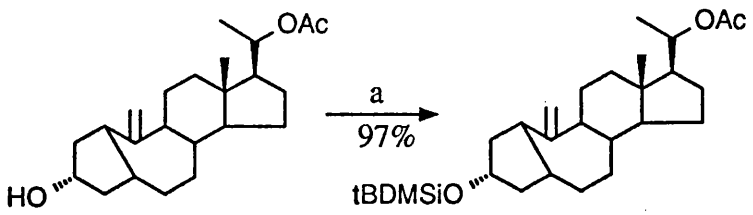


129 **130** **131**

Reagents and conditions: a. DEAD, PPh_3, HCO_2H, THF; b. K_2CO_3, MeOH, rt, 1 hr.

Scheme 92

Protection of the 3α-hydroxyl group of **131** was performed as usual to give the 3α-silyloxy steroid (**124**) in a quantitative yield (Scheme 93).



131 **124**

Reagents and conditions: a. tBDMSiCl, Im, DMF, rt, 1 hr.

Scheme 93

The target compound 5(10→1βH)*abeo*pregnan-3α-ol-20-one (**128**) may then be prepared as described earlier in this chapter. The overall yield of **128** *via* this second route, whereby ring closure was carried out prior to inversion of configuration at C(3), was 17% from pregnenolone, over 14 steps (or 26% from the 5,10-secosteroid (**39**)).

2.6 Conformational analysis of A and B rings of 5,10-secosteroids

It has been generally recognized that in studies on structure-activity relations it is very important to include the three-dimensional structure information. For that reason conformational analysis together with molecular graphics have now become

key tools in computer-assisted drug design.[164]

Chemical structures used in studies on biological activity are usually specified on different levels of precision and sophistication. The *constitution* of a molecule simply specifies its elemental composition and the atomic topology. The *configuration* gives the spacial attachment of the atoms, while the molecular *conformation* designates the arrangement of the atoms in a molecule with a given configuration in three-dimensional space. Of critical importance are the effects on attractive and non-attractive lipophilic, polar, hydrogen bonded and charged interactions with the receptor.

The borderline between configuration and conformation is usually defined by stating that configurational isomers can only be converted by breaking and mending chemical bonds, whereas conformational isomers (*conformers*) are interconverted solely by rotation about single bonds simultaneously with co-operative geometry deformations. The barrier height of the potential energy of a molecule that experiences a conformational conversion is relatively low. Therefore, in solution some of the interconversions may easily take place, even at room temperature, so that different conformers will co-exist in a dynamic manner. Moreover, a conformer which remains within a certain potential well may exhibit a considerable conformational flexibility.

X-ray diffraction in single crystals yields the molecular geometry, and the molecular packing and nuclear magnetic resonance (NMR) is used to give additional information about the conformation of molecules in solution. The X-ray structures can be used as starting geometries in molecular mechanics studies. This computational technique allows a relative energy to be associated with each conformation and it has the ability to minimize the energy with respect to variations of the molecular geometry. An indispensible reference on structural

data has been compiled in the "Atlas of Steroid Structure".[165] Within the Born-Oppenheimer approximation, the Schrödinger equation for a molecule can be separated into a part describing the motions of the nuclei. Molecular mechanics studies the latter set of motions, using empirical potential functions to describe the forces between nuclei due to the electrons.

Molecular mechanics makes it feasible to explore the multi-dimensional potential energy surface that describes the energy of the molecule instead of studying just a few selected geometries. Within its limit of applicability, the method is capable of satisfactorily reproducing experimentally determined geometries and relative potential energies.

The concepts behind molecular mechanics are conceived in plain terms with respect to covalent bonds, charge interactions and forces between non-bonded atoms. The molecular energy is obtained from the atomic coordinates as a simple sum of a series of classical potential energy functions. Required is a set of empirical functions, known as a *force field.* An algorithm is used for minimizing the potential energy by adjusting the atomic coordinates. Unfortunately, the success of the molecular mechanics method depends on experimental data, and the reliability can be no better than that of the data used for the initial parameterization of the force field.

The structural model is expressed mathematically in a set of coordinates. From such a model the potential (steric) energy can be calculated. The steric energy is then minimized by varying the coordinates in an iterative process. To compare the relative energies of different conformers, and to get an idea about the height of an interconversion barrier, geometry optimization with respect to energy minimization is a necessity. Several techniques have been developed to find the geometry for which the energy is simultaneously a minimum with respect to all

coordinates.[166]

Intermolecular interactions and molecular flexibility

Hydrogen bonds play a crucial role in intermolecular steroid-receptor interactions and they account partly for the packing forces in the solid state. The *head-tail* designation is used to describe hydrogen bonding in the crystal, which refers to the edges of the steroid molecular corresponding to C(3) and C(17) respectively or to substituents on these positions. For van der Waals interactions it is illustrative to consider the shape of the molecule.

The molecular flexibility of a steroid molecule may be simulated by molecular mechanics. The positions of certain atoms may be changed step-wise in such a way that the molecule may be bent towards the α- or β-side. Minimisation will then give the minimum energy conformation of the molecule.

The configuration of anaesthetically potent steroid molecules may mimic the actual situation at the receptor site, where presumably also very favourable hydrogen bond interactions occur.

Conformational analysis of six- and ten-membered rings[167]

Cyclic hydrocarbons have long been a major target of conformational analysis.[168] Pseudorotation[169] is the mode of interconversion between the different twist-boat forms of cyclohexane as well as between different symmetrical conformers of larger ring hydrocarbons. Pseudorotation usually surmounts low-energy barriers and is associated with a simultaneous change of all ring torsion angles without severe distortions in bond angles. Another mode of conformational interconversion, the symmetrical mode, usually crosses higher energy barriers than pseudorotation. Examples of the symmetrical mode are transitions from the cyclohexane chair form to the twist-boat form retaining an axis of symmetry, and

to the boat form retaining a plane of symmetry.[170]

Six-membered rings

The twist-boat (TB) conformer interconverts to its enantiomer (via pseudorotation) through the boat conformation. The boat conformation exhibits a very low energy barrier. The chair to twist-boat (C-TB) interconversion (symmetrical mode) courses a fairly high energy barrier through a so-called half-chair conformation. Both interconversions exhibit the very same kind of coordinated atomic movement. This atomic movement has been termed *kayaking*, because it involves three ring bonds A-B-C-D following a moving pattern similar to the pattern exhibited by the movement of the kayaker's arms and the paddle. Kayaking is associated with characteristic torsion angle differences. There is a significant change in the middle torsion angle (B-C) and a smaller change in both the other torsion angles (A-B and C-D) that is opposite to the change in the central torsion angle. The TB-TB interconversion exhibits two kayaking patterns. The C-TB interconversion exhibits one kayak pattern. This particular version of kayaking is coded "K3"

Ten-membered rings

Only a few low-energy cyclodecane conformations have been discussed in detail.[170,171] The symmetrical boat-chair-boat (BCB) form has been found to be the minimum energy conformer, cyclodecane favouring the BCB form as much as cyclohexane favours the chair form.[171] The BCB form pseudorotates with itself and has been found in the crystal structure of several cyclodecane derivatives. It may be that crystal packing effects gives rise to the crystals favouring the BCB form.

The five lowest energy conformers and their relative strain energies (in KJ/mol) are: BCB (0), TBCC (1.8), TBC (4.7), TCCC (4.8), and BCC (6.4). The TCCC

conformer is the crown form of cyclodecane, whereas the CCC conformer is neither a minimum nor a saddle point.[171]

The higher energy interconversions are dominated by the K3-kayaking mode, but cyclodecane displays new versions of the basic kayaking and flapping motions; handle flapping and single corner flapping. Handle flapping almost rigidly flaps a ring torsion angle A-B-C-D about the hinge comprised of the line AD. The new mode of kayaking is termed K5-kayaking, and is different from K3-kayaking in that it involves five (A′-A-B-C-D-D′), rather than three ring bonds (A-B-C-D).

In summary, the different modes of conformational interconversion associated with the lowest barriers between minimum energy conformers display patterns of well-coordinated atomic movement. It was found that 97% of the 115 conformational interconversions in cyclohexane to cyclodecane exhibit the same basic atomic movements.[167] Each interconversion, no matter how complex, can be separated into a few basic modes; termed kayaking and flapping.

Conformations of the ten-membered ring in 5,10-secosteroids

X-ray analysis of the E-isomer of the 5,10-secosteroids (3β-epimer) showed the cyclodecenone ring to adopt an extended crown conformation, of type A_1, (Figure 26), of approximate symmetry 2/m (C_{2h}).[172] Similar shapes have been reported for the 10-membered ring in both the 1:1 silver nitrate adduct of germacatriene[173] and elephantol p-bromobenzoate.[174]

The flexibility of the 10-membered ring makes its geometry very sensitive to even small perturbations, for example, substituent effects or crystal packing forces.

From ^1H and ^{13}C NMR, in solution, the 10-membered ring exists in at least two distinct forms, the predominant one corresponding to the solid state conformation

of A_1, and the minor most likely to be B_2 (Figure 26). Although the results show only the two most populated conformations, the actual time-dependent spatial arrangement is much more complicated. Conformations with relative populations of a few percent affect the NMR spectra only slightly but might be important in a particular chemical reaction.

Additional effects such as the transannular interaction of the double bond with the carbonyl group have been postulated to explain the unexpected thermodynamic stability of the 'crown'-type conformation.

Four possibilities were assumed of orientating the olefinic methyl group and the C(5) carbonyl group above, or below, the plane of the 10-membered ring[172] (Figure 26). Other conformations distinctly different from the four proposed lead to stronger steric interactions. A discussion of conformations of medium sized monocyclic carbonyl compounds in solution has been published by Anet *et al.*[175]

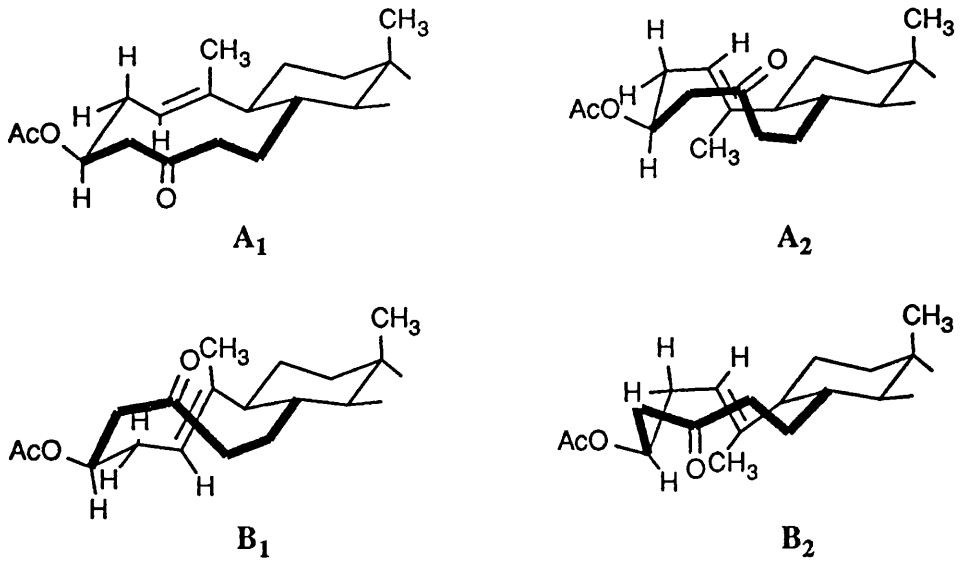


Figure 26. Conformations; i) A_1: major conformation in solution and solid state conformation, ii) A_2, iii) B_1, iv) B_2 : minor conformation in solution.

Two classes of possible conformations may be distinguished according to the

chemical shifts of the olefinic proton[83] : in class A; H-C(1) is in the shielding region of the keto group, in class B; it is unaffected.

The four conformations of Figure 26 are characterized as follows:

A_1 (*crown conformation = solid state conformation*). H-C(1) is in the shielding region of the keto group.

A_2 (*ground-state cyclodecane conformation*). H-C(1) is in the shielding region of the keto group. However, the olefinic methyl group and C(6) are now in position α.

B_1 (*ground-state conformation of (E)-cyclodecene-$AgNO_3$-complex*[176]). The olefinic methyl group at C(10) is in the shielding region of the keto group and H-C(1) is not influenced by the latter. The olefinic methyl group is above the plane of the ring.

B_2 (*conformation proposed in reference* 161i). As in B_1 the olefinic methyl group is in the shielding region of the ketone.

Conformation B_2 has a slightly higher energy than conformation A_1, of about 1 Kcal/mol[172] (or 4.18 KJ/mol). The conformation B_2, postulated as the minor component in solution, represents according to calculations the least stable of the discussed set of four (Figure 26). The relative rigidity of the system and the transannular electronic interaction between the double bond and the carbonyl group could account for the higher than expected stability of B_2.

Proton NMR experiments carried out on the 5,10-secosteroid (**57**) supported the existence of various conformations of the 10-membered ring in solution. Looking

at the 360 MHz data, the signal from 3β looked different in $CDCl_3$ to that in C_6D_6, presumably due to different couplings to neighbouring protons. Vicinal couplings are not expected to be solvent dependent, thus it was concluded that there was more than one conformation in solution. Two or more conformations are in fast exchange on the NMR timescale, and the equilibrium between different conformers is perturbed by solvation effects. Some of the results of the NMR studies are included in the Appendix.

The geometry of the double bond deviates only slightly from planarity, in contrast to other (**E**)-cyclodecene structures. The geometrical prerequisite for this is the short transnnular distance C(1).....C(5) of only approximately 2.84Å, considerably less than the sum of the van der Waals radii. Apart from the absence of steric hinderance between the two sp^2-carbon atoms C(1) and C(5), the short transannular interaction of the olefinic bond and the carbonyl double bond can be explained by the π-interaction of the type reported for (**E**)-cyclodec-5-enone[177] and analogous cases.[178]

The two deduced conformations can be correlated with the stereochemical course of different chemical transformations described for compounds of type **39**;
1. Reduction of the C(5)-oxo group.[179] In the two conformations, A_1 and B_2, a preferential attack at the C(5) carbonyl atom from the less hindered front side could be expected. Consequently the (5S)-alcohol is formed.
2. Acid catalysed cyclization.[83] When treated with acid in aprotic solvents the (**E**)-5,10-seco-enone is cyclized to a 9:1 mixture of two isomeric compounds[172] (Figure 27). The *trans*-compound can be stereochemically correlated with B_2 and the *cis*-isomer with A_1.

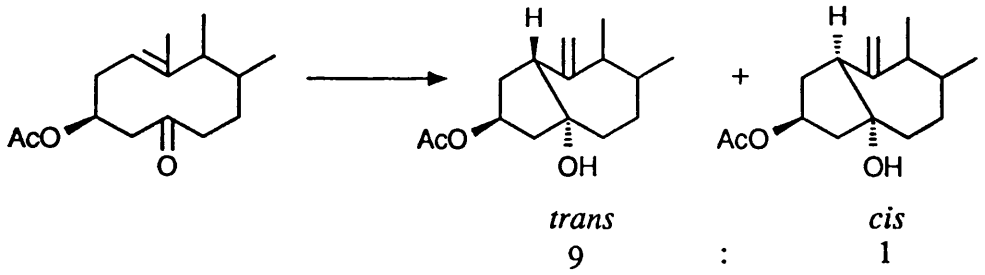


Figure 27

3. Thermal cyclization.[161] An intramolecular synchronous (symmetry allowed) process can only be formulated if B_2 is the involved conformation (Figure 28).

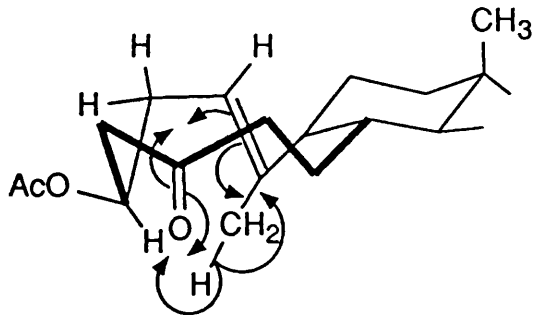


Figure 28. Transition state conformation for the thermal cyclization

The π-interaction between the olefinic bond and the ketone may be the reason for the unsuccessful attempts to reduce the C(1)-C(10) double bond by, for example, hydrogenation.

The increased rate of hydrolysis of the C(3) esters can be explained in terms of the relatively unhindered environment of the C(3) substituents in the 'crown' conformation, A_1.

The six-membered ring, ring C, assumes the normal chair conformation, and the five-membered ring, ring D, is intermediate between a C(13) envelope and a C(16) half chair.

The resonance signal, in the NMR spectrum, of the *trans*-isomer is shifted upfield by approximately 0.54 ppm with respect to the *cis*-isomer. This is due to the olefinic proton laying just above the carbonyl double bond of the keto group, i.e., in the region of positive shielding. It is assumed that the shielding influence of the C(5) carbonyl group causes a high field shift of the H-C(1) resonance in the main conformation. This influence is not operative for the minor conformation, where the keto group and the vinylic proton are anti-parallel to each other. Ring-flipping does not change the relative positions.

The olefinic hydrogen of the 5,10-seco-enone has a similar orientation as the olefinic hydrogen of *trans*-5-cyclodecenone. It is coupled to the C(19) methyl group, but the coupling is unresolvable on our NMR timescale.

The change in the configuration of the C(3) acetoxy group from α to β leads to inversion of the OC(5) and $H_3C(19)$ moieties between α and β positions for both conformations.[97] This behaviour indicates that the energy difference between the two types of conformations is small compared to the energy difference between an axial and an equatorial acetoxy group.

Molecular mechanics techniques have been used to investigate the conformational space of a set of steroid analogues.

An initial conformation of the 5,10-secosteroid (**39**) was built from its internal coordinates (bond length, angle, and torsion) derived from X-ray defraction reference using the Insight Biosym molecular modelling suite.[180] This structure was then minimised (Figure 29) and the resultant structure used as a template for the building of the steroid analogues. All of these structures were minimised and their conformations compared. The final configurations corresponded quite well to the configurations found in the crystal structures.[97,172] The structures were refined

with hydrogen atoms. Any differences in X-ray structures and models might well be sought in a transannular attraction of the olefinic and C(5) carbonyl moieties, which is not represented in the force field.

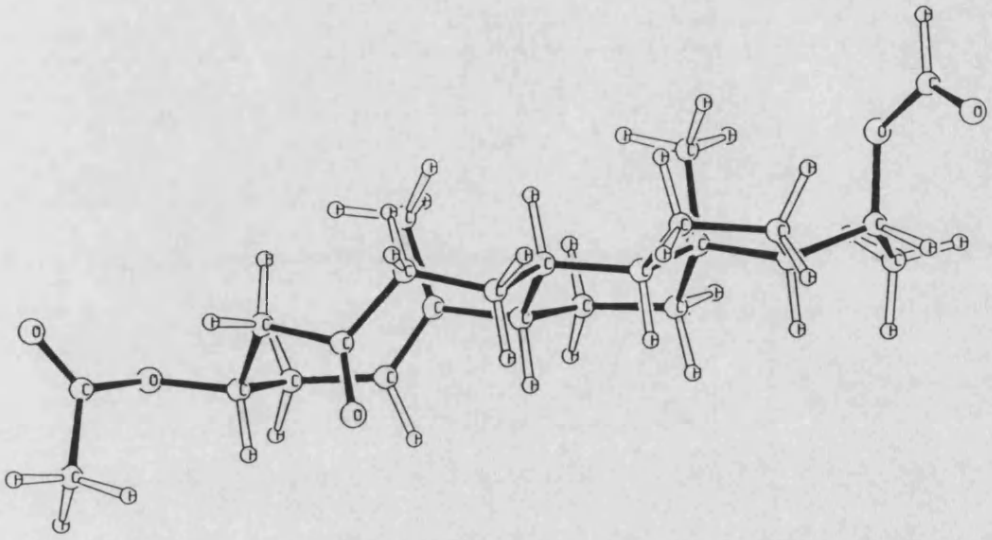


Figure 29. Minimised conformation of **39** (C(20) acetate replaced by C(20) formate)

A molecular dynamics simulation was performed upon **39** in order to investigate the conformational space available to the 10-membered ring. The structure was given enough energy to initiate ring flipping at C(2)-C(3)-C(4) and then allowed to adopt its most stable conformation (Figure 29). The 10-membered ring assumed the now familiar crown-conformation with the C(3) acetoxy group in an equatorial position. The C(19) methyl and C(5) carbonyl are in a *trans*-arrangement, with the C(5) carbonyl moiety shielding the H-C(1) proton.

A molecular dynamics simulation was performed on compound **44** to investigate the possibility of intramolecular hydrogen bonding between the C(3) hydroxyl proton and the C(5) carbonyl oxygen, via a favourable six-membered ring.

However, the crown conformation is of a lower energy and is the adopted one (Figure 30).

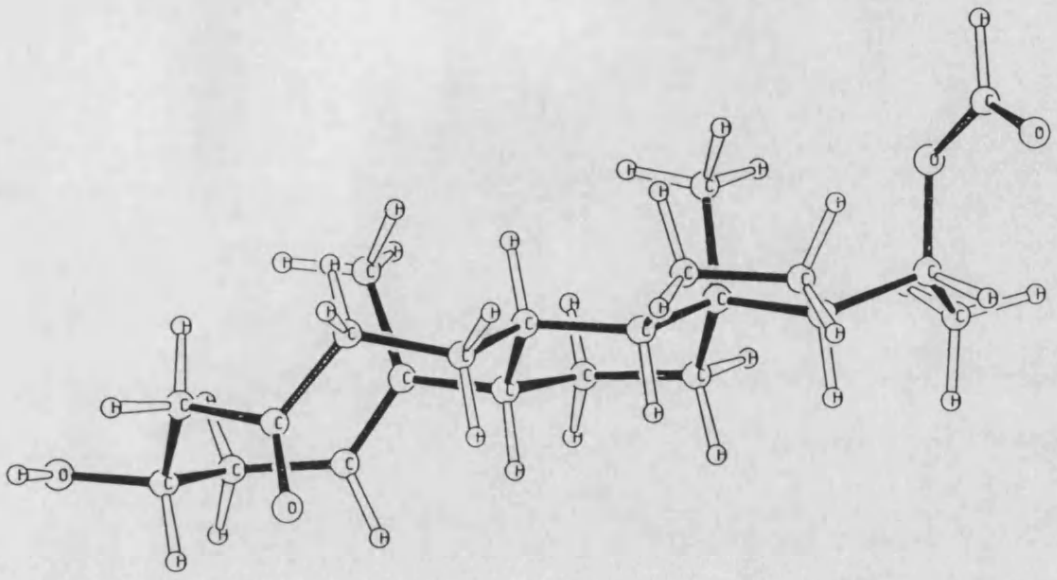


Figure 30. Minimised conformation of **44** (C(20) acetate replaced by C(20) formate)

The results of the simulation were analysed using FOCUS[181] and an atom-atom distance map was produced for the two hydrogen bonding atoms, shown in Figure 31. These show that the hydroxyl proton is never within an excepted hydrogen bonding distance of the carbonyl oxygen, i.e., 2.5Å.

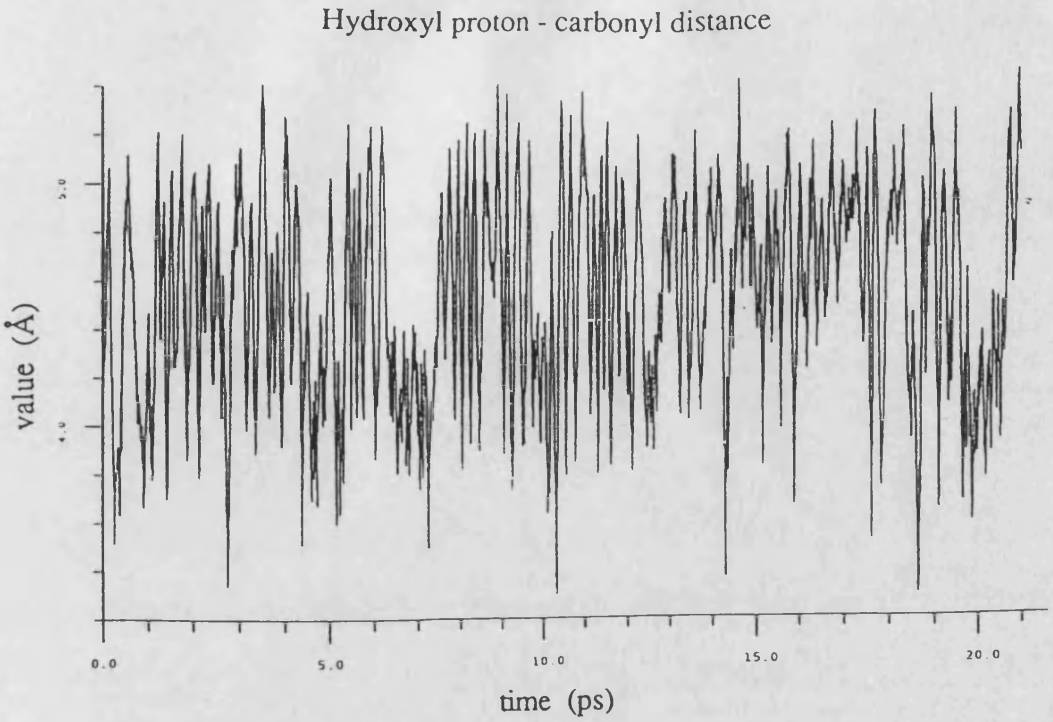


Figure 31. C(3) hydroxyl proton - C(5) carbonyl oxygen distance for **44**

Molecular mechanics techniques were also used to show the favoured (5S)-configuration of the C(5) hydroxyl, after reduction of the C(5) oxo group (Figure 32). The preferred conformations of rings C and D, discussed earlier, can also be seen clearly.

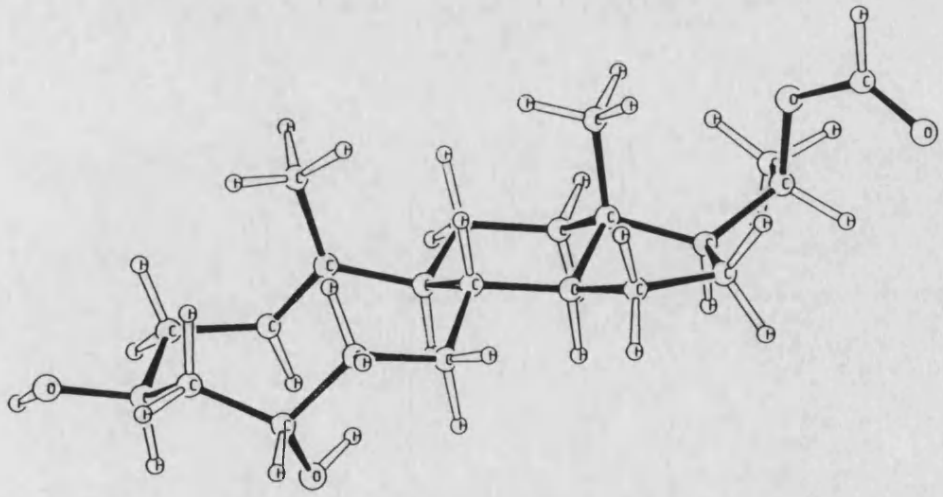
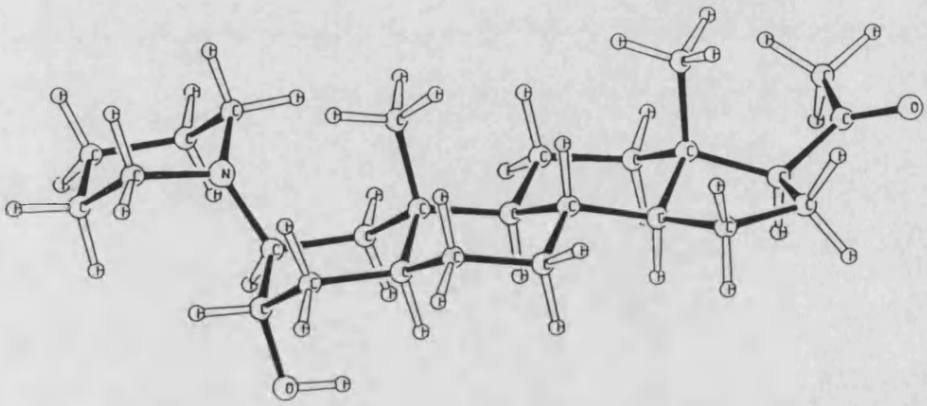
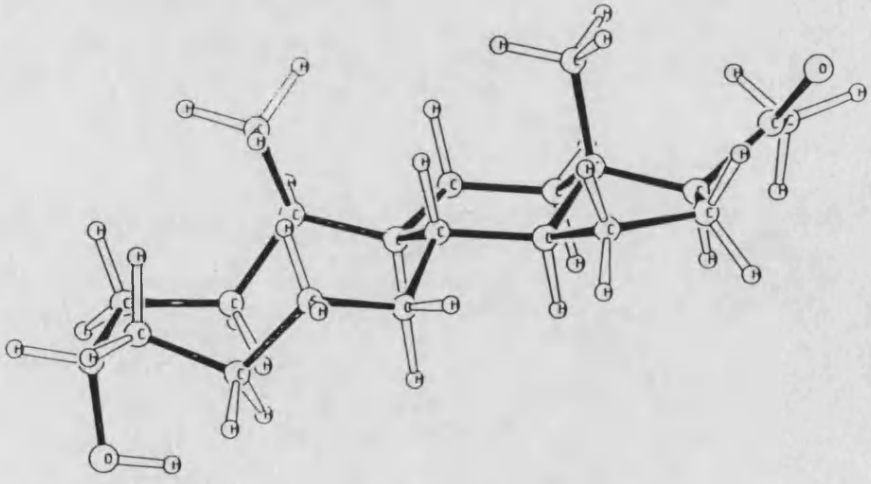


Figure 32. Minimised conformation of C(5) hydroxy-secosteroid

Two more structures minimised were the anaesthetically potent 2-morpholiny-1-3α-hydroxy steroid (**132**) and the target secosteroid (107), in order to compare the relative positions of the important C(3α) hydroxyl groups (Figure 33). Both C(3) hydroxyls are in a similar position, with the secosteroid hydroxyl possessing a greater flexibility and thus more free to adopt a favoured conformation at the receptor site. Hence, the secosteroid, **107** looked promising for a new anaesthetically potent steroid. It has up to now not undergone biological evaluation.



132



107

Figure 33. Minimised conformation of the anaesthetically active steroid (**132**) and the secosteroid (**107**)

Transannular cyclisations

It has been noted that only the *trans*-secosteroid readily undergoes transannular cyclisations.[83]

In the stable conformation, the *trans*-seco-ketone would be expected to undergo transannular reactions with participation of the C(1)-C(10) double bond, because of the favourable position of the carbonyl group with respect to the olefinic bond. However, the *cis*-isomer, with the double bond and carbonyl group on opposite sides of the ring (Figure 18), should not favour intramolecular cyclisations. In order to undergo transannular cyclisation the molecular of the *cis*-seco-ketone would first have to change to a less stable conformation.

In fact, the unsaturated *cis*- and *trans*-isomers were seen to behave differently towards reagents which effect or participate in reactions involving bond formation across the 10-membered ring, and that the trans-isomer is far more reactive than the *cis*-isomer.[83]

It was found that both the C(3α) and C(3β) epimers underwent cyclisations, involving C(1)-C(5) bond formation to give A-nor-B-homo derivatives, of the type **88, 109,** and **124**, with the same configuration at C(1) and C(5), namely with the *trans*-1β,5α-configuration,[97]and the seven-membered ring in a twist-chair conformation.[161i] In all these internal ring closures the C(3β) acetate was considerably more reactive than the C(3α) epimer, suggesting that the cyclisations proceed via a conformation with the geometry of B_2, shown in Figure 26, which resembles the ground state conformation of *trans*-cyclodecene (in its complex with silver nitrate).[176]

The *trans*-1β,5α-configuration was deduced by Akhtar and Marsh[90] from the facts

that analysis of the infrared spectra revealed, a) the C(5) alcohols containing a C(10) methylene group have a band corresponding to a hydrogen bonded C(5) hydroxy (this intramolecular hydrogen bond being formed with the π-electrons of the exocyclic methylene C(10)-C(19) double bond, which disappears when the C(10) methylene group is reduced to a methyl group, b) that the 10-methylen-e-3β,5α-diol shows a free hydroxyl group, and c) that the stereoisomeric 10-methylene-3α,5α-diol has no band corresponding to a free hydroxyl group. Moreover, the saturated 10-methyl-3α,5α-diol also forms an intramolecular hydrogen bond between the two (therefore *cis*) hydroxyl groups.

Our research has shown that, from the X-ray of **117**, there exists intermolecular hydrogen bonding between the two hydroxyl groups in the solid state, giving a herringbone arrangement (Figure 25), discussed earlier.

Another cyclisation, involving C(1)-C(3) bond formation is the solvolysis of the epimeric C(3) tosylates.[104ii] Both compounds react in the same way, i.e. affording cyclopropane derivatives, but whereas the C(3β) tosylate was converted to the 1β,3α-cyclo-5,10-secosteroid, of the type **44A**, the C(3α) tosylate cyclizes to the 1α,3β-cyclo-5,10-secosteroid with the opposite configuration at the junction carbon atoms C(1) and C(3). Thus, the two cyclisations proceed *via* similar, but different, intermediate geometries.[97]

2.7 Conclusion

The secosteroids, **60** and **65**, and the *abeo*steroids, **117** and **128**, all possess highly constrained modified ring AB portions, i.e., the 'crown' conformation in the cases of **60** and **65**. The overall conformation appears to be governed by the constraints imposed by the individual substituents present. As a result they all proved to be

inactive as $GABA_A$ agonists, suggesting an unfavourable position, with respect to the receptor, adopted by the 3α-hydroxyl in the cases of the tested secosteroids and *abeo*steroids.

Surprisingly, in the cases of **65** and **117**, neither the 3α-hydroxyl nor the 5α-hydroxyl were in a favourable position for interaction with the $GABA_A$ receptor.

Sufficient quantities of the saturated secosteroid, **107**, have thus far been unavailable for biological evaluation.

Future work should be directed towards synthesis of a sufficient amount of **107** to allow biological evaluation, with respect to the $GABA_A$ receptor, and also synthesis of the BC and CD secosteroids. Also synthesis of the **Z**-isomer of **39** may lead to a shorter synthesis of **107**, as protection of the $\Delta^{1,10}$ double bond may not be necessary due to its lower affinity for transannular reactions.

EXPERIMENTAL

EXPERIMENTAL

3.1 Instrumentation and experimental techniques

All solvents were dried and distilled before use. Petrol refers to petroleum ether of 60-80°C boiling range and ether to diethyl ether. Tetrahydrofuran was pre-dried over sodium wire and then refluxed over sodium benzophenone ketyl under a nitrogen atmosphere until anhydrous. This was redistilled immediately prior to use. All other solvents and reagents were purified using the procedures described in *Purification of Laboratory Chemicals*.[182]

Thin layer chromatography (tlc) was used extensively as a qualitative guide during reactions and for assessing the purity of compounds. Merck DC-alufolien kieselgel 60 F_{254} sheets, containing fluorescent indicator, were used for this purpose. Visualisation of compounds was achieved by illumination under short wavelength (254 nm) ultraviolet light, when possible. Plates were developed by treatment with either a 0.5% (w/v) aqueous solution of potassium permanganate or a 7% (w/v) methanolic solution of phosphomolybdic acid (PMA) or a 3% (w/v) solution of anisaldehyde in ethanol (Anis) or a solution of sulphuric acid in methanol, normally followed by warming of the tlc plate.

Normal phase medium pressure flash chromatography was routinely employed using Amicon Matrex or Merck 9385 silica gel. Columns were packed as a slurry in the eluting solvent and the material to be chromatographed introduced directly as a solution in the eluting solvent or pre-absorbed onto silica and then applied as a thin layer to the top of the column. A pressure gradient was developed using a small hand bellow.

Glassware used for moisture sensitive reactions was heated in an oven at 120°C overnight and then allowed to cool in a desicator over calcium chloride. Flasks and stirrer bars were additionally flame dried under a stream of dry nitrogen prior to use.

Solvents were evaporated with a Buchi rotary evaporator using a water aspirator or a vacuum pump as required, and a water bath temperature of <40°C to avoid unnecessary heating.

Melting points (m.p.) were determined on commercially available apparatus (electrothermal MKII or Gallenkamp) and are uncorrected. Elemental micro-analyses were carried out using a Carlo Erba 1106 Elemental Analyser. Optical rotations were measured using a Perkin-Elmer 141 polarimeter with concentrations expressed in $g/100cm^3$.

Infrared spectra were recorded in the range 4000-600cm^{-1} using a Perkin-Elmer 1310 spectrophotometer with peaks reported (υ_{max}) in wave numbers (cm^{-1}), and the abbreviations br (broad), s (strong), vs (very strong), sh (sharp) used to describe the peaks. Samples were prepared as liquid films, nujol mulls or chloroform solutions as indicated.

Proton magnetic resonance spectra were recorded on a Jeol GX FT 270 (270 MHz) spectrometer, although where indicated a Jeol GX FT 400 (400 MHz) instrument was used, and some spectra were run at 200 MHz at the Organon laboratories, Newhouse. Carbon-13 magnetic resonance spectra were recorded on a Jeol GX FT 270 spectrometer operating at 67.8 MHz and using 90 and 135 DEPT pulse sequences to aid multiplicity determination. Chemical shifts (δ) are expressed in parts per million (ppm) downfield from the internal standard tetramethylsilane. The multiplicities of the resonances are denoted by s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet) and m (multiplet). The abbreviation br (broadened) is used to indicate

signficant broadening, whether due to rapid exchange or unresolved fine coupling. Homonuclear decoupling experiments and 2D homonuclear shift correlated (COSY) spectra were used to confirm proton assignments when required.

Mass spectra were recorded using a VG Analytical 7070E instrument with a VG 2000 data system. Electron ionisation (E.I.) spectra were produced using an ionising potential of 70ev. Chemical ionisation (C.I.) was employed using *iso*-butane as the reagent gas.

3.2 Experimental procedure

Preparation of pregna-1(2),4(5)-diene-3,20-dione (**4**)

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, DDQ, (4.54g, 19.2mM) was added to a stirred solution of *progesterone* (5.0g, 16mM) in toluene (150 cm^3). The reaction mixture was refluxed under nitrogen for 24h. After cooling it was filtered and the filtrate washed with 1% potassium hydroxide solution (30 cm^3), and water (2 x 30 cm^3). The organic layer was dried over magnesium sulphate, evaporated *in vacuo*, and purified by column chromatography on silica gel eluting with ethyl acetate-petrol (1:4) to give the *diene* (**4**) (2.72g, 55%) as an off-white solid, m.p. 140-141°C (R_f = 0.54 ethyl acetate-petrol (1:1)); υ_{max} (NUJOL)/cm^{-1} 1692 s ((20)C=O), 1663 s ((3)C=O), 1624 (C=C), 1602 (C=C); δ_H (270 MHz, $CDCl_3$) 7.06 (1H, dd, J 2.8, $J_{1,2}$ 10.3 Hz, H-C(1)), 6.25 (1H, dd, $J_{2,4}$ 1.9, $J_{2,1}$ 10.3 Hz, H-C(2)), 6.08 (1H, t, $J_{4,2}$ 1.9 Hz, H-C(4)), 2.13 (3H, s, H_3C(21), 1.25 (3H, s, H_3C(19)), 0.71 (3H, s, H_3C(18)); *m/z* (C.I.) 313 ([M^+ + 1], 100%), 205 (11), 279 (1), 269 (4), 227 (2); Found: C, 80.2; H, 8.88. $C_{21}H_{28}O_2.{}^1/_8\ H_2O$ requires C, 80.2; H, 8.99%.

Data for progesterone; m.p. 128-130°C (R_f = 0.57, ethyl acetate-petrol (1:1)); υ_{max} (NUJOL)/cm^{-1} 1697 s (C=O), 1660 s (C=O), 1614 (C=C); δ_H (270 MHz, $CDCl_3$) 5.75 (1H, s, H-C(4)), 2.13 (3H, s, H_3C(21)), 1.20 (3H, s, H_3C(19)), 0.68 (3H, s, H_3C(18)).

20-Ethylenedioxypregna-1(2),4(5)-dien-3-one (5)

To a stirred solution of the *dione* (4) (1.53g, 4.9mM) in triethyl orthoformate (8.5 cm^3, 51.0mM) was added ethylene glycol (3.8 cm^3, 7.5mM) and a catalytic amount of p-TSA (20mg). The reaction mixture was refluxed for 3h. After cooling it was diluted with ethyl acetate (50cm^3) and washed with sodium bicarbonate solution (10cm^3 of 1N) and water (2 x 15cm^3). The organic layer was dried over magnesium sulphate, and concentrated *in vacuo*. Recrystallisation from methanol gave the *acetal* (5) (1.20g, 83%) as an off-white solid, m.p. 185-186°C (R_f = 0.66, ethyl acetate-petrol (1:1)); υ_{max} (NUJOL)/cm^{-1} 1655 s (C=O), 1630 (C=C), 1610 (C=C), 1047 (C-O); δ_H (270 MHz, $CDCl_3$) 7.06 (1H, dd, *J* 3.0, $J_{1,2}$ 10.1 Hz, H-C(1)), 6.23 (1H, dd, *J* 1.9, $J_{2,1}$ 10.1 Hz, H-C(2)) 6.07 (1H, t, *J* 1.6 Hz, H-C(4)), 4.04-3.85 (4H, m, OCH_2CH_2O), 1.29 (3H, s, $H_3C(21)$), 1.23 (3H, s, $H_3C(19)$), 0.84 (3H, s, $H_3C(18)$); *m/z* (C.I.) 357 ([M^+ + 1], 36%), 341 (3), 313 (3), 295 (2), 271 (2), 255 (2); Found: C, 76.0; H, 9.05. $C_{23}H_{32}O_3.{}^2/_5\ H_2O$ requires C, 76.0; H, 9.03%.

1α-Phenylthio-20-ethylenedioxypregnan-4(5)-en-3-one (7)

20-Ethylenedioxypregna-1(2),4(5)-diene-3-one (5) (50mg, 0.14mM) was added to a stirred solution of thiophenol (0.15cm^3, 0.14mM) and triethylamine (2µl, 14 µM) in THF (2cm^3). The reaction mixture was stirred at room temperature for 24h. This was then diluted with methylene chloride (10cm^3), washed with aqueous sodium hydroxide (2cm^3 of 1N) and water (2 x 4 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. Purification by column chromatography eluting with ether-petrol (4:1) gave the *thiophenol* (7) (53mg, 81%) as a yellow solid, m.p. 143-145°C (R_f = 0.67, ether-petrol (7:3)); υ_{max} (NUJOL)/cm^3 1673 s (C=O), 1580 (C-C, Ar), 1470 (C-C, Ar), 950 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 7.38-7.30 (5H, m, Ar), 5.85-5.78 (1H, m, H-C(4)), 3.98-3.82 (4H, m, OCH_2CH_2O), 1.36 (3H, s, $H_3C(21)$), 1.31 (3H, s, $H_3C(19)$), 0.83 (3H, s, $H_3C(18)$); *m/z* (E.I.) 466 (M^+, 2%), 456 (2), 431 (3), 110 (10), 87 (100); Found : C, 74.2; H, 8.2. $C_{29}H_{38}SO_3$ requires C, 74.6;

H, 8.2%.

1-trimethylsilyl-20-Ethylenedioxypregnan-4(5)-en-3-one (9)

To a stirred solution of hexamethylsilane (0.34cm^3, 1.7mM) in HMPA (3cm^3) at 0°C, under nitrogen, was added methyl lithium (1.2cm^3, 1.7mM) to give a deep red solution, indicating the formation of the silyl lithium. After stirring at 0°C for 20 min the reaction mixture was diluted with THF (3cm^3) and copper(I) cyanide (0.15g, 1.7mM) added to give a black solution which was stirred for a further 20 min at 0°C. The cuprate solution was then cooled to -78°C after which a solution of *20-ethylenedioxypregna-1(2),4(5)-dien-3-one* (5) (100mg, 0.2mM) in THF (2cm^3) was added. The reaction mixture was stirred under nitrogen at -78°C for 1h and at 0°C for a further 3h. It was then stirred at room temperature for 96h after which it was quenched by the addition of saturated ammonium chloride solution (5cm^3) and diluted with petrol (10cm^3). After filtration through glass wool the filtrate was washed with water (3 x 5 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with ethyl acetate-petrol (1:4) to give *isomer A* of 9 (20mg, 17%) as a colourless oil (R_f = 0.81, ethyl acetate-petrol (1:1)); δ_H (270 MHz, $CDCl_3$) 5.70 (1H, s, H-C(4)), 3.78-3.70 (4H, m, OCH_2CH_2O), 1.34 (3H, s, H_3C(19)), 0.70 (3H, s, H_3C(18)), 0.05 (9H, s, $(CH_3)_3Si$).

Further elution gave *isomer B* of (9) (10mg, 8%) as a colourless oil (R_f = 0.79, ethyl acetate-petrol (1:1)); δ_H (270 MHz, $CDCl_3$) 5.79 (1H, s, H-C(4)), 3.79-3.70 (4H, m, OCH_2CH_2O), 1.34 (3H, s, H_3C(19), 0.71 (3H, s, H-C(18)), 0.05 (9H, s, $(CH_3)_3Si$).

β-*Oestradiol* (**11**)

β-*Oestradiol diacetate* (**10**) (15.0g, 42.1mM) and potassium carbonate (15.0g, 108mM) were stirred together in methanol (200cm^3) at 70-75°C for 90 min. After this water,(500cm^3) was added to the reaction mixture followed by sodium chloride to

aid precipitation. The solid was filtered off, dissolved in methylene chloride ($200cm^3$), washed with water (2 x 50 cm^3) and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and recrystallisation from methanol gave β-*oestradiol* (**11**) (11.4g, 99%) as a white solid, m.p. 179-180°C (lit. 178-179°C) (R_f = 0.29, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3423 br (OH), 3180 br (OH), 1608 (C-C, Ar), 1586 (C-C, Ar), 873 (C-H, Ar), 819 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 7.07 (1H, d, $J_{2,1}$ 8.4 Hz,H-C (2)), 6.58 (1H, dd, $J_{1,4}$ 2.7, $J_{1,2}$ 8.4 Hz, H-C(1)), 6.52 (1H, d, $J_{4,1}$ 2.6 Hz, H-C(4)); 3.64 (1H, t, $J_{17,16}$ 8.4 Hz, H-C(17)), 0.74 (3H, s, H_3C(18)); *m/z* (E.I.) 272 (M^+, 100%), 213 (33), 160 (29),133 (20); Found : C, 76.1, H, 9.33. $C_{18}H_{24}O_2.{}^3/_4H_2O$ requires C, 76.1; H, 8.92%.

3-Methoxyoestra-17β-ol (**12**)

β-*Oestradiol* (**11**) (15.0g, 55.1mM) in THF ($100cm^3$) was added dropwise to 80% sodium hydride (1.25g, 61.0mM), previously washed with petrol (3 x 20 cm^3), in THF (50 cm^3) and stirred under nitrogen. The reaction mixture was stirred at 0°C for 30 min, until the evolution of hydrogen ceased, and then methyl iodide (3.8 cm^3, 60.5mM) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 6h. Saturated ammonium chloride solution (25 cm^3) was added to quench, and the reaction mixture extracted with methylene chloride (100 cm^3), washed with water (2 x 50 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by column chromatography eluting with ethyl acetate-petrol (1:4) and recrystallisation from methanol gave the *methyl ether* (**12**) (11.5g, 73%) as a white solid, m.p. 108-109°C (R_f = 0.53, ethyl acetate-petrol (3:7); υ_{max} (NUJOL)/cm^{-1} 3440 br (OH), 1609 (C-C, Ar), 1576 (C-C, Ar), 1250 (O-CH_3), 870 sh (C-H, Ar), 818 sh (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 7.20 (1H, d, $J_{2,1}$ 8.6 Hz, H-C(2)), 6.72 (1H, dd, $J_{1,4}$ 3.0, $J_{1,2}$ 8.3 Hz, H-C(1)), 6.64 (1H, d, $J_{4,2}$ 2.7 Hz, H-C(4)), 3.77 (3H, s, OCH_3), 3.72 (1H, t, $J_{7,16}$ 7.1 Hz, H-C(17)), 0.77 (3H, s, H_3C(18)); *m/z* (E.I.) 286 (M^+, 15%), 258 (1), 227 (3), 186 (5), 160 (4), 45 (49), 31 (100); Found : C, 75.3; H, 9.04. $C_{19}H_{26}O_2.H_2O$ requires C, 75.0; H, 9.21%.

3-Methoxyoestr-17-one (**13**)

Method 1

A solution of chromium trioxide (96 mg, 0.96mM) and sulphuric acid solution (0.2 cm^3 of 2N) in water (0.2 cm^3) was added to a stirred solution of *3-methoxyoestr-17β-ol* (**12**) (250 mg, 0.87mM) in acetone (5 cm^3). The reaction mixture was stirred at room temperature for 6h. Water (10 cm^3) was added and the reaction mixture extracted with methylene chloride (2 x 20 cm^3). The organic extracts were dried over magnesium sulphate, concentrated *in vacuo* and purified by column chromatography eluting with ethyl acetate-petrol (1:9) to give the *ketone* (**13**) (50 mg, 20%) as a white solid, m.p. 176-177°C (R_f = 0.29, ethyl acetate-petrol (1:9)); υ_{max} (NUJOL)/cm^{-1} 1736 s (C=O), 1608 s (C-C, Ar), 1579 s (C-C, Ar), 1503 s (C-C, Ar), 1245 (O-CH_3), 846 (C-H, Ar), 823 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 7.21 (1H, d, $J_{2,1}$ 8.6 Hz, H-C(2)), 6.72 (1H, dd, $J_{1,4}$ 2.8, $J_{1,2}$ 8.6 Hz, H-C(1)), 6.65 (1H, d, $J_{4,1}$ 2.8 Hz, H-C(4)), 3.78 (3H, s, OCH_3), 2.89 (2H, t, $J_{16,15}$ 4.3 Hz H_2-C(16)), 0.91 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 220.69 (C-17), 157.52 (C-3), 137.62 (C-10), 131.93 (C-5), 126.20 (C-1), 113.80 (C-2), 111.48 (C-4); *m/z* (E.I.) 284 (M^+, 100%), 256 (4), 227 (12), 199 (39), 160 (38), 91 (22), 57 (40); Found : C, 80.7; H, 8.55, $C_{19}H_{24}O_2$ requires C, 80.3; H, 8.45%.

Method 2

Pyridinium chlorochromate, PCC, (207 mg, 0.96mM) was added to a stirred solution of *3-methoxyoestr-17β-ol* (**12**) (250 mg, 0.87mM) in methylene chloride (5 cm^3). The reaction mixture was stirred at room temperature under nitrogen for 4h. Water (5 cm^3) was added, the organic layer separated, and the aqueous layer extracted with diethyl ether (2 x 10 cm^3). The combined organic extracts were dried over magnesium sulphate, evaporated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:4) to give the *ketone* (**13**) (97 mg, 39%) as a white

solid, m.p. 176-177°C (R_f = 0.29, ethyl acetate-petrol (1:9)).

Method 3

3-Methoxyoestr-17β-ol (**12**) (300 mg, 1.05mM), TPAP (18 mg, 5 mol%) and NMO (184 mg, 1.57mM) were stirred together in methylene chloride (5 cm^3), in the presence of ground molecular sieves, at room temperature for 12h. Silica gel was added to the reaction mixture and the solvent removed *in vacuo*. Column chromatography, eluting with ethyl acetate-petrol (1:4) gave the *ketone* (**13**) (231 mg, 77%) as a white solid, m.p. 176-177°C (R_f = 0.29, ethyl acetate-petrol (1:9)).

3-Methoxy-17β-ethyloestr-17(19)-ene (**14**)

Sodium hydride (3.00 g, 125mM) was added to dimethyl sulphoxide (150 cm^3), freshly distilled and dried, and stirred for 45 min at 75°C, under nitrogen to give a light green solution. After cooling to room temperature ethyl triphenylphosphonium bromide (46.4g, 125mM) in dimethyl sulphoxide (100 cm^3) was added to give a red solution, followed by *3-methoxyoestr-17-one* (**13**) (9.60 g, 25.0mM) in dimethyl sulphoxide (50 cm^3). The reaction mixture was warmed to 55-60°C and stirred under nitrogen for 2h. After cooling the reaction mixture was poured into saturated brine (500 cm^3). The solid was filtered off, washed with water (400 cm^3) and dissolved in methylene chloride (300 cm^3). Water (100cm^3) was added and the organic layer separated and washed with water (100 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The solid was dissolved in methylene chloride (30 cm^3) and applied to a silica column eluting with methylene chloride and the solvent evaporated *in vacuo*. Purification by recrystallisation from methanol gave the *alkene* (**14**) (8.12g, 82%) as a white solid, m.p. 76-77°C (R_f = 0.84 ethyl acetate-petrol (1:9)); υ_{max} (NUJOL)/cm^{-1} 1676 (C=C), 1610 s (C-C, Ar), 1574 s (C-C, Ar), 1254 (O-CH_3), 872 (C-H, Ar), 812 (C-H, Ar); δ_H (400 MHz, $CDCl_3$) 7.21 (1H, d, $J_{2,1}$ 8.3 Hz, H-C(2)), 6.71 (1H, dd, $J_{1,4}$ 2.8, $J_{1,2}$ 8.5 Hz, H-C(1)), 6.63 (1H, d, $J_{4,1}$ 2.8 Hz, H-C(4)), 5.15 (1H, qt, $J_{20,16'}$ 1.8, $J_{20,16''}$ 2.1, $J_{20,21}$ 7.0 Hz, H-C(20)), 3.78 (3H, s, OCH_3), 1.69 (3H,

dt, $J_{21,16'}$ 1.8, $J_{21,16''}$ 2.1, $J_{21,20}$ 7.0 Hz, $H_3C(21)$), 0.91 (3H, s, $H_3C(18)$); *m/z* (E.I.) 296 (M^+, 6%), 262 (24), 215 (5), 183 (22), 154 (100), 76 (18); Found : C, 84.9; H, 9.60. $C_{21}H_{28}O$ requires C, 85.1; H, 9.46%.

3-Methoxy-17β-(1-hydroxyethyl)oestrane (**15**)

To a stirred solution of the *alkene* (**14**) (250 mg, 0.84mM) in THF (10 cm^3) was added diborane in THF (1.70 cm^3, 0.90mM) under nitrogen. After stirring at room temperature, for 1h, 10% sodium hydroxide solution (7 cm^3) was added dropwise. After cooling to 0°C, 30% hydrogen peroxide (4.50 cm^3) was added dropwise and the reaction mixture stirred for a further 1h at 0°C under nitrogen. The reaction mixture was then allowed to separate and the THF layer treated with sodium metabisulphite solution (5 cm^3), washed with water (2 x 5 cm^3) and dried over anhydrous sodium sulphate. The solvent was evaporated at reduced pressure and purification by column chromatography, eluting with ethyl acetate-petrol (3:7) gave the *20-hydroxyl* (**15**) (212 mg, 80%) as a white solid, m.p. 105-106°C (R_f = 0.32, ethyl acetate-petrol (3:7); υ_{max} (NUJOL)/cm^{-1} 3483 br (OH), 1602 s (C-C,Ar), 1578 s (C-C, Ar), 1226 (O-CH_3), 897 (C-H, Ar), 814 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 7.21 (1H, d, $J_{2,1}$ 8.4 Hz, H-C(2)), 6.71 (1H, dd, $J_{1,4}$ 2.7, 8.6 Hz, H-C(1)), 6.63 (1H, d, $J_{4,1}$ 2.7 Hz, H-C(4), 3.80-3.68 (1H, m, H-C(20)), 3.78 (3H, s, OCH_3), 1.26 (3H, d, $J_{21,20}$ 6.2 Hz, $H_3C(21)$), 0.70 (3H, s, $H_3C(18)$); δ_C (400 MHz, $CDCl_3$) 157.36 (C-3), 137.95 (C-10), 132 .74 (C-5), 126.20 (C-1), 113.70 (C-2), 111.38 (C-4), 70.31 (C-20); *m/z* (E.I.) 314 (M^+, 100%), 296 (9), 286 (4), 227 (18), 119 (13), 173 (40), 57 (55); Found : C, 79.5; 9.78. $C_{21}H_{30}O_2.{}^1/_6\ H_2O$ requires C, 79.5; H, 9.46%.

3-Methoxyoestra-2(3),5(10)-dien-17β-ol (**16**)

Distilled ether (20 cm^3) and liquid ammonia (50 cm^3) were stirred at room temperature using an overhead mechanical stirrer and a cold finger for 10 min. To this was added lithium shot (1.5 g) to give a blue solution which was stirred for 5 min after which *3-methoxyoestra-17β-ol* (**12**) (1.0 g, 3.50mM) in ether (20 cm^3) was added

dropwise and the reaction mixture stirred for a further 90 min. Ethanol (15 cm^3) was added dropwise, with extreme caution, followed by addition of water (40 cm^3) dropwise to quench, after allowing half of the ammonia to evaporate off. The reaction mixture was extracted with methylene chloride (60 cm^3) washed with water (2 x 15 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:9) gave the *diene* (**16**) (630 mg, 63%) as a white solid, m.p. 96-97°C (R_f = 0.48, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3344 br (OH), 1696 (C=C), 1665 (C=C), 1225 (O-CH_3); δ_H (270 MHz, $CDCl_3$) 5.83 (1H, s, H-C(2)), 3.67 (1H, t, $J_{17,16}$ 8.5 Hz, H-C(17)), 3.49 (3H, s, OCH_3), 0.81 (3H, s, H_3C(18)); *m/z* (E.I.) 288 (M^+, 56%), 260 (4), 227 (3), 201 (5), 122 (100), 85 (33), 57 (81).

Oestr-5(10)-en-17β-ol-3-one (**17**)

The *protected enol* (**16**) (100 mg, 0.35mM) and oxalic acid (3 cm^3 of 0.1M solution) were stirred together in methanol (10 cm^3) at 30°C for 40 min. Methylene chloride (20 cm^3) was added to the reaction mixture and the organic layer washed with saturated sodium bicarbonate solution (10 cm^3) and water (2 x 10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. Recrystallisation from ethyl acetate gave the *C(3) ketone* (**17**) (80 mg, 84%) as a white solid, m.p. 176-177°C (R_f = 0.26, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3385 br (OH), 1675 s (C=O), 1640 (C=C); δ_H (270 MHz, $CDCl_3$) 3.69 (1H, t, $J_{17,16}$ 8.3 Hz, H-C (17)), 0.77 (3H, s, H_3C (18)); *m/z* (Low eV E.I.) 274 (M^+, 100%), 256 (16), 230 (18), 110 (6).

3-Methoxy-19-nor-pregna-2(3),5(10)-dien-20β-ol (**18**)

Distilled ether (50 cm^3) and liquid ammonia (100 ml, approx. 50 mol. equiv) were stirred at room temperature using a overhead mechanical stirrer. To this was added lithium shot (4.0 g) to give a blue solution. After stirring for 5 min *3-methoxy-17β-(1-hydroxyethyl)oestrane* (**15**) (3.9 g, 12.4mM) in ether (50 cm^3) was added dropwise and the reaction mixture stirred for a further 90 min. Then, ethanol (50 cm^3)

was added dropwise, with extreme caution, followed by water (50 cm^3) to quench, after allowing half of the ammonia to evaporate off. The reaction mixture was extracted with methylene chloride (2 x 100 cm^3), washed with water (2 x 40 cm^3), dried over magnesium chloride and concentrated *in vacuo*. The product was isolated crude and used as thus in the next step, the synthesis of *19-nor-pregn-5(10)-e-n-20β-ol-3-one* (**19**).

Crude yield of *3-methoxy-19-nor-pregna-2(3),5(10)-dien-20β-ol* (**18**) was 5.5g. (R_f = 0.64 ethyl acetate-petrol (3:7)), δ_H (270 MHz, $CDCl_3$) 5.88-5.80 (1H, m, H-C(2)), 3.78 (3*H*, s, OCH_3), 3.74-3.68 (1H, m, H-C(20)), 0.82 (3H, s, H_3C(18)).

19-Nor-pregn-5(10)-en-20β-ol-3-one (**19**)

3-Methoxy-19-nor-pregna-2(3), 5(10)-dien-20β-ol (**18**) (100 mg, 0.32mM) and oxalic acid (3 cm^3 of 0.1M solution) were stirred in methanol (10 cm^3) at 30°C for 45 min. Water (10 cm^3) was added to the reaction mixture and it was extracted with methylene chloride (2 x 15 cm^3). The organic extracts were combined and washed with sodium bicarbonate solution (10 cm^3 of 2N), and water (2 x 10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give the $\Delta^{5,10}$ *alkene* (**19**) (75 mg, 79%) as a white solid, m.p. 176-177°C (R_f = 0.32, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3426 br (OH), 1698 s (C=O), 1661 (C=C); δ_H (270 MHz, $CDCl_3$) 3.78-3.66 (1H, m, H-C(20)), 1.23 (3H, d, $J_{21,20}$ 6.4 Hz, H_3C(21)), 0.69 (3H, s, H_3C(18)); *m/z* (low eV E.I.) 302 (M^+, 100%), 284 (6), 257 (13), 243 (12), 215 (3), 119 (5); Found : C, 75.7; H, 9.78. $C_{20}H_{30}O_2.{}^5/_6\ H_2O$ requires C, 75.7; H, 9.46%.

Further eluting with ethyl acetate-petrol (1:9) gave *19-nor-pregn-4(5)-e-n-20β-ol-3-one* (**19A**) (10 mg, 10%) as a white solid, m.p. 95-97°C (R_f = 0.15, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3421 br (OH), 1656 s (C=O), 1611 (C=C); δ_H (270 MHz, $CDCl_3$) 5.83 (1H, s, H-C(4)), 3.78-3.66 (1H, m, H-C(20)), 1.25 (3H, d,

$J_{21,20}$ 6.2 Hz, $H_3C(21)$), 0.73 (3H, s, $H_3C(18)$); δ_C (400 MHz, $CDCl_3$) 200.02 (C-3), 166.98 (C-5), 124.33 (C-4), 70.01 (C-20); *m/z* (E.I.) 302 (M^+, 38%), 284 (50), 269 (11), 217 (100), 110 (94), 57 (57); Found : C, 77.3; H, 10.0. $C_{20}H_{30}O_2.{}^1/_2\ H_2O$ requires C, 77.2; H, 9.65%.

3-Ethylenedioxy-19-nor-pregn-5(10)-en-20β-ol (**20**)

19-Nor-pregn-5(10)-en-20β-ol-3-one (**19**) (1.70 g, 5.63mM), ethylene glycol (3.14 cm^3, 56.3mM) and a catalytic amount of p-TSA (20 mg) were stirred together in toluene (100 cm^3) at 70°C for 2h with a Dean and Stark trap. After this the reaction mixture was diluted with methylene chloride (100 cm^3), washed with water (2 x 50 cm^3) and dried over anhydrous sodium sulphate. The solvent was removed *in vacuo* and the residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:4) to give the *acetal* (**20**) (932 mg, 48%) as a white solid, m.p. 124-125°C (R_f = 0.41, ethyl acetate-petrol (3:7)), υ_{max} (NUJOL)/cm^{-1} 3497 sh (OH), 1672 (C=C), 1107 s (C-O); δ_H (200 MHz, $CDCl_3$) 4.01-3.89 (4H, m, OCH_2CH_2O), 3.68 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.3 Hz, H-C(20)), 1.25 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.68 (3H, s, $H_3C(18)$); δ_C (400 MHz, $CDCl_3$) 128.95 (C-10), 125.07 (C-5), 107.79 (C-3), 69.83 (C-20), 63.92 (OCH_2), 63.72 (OCH_2); *m/z* (low eV E.I.) 346 (M^+, 57%), 284 (22), 115 (9), 99 (100), 63 (7), 55 (10); Found : C, 76.1; H, 10.2. $C_{22}H_{34}O_3$ requires C, 76.3; H, 9.83%.

20-Ethylenedioxypregnenolone (**23**)

Pregnenolone (2.0 g, 6.3mM), p-toluenesulphonic acid (60 mg, 0.3mM) and ethylene glycol (70 cm^3) were placed in a rbf. Ethylene glycol and water were co-distilled from the reaction vessel at 60±2°C and 1 mmHg pressure over a two hour period. The reaction mixture was basified by the addition of saturated sodium hydrogen carbonate solution (20 cm^3) and the white solid filtered off, washed with water (2 x 100 cm^3) and dried under vacuum. Purification by recrystallisation from ethyl acetate-petrol gave the *acetal* (**23**) (1.32 g, 58%) as a white solid, m.p. 155-157°C (lit.[87] 156-158°c),

(R_f = 0.38, ethyl acetate-petrol (1:2)); υ_{max} (NUJOL)/cm^{-1} 3460 br (OH), 1631 (C=C), 1067 s (C-O); δ_H (270 MHz, $CDCl_3$) 5.48 (1H, d, $J_{6,7}$ 5.0 Hz, H-C(6)), 3.95-3.84 (4H, m, OCH_2CH_2O), 3.54-3.46 (1H, m, H_α-C(3)), 1.29 (3H, s, H_3C(21)), 1.03 (3H, s, H_3C(19)), 0.65 (3H, s, H_3C(18)); *m/z* (C.I.) 361 ([M^+ + 1], 6%), 343 (1), 317 (2), 299 (4), 281 (1), 87 (100).

3β-Acetoxy-20-ethlenedioxypregn-5(6)-ene (24)

To a stirred solution of *pregnenolone* (20 g, 63.3mM), triethylamine (10.6 cm^3, 76.2mM) and DMAP (770 mg, 6.3mM) in THF (500 cm^3) was added acetic anhydride (60 cm^3, 636mM). The reaction mixture was stirred at room temperature for 90 min. The mixture was poured in water, saturated with sodium chloride, (500 cm^3), and the resulting white solid filtered off, washed with water (200 cm^3) and dissolved in methylene chloride (500 cm^3). The organic solution was washed with water (2 x 100 cm^3), dried over magnesium sulphate and concentrated *in vacuo* to give the *3β-acetate* (crude yield, 23.1g) as a white solid, (R_f = 0.65, ethyl acetate-toluene (1:2)). The *3β-acetate* was used crude in the next step.

Crude *3β-acetoxypregn-5(6)-en-20-one* (23.1 g, 64.5mM), ethylene glycol (35.9 cm^3, 645mM) and p-TSA (1.23 g, 6.45mM) were dissolved in triethylorthoformate (200 cm^3) and the mixture refluxed for $2^1/_2$ h. After cooling the reaction mixture was poured into water, saturated with NaCl (500 cm^3), and the resulting solid filtered off and dissolved in methylene chloride (500 cm^3). The organic solution was washed with water (2 x 200 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was recrystallised from methanol to give the *20-acetal* (24) (27.3 g, 93% overall) as a white solid, m.p. 123-124°C (R_f = 0.55, methanol-methylene chloride-hexane (2:3:13)); υ_{max} (NUJOL)/cm^{-1} 1727 s (C=O), 1645 (C=C), 1270 s (C-O); δ_H (270 MHz, $CDCl_3$) 5.24-5.13 (1H, m, H_α-C(3)), 4.01-3.89 (4H, m, OCH_2CH_2O), 3.88-3.82 (1H, m, H-C(6)), 2.01 (3H, s, H_3CCO), 1.26 (3H, s, H_3C(21)), 1.02 (3H, s, H_3C(19)), 0.75 (3H, s, H_3C(18)).

20-Ethylenedioxy-5α.6α-epoxypregnan-3β-ol (25)

To a stirred solution of *20-ethylenedioxypregnenolone* (23) (1.89 g, 5.0mM) in methylene chloride (40 cm^3) was added 80% m-chloroperoxybenzoic acid (1.89 g, 6.0mM). The reaction mixture was stirred at room temperature under nitrogen for 45 min. After which 25% sodium sulphite solution and 25% sodium bicarbonate solution were added cautiously, until the starch-iodide test gave a negative result. The organic layer was separated and washed with sodium bicarbonate (10 cm^3 of 1N) and water (2 x 10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:2) to give the *epoxide* (25) (1.69 g, 90%) as a white solid, m.p. 198-199°C (R_f = 0.21, ethyl acetate-petrol (3:10)); υ_{max} (NUJOL)/cm^{-1} 3442 sh (OH), 1254 (C-O); δ_H (270 MHz, $CDCl_3$) 4.00-3.82 (5H, m, H_α-C(3) + OCH_2CH_2O), 2.90 (1H, d, $J_{6,7}$ 4.4 Hz, Hβ-C(6)), 1.28 (3H, s, H_3C(21)), 1.06 (3H, s, H_3C 19)), 0.71 (3H, s, H_3C(18)); *m/z* (C.I.) 377 ([M^+ + 1], 8%), 359 (7), 315 (3), 285 (3), 95 (4), 87 (100); Found : C, 73.2; H, 9.77. $C_{23}H_{36}O_4$ requires C, 73.4; H, 9.57%.

3β-Acetoxy-20-ethylenedioxy-5α-6α-epoxypregnane (26)

To a stirred solution of *3β-acetoxy-20-ethylenedioxypregnen* (24) (24.12 g, 0.06M) in methylene chloride (300 cm^3) was added, portionwise, 80% m-chloroperoxybenzoic acid (15.5 g, 0.072M). The reaction mixture was stirred at room temperature for 1h. After this 25% sodium sulphite solution and 25% sodium bicarbonate solution were added to the reaction mixture with stirring until the starch-iodide test gave a negative result. The organic layer was separated and washed with sodium bicarbonate solution (50 cm^3 of 1N) and water (2 x 50 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. Purification by recrystallisation from ethyl acetate-petrol gave the *epoxide* (26) (24.31 g, 97%) as a white solid, m.p. 184-186°C (R_f = 0.42, ethyl acetate-petrol (1:3)); υ_{max} (NUJOL)/cm^{-1} 1729 s (C=O), 1248 (C-O), 1047 (C-O); δ_H (200 MHz, $CDCl_3$) 5.02-4.85 (1H, m, H_α-C(3)), 4.01-3.83 (4H, m, OCH_2CH_2O), 2.90

(1H, d, $J_{17,16}$ 4.76 Hz, H-C(17)), 1.29 (3H, s, $H_3C(21)$), 1.09 (3H, s, $H_3C(19)$), 0.71 (3H, s, $H_3C(18)$).

20-Ethylenedioxypregnan-3β,5α-diol (**27**)

To a stirred suspension of lithium aluminium hydride (6.8 g, 0.18M) in THF (500 cm^3) was added the *epoxide* (**26**) (25.0 g, 0.06M), dropwise in THF (200 cm^3). The reaction mixture was stirred with overhead mechanical stirring at room temperature, under nitrogen, for 24h. 1% Potassium hydroxide solution was added cautiously to quench, while stirring. The resulting white precipitate was filtered through celite. The filtrate was diluted with methylene chloride (500 cm^3) and washed with water (2 x 100 cm^3), dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was recrystallised from ethyl acetate-petrol to give the *diol* (**27**) (21.25 g, 94%) as a white solid, m.p. 203-205°C (R_f = 0.24, ethyl acetate); υ_{max}(NUJOL)/cm^{-1} 3389 sh (OH), 3296 br (OH), 1047 (C-O); δ_H (270 MHz, $CDCl_3$) 4.04-3.80 (5H, m, H_α-C(3) + OCH_2CH_2O), 1.29 (3H, s, $H_3C(21)$), 0.99 (3H, s, $H_3C(19)$), 0.75 (3H, s, $H_3C(18)$); *m/z* (C.I.) 379 ([M^+ + 1], 6%), 361 (7), 343 (1), 317 (1), 301 (4), 159 (1), 87 (100), 69 (10).

3β-Acetoxy-20-ethylenedioxypregnan-5α-ol (**28**)

The *diol* (**27**) (21.0 g, 56.0mM) was added to a stirred solution of acetic anhydride (8.4 cm^3, 89.0mM) in pyridine (300 cm^3). The reaction mixture was stirred at room temperature for 24h. After this it was poured into saturated brine (1000 cm^3) and the white solid filtered off. The solid was washed with dil. HCl and water and then dissolved in methylene chloride (200 cm^3). Water (100 cm^3) was added, the organic layer separated and washed with water (100 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was recrystallised from methanol to give *3β-acetoxy-20-ethylenedioxypregnan-5α-ol* (**28**) (22.05 g, 94%) as a white solid, m.p. 200-201°C (R_f = 0.19, ethyl acetate-petrol (1:3)); $[\alpha]^{22}_D$ = +4.1° (C = 0.462 in CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 3465 br (OH), 1726 s (C=O), 1247 (C-O); δ_H (270

MHz, $CDCl_3$) 5.28-5.14 (1H, m, H_α-C(3)), 4.08-3.81 (4H, m, OCH_2CH_2O), 2.02 (3H, s, H_3CCO), 1.30 (3H, s, $H_3C(21)$), 1.02 (3H, s, $H_3C(19)$), 0.77 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 170.61 (C=O), 111.92 (C-20), 74.94 (C-5), 70.84 (C-3), 65.15 + 63.15 (OCH_2CH_2O); *m/z* (C.I.) 421 ([M^+ + 1], 5%), 403 (2), 361 (5), 343 (3), 299 (4), 251 (2), 159 (1), 87 (100); Found : C, 69.2; H, 9.41. $C_{25}H_{40}O_5.{}^3/_4\ H_2O$ requires C, 69.2; H, 9.57%.

3β-Acetoxypregnan-5α-ol-20-one (29)

Aqueous sulphuric acid solution (10 ml of 1N) was added dropwise to a stirred solution of the *acetal* (28) (3.0 g, 7.14mM) in acetone (200 cm^3). The reaction mixture was stirred at room temperature for 30 mins. The mixture was concentrated at reduced pressure and water (50 cm^3) was added. The solution was extracted with methylene chloride (100 cm^3) and the organic layer washed with sodium carbonate solution (20 ml of 1N) and water (2 x 30 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was recrystallised from ethyl acetate to give the *ketone* (29) (2.23 g, 83%) as white needles, m.p. 199-200°C (R_f = 0.17, ethyl acetate-petrol (1:3)); υ_{max} (NUJOL)/cm^{-1} 3419 br (OH), 1725 s (C=O), 1682 s ((20)C=O), 1243 (C-O); δ_H (270 MHz, $CDCl_3$) 5.20-5.10 (1H, m, H_α-C(3)), 2.55 (1H, t, $J_{17,16}$ 8.1 Hz, H_α-C(17)), 2.11 (3H, s, $H_3C(21)$), 2.02 (3H, s, H_3CCO), 1.00 (3H, s, $H_3C(19)$), 0.60 (3H, s, $H_3C(18)$); δ_C (400 MHz, $CDCl_3$) 209.63 (C-20), 170.65 (C=O), 74.81 (C-5), 70.75 (C-3); *m/z* (C.I.) 377 ([M^+ + 1], 38%), 359 (32), 336 (10), 317 (25), 299 (100), 281 (10); Found : C, 72.7; H, 9.68. $C_{23}H_{36}O_4.{}^1/_4\ H_2O$ requires C, 72.5; H, 9.59%.

(E)-3β-Acetoxy-5,10-secopregn-1(10)-ene-5,20-dione (30)

Ceric ammonium nitrate (326 mg, 0.60mM) in water (1 cm^3) was added to *3β-acetoxy-20-ethylenedioxypregnan-5α-ol* (28) (100 mg, 0.24mM) stirred at 80°C in acetonitrile (2 cm^3), to give a bright red colour. The reaction mixture was stirred at 80°C for 3 min, after which it had become decolourised. It was immediately poured

into ice/25% sodium bicarbonate solution (10 cm^3) and extracted with methylene chloride (3 x 10 cm^3), dried over anhydrous sodium sulphate, and evaporated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:5). The first elute gave *3β-acetoxypregnan-5αp-ol-20-one* (**29**) (37 mg, 37%) as a white solid, m.p. 199-200°C (R_f = 0.17, ethyl acetate-petrol (1:3)).

The next eluate gave *(E)-3β-acetoxy-5,10-secopregn-1(10)-ene-5,20-dione* (**30**) (22 mg, 22%) as a white solid, m.p. 208-209°C (R_f = 0.36, ethyl acetate-petrol (1:4); $[\alpha]^{22}_D$ = + 62.5° (C = 1.12, CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 1732 s (C=O), 1714 s ((20)C=O), 1698 ((5)C=O), 1645 (C=C), 1246 (C-O); δ_H (270 MHz, $CDCl_3$) 5.48-5.32 (1H, m, H_α-C(3)), 4.90-4.80 (1H, m, H_E-C(1)), 2.12 (3H, s, H_3C(21)), 2.04 (3H, s, H_3CCO), 1.75 (3H, s, H_3C(19)), 0.66 (3H, s, H_3C(18)); *m/z* (C.I.) 375 ([M^+ + 1], 28%), 259 (10), 315 (68), 297 (100), 281 (10), 260 (12), 159 (10), 81 (15); Found : C, 74.1; H, 9.43. $C_{23}H_{34}O_4$ requires C, 73.8; H, 9.09%.

(E)-3β-Hydroxy-5,10-secopregn-1(10)-ene-5,20-dione (**31**)

(E)-3β-Acetoxy-5,10-secopregn-1(10)-ene-5,20-dione (30) (500 mg, 1.20mM) was added to a stirred solution of potassium carbonate (500 mg, 3.62mM) in methanol (10 cm^3). The reaction mixture was stirred at room temperature for 30 min. Water (15 cm^3) was added and the reaction mixture extracted with methylene chloride (2 x 20 cm^3). The combined organic extracts were washed with dil. HCl (10 cm^3) and water (10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:1) gave the *3β-hydroxy dione* (**31**) (449 mg, 87%) as a white solid, m.p. 168-169°C (R_f = 0.32, ethyl acetate-petrol (1:1)); $[\alpha]^{22}_D$ = + 40.0° (C=0.70, ethyl acetate); υ_{max} (NUJOL)/cm^{-1} 3370 br (OH), 1694 s ((20)C=O), 1679 s ((5)C=O), 1664 (C=C); δ_H (270 MHz, $CDCl_3$) 4.89-4.80 (1H, m, H_E-C(1)), 4.48-4.34 (1H, m, H_α-C(3)), 2.12 (3H, s, H_3C(21)), 1.74 (3H, s, H_3C(19)), 0.66 (3H, s, H_3C(18)); *m/z* (C.I.) 333 ([M^+ + 1], 51%), 3.15 (100), 297 (99), 279 (11), 271 (18), 257 (18), 231 (5), 85 (30); Found :

C, 75.5; H, 9.76. $C_{21}H_{32}O_3$ requires C, 75.9; h, 9.64%.

3β-Acetoxy-20-ethylenedioxy-5,10-secopregn-1(10)-en-5-one **(32 and 33)**

Method 1

A stirred suspension of *3β-acetoxy-20-ethylenedioxypregnan-5α-ol* (**28**) (5.0 g, 11.9mM), yellow mercuric oxide (8.0 g, 37.0mM) and iodine (8.0 g, 31.5mM) in carbon tetrachloride (80 cm^3) was irradiated for 2.5 h. at room temperature with a 500W tungsten lamp. The solid was removed by filtration and the filtrate washed with aqueous sodium thiosulphate solution (30 cm^3), aqueous sodium bicarbonate solution (30 cm^3) and water (2 x 20 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate-petrol (1:19). The first eluate gave *(Z)-3β-acetoxy-20-ethylenedioxy-5,10-secopregn-1(10)-en-5- one* (**33**) (407 mg, 8%) as a white solid, m.p. 138-139°C (R_f = 0.50, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 1727 s (C=O), 1699 s ((5)C=O), 1647 (C=C), 1245 (C-O); δ_H (270 MHz, $CDCl_3$) 5.46-5.33 (1H, m, H_Z-C(1)), 5.32-5.21 (1H, m, H_α-C(3)), 4.06-3.84 (4H, m, OCH_2CH_2O), 2.02 (3H, s, H_3CCO), 1.74 (3H, s, $H_3C(19)$), 1.28 (3H, s, $H_3C(21)$), 0.59 (3H, s, $H_3C(18)$); *m/z* (C.I.) 419 ([M^+ + 1], 7%), 403 (2), 375 (2), 360 (10), 341 (5), 297 (10), 269 (5), 87 (100).

The next eluate afforded a mixture of products (2.05 g,) which upon recrystallisation from ethyl acetate-petrol gave *(E)-3β-acetoxy-20-ethylenedioxy-5,10-secopreg-n-1(10)-en-5-one* (**32**) (695 mg, 14%) as a white solid, m.p. 133-135°C (R_f = 0.38, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 1729 s (C=O), 1702 s ((5)C=O), 1643 (C=C), 1243 (C-O); δ_H (270 MHz, $CDCl_3$) 5.48-5.32 (1H, m, H_α-C(3)), 4.92-4.80 (1H, m, H_E-C(1)), 2.02 (3H, s, H_3CCO), 1.75 (3H, s, $H_3C(19)$), 1.30 (3H, s, $H_3C(21)$), 0.61 (3H, s, $H_3C(18)$); *m/z* (C.I.) 419 ([M^+ + 1] missing), 375 ([M^+ + 1 - CH_3CHO], 30%), 359 (11), 341 (3), 315 (67), 299 (47), 297 (100), 279 (8), 122 (11), 81 (14);

Found : C, 70.9; H, 9.25. $C_{25}H_{38}O_5.{}^1/_4\ H_2O$ requires C, 71.0; H, 9.11%; and starting material, *3β-acetoxy-20-ethylenedioxypregnan-5α-ol* (28) (410 mg, 8%).

Further eluates gave *(E)-3β-acetoxy-5,10-secopregn-1(10)-ene-5,20-dione* (**30**)(1.14 g, 26%) as a white solid, m.p. 208-209°C (R_f = 0.36, ethyl acetate-petrol (1:4)); $[\alpha]^{22}_D$ = + 62.5° (C = 1.12, CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 1732 s (C=O), 1714 s ((20)C=O), 1698 ((5)C=O), 1645 (C=C), 1246 (C-O); δ_H (270 MHz, $CDCl_3$) 5.48-5.32 (1H, m, H_α-C(3)), 4.90-4.80 (1H, m, H_E-C(1)), 2.12 (3H, s, H_3C(21)), 2.04 (3H, s, H_3CCO), 1.75 (3H, s, H_3C(19)), 0.66 (3H, s, H_3C(18)); *m/z* (C.I.) 375 ([M^+ + 1], 28%), 359 (10), 315 (68), 297 (100), 281 (10), 260 (12), 159 (10), 81 (15); Found : C, 74.1; H, 9.43. $C_{23}H_{34}O_4$ requires C, 73.8; H, 9.09%.

Method 2

To a refluxing solution of lead tetraacetate (232 mg, 0.52mM), recrystallised from acetic acid and sucked dry under vacuum over phosphoric acid, in toluene (3 cm^3) was added a solution of *3β-acetoxy-20-ethylenedioxypregnan-5α-ol* (**28**) (200 mg, 0.48mM) in toluene (2 cm^3). Calcium carbonate (200 mg) was added and the reaction mixture was refluxed for 48 h after which starch-iodide paper indicated that there was no oxidant left. After cooling the reaction mixture was diluted with water (5 cm^3) and extracted with ether (2 x 15 cm^3). The combined organic extracts were dried over magnesium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give (Z)-*3β-acetoxy-20-ethylenedioxy-5,10-secopregn-1(10)-en-5-one* (**33**) (19 mg, 10%) as a white solid, m.p. 138-140°C (R_f = 0.50, ethyl acetate-petrol (1:4)).

Preparation of 5α,6α-epoxypregnan-3β-ol-20-one (34)

50% m-Chloroperoxybenzoic acid (13.1 g, 37.9mM) was slowly added to a stirred solution of *pregnenolone* (10 g, 31.6mM) in methylene chloride (200 cm^3). The reaction mixture was stirred at room temperature under nitrogen for 1h. 25% Sodium

sulphite solution and 25% sodium bicarbonate solution were added cautiously, with stirring, until the starch-iodide test gave a negative result. The solution was then extracted with methylene chloride (2 x 150 cm^3), and the organic extracts washed with water (2 x 50 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. Column chromatography on silica gel, eluting with ethyl acetate-petrol (1:2) gave the *epoxide* (**34**) (10.3 g, 98%) as a white solid, m.p. 177-178°C (R_f = 0.17, ethyl acetate-petrol (1:2)); υ_{max} (NUJOL)/cm^{-1} 3438 br (OH), 1684 (C=O), 1230; δ_H (270 MHz, $CDCl_3$) 4.01-3.82 (1H, m, H_α-C(3)), 2.94 (1H, d, $J_{6,7}$ 5.0 Hz, H_β-C(6)), 2.51 (1H, t, *J* 8.4 Hz, H-C(17)), 2.11 (3H, s, H_3C(21)), 1.06 (3H, s, H_3C(19)), 0.55 (3H, s, H_3C(18)); *m/z* (E.I.) 332 (M^+, 20%), 314 (10), 299 (6), 156 (27), 71 (38), 43 (100); Found : C, 75.3; H, 9.63. $C_{21}H_{32}O_3$ requires C, 75.9; H, 9.64%.

Data for pregnenolone; m.p. 190-192°C (R_f = 0,33, ethyl acetate-petrol (1:2)); $[\alpha]^{20}_D$ = + 33.3° (C = 1.17 in EtOH); υ_{max} (NUJOL)/cm^{-1} 3507 br (OH), 1684 (C=O), 1643 (C=C); δ_H (270 MHz, $CDCl_3$) 5.36-5.34 (1H, m, H-C(6)), 3.58-3.48 (1H, m, H_α-C(3)), 2.54 (1H, t, *J* 8.9 Hz, H-C(17)), 2.12 (3H, s, H_3C(21)), 1.01 (3H, s, H_3C(19)), 0.63 (3H, s, H_3C(18)); *m/z* (E.I.) 316 (M^+, 41%), 298 (29), 283 (23), 213 (17), 105 (30), 57 (54), 43 (100).

Pregna-3β-5α-20β-triol (**35**)

Method 1

To an overhead mechanically stirred solution of the *epoxide* (**34**) (10.5 g, 31.6mM) in THF (300 cm^3) was added, cautiously, lithium aluminium hydride (5.8 g, 158mM). The reaction mixture was refluxed under nitrogen for 5h and stirred at room temperature for a further 3h. It was then quenched by the addition of 1% potassium hydroxide solution. The resulting white precipitate was filtered through celite. The filtrate was extracted with methylene chloride. The organic extracts were washed with water (2 x 50 cm^3), dried over anhydrous sodium sulphate and concentrated *in*

vacuo. Recrystallisation from ethyl acetate gave the *triol* (35) (8.7 g, 82%) as a white solid, m.p. 229-230°C (R_f = 0.48, methanol-CH_2Cl_2 (1:20)); $[\alpha]^{22}_D$ = + 1.0° (C=1.00 in EtOH); υ_{max} (NUJOL)/cm^{-1} 3406 (OH), 3384 (OH), 3316 (OH), 1240 (C-O); δ_H (270 MHz, $CDCl_3$) 4.12-3.98 (1H, m, H_α-C(3)), 3.76-3.63 (1H, m, H_β-C(20)), 1.12 (3H, d, $J_{21,20}$ 6.2 Hz, $H_3C(21)$), 0.98 (3H, s, $H_3C(19)$), 0.74 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 74.75 (C-5), 69.93 (C-3), 66.59 (C-20); *m/z* (E.I.) 336 (M^+, 1%), 318 (59), 300 (30), 285 (21), 283 (3), 264 (59), 232 (80), 41 (100); (C.I.) 337 ([M^+ + 1], 2%), 319 (39), 301 (100), 283 (32); Found : C, 67.8; H, 11.0. $C_{21}H_{36}O_3.2H_2O$ requires C, 67.7; H, 10.8%.

Method 2

In a similar procedure *5α,6α-epoxypregna-3β,20β-diol* (37) (10.0 g, 29.9mM) and lithium aluminium hydride (5.5 g, 149mM) were refluxed together in THF (400 cm^3), with overhead mechanical stirring, for 5h. After cooling the reaction mixture was quenched by the addition of 1% potassium hydroxide solution. The resulting white precipitate was filtered through celite, and the filtrate diluted with methylene chloride (400 cm^3). The organic solution was washed with water (2 x 250 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was recrystallised from ethyl acetate to give the *3β,5α,20β-triol* (35) (9.36 g, 93%) as a white solid, m.p. 228-230°C (R_f = 0.48, methanol-methylene chloride (1:20)).

Pregnen-3β,20β-diol (36)

Lithium aluminium hydride (18 g, 0.47M) was added portionwise to a solution of *pregnenolone* (100 g, 0.32M) in THF (1000 ml) with overhead mechanical stirring. Hydride was added with caution. The mixture was stirred at room temperature for 3h and then quenched with 1% potassium hydroxide solution whilst cooling in an ice bath. The white precipitate formed was filtered through celite and the filtrate was concentrated *in vacuo*. The residue was taken up in methylene chloride (700 cm^3) and washed with water (2 x 100 cm^3), dried over anhydrous sodium sulphate and

concentrated *in vacuo*. Purification by recrystallisation from ethyl acetate-petrol gave the *diol* (**36**) (99.6 g, 99%) as a white solid, m.p. 204-205°C (R_f = 0.15, ethyl acetate-petrol (1:2)); υ_{max} (NUJOL)/cm^{-1} 3409 (OH), 3272 (OH), 1643 (C=C); δ_H (270 MHz, $CDCl_3$) 5.38-5.31 (1H, m, H-C(6)), 3.76-3.70 (1H, m, H_α-C(20)), 3.53-3.49 (1H, m, H_α-C(3)), 1.13 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 1.01 (3H, s, $H_3C(19)$), 0.75 (3H, s, $H_3C(18)$); *m/z* (E.I.) 318 (M^+, 100%), 300 (67), 285 (36), 267 (45), 189 (92), 107 (87), 43 (96); Found : C, 77.7; H, 10.9. $C_{21}H_{34}O_2.{}^1/_3H_2O$ requires C, 77.5; H, 10.7%.

5α,6α-Epoxypregna-3β,20β-diol (**37**)

60% m-Chloroperoxybenzoic acid (104.2 g, 0.30M) was added portionwise to a stirred solution of *pregnendiol* (**36**) (80 g, 0.25M) in methylene chloride (800 cm^3). The reaction mixture was stirred at room temperature for 2h. 25% Sodium sulphite solution and 25% sodium bicarbonate solution were added cautiously, with stirring, until the starch-iodide test gave a negative result. The solution was then separated and the organic layer washed with sodium bicarbonate (2 x 200 cm^3) and water (2 x 100 cm^3). The organic extracts were dried over anhydrous sodium sulphate and the solvent removed *in vacuo*. The residue was recrystallised from ethyl acetate-petrol to give the *epoxide* (**37**) (68.1 g, 81%) as a white solid, m.p. 198-199°C (R_f = 0.08, ethyl acetate-petrol (1:2)); υ_{max} (NUJOL)/cm^{-1} 3483 br (OH), 3419 br (OH), 1279 (C-O); δ_H (270 MHz, $CDCl_3$) 3.97-3.84 (1H, m, H_α-C(3)), 3.77-3.64 (1H, m, H_α-C(20)), 2.91 (1H, d, $J_{6,7}$ 4.4 Hz, Hβ-C(6)), 1.12 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 1.07 (3H, s, $H_3C(19)$), 0.70 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 70.46 (C-3), 69.38 (C-6), 68.66 (C-20), 65.70 (C-5); *m/z* (Low eV E.I.) 334 (M^+, 100%), 316 (74), 298 (47), 272 (28), 120 (51), 91 (58); Found : C, 74.8; H, 10.4. $C_{21}H_{34}O_3$ requires C, 75.4; H, 10.2%.

3β,20β-Diacetoxypregnan-5α-ol (**38**)

Pregnan-3β,5α,20β-triol (**35**) (7.0 g, 20.8mM) and acetic anhydride (5.9 cm^3, 62.4mM) were stirred together in pyridine (150 cm^3) at room temperature for 24h.

The reaction mixture was then poured into water and salt added to aid precipitation. The resulting white solid was filtered off and washed with dil. HCl and water. The solid was then dissolved in methylene chloride(150 cm^3), washed with water (2 × 50 cm^3) and dried over anhydrous sodium sulphate. The solvent was removed *in vacuo* and purification by recrystallisation from methanol yielded the *diacetate* (**38**) (8.66 g, 99%) as a white solid, m.p. 207-208°C (R_f = 0.40, ethyl acetate-toluene (1:4)); υ_{max} (NUJOL)/cm^{-1} 3544 br (OH), 1728 vs (C=O), 1244 (C-O); δ_H (270 MHz, $CDCl_3$) 5.23-5.09 (1H, m, H_α-C(3)), 4.89-4.81 (1H, m, H_α-C(20)), 2.02 (3H, s, $COCH_3$), 2.01 (3H, s, $COCH_3$), 1.14 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.99 (3H, s, $H_3C(19)$), 0.62 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 170.61 (C=O), 170.45 (C=O), 74.96 (C-5), 72.82 (C-3), 70.79 (C-20); *m/z* (E.I.) 402 ([M^+ - H_2O], 9%), 360 (6), 342 (31), 306 (32), 282 (21), 81 (29), 43 (100); (C.I.) 403 ([M^+ + 1 - H_2O], 11%), 361 (15), 343 (77), 301 (33), 283 (100); Found : C, 70.6; H, 9.52. $C_{25}H_{40}O_5.{}^1/_4H_2O$ requires C, 70.7; H, 9.54%; $[\alpha]^{22}_D$ = -3.5° (C=1.0 in CH_2Cl_2).

3β,20β-Diacetoxy-5,10-secopregn-1(10)-en-5-one (**39** and **40**)

Method 1

Ceric ammonium nitrate (11.42 g, 20.8mM) in water (20 cm^3) was added to a stirred solution of *3β,20β-diacetoxypregnan-5α-ol* (**38**) (3.50 g, 8.3mM) in acetonitrile (50 cm^3) at 80°C. The reaction mixture was stirred at 80°C for 3 min after which it turned colourless from an initial deep red colour. The reaction mixture was immediately poured into ice/25% aq. sodium bicarbonate (200 cm^3) and after cooling extracted with methylene chloride (3 x 100 cm^3). The combined organic extracts were washed with water (2 x 50 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (**39**) (2.86 g, 82%) as a white solid, recrystallised from ethyl acetate-petrol, m.p. 155-156°C (R_f = 0.36, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 1727 s (C=O), 1704 s ((5)C=O), 1631

(C=C), 1240 (C-O); δ_H (270 MHz, $CDCl_3$) 4.86-4.80 (2H, m, H_E-C(1) + H-C(20)), 4.49-4.33 (1H, m, H_β-C(3)), 2.03 (3H, s, H_3CCO), 2.01 (3H, s, H_3CCO), 1.73 (3H, s, $H_3C(19)$), 1.14 (3H, d, $J_{21,20}$ 6.2 Hz, $H_3C(21)$), 0.67 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 201.63 (C-5), 170.79 (C=O), 170.41 (C=O), 150.77 (C-10), 114.24 (C-1), 74.15 (C-3), 72.63 (C-20); *m/z* ((+) FAB in NBA) 419 ([M^+ + 1], 24%), 403 (8), 359 (53), 341 (85), 315 (6), 299 (39), 281 (100), 253 (9); Found : C, 72.2; H, 9.39. $C_{25}H_{38}O_5$ requires C, 71.8; H, 9.09%.

Method 2

A stirred suspension of *3β,20β-diacetoxypregnan-5α-ol* (38) (26.0 g, 61.9mM), yellow mercuric oxide (41.6 g, 186mM) and iodine (42.0 g, 190mM) in carbon tetrachloride (600 cm^3) was irradiated for 3h at room temperature with a 500W tungsten lamp. The solid was removed by filtration and the filtrate washed successively with sodium thiosulphate solution (200 cm^3), sodium bicarbonate solution (200 cm^3) and water (2 x 100 cm^3), dried over magnesium sulphate and evaporated under reduced pressure. Purification of the residue by column chromatography, eluting with ethyl acetate-petrol (1:9) gave *(Z)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (40) (2.81 g, 11%) as a white solid, m.p. 124-125°C (R_f = 0.44, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 1729 s (C=O), 1702 s ((5)C=O), 1642 (C=C), 1242 (C-O); δ_H (270 MHz, $CDCl_3$) 5.36-5.26 (1H, m, H_Z-C(1)), 4.84-4.76 (1H, m, H-C(20)), 4.40-4.29 (1H, m, H_α-C(3)), 2.03 (3H, s, H_3CCO), 2.00 (3H, s, H_3COO), 1.76 (3H, s, $H_3C(19)$), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.71 (3H, s, $H_3C(18)$); *m/z* (C.I.) 419 ([M^+ + 1], 2%), 401 (3), 359 (48), 341 (60), 299 (69), 281 (100), 271 (4), 149 (12), 89 (48).

Further elutes gave *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (39) (12.42 g, 48%) as a white solid, m.p. 155-156°C (R_f = 0.36, ethyl acetate-petrol (1:4)).

3β-Benzoxypregnan-5α,20β-diol (**41**)

To a sitred suspension of sodium hydride (613 mg, 25.5mM), washed several times with petrol prior to use, in THF (50 cm^3) was added the *triol* (**35**) (2.86 g, 8.5mM) in THF (10 cm^3) dropwise, under nitrogen at 0°C. The reaction mixture was stirred for 15 min, until frothing ceased, after which benzyl bromide (1.11 cm^3, 9.4mM) was added dropwise. The reaction mixture was stirred for a further 6h at 0°C and then 12h at room temperature. Water was added to quench and the THF removed *in vacuo*. The residue was diluted with methylene chloride (100 cm^3) and washed with water (2 x 20 cm^3). The organic layer was dried over anhydrous sodium sulphate, evaporated under reduced pressure, and purified by column chromatography, eluting with ethyl acetate-petrol (1:10) to give the *monobenzyl ester* (**41**) ((2.65 g, 73%) as a white solid, m.p. 175-176°C (R_f = 0.17, ethyl acetate-petrol (3:17)); υ_{max} (NUJOL)/cm^{-1} 3423 br (OH), 3373 br (OH), 1497, 1443 s (C-C, Ar), 739, 697 s (C-H, Ar); δ_H (400 MHz, $CDCl_3$) 7.34-7.22 (5H, m, Ar), 4.54 (2H, q, *J* 11.8, *J* 16.9 Hz, $PhC\underline{H}_2O$), 3.88-3.80 (1H, m, H_α-C(3)), 3.71 (1H, dq, $J_{20,21}$ 5.8 $J_{20,17}$ 10.98 Hz, H_α-C(20)), 1.13 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 1.00 (3H, s, H_3C(19)), 0.74 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 139.18 (C-Ar), 128.18 (C-Ar), 127.86 (C-Ar), 127.60 (C-Ar), 127.53 (C-Ar), 127.31 (C-Ar), 75.18 (C-5), 77.41 ($ArCH_2O$), 74.47 (C-3), 70.21 (C-20); *m/z* (C.I.) 409 ([M^+ + 1 - H_2O], 17%), 408 (30), 391 (70), 319 (48), 301 (100), 299 (88), 283 (81), 107 (20); Found : C, 77.9; H, 9.82. $C_{28}H_{42}O_3.{}^1/_4H_2O$ requires C, 78.0; H, 9.87%.

3β-Benzoxy-20β-acetoxypregnan-5α-ol (**42**)

The *diol* (**41**) (100 mg, 0.23mM) was added to a stirred solution of acetic anhydride (0.033 cm^3, 0.35mM) in pyridine (3 cm^3). The reaction mixture was stirred at room temperature for 24h, after which it was poured into saturated brine (20 cm^3). The resulting white solid was filtered off and washed with dil. HCl and water. The solid was then dissolved in methylene chloride (20 cm^3), washed with water (2 x 5 cm^3) and dried over anhydrous sodium sulphate. The solvent was removed *in vacuo* and

purification by column chromatography on silica gel, eluting with ethyl acetate-petrol (1:9) gave the *acetate* (**42**) (100 mg, 91%) as a white solid, m.p. 184-185°C (R_f = 0.53, ethyl acetate-petrol (3:17)); υ_{max} (NUJOL)/cm^{-1} 3464 sh (OH), 1703 s (C=O), 1265 s (C-O), 754, 703 (C-H, Ar); δ_H (400 MHz, $CDCl_3$) 7.34-7.23 (5H, m, Ar), 4.83 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.4 Hz, H_α-C(20)), 4.54 (2H, q, *J* 11.8, *J* 16.8 Hz, $PhCH_2O$), 3.86-3.79 (1H, m, H_α-C(3)), 2.01 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 5.8 Hz $H_3C(21)$), 0.99 (3H, s, $H_3C(19)$), 0.62 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 170.48 (C=O), 139.14 , 128.33, 127.58, 127.38 (all C-Ar), 75.22 (C-5), 74.43 ($PhCH_2O$), 72.86 (C-3), 70.23 (C-20); *m/z* (E.I.) 450 ([M^+-H_2O], 5%), 432 (1), 408 (1), 390 (3), 359 (5), 299 (47), 149 (59), 71 (59), 57 (100); Found : C, 76.0; H, 9.56. $C_{30}H_{44}O_4.{}^1/_4H_2O$ requires C, 76.2; H, 9.42%.

(E)-3β-Benzoxy-20β-acetoxy-5,10-seco-1(10)-pregnen-5-one (**43**)

To a stirred solution of *3β-benzoxy-20β-acetoxypregnan-5α-ol* (**42**) (250 mg, 0.53mM) in acetonitrile (10 cm^3) at 80°C was added a solution of ceric ammonium nitrate (732 mg, 1.34mM) in water (3 cm^3) to give a deep red colour. After 3 min the solution had decolourised and was immediately poured into ice/25% sodium carbonate solution (25 cm^3). After cooling the solution was extracted with methylene chloride (3 x 30 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give the *secosteroid* (**43**) (114 mg, 46%) as a white solid, m.p. 102-103°C (R_f = 0.52, ethyl acetate-petrol (3:17)); υ_{max} (NUJOL)/cm^{-1} 1730 s (C=O), 1702 s ((5)C=O), 1662 (C-C, Ar), 1450 (C-C, Ar), 1246 (C-O), 740 (C-H, Ar), 696 (C-H, Ar); δ_H (400 MHz, $CDCl_3$) 7.35-7.25 (5H, m, Ar), 4.83 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.4 Hz, H_α-C(20)), 4.84-4.75 (1H, m, H_E-C(1)), 4.57 (1H, d, *J* 2.1 Hz, ArCH), 4.54 (1H, d, *J* 4.6 Hz, ArCH), 4.16-4.08 (1H, m, H_α-C(3)), 2.01 (3H, s, H_3CCO), 1.73 (3H, s, $H_3C(19)$), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.67 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 207.98 (C-5), 170.43 (C=O), 139.84 (C, Ar), 138.32 (C-10), 128.37 (C, Ar), 128.28 (C, Ar), 127.60 (C, Ar), 127.53 (C, Ar), 127.33 (C-1), 123.87 (C, Ar), 80.38 (C-3),

72.68 (C-20), 71.22 ($ArCH_2$); Found : C, 75.1; H, 9.12. $C_{30}H_{42}O_4.{}^3/_4H_2O$ requires C, 75.1; H, 9.07%.

(E)-20β-Acetoxy-5,10-secopregn-1(10)-en-3β-ol-5-one (44)

Method 1

(E)-3β,20β-Diacetoxy-5,10-secopregn-1(10)-en-5-one (39) (1.50 g, 3.59mM) and potassium carbonate (1.50 g, 10.9mM) were stirred together in methanol (25 cm^3) at room temperature for 10 min. Water (25 cm^3) was added and the solution extracted with methylene chloride (3 x 30 cm^3). The combined organic extracts were washed with dil. HCl (15 cm^3) and water (2 x 15 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (3:7) gave the *3β-hydroxy acetate* (44) (1.31 g, 97%) as a white solid, m.p. 131-132°C (R_f = 0.23, ethyl acetate-petrol (3:7)); $[\alpha]^{22}_D$ = +6.6° (C = 1.00, ethyl acetate); υ_{max} (NUJOL)/cm^{-1} 3484 sh (OH), 3205 br (OH), 1726 s (C=O), 1703 s ((5)C=O), 1659 (C=C), 1239 (C-O); δ_H (270 MHz, $CDCl_3$) 4.89-4.80 (1H, m, H_E-C(1)), 4.47-4.35 (1H, m, H_α-C(3)), 3.80-3.65 (1H, m, H-C(20)), 2.78-2.68 (1H, m, H_α-C(4)), 1.74 (3H, s, H_3C(19)), 1.15 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 0.80 (3H, s, H_3C(18)); m/z (C.I.) 377 ([M^+ + 1], 5%), 359 (12), 341 (6), 332 (1), 317 (25), 299 (75), 281 (24); Found C, 73.3; H, 9.88. $C_{23}H_{36}O_4$ requires C, 73.4; H, 9.57%.

Method 2

A solution of 10% palladium on activated carbon (3 mg, 10% w/w) and *3β-benzoxy-20β-acetoxy-5,10-secopregn-1(10)-en-5-one* (43) (30 mg, 0.6mM) in ethanol (3 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was stirred under an atmosphere of hydrogen at atmospheric pressure for 12h. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was dissolved in methylene chloride (10 cm^3) and washed with water (2 x 4 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was

purified by column chromatography, eluting with ethyl acetate-petrol (2:3) to give the *3β-alcohol* (**44**) (23 mg, 95%) as a white solid, m.p. 131-132°C (R_f = 0.23, ethyl acetate-petrol (3:7)).

3β,20β-Dibenzoxypregnan-5α-ol (**45**)

To a stirred suspension of tetrabutylammonium iodide (22 mg, 0.06mM) and sodium hydride (36 mg, 1.5mM), washed several times with petrol prior to use, in THF (8 cm^3) was added the *triol* (**35**) (200 mg, 0.6mM) in THF (3 cm^3) dropwise, under nitrogen at 0°C. The reaction mixture was stirred at 0°C for 15 min, until frothing ceased, after which benzyl bromide (0.15 cm^3, 1.25mM) was added dropwise and then stirred at room temperature, under nitrogen, for 24h. Water was added to quench and the organic solvent removed *in vacuo*. The residue was diluted with methylene chloride (20 cm^3) and washed with water (2 x 10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:19) gave the *dibenzyl ester* (**45**) (162 mg, 47%) as a white solid, m.p. 109-110°C (R_f = 0.45, ethyl acetate-petrol (1:19)); υ_{max} (NUJOL)/cm^{-1} 3471 br (OH), 1604, 1496, 1452 s (C-C, Ar), 733, 694 s (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 7.33-7.25 (10H, m, Ar), 4.54 (2H, d, J_{gem} 2.4 Hz, $PhCH_2O$-C(3)), 4.47 (2H, dd, J_{gem} 11.3, J 64.2 Hz, $PhCH_2O$-C(20)), 3.91-3.80 (1H, m, H_α-C(3)), 3.50-3.38 (1H, m, H_α-C(20)), 1.14 (3H, d, *J* 6.1 Hz, H_3C(21)), 0.99 (3H, s, H_3C(19)), 0.65 (3H, s, H_3C(18)); *m/z* (E.I.) 516 (M^+ missing), 408 ([M^+-$PhCH_2OH$], 1%), 380 (1), 304 (5), 279 (3), 149 (22), 105 (100), 77 (92); Found : C, 79.4; H, 9.27. $C_{35}H_{48}O_3.\ ^3/_4H_2O$ requires C, 79.3; H, 9.35%.

(E)-3β-20β-Dibenzoxy-5,10-seco-1(10)-pregnen-5-one (**46**)

Ceric ammonium nitrate (611 mg, 1.11mM) in water (2 cm^3) was added to *3β,20β-dibenzoxypregnan-5α-ol* (**45**) (230 mg, 0.45mM) in acetonitrile (10 cm^3), stirred at 80°C. The reaction mixture was stirred at 80°C for 3 min, after which it had become clear, and was then poured immediately into ice/25% sodium bicarbonate

solution (25 cm^3). The solution was allowed to cool and then extracted with methylene chloride (3 x 25 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. Purification by column chromatography on silica gel, eluting with ethyl acetate-petrol (1:9) gave the *dibenzyl protected secosteroid* (46) (160 mg, 70%) as a white solid, m.p. 122-123°C (R_f = 0.38, ethyl acetate-petrol (1:9)); υ_{max} (NUJOL)/cm^{-1} 1702 s (C=O), 1657 (C=C), 1494 (C-C, Ar), 1452 (C-C, Ar), 754 (C-H, Ar), 732 (C-H, Ar), 697 (C-H, Ar); δ_H (400 MHz, $CDCl_3$) 7.35-7.24 (10H, m, Ar), 4.83-4.74 (1H, m, H_E-C(1)), 4.63-4.33 (4H, m, $PhCH_2$ x 2), 4.16-4.08 (1H, m, H_α-C(3)), 3.43 (1H, dq, $J_{20,21}$ 5.8, $J_{20,17}$ 9.8 Hz, H_α-C(20)), 1.73 (3H, s, H_3C(19)), 1.14 (3H, d, $J_{21,20}$ 5.8 Hz, H_3C(21)), 0.69 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 208.01 (C-5), 140.16 (C-10), 138.90 (C, Ar), 138.39 (C, Ar), 128.42 (C, Ar), 128.22 (C, Ar), 127.85 (C, Ar), 127.65 (C, Ar), 127.60 (C, Ar), 127.28 (C, Ar), 123.65 (C-1), 80, 49 (C-20), 77.61 (C-3), 71.27 ($PhCH_2O$), 69.99 ($PhCH_2O$); *m/z* (C.I.) 515 ([M^+ + 1], 3%), 497 (2), 479 (1), 423 (5), 407 (16), 389 (15), 279 (25), 205 (32), 107(70), 91 (100); Found : C, 80.2; H, 9.30. $C_{35}H_{46}O_3.{}^1/_2H_2O$ requires C, 80.3; H, 8.99%.

(E)-3β,20β-Dihydroxy-5,10-secopregn-1(10)-en-5-one (47)

Method 1

To a stirred solution of potassium carbonate (2.0 g, 14.5mM) in methanol (30 cm^3) was added *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (39) (950 mg, 2.27mM). The reaction mixture was stirred at reflux for 24h, under nitrogen. Water (20 cm^3) was added and the solution was extracted with methylene chloride (2 x 50 cm^3). The combined organic extracts were washed with dil. HCl (20 cm^3) and water (20 cm^3), dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by column chromatography eluting, with ethyl acetate-petrol (1:1) to give the *(E)-3β,20β-diol* (47) (615 mg, 81%) as a white solid, m.p. 241-242°C (R_f = 0.24, ethyl acetate-petrol (1:1)); $[\alpha]^{22}_D$ = -31.3° (C = 0.8, methanol); υ_{max} (NUJOL)/cm^{-1} 3560 br (OH), 3395 br (OH), 1695 s (C=O), 1650

(C=C); δ_H (270 MHz, $CDCl_3$) 4.87-4.79 (1H, m, H_E-C(1)), 4.46-4.32 (1H, m, H_α-C(3)), 3.77-3.67 (1H, m, H-C(20)), 1.76 (3H, s, H_3C(19)), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, H_3C(21)), 0.79 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 206.51 (C-5), 137.99 (C-10), 122.93 (C-1), 71.10 (C-3), 67.90 (C-20); *m/z* (low eV E.I.) 334 (M^+, 100%), 316 (43), 298 (15), 277 (37), 248 (51), 233 (29), 218 (19); Found : C, 75.2; H, 10.3. $C_{21}H_{34}O_3$ requires C, 75.4; H, 10.2%.

Method 2

A solution of 10% palladium on activated carbon (6 mg, 20% w/w) and *(E)-3β,20β-dibenzoxy-5,10-secopregn-1(10)-en-5-one* (46) (30 mg, 0.06mM) in ethanol (3 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was stirred under an atmosphere of hydrogen at atmospheric pressure for 12h. The mixture was filtered and the filtrate concentrated *in vacuo*. The residue was dissolved in methylene chloride (5 cm^3) and washed with water (2 x 3 cm^3), dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:1) to give the *3β,20β-diol* (**47**) (18mg, 93%) as a white solid, m.p. 241-242°C (R_f = 0.24, ethyl acetate-petrol (1:1)).

(E)-3α-Formoxy-20β-5,10-secopregn-1(10)-en-5-one (54)

Diethylazodicarboxylate (0.63 cm^3, 3.99mM) was added dropwise to a stirred solution of *20β-acetoxy-5,10-secopregn-1(10)-en-3β-ol-5-one* (**44**) (1.0g, 2.66mM), triphenyl phosphine (2.09g, 7.98mM) and formic acid (0.15 cm^3, 3.99mM) in THF (75 cm^3) at 0°C. The reaction mixture was stirred at 0-10°C for 4h. Water (50 cm^3) was added to the mixture and it was extracted with methylene chloride (2 x 100 cm^3). The combined organic extracts were washed with aq. sodium bicarbonate (25 cm^3) and water (2 x 50 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give *20β-acetoxy-1,3(10→1βH, 5→3αH)abeopregn-10(19)-en-5-one* (**55**)

(133mg, 14%), which was recrystallised from ethyl acetate to give colourless prisms, m.p. 135-137°C (R_f = 0.81, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3071 (C-C, 3' ring), 1736 s (C=O), 1698 s ((5)C=O), 1634 (C=C); δ_H (270 MHz, $CDCl_3$) 4.84 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.3 Hz, H-C(20)), 4.61 (1H, s, H-C(19)), 4.52 (1H, s, H-C(19)), 3.05 (1H, dd, $J_{1,2'}$ 5.7 $J_{1,2''}$ 15.4 Hz, H-C(1)), 2.78 (1H, dddd, *J* 2.1, J 6.3, *J* 12.4, *J* 19.1 Hz, H-C(3)), 2.41-2.33 (1H, m, H_α-C(4)), 2.01 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.66 (3H, s, $H_3C(18)$); *m/z* (E.I.) 358 (M^+, 55%), 343 (10), 329 (8), 298 (11), 283 (12), 265 (10), 159 (26), 147 (36); Found : C, 77.2; H, 9.61. $C_{23}H_{34}O_3$ requires C, 77.1; H, 9.50%.

Further elutes gave *(E)-3α-formoxy-20β-acetoxy-5,10-secopregn-1(10)-en-5-one* (**54**) (721mg, 67%) as a white solid, m.p. 122-123°C (R_f = 0.71, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 1729 s (C=O), 1710 s (HC=O), 1704 s ((5)C=O), 1635 (C=C), 1242 (C-O); δ_H (270 MHz, $CDCl_3$) 8.00 (1H, s, HCO), 5.55-5.45 (1H, m, H_β-C(3)), 4.92-4.78 (2H, m, H_E-C(1) + H-C(20)), 2.01 (3H, s, H_3CCO), 1.75 (3H, s, $H_3C(19)$), 1.13 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.66 (3H, s, $H_3C(18)$); *m/z* (C.I.) 405 ([M^++1], 6%), 387 (5), 359 (68), 345 (43), 327 (95), 299 (69), 281 (100); Found : C, 71.1; H, 9.00. $C_{24}H_{36}O_5$ requires C, 71.3; H, 8.91%.

(E)-20β-Acetoxy-5,10-secopregn-1(10)-en-3α-ol-5-one (**56**)

Potassium carbonate (3.0g, 21.7mM) was added to a stirred solution of *(E)-3α-formoxy-20β-acetoxy-5,10-secoprepregn -1(10)-en-5-one* (**54**) (3.0g, 7.43mM) in methanol (100 cm^3). The reaction mixture was stirred at room temperature for 1h. The mixture was diluted with water (50 cm^3) and extracted with methylene chloride (2 x 100 cm^3). The combined organic extracts were washed with dil. HCl (30 cm^3) and water (2 x 40 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (3:7) to give the *3α-hydroxyl* (**56**) (2.74g, 98%) as a white solid, m.p. 149-150°C (R_f = 0.23, ethyl acetate-petrol (3:7)); $[\alpha]^{22}_D$ = + 6.0 (C=1.00, ethlyl

acetate); υ_{max} (NUJOL)cm^{-1} 3424 br (OH), 1726 s (C=O), 1704 s (C=O); δ_H (270 MHz, $CDCl_3$) 4.87-4.80 (2H, m, H_E-C(1)+H-C(20)), 4.44-4.34 (1H, m, H_β-C(3)), 2.01 (3H, s, H_3CCO), 1.73 (3H, s, $H_3C(19)$), 1.14 (3H, d, $J_{21,20}$ 5.9 Hz, $H_3C(21)$), 0.67 (3H, s, $H_3C(18)$); δ_H ($CDCl_3$) 207.64 (C-5), 170.21 (C=O), 139.83 (C-10), 123.77 (C-1), 120.61 (C-20), 70.79 (C-3); *m/z* (C.I.) 377 ([M^++1], 10%), 359 (16), 341 (6), 317 (31), 299 (100), 281 (33), 259 (4); Found : C, 71.2; H, 9.52. $C_{23}H_{36}O_4.{}^5/_8H_2O$ requires C, 71.3; H, 9.62%.

(E)-3α-Tertbutyldimethylsiloxy-20β-acetoxy-5,10-secopregn-1(10)-en-5-one (**57**) *(E)-20β-acetoxy-5,10-secopregn-1(10)-en-3α-ol-5-one* (56) (100mg, 0.27mM) in DMF (1 cm^3) was added dropwise to a stirred solution of *tert*-butyldimethylsilyl chloride (44mg, 0.29mM) and imidazole (20mg, 0.29mM) in DMF (2 cm^3) under nitrogen. The reaction mixture was stirred at room temperature under nitrogen for 30 min after which the DMF was removed *in vacuo*. Methylene chloride (20 cm^3) was added to the residue, and it was washed with dil. HCl (2 cm^3) and water (2 x 5 cm^3), dried over magnesium sulphate and evaportated at reduced pressure. Purification by column chromatography, eluting with ethyl acetate-petrol (1:9) gave the *C-3 silyl ether* (**57**) (119mg, 92%) as a white solid, m.p. 113-114°C (R_f = 0.71, ethyl acetate-petrol (3:17)); υ_{max} (NUJOL)/cm^{-1} 1728 s (C=O), 1700 s ((5)C=O), 1658 (C=C), 1069 (O-Si); δ_H (270 MHz, $CDCl_3$) 4.86-4.78 (2H, m, H_E-C(1) + H-C(20)), 4.45-4.31 (1H, m, H_β-C(3)), 2.00 (3H, s, H_3CCO), 1.72 (3H, s, $H_3C(19)$), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.89 (9H, s, $(H_3C)_3CSi$), 0.66 (3H, s, $H_3C(18)$), 0.10 (6H, s, $(H_3C)_2Si$); *m/z* (C.I.) 491 ([M^++1], 19%), 473 (11), 431 (46), 413 (70), 373 (21), 359 (15), 341 (37), 281 (100); Found : C, 71.2; H, 10.5. $C_{29}H_{50}SiO_4$ requires C, 71.0; H, 10.2%.

(E)-3α-Tertbutyldimethylsiloxy-5,10-secopregn-1(10)-en-20β -ol-5-one (**58**) Potassium carbonate (100mg, 0.72mM) was added to a stirred solution of *(E)-3α-terbutyldimethylsiloxy-20β-acetoxy-5,10-secopregn-1 (10)-en-5-one* (**57**)

(50mg, 0.10mM) in methanol (2 cm^3) at room temperature. The reagents were heated to reflux for 24h. After cooling the reaction mixture was diluted with water (5 cm^3) and extracted with methylene chloride (3 x 10 cm^3). The combined organic extracts were washed with dil. HCl (5 cm^3) and water (2 x 10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give the *20β-hydroxyl silyl ether* (**58**) (43mg, 94%) as a white solid, m.p. 163-164°C (R_f = 0.37 ethyl acetate-petrol (1:9)); υ_{max} (NUJOL)/cm^{-1} 3405 br (OH), 1691 s (C=O), 1658 (C=C), 1066 (O-Si); δ_H (270 MHz, $CDCl_3$) 4.83-4.78 (1H, m, H_E-C(1)), 4.40-4.33 (1H, m, H_β-C(3)), 3.70 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 9.8 Hz, H-C(20)), 1.74 (3H, s, H_3C(19)), 1.13 (3H, d, $J_{21,20}$ 6.2 Hz, H_3C(21)), 0.89 (9H, s, $(H_3C)_3CSi$), 0.79 (3H, s, H_3C(18)), 0.10 (6H, s, $(H_3C)_2Si$); *m/z* (C.I.) 449 ([M^++1], 17%), 431 (46), 413 (31), 391 (19), 317 (19), 299 (100); Found : C, 71.1; H, 10.8. $C_{27}H_{48}SiO_3.{}^1/_2H_2O$ requires C, 70.9; H, 10.7%.

(E)-3α-Tertbutyldimethylsiloxy-5,10-secopregn-1(10)-ene-5,20-dione (**59**)
Pyridinium chlorochromate, PCC, (310mg, 1.44mM) was added to a stirred solution of *(E)-3α-tertbutyldimethylsiloxy-5,10-secopregn-1(10)-en-20β -ol-5-one* (**58**) (430mg, 0.96mM) in methylene chloride (20 cm^3). The reaction mixture was stirred at room temperature under nitrogen for 3h. Silica gel was then added to the reaction mixture, the solvent evaporated *in vacuo* and purification by column chromatography, eluting with ethyl acetate-petrol (1:9) gave the *5,20-dione* (**59**) (409mg, 96%) as a white solid, m.p. 142-143°C (R_f = 0.49, ethyl acetate-petrol (1:9)); υ_{max} (NUJOL)/cm^3 1707 s (C=O), 1693 s (C=O), 1663 (C=C), 1078 (O-Si); δ_H (270 MHz, $CDCl_3$) 4.92-4.75 (1H, m, H_E-C(1)), 4.46-4.30 (1H, m, H_β-C(3)), 2.11 (3H, s, H_3C(21)), 1.73 (3H, s, H_3C(19)), 0.89 (9H, s, $(H_3C)_3CSi$), 0.65 (3H, s, H_3C(18)), 0.10 (6H, s, $(H_3C)_2CSi$); *m/z* (C.I.) 447 ([M^++1], 71%), 429 (25), 389 (13), 315 (19), 297 (100); Found : C, 72.3; H, 10.4. $C_{27}H_{46}SiO_3$ requires C, 72.6; H, 10.3%.

(E)-3α-Hydroxy-5,10-secopregn-1(10)-ene-5,20-dione (60)

1M TBAF in THF (0.92 cm^3, 0.91mM) was added dropwise to a stirred solution of *(E)-3α-tertbutyldimethylsiloxy-5,10-secopregn-1(10)-ene-5, 20-dione* (59) (340mg, 0.76mM) in THF (10 cm^3). The reaction mixture was stirred at room temperature for 2h. Methylene chloride (30 cm^3) was added and the solution washed with dil. HCl (5 cm^3) and water (2 x 10 cm^3). The organic layer was dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography eluting with ethyl acetate-petrol (3:7), followed by recrystallisation from ethyl acetate to give the *3α-hydroxy dione* (60) (212mg, 84%) as flat crystals, m.p. 182-184°C (R_f = 0.17, ethyl acetate-petrol (3:7)); $[\alpha]^{22}_D$ = +79.3° (C=1.04, CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 3370 br (OH), 1692 s (C=O), 1678 s (C=O), 1640 (C=C); δ_H (270 MHz, $CDCl_3$) 4.86-4.82 (1H, m, H_E-C(1)), 4.60 (1H, d, *J* 7.7 Hz, HO-C(3)), 4.48-4.28 (1H, m, H_β-C(3)), 2.12 (3H, s, H_3C(21)), 1.74 (3H, s, H_3C(19)), 0.66 (3H, s, H_3C(18)); *m/z* (low eV E.I.) 332 (M^+, 100%), 314 (60), 246 (80), 233 (44), 149 (13), 99 (18); Found : C, 75.9; H, 9.84. $C_{21}H_{32}O_3$ requires C, 75.9; H, 9.64%; UV_{max} = 293nm.

(E)-20β-Acetoxy-5,10-secopregn-1(10)-ene-3α-5α-diol (61)

Sodium borohydride (384mg, 10.1mM) was added portionwise to a stirred solution of *(E)-20β-acetoxy-5,10-secopregn-1(10)-en-3α-ol-5-one* (56) (1.90g, 5.05mM) in ethanol (100 cm^3). The reaction mixture was stirred at room temperature for 12h. Water (20 cm^3) was added and the solution extracted with methylene chloride (2 x 100 cm^3). The combined organic extracts were dried over anhydrous sodium sulphate and evaporated *in vacuo*. The residue was purified by column chroamtography eluting with ethyl acetate-petrol (1:1) to give the *3α,5α-diol* (61) (1.24g, 65%) as a white solid, m.p. 68-69°C (R_f = 0.23, ethyl acetate-petrol (1:1)); $[\alpha]^{22}_D$ = -18.0° (C=0.64, ethyl acetate); υ_{max} (NUJOL)/cm^{-1} 3375 br (OH), 1729 (C=O), 1665 (C=C); δ_H (270 MHz, $CDCl_3$) 5.47-5.45 (1H, m, H_E-C(1)), 4.88-4.81 (1H, m, H-C(20)), 4.29-4.22 (1H, m, H_β-C(3)), 4.22-4.15 (1H, m, H_β-C(5)), 2.55-2.51 (1H, m, H_α-C(4)), 2.01 (3H, s, H_3CCO),1.69 (3H, brs, H_3C(19)), 1.15 (3H, d, $J_{21,20}$ 6.4 Hz, H_3C(21)), 0.66 (3H, s,

$H_3C(18)$); *m/z* (E.I.) 378 (M^+ missing), 360 ([M^+ - H_2O], 4%), 342 (4), 318 (4), 306 (6), 300 (5), 285 (7), 267 (4), 218 (10); Found : C, 71.9; H, 10.1. $C_{23}H_{38}O_4.{}^1/_3H_2O$ requires C, 71.9; H, 10.1%.

(E)-3α,5α-(Ditertbutyl)siloxy-20β-acetoxy-5,10-secopregn-1(10)-ene (**62**)

2,6-Lutidine (0.34 cm^3, 2.94mM) was added dropwise to a stirred solution of *(E)-20β-acetoxy-5,10-secopregn-1(10)-ene-3α,5α-diol* (**61**) (370mg, 0.98mM) and di-*tert*butylsilylbis(trifluoromethanesulphonate) (0.43 cm^3, 1.17mM) in methylene chloride (10 cm^3) at 0°C under nitrogen. The reaction mixture was stirred at 0°C for 10 min after which silica gel was added. The solvent was removed under reduced pressure and the silica applied to a column. Column chromatography, eluting with ethyl acetate-petrol (1:9) gave the *bissilyl ether* (**62**) (390mg, 77%) as a colourless oil (R_f = 0.85, ethyl acetate-petrol (3:17)); υ_{max} (FILM)/cm^{-1} 1733 s (C=O), 1658 (C=C), 1059 (O-Si); δ_H (270 MHz, $CDCl_3$) 5.57-5.53 (1H, m, H_E-C(1)), 4.87-4.80 (1H, m, H-C(20)), 4.53-4.50 (1H, m, H_β-C(3)), 4.31-4.25 (1H, m, H_β-C(5)), 2.48 (2H, dd, *J* 4.4, *J* 9.3 Hz, H_2-C(4)), 2.02 (3H, s, H_3CCO), 1.69 (3H, s, $H_3C(19)$), 1.15 (3H, d, $J_{21,20}$ 6.4 Hz, $H_3C(21)$), 1.02 (9H, s, $(H_3C)_3CSi$), 1.00 (9H, s, $(H_3C)_3CSi$), 0.65 (3H, s, $H_3C(18)$); *m/z* (C.I.) 519 ([M^++1], 4%), 459 (29), 401 (8), 343 (6), 283 (34), 239 (6), 205 (100); Found : C, 68.0; H, 10.6. $C_{31}H_{54}SiO_4.1{}^2/_3H_2O$ requires C, 67.9; H, 10.5%.

(E)-3α,5α-(Ditertbutyl)siloxy-5,10-secopregn-1(10)-en-20β-ol (**63**)

Potassium carbonate (500mg, 3.62mM) was added to a stirred solution of *(E)-3α,5α-(ditertbutyl)siloxy-20β-acetoxy-5,10-secopregn-1 (10)-ene* (**62**) (370mg, 0.71mM) in methanol (20 cm^3) and the mixture refluxed for 24h. After cooling the mixture was diluted with water (10 cm^3) and the solution extracted with methylene chloride (3 x 20 cm^3). The combined organic extracts were washed with dil. HCl (5 cm^3) and water (2 x 10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:19) to give the *20β-hydroxyl bissilyl ether* (**63**) (314mg, 92%) as a

colourless oil (R_f = 0.44, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 3420 br (OH), 1658 (C=C), 1059 (O-Si); δ_H (270 MHz, $CDCl_3$) 5.60-5.50 (1H, m, H_E-C(1)), 4.60-4.50 (1H, m, H_β-C(3)), 4.35-4.25 (1H, m, H_β-C(5)), 3.80-3.70 (1H, m, H-C(20)), 2.55-2.45 (2H, m, H_2-C(4)), 1.69 (3H, s, H_3C(19)), 1.15 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 1.07 (9H, s, $(H_3C)_3CSi$), 1.03 (9H, s, $(H_3C)_3CSi$), 0.78 (3H, s, H_3C(18)); *m/z* (C.I.) 477 ([M^++1], 37%), 459 (80), 419 (90), 401 (11), 301 (69), 283 (100), 257 (20).

(E)-3α,5α-(Ditertbutyl)siloxy-5,10-secopregn-1(10)-en-20-o ne (**64**)

Pyridinium chlorochromate (183mg, 0.85mM) was added to a stirred solution of *(E)-3α,5α-(ditertbutyl)siloxy-5,10-secopregn-1(10)-en-20β- ol* (**63**) (270mg, 0.57mM) in methylene chloride (15 cm^3) and the mixture stirred at room temperature, under nitrogen, for 2.5h. The reaction mixture was preabsorbed onto silica gel and applied to a column. Column chromatography, eluting with ethyl acetate-petrol (1:19) gave the *20-ketone* (**64**) (247mg, 92%) as a colourless oil (R_f = 0.52, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 1706 s (C=O), 1657 (C=C), 1059 (O-Si); δ_H (270 MHz, $CDCl_3$) 5.56 (1H, dd, *J* 6.0, *J* 10.2 Hz, H_E-C(1)), 4.53-4.51 (1H, m, H_β-C(3)), 4.30-4.23 (1H, m, H_β-C(5)), 2.55-2.46 (2H, m, H_2-C(4)), 2.12 (3H, s, H_3C(21)), 1.70 (3H, s, H_3C(19)), 1.05 (9H, s, $(H_3C)_3CSi$), 1.03 (9H, s, $(H_3C)_3CSi$), 0.64 (3H, s, H_3C(18)); *m/z* (C.I.) 475 ([M^++1], 100%), 457 (14), 417 (75), 391 (11), 281 (17), 255 (7), 239 (21).

(E)-3α,5α-Dihydroxy-5,10-secopregn-1(10)-en-20-one (**65**)

To a stirred solution of *(E)-3α,5α-(ditertbutyl)siloxy-5,10-secopregn-1(10)-en-20-o ne* (**64**) (200mg, 0.42mM) in THF (10 cm^3) was added hydrogen fluoride-pyridine (0.06 cm^3, 0.51mM). The reaction mixture was stirred at room temperature under nitrogen for 1h. After this the reaction mixture was diluted with methylene chloride (30 cm^3) and washed with aq. copper sulphate (2 x 10 cm^3), aq. sodium bicarbonate (10 cm^3) and water (2 x 10 cm^3). The organic layer was dried over anhydrous sodium sulphate,

evaporated under reduced pressure, and purified by column chromatography, eluting with ethyl acetate-petrol (3:1) to give the *3α,5α-diol* (65) (80mg, 57%) as a white solid, m.p. 131-133°C (R_f = 0.36, ethyl acetate-petrol (3:1)); υ_{max} (NUJOL)/cm^{-1} 3351 br (OH), 3222 br (OH), 1703 s (C=O), 1690 (C=C); δ_H (270 MHz, $CDCl_3$) 5.46-5.40 (1H, m, H_E-C(1)), 4.32-4.26 (1H, m, H_β-C(3)), 4.24-4.16 (1H, m, H_β-C(5)), 2.56-2.50 (2H, m, H_2-C(4)), 2.12 (3H, s, H_3C(21)), 1.70 (3H, s, H_3C(19)), 0.70 (3H, s, H_3C(18)); *m/z* (C.I.) 335 ([M^++1], 7%), 317 (11), 299 (15), 281 (4), 255 (6) 245 (3); Found : C, 73.3; H, 9.95. $C_{21}H_{34}O_3.{}^1/_2H_2O$ requires C, 73.4; H, 10.2%; UV_{max} = 290nm.

3α-Benzoylpregnenolone (66)

To a stirred solution of *pregnenolone* (3g, 9.5mM), benzoic acid (1.27g, 10.4mM) and triphenylphosphine (5.47g, 20.9mM) in THF (100 cm^3) was added diethyl azodicarboxylate, DEAD, (1.64 cm^3, 10.4mM) dropwise at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred under nitrogen for 6h. Half of the THF was removed *in vacuo* and water (100 cm^3) was added to the residue. Ethyl acetate (50 cm^3) was added and the organic layer was separated, and the aqueous layer was extracted with diethyl ether (2 x 25 cm^3). The combined organic extracts were dried over magnesium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:20) to give the *benzoyl ester* (66) (2.7g, 68%) as a white solid, m.p. 191-192°C (R_f = 0.45, ethyl acetate-petrol (3:37)); υ_{max} (NUJOL)cm^{-1} 1699 s (C=O), 1670 (C=C), 711 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 8.07-7.39 (5H, m, Ar), 5.32 (1H, t, $J_{6,7}$ 2.7 Hz, H-C(6)), 5.26-5.24 (1H, m, H_βC(3)), 2.13 (3H, s, H_3C(21)), 1.06 (3H, s, H_3C(19)), 0.65 (3H, s, H_3C(18)); *m/z* (C.I.) 421 ([M^++1], 41%), 403 (3), 299 (100), 281 (17), 257 (6); Found : C, 80.3; H, 8.73. $C_{28}H_{36}O_3$ requires C, 80.0; H, 8.57%.

3α-Pregnenolone (**67**)

Method 1

3α-Benzoylpregnenolone (**66**) (250mg, 0.60mM) in methylene chloride (1 cm^3) was added to a stirred solution of sodium hydroxide (250mg, 6.1mM) in methanol (5 cm^3). The reaction mixture was stirred at 80°C for 1h. After allowing the mixture to cool it was diluted with methylene chloride (20 cm^3) and washed with dil. HCl (2 x 15 cm^3), with care, and water (2 x 10 cm^3). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:5) to give *3α-pregnenolone* (**67**) (130mg, 69%) as a white solid, m.p. 114-115°C (R_f = 0.33, ethyl acetate-petrol (1:2)); $[\alpha]^{22}_D$ = + 16.2° (C=1.05 in CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 3381 br (OH), 1703 s (C=O), 1665 (C=C); δ_H (270 MHz, $CDCl_3$) 5.42-5.39 (1H, m, H-C(6)), 4.05-3.98 (1H, m, H_β-C(3)), 2.13 (3H, s, H_3C(21)), 1.01 (3H, s, H_3C(19)), 0.63 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 209.40 (C-20), 138.58 (C-5), 123.27 (C-6), 71.44 (C-3); *m/z* (E.I.) 316 (M^+, 21%), 298 (38), 283 (22), 261 (15), 145 (22), 71 (59), 57 (100); Found : C, 79.4; H, 10.3. $C_{21}H_{32}O_2$ requires C, 79.7; H, 10.1%.

Method 2

3α-Pregnenolone formate (**68**) (10g, 0.029M) in methylene chloride (50 cm^3) was added to a stirred solution of potassium carbonate (10g, 0.072M) in methanol (750 cm^3). The reaction mixture was stirred at room temperature for 30 min, after which water (200 cm^3) was added. The mixture was then extracted with methylene chloride (2 x 500 cm^3). The organic layer was washed with dil. HCl (2 x 50 cm^3) and water (2 x 50 cm^3), and dried over anhydrous sodium sulphate, evaporated under reduced pressure and purified by crystallisation from ethyl acetate-petrol to give *3α-pregnenolone* (**67**) (7.9g, 86%) as a white solid, m.p. 114-115°C. (R_f = 0.33, ethyl acetate-petrol (1:2)).

3α-Pregnenolone formate (**68**)

To a stirred solution of *pregnenolone* (10g, 32.0mM), formic acid (2.0 cm^3, 51.0mM) and triphenylphosphine (18.25g, 70.0mM) in THF (250 cm^3) was added diethyl azodicarboxylate (10.0 cm^3, 64.0mM) dropwise at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred under nitrogen for 4h. It was then concentrated at reduced pressure and the residue dissolved in methylene chloride (150 cm^3). Water (50 cm^3) was added, the organic layer was separated, and the aqueous layer was extracted with diethyl ether (2 x 30 cm^3). The combined organic extracts were washed with brine (50 cm^3), dried over magnesium sulphate, and evaporated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:10) to give the *formyl ester* (**68**) (7.95g, 73%) as a white solid, recrystallised from ethyl acetate, m.p. 153-154°C (R_f = 0.68, ethyl acetate-petrol (3:7)); $[\alpha]^{22}_D$ = + 11.1 (C=1.14 in EtOH); υ_{max} (NUJOL)/cm^{-1} 1714 s (HC=O), 1699 s ((20)C=O), 1676 (C=C); δ_H (200 MHz, $CDCl_3$) 8.06 (1H, s, HCOO), 5.36-5.29 (1H, m, H-C(6)), 5.21-5.12 (1H, m, H_β-C(3)), 2.13 (3H, s, H_3C(21)), 1.04 (3H, s, H_3C(19)), 0.66 (3H, s, H_3C(18)); *m/z* (C.I.) 345 ([M^++1], 30%), 327 (11), 299 (100), 281 (13), 255 (5), 147 (5), 85 (8); Found : C, 76.5; H, 9.45. $C_{22}H_{32}O_3$ requires C, 76.7; H, 9.30%.

5α,6α-Epoxypregnan-3α-ol-20-one (**69**)

60% m-Chloroperoxybenzoic acid (1.6g, 7.2mM) was added to a stirred solution of *3α-pregnenolone* (**67**) (2.0g, 6.0mM) in methylene chloride (50 cm^3). The reaction mixture was stirred at room temperature under nitrogen for 1h. After this 25% sodium sulphite solution and 25% sodium bicarbonate solution were added cautiously, with stirring, until the starch-iodide test gave a negative result. The solution was then extracted with methylene chloride (2 x 50 cm^3). The organic extracts were combined and dried over anhydrous sodium sulphate, concentrated *in vacuo* and purified by column chromatography, eluting with ethyl acetate-toluene

(1:2) to give the *epoxide* (**69**) (1.77g, 84%) as a white solid, m.p. 118-119°C (R_f = 0.20, ethyl acetate-petrol (1:2)); υ_{max} (NUJOL)/cm^{-1} 3401 br (OH), 1696 s (C=O), 1248 (C-O); δ_H (200 MHz, $CDCl_3$) 4.15-4.05 (1H, m, H_β-C(3)), 2.90 (1H, d, $J_{6,7}$ 5.1 Hz, H_β-C(60), 2.12 (3H, s, H_3C(21)), 1.07 (3H, s, H_3C(19)), 0.58 (3H, s, H_3C(18)); δ_C (400 MHz, $CDCl_3$) 209.50 (C-20), 67.75 (C-3), 65.28 (C-5), 63.23 (C-6); *m/z* (E.I.) 332 (M^+, 13%), 314 (11), 301 (7), 281 (6), 211 (10), 71 (32), 43 (100); Found : C, 73.6; H, 9.25. $C_{21}H_{32}O_3$ + $^1/_2$EtOAc requires C, 73.4; H, 9.57%.

Pregnan-3α,5α,20β-triol (**70**)

To a stirred suspension of lithium aluminium hydride (858mg, 22.6mM) in THF (25 cm^3) was added the *epoxide* (**69**) (1.5g, 4.5mM) in THF (25 cm^3) dropwise. The reaction mixture was refluxed for 4h. After allowing the reaction mixture to cool to room temperature 1% potassium hydroxide solution was added to quench. The resulting white precipitate was filtered through celite and the filtrate concentrated *in vacuo*. The residue was dissolved in methylene chloride (50 cm^3) and washed with water (2 x 15 cm^3). The organic layer was dried over anhydrous sodium sulphate, evaporated under reduced pressure, and purified by column chromatography, eluting with ethyl acetate-petrol (2:1) to give the *triol* (**70**) (577mg, 38%) as a white solid, m.p. 261-262°C (R_f = 0.48, methanol-CH_2Cl_2 (1:19)); υ_{max} (NUJOL)/cm^{-1} 3436 sh (OH); δ_H (200 MHz, $CDCl_3$) 4.12-4.04 (1H, m, H_β-C(3)), 3.80-3.67 (1H, m, H_α-C(20)), 1.15 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 0.95 (3H, s, H_3C(19)), 0.74 (3H, s, H_3C(18)); *m/z* (C.I.) 337 ([M^++1], 1%), 319 (24), 301 (100), 283 (29), 264 (13); Found : C, 75.2; H, 11.0. $C_{21}H_{36}O_3$ requires C, 75.0; H, 10.7%.

3α,20β-Diacetoxypregnan-5α-ol (**71**)

Pregnan-3α,5α,20β-triol (**70**) (500mg, 1.5mM) and acetic anhydride (0.42 cm^3, 0.5mM) were stirred together in pyridine (5 cm^3) at room temperature for 24h. The reaction mixture was then poured into saturated brine (25 cm^3) and the resulting white solid filtered off. The solid was washed with dil. HCl and water, and then dissolved

in methylene chloride(20 cm^3). Water (5 cm^3) was then added, the organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 x 15 cm^3). The combined organic extracts were dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:5) to give the *diacetate* (**71**) (606mg, 97%) as a white solid, m.p. 172-173°C (R_f = 0.40, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 3577 sh (OH), 1728 s (C=O), 1246 (C-O); δ_H (270 MHz, $CDCl_3$) 5.23-5.18 (1H, m, H_β-C(3)), 4.84 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.3 Hz, H_α-C(20)), 2.07 (3H, s, H_3CCO), 2.02 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.95 (3H, s, $H_3C(19)$), 0.63 (3H, s, $H_3C(18)$); *m/z* (E.I.) 420 (M^+ missing), 402 ([M^+ - H_2O], 10%), 358 (6), 342 (54), 306 (60), 282 (26); Found : C, 71.7; H, 9.91. $C_{25}H_{40}O_5$ requires C, 71.4; H, 9.52%.

(E)-3α,20β-Diacetoxy-5,10-secopregn-1(10)-en-5-one (72)

Ceric ammonium nitrate (754mg, 1.38mM) in water (3 cm^3) was added to a stirred solution of *3α,20β-diacetoxypregnan-5α-ol* (**71**) (230mg, 0.55mM) in acetonitrile (10 cm^3) at 80°C. After 3 min at 80°C the mixture was immediately poured into ice/25% sodium bicarbonate solution (50 cm^3) and the solution extracted with methylene chloride (2 x 50 cm^3). The combined organic extracts were washed with water (2 x 25 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (3:17) to give *(E)-3α,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (**72**) (85mg, 37%) as a white solid, m.p. 138-139°C (R_f = 0.36, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 1731 s (C=O), 1702 s (C=O), 1643 (C=C), 1246 (C-O); δ_H (270 MHz, $CDCl_3$) 5.27-5.22 (1H, m, H_β-C(3)), 4.93-4.79 (2H, m, H_E-C(1) + H-C(20)), 2.05 (3H, s, H_3CCO), 2.03 (3H, s, H_3CCO), 1.74 (3H, s, $H_3C(19)$), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.71 (3H, s, $H_3C(18)$); *m/z* (E.I.) 418 (M^+, missing), 358 (M^+ - AcOH, 39%), 340 (7), 298 (8), 280 (14), 147 (16), 121 (18), 43 (100).

3α-Benzoyl-20-ethylenedioxypregnan-5α-ol (73)

To a stirred solution of benzoic acid (360mg, 3.20mM), triphenylphosphine (840mg, 3.20mM) and *20-ethylenedioxypregnan-3β,5α-diol* (27) (1.0g, 2.66mM) in THF (100 cm^3) under nitrogen was added diethylazodicarboxylate (0.5 cm^3, 3.20mM) in THF (50 cm^3) dropwise. The initial orange solution became colourless and the reaction mixture was stirred at room temperature for 12h, after which it was concentrated *in vacuo*, diluted with ether (50 cm^3) and washed successively with ammonium chloride solution (20 cm^3), saturated sodium bicarbonate solution (20 cm^3), and water (2 x 20 cm^3). The organic extract was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue was recrystallised from petrol to give the *3α-benzoate* (73) (500mg, 40%) as a white solid, m.p. 192-193°C (R_f = 0.62, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 3590 br (OH), 1710 s (C=O), 1635 s (C-C, Ar), 1251 (C-O), 907 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 8.00-7.45 (5H, m, Ar), 5.47-5.43 (1H, m, H_β-C(3)), 3.98-3.82 (4H, m, OCH_2CH_2O), 3.20 (1H, brs, HO-C(5)), 1.28 (3H, s, H_3C(21)), 1.06 (3H, s, H_3C(19)), 0.67 (3H, s, H_3C(18)); *m/z* (C.I.) 483 ([M^++1], 3%), 465 (2), 87 (100).

20-Ethylenedioxypregna-3α,5α-diol (74)

3α-Benzoyl-20-ethylenedioxypregnan-5α-ol (73) (70mg, 0.15mM) was added to a stirred solution of sodium hydroxide (24mg, 0.58mM) in 1:1 mixture of methanol and methylene chloride (10 cm^3). The reaction mixture was stirred vigorously at room temperature for 3h, after which it was diluted with methylene chloride (10 cm^3) and washed with saturated sodium bicarbonate solution (2 x 5 cm^3) and water (2 x 5 cm^3). The organic extract was dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:2) to give the *3α,5α-diol* (74) (41mg, 76%) as a white solid, m.p. 182-184°C (R_f = 0.24, ethyl acetate); υ_{max} ($CHCl_3$ sol.)/cm^{-1} 3390 br (OH), 1254 S (C-O); δ_H (270 MHz, $CDCl_3$) 4.14-4.06 (1H, m, H_β-C(3)), 3.98-3.83 (4H, m, OCH_2CH_2O), 1.29 (3H, s, H_3C(21)), 1.02 (3H, s, H_3C(19)), 0.74 (3H, s, H_3C(18));

m/z (C.I.) 379 ([M^++1], 5%), 87 (100).

3α,5α-Dihydroxypregnan-20-one (75)

20-Ethylenedioxypregnan-3α,5α-diol (74) (300mg, 0.8mM) and aq. sulphuric acid solution (2 cm^3 of 1N) were refluxed in methanol (30 cm^3) for 30 min. After cooling the reaction mixture was diluted with water (20 cm^3) and extracted with methylene chloride (30 cm^3). The organic layer was washed with sodium bicarbonate solution (20 cm^3) and water (2 x 10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was recrystallised from ethyl acetate-petrol to give the *diol* (75) (130mg, 50%) as white needles, m.p. 182-183°C (R_f = 0.39, ethyl acetate-petrol (1:1)); $[\alpha]^{22}_D$ = + 105.6° (C=0.973 in $CHCl_3$); υ_{max} (NUJOL)/cm^{-1} 3319 sh (OH), 3207 sh (OH), 1697 s (C=O); δ_H (200 MHz, $CDCl_3$) 4.14-4.06 (1H, m, H_β-C(3)), 2.90-2.76 (1H, brs, O-H), 2.55 (1H, t, $J_{17,16}$ 8.4 Hz, H-C(17)), 2.12 (3H, s, H_3C(21)), 0.92 (3H, s, H_3C(19)), 0.60 (3H, s, H_3C(18)); δ_C (400 MHz, $CDCl_3$) 209.76 (C-20), 74.82 (C-5), 67.78 (C-3); *m/z* (C.I.) 335 ([M^++1], 42%), 317 (100), 299 (88), 281 (11), 262 (18); Found : C, 75.3; H, 10.1. $C_{21}H_{34}O_3$ requires C, 75.4; H, 10.2%.

Attempted hydrogenation of (E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one (39)

Method 1

A solution of *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (39) (250mg, 0.60mM) and 10% palladium on activated carbon (25mg, 10% w/w) in ethanol (20 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was stirred under an atmosphere of hydrogen at atmospheric pressure for 1 week. After filtration, the solution was worked up as normal. No saturated product was isolated, just unreacted starting material (245mg, 98%).

Method 2

30% Hydrogen peroxide (0.045 cm^3, 0.45mM) was added to a stirred solution of *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (**39**) (60mg, 0.14mM), hydrazine (0.023 cm^3, 0.74mM) and a trace of copper (II) sulphate in ethanol (5 cm^3). The reaction mixture was stirred at 0°C for 2h and a further 24h at room temperature, under nitrogen. Water (10 cm^3) was added to quench and the mixture extracted with methylene chloride (2 x 15 cm^3). After normal work up, no saturated product was isolated, just unreacted starting material (47mg, 78%).

Method 3

A stirred solution of *(E)-3β-20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (**39**) (100mg, 0.24mM), palladium (II) hydroxide (25mg, 25% w/w) and freshly distilled cyclohexene (1 cm^3) in ethanol (5 cm^3) was refluxed for 24h. After cooling the mixture was filtered, and the filtrate concentrated *in vacuo*. The residue was dissolved in methylene chloride (10 cm^3), and the solution washed with water (2 x 5 cm^3), dried over magnesium sulphate and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:4) to give no saturated product, just unreacted starting material (97mg, 97%).

Preparation of pregnanolone (**77**)

10% Palladium on activated carbon (250mgs, 25% w/w) was added under nitrogen to *pregnenolone* (1g, 3.16mM) in ethanol (50 cm^3). After degassing the mixture for 15 min it was stirred under an atmosphere of hydrogen at atmophseric pressure for 2.5h. The catalyst was removed via filtration through celite, and the filtrate concentrated *in vacuo*. The resulting colourless oil was diluted using ethyl acetate (50 cm^3), washed with water (2 x 10 cm^3), and the organic extract dried over anhydrous sodium sulphate. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel, eluting with ethyl acetate-petrol (1:4) to give

pregnanolone (77) (894mg, 89%) as a white solid, m.p. 204-205°C (R_f = 0.51, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3389 br (OH), 1678 (C=O); δ_H (270 MHz, $CDCl_3$) 3.66-3.54 (1H, m, H_α-C(3)), 2.52 (1H, t, *J* 9.0 Hz, H-C(17)), 2.11 (3H, s, H_3C(21)), 0.71 (3H, s, H_3C(19)), 0.60 (3H, s, H_3C(18)); *m/z* (E.I.) 318 (M^+, 34%), 300 (22), 285 (10), 215 (28), 107 (31), 43 (100); Found : C, 79.1; H, 11.0. $C_{21}H_{34}O_2$ requires C, 79.2; H, 10.7%.

20β-Acetoxy-5(10→1βH)abeopregnane (78)

A solution of *3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (39) (1.0g, 2.39mM) and 10% palladium on carbon (100mg, 10% w/w) in methylene chloride (100 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was then stirred under an atmosphere of hydrogen at atmospheric pressure for 72h. The mixture was filtered and the filtrate washed with water (2 x 30 cm^3), dried over magnesium sulphate, and purified by column chromatography, eluting with ethyl acetate-petrol (1:49) to give *20β-acetoxy-5(10→1βH)abeopregnane* (78) (529mg, 64%) as a white solid, m.p. 73-74°C (R_f = 0.82, ethyl acetate-petrol (1:9)); υ_{max} (NUJOL)/cm^{-1} 1734 s (C=O), 1244 (C-O); δ_H (270 MHz, $CDCl_3$) 4.88-4.78 (1H, m, H-C(20)), 2.02 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 0.94 (3H, d, $J_{19,10}$ 7.5 Hz, H_3C(19)), 0.65 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 170.51 (C=O), 72.96 (C-20); *m/z* (E.I.) 346 (M^+, 6%), 286 (70), 271 (13), 257 (15), 232 (15), 218 (35), 43 (100); Found : C, 79.4; H, 11.1. $C_{23}H_{38}O_2$ requires C, 79.7; H, 11.0%.

5(10→1βH)Abeopregnan-20β-ol (79)

A solution of *20β-acetoxy-5(10→1βH)abeopregnane* (78) (300mg, 0.87mM) and potassium carbonate (300mg, 2.17mM) in methanol (15 cm^3) was stirred at room temperature for 96h. Water (5 cm^3) was added, and the mixture extracted with methylene chloride (2 x 30 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:19) to give *5(10→1βH)abeopregnan-20β-ol* (79) (253mg,

96%) as a white solid, m.p. 73-74°C (R_f = 0.52, ethyl acetate-petrol (1:9)); υ_{max} (NUJOL)/cm^{-1} 3385 br (OH), 1246 (C-O); δ_H (270 MHz, $CDCl_3$) 3.73 (1H, dq, $J_{20,21}$ 6.0, $J_{20,17}$ 9.7 Hz, H-C(20)), 1.13 (3H, d, $J_{21,20}$ 6.2 Hz, $H_3C(21)$), 0.94 (3H, d, $J_{19,10}$ 7.3 Hz, $H_3C(19)$), 0.77 (3H, s, $H_3C(18)$); *m/z* (C.I.) 305 ([M^++1], 1%), 287 (100), 271 (5), 259 (7), 218 (61), 151 (30), 123 (23), 95 (20); Found : C, 82.1; H, 12.2. $C_{21}H_{36}O.^1/_6H_2O$ requires C, 82.1; H, 11.8%.

Attempted preparation of 3β,20β-dihydroxy-5,10-secopregn-1(10)-en-5-one (**47**)

3β,20β-Diacetoxy-5,10-secopregn-1(10)-en-5-one (39) (1.0g, 2.39mM) and potassium hydroxide (147mg, 2.63mM) were refluxed together in diethylene glycol (20 cm^3) for 2h. After cooling, water (20 cm^3) was added and the reaction mixture extracted with methylene chloride (3 x 25 cm^3). The combined organic extracts were washed with dil. HCl (20 cm^3) and water (2 x 20 cm^3), dried over anhydrous sodium sulphate, and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:1) to give *5(10→1βH)abeopregn-10(19)-ene-3β,5α,20β-triol* (**80**) (199mg, 25%) as a colourless oil, (R_f = 0.20, ethyl acetate-petrol (1:1)); υ_{max} (FILM)/cm^{-1} 3410 br (OH), 1628 (C=C); δ_H (270 MHz, $CDCl_3$) 5.14 (1H, s, H-C(19)), 4.99 (1H, s, H-C(19)), 4.52-4.42 (1H, m, H_α-C(3)), 3.79-3.68 (1H, m, H-C(20)), 3.22-3.12 (1H, m, H-C(1)), 1.13 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.80 (3H, s, $H_3C(18)$); *m/z* (E.I.) 334 (M^+, 75%), 316 (100), 298 (29), 272 (18), 248 (34), 233 (14), 165 (21), 155 (13), 147 (10).

3β,20β-Acetoxy-5-(propyl dithiane)-5,10-secopregn-1(10)-ene (**81**)

1,3-Propane dithiol (0.48 cm^3, 4.79mM) was added to a stirred solution of *3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (39) (2.0g, 4.79mM) and p-TSA (1.0g, 5.8mM), recrystallised from water, in acetic acid (20 cm^3). The reaction mixture was stirred at room temperature for 12h. Water (10 cm^3) was added and the solution extracted with methylene chloride (3 x 25 cm^3), dried over magnesium sulphate and evaporated under reduced pressure. Purification by column

chromatography, eluting with ethyl acetate-petrol (1:9) gave the *dithiane* (**81**) (192mg, 8%) as a yellow/brown solid, m.p. 92-93°C (R_f = 0.58, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 1730 s (C=O), 1641 (C=C); δ_H (270 MHz, $CDCl_3$) 5.69-5.50 (1H, m, H_α-C(3)), 4.83-4.64 (2H, m, H_E-C(1) + H-C(20)), 2.62-2.15 (6H, m, $SCH_2CH_2CH_2S$), 1.99 (3H, s, H_3CCO), 1.96 (3H, s, H_3CCO), 1.76 (3H, s, H_3C(19)), 1.08 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 0.57 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 171.21 (C=O), 170.70 (C=O), 141.52 (C-10), 123.97 (C-1), 72.11 (C-20), 72.06 (C-3); *m/z* ((+)FAB in NBA) 508 (M^+, 13%), 449 (19), 401 (16), 341 (91), 281 (100), 253 (6); ((-)FAB in NBA) 507 ([M^+-1], 100%), 470 (9), 433 (36), 391 (17), 373 (15), 322 (24), 302 (21), 273 (17); Found : C, 61.3; H, 8.25. $C_{28}H_{44}S_2O_4.2^1/_4H_2O$ requires C, 61.3; H, 8.84%.

3β,19-Dihydroxyandrost-5(6)-en-17-one (**83**)

Potassium carbonate (5.0g, 36.2mM) was added to a stirred solution of *3β-acetoxyandrost-5(6)-en-19-ol-17-one* (**82**) (5.0g, 14.5mM) in methanol (100 cm^3). The reaction mixture was stirred at room temperature for 3h. Water (50 cm^3) was added and the solution was extracted with methylene chloride (100 cm^3). The organic layer was washed with dil. HCl (20 cm^3) and water (2 x 25 cm^3), dried over anhydrous sodium sulphate and concentrated at reduced pressure. The residue was purified by column chromatography eluting, with ethyl acetate-petrol (3:2) to give the *diol* (**83**) (3.8g, 87%) as a white solid, which on recrystallisation from ethyl acetate gave needles, m.p. 229-231°C (R_f = 0.25, ethyl acetate-petrol (3:2)); υ_{max} (NUJOL)/cm^{-1} 3342 br (OH), 1732 s (C=O), 1666 (C=C); δ_H (270 MHz, DMSO-d_6) 5.48 (1H, brs, H-C(6)), 4.61 (1H, d, *J* 4.6 Hz, H-OC(3)), 4.37 (1H, t, *J* 4.7 Hz, H-OC(19)), 3.68 (1H, dd, *J* 4.5, J_{gem} 11.3 Hz, H-C(19)), 3.40 (1H, dd, *J* 5.3, J_{gem} 11.3 Hz, H-C(19)), 3.34-3.22 (1H, m, H_α-C(3)), 0.83 (3H, s, H_3C(18)); δ_C (DMSO-d_6) 219.91 (C-17), 138.20 (C-5), 122.99 (C-6), 70.09 (C-3), 61.80 (C-19); *m/z* (C.I.) 305 ([M^++1], 23%), 287 (100), 269 (64), 256 (94), 223 (10), 145 (12), 69 (15); Found : C, 74.9; H, 9.43. $C_{19}H_{28}O_3$ requires C, 75.0; H, 9.21%.

3β,19-Dihydroxy-17-(1,3-propane dithiane)-androst-5(6)-ene (84)

To a stirred solution of *3β,19-dihydroxyandrost-5(6)-en-17-one* (83) (1.0g, 3.29mM) and 1,3-propanedithiol (0.36 cm^3, 3.62mM) in methanol (5 cm^3) was added boron trifluoride etherate (1.0 cm^3, 8.13mM). The reaction mixture was stirred at room temperature for 30 min and then the resulting white precipitate was filtered off and washed with water (15 cm^3) and methanol (10 cm^3). The white solid was dried under vacuum and then recrystallised from ethyl acetate to give the *propanedithiane* (84) (912mg, 70%) as a white solid, m.p. 189-191°C (R_f = 0.28, ethyl acetate-petrol 1:1)); υ_{max} (NUJOL)/cm^{-1} 3355 br (OH), 1666 (C=C), 1365 (C-5); δ_H (270 MHz, $CDCl_3$) 5.78-5.72 (1H, m, H-C(6)), 3.87 (1H, d, J_{gem} 11.7 Hz, H-C(19)), 3.63 (1H, d, J_{gem} 11.7 Hz, H-C(19)), 3.03-2.34 (6H, m, $SCH_2CH_2CH_2S$), 1.06 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 135.59 (C-5), 126.89 (C-6), 71.23 (C-3), 63.20 (C-17), 62.75 (C-19), 42.17 (S-CH_2), 39.62 (S-CH_2); *m/z* (C.I.) 395 ([M^++1], 60%), 377 (35), 365 (20), 347 (19), 289 (22), 271 (100), 241 (39), 149 (33), 107 (40); Found : C, 64.1; H, 8.92. $C_{22}H_{34}S_2O_2.H_2O$ requires C, 64.1; H, 8.74%.

3β,19-Diacetoxy-17-ethanedithianeandrost-5(6)-ene (85)

To a stirred solution of *3β-acetoxyandrost-5(6)-en-19-ol-17-one* (82) (890mg, 2.58mM) and ethane dithiol (0.23 cm^3, 2.84mM) in acetic acid (15 cm^3) was added boron trifluoride etherate (1.0 cm^3, 8.13mM). The reaction mixture was stirred at room temperature for 2h. Diethyl ether (25 cm^3) was added, together with water (10 cm^3). The organic layer was separated and washed with water (2 x 10 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate-petrol (1:19) to give the *dithiane* (85) (492mg, 41%) as a white solid, m.p. 93-94°C (R_f = 0.52, ethyl acetate-petrol (1:19)); υ_{max} (NUJOL)/cm^{-1} 1729 s (C=O), 1722 s (C=O), 1666 (C=C), 1244 (C-O); δ_H (270 MHz, $CDCl_3$) 5.64-5.60 (1H, m, H-C(6)), 4.68-4.56 (1H, m, H_α-C(3)), 4.50 (1H, d, J_{gem} 11.8 Hz, H-C(19)), 3.96 (1H, d, J_{gem} 11.8 Hz, H-C(19)),

3.33-3.07 (4H, s, SCH_2CH_2S), 2.04 (3H, s, H_3CCO), 2.02 (3H, s, H_3CCO), 0.95 (3H, s, $H_3C(18)$); *m/z* (C.I.) 465 ([M^++1], 25%), 436 (4), 421 (1), 405 (100), 371 (5), 345 (31), 251 (16), 237 (10); Found : C, 63.7; H, 7.87. $C_{25}H_{36}S_2O_4.{}^1/_3H_2O$ requires C, 63.8; H, 7.80%.

3β,20β-Diacetoxy-5,10-secopregn-1(10)-ene (86)

Method 1

Sodium iodide (3.50g, 23.2mM) and sodium acetate (400mg, 4.82mM) were stirred in acetic acid (20 cm^3). After cooling in an ice-bath zinc dust (5.0g, 76.9mM) was added to the reaction mixture followed by the dropwise addition of *1β,10α-epoxy-3β,20β-diacetoxy-5,10-secopregnane* (95) (460mg, 1.10mM) in acetic acid (15 cm^3). The reaction mixture was allowed to warm to room temperature and stirred for 5 days. The reaction mixture was diluted with water (50 cm^3) and extracted with methylene chloride (3 x 50 cm^3). The combined organic extracts were washed with water (2 x 30 cm^3), dried over magnesium sulphate and concentrated under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:39) to give *3β,20β-diacetoxy-5,10-secopregn-1(10)-ene* **(86)** (182mg, 73% corrected recovered for starting material) as a colourless oil, (R_f = 0.65, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 1733 s (C=O), 1654 (C=C), 1241 (C-O); δ_H (270 MHz, $CDCl_3$) 5.42-5.35 (1H, m, H_Z-C(1)), 5.09-4.98 (1H, m, H_α-C(3)), 4.84 (1H, dq, $J_{20,21}$ 6.1 $J_{20,17}$ 10.3 Hz, H-C(20)), 4.81 (1H, m, H_E-C(1)), 2.03 (3H, s, H_3CCO), 2.02 (3H, s, H_3CCO), 1.65 (3H, s, $H_3C(19)$), 1.15 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.64 (3H, s, $H_3C(18)$). *m/z* (C.I.) 405 ([M^++1], 1%), 363 (38), 345 (17), 303 (100), 285 (95), 273 (6).

The next elutes gave *1β,10α-epoxy-3β,20β-diacetoxy-5,10-secopregnane* **(95)** (204mg, 43%). Further elutes gave *3β,20β-diacetoxy-5,10-secopregn-10(19)-e-n-1α-ol* **(98)** (58mg, 13%) as a colourless oil, (R_f = 0.09, ethyl acetate-petrol (1:9));

υ_{max} (FILM)/cm^{-1} 3386 br (OH), 1728 s (C=O), 1646 (C=C), 1243 (C-O); δ_H (270 MHz, $CDCl_3$) 5.40 (1H, s, H-C(19)), 5.06 (1H, s, H-C(19)), 4.89-4.76 (2H, m, H_α-C(3) + H-C(20)), 4.13 (1H, dd, $J_{1,2'}$ 7.1 $J_{1,2''}$ 14.3 Hz, H-C(1)), 2.08 (3H, s, H_3CCO), 2.04 (3H, s, H_3CCO), 1.16 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.70 (3H, s, $H_3C(18)$); *m/z* (C.I.) 421 [M^++1], 1%), 403 (5), 361 (30), 343 (22), 301 (90), 283 (100), 271 (7).

Method 2 **(Scheme 51)**

A suspension of *(E)-3β,20β-diacetoxy-5(propyl dithiane)-5,10-secopregn-1(10)-ene* **(81)** (73mg, 0.14mM) and W-2 raney nickel (1.74g, 29.5mM) in ethanol (20 cm^3) was refluxed for 1h. After cooling,the reaction mixture was filtered over celite and the filtrate concentrated under reduced pressure. The residue was dissolved in methylene chloride (20 cm^3) and washed with water (2 x 10 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:49) gave *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-ene* **(86)** (43mg, 71%) as a colourless oil (R_f = 0.65, ethyl acetate-petrol (1:9)).

(E)-3β,20β-Diacetoxy-5,10-secopregn-1(10)-en-5α-ol (87)

3β,20β-Diacetoxy-5,10-secopregn-1(10)-en-5-one (39) (1.0g, 2.39mM) in ethanol (10 cm^3) was added dropwise to a stirred solution of sodium borohydride (109mg, 2.87mM) in ethanol (15 cm^3) at 0°C. The reaction mixture was stirred at 0°C for 2h and then allowed to warm to room temperature and stirred for a further 7h. Water (10 cm^3) was added to quench and the reaction mixture extracted with methylene chloride (3 x 25 cm^3). The combined organic extracts were dried over anhydrous sodium sulphate, evaporated under reduced pressure, and purified by column chromatography, eluting with ethyl acetate-petrol (1:3) to give the *5α-hydroxyl* **(87)** (770mg, 77%) as a white solid, m.p. 123-124°C (R_f = 0.32, ethyl acetate-toluene (1:2)); $[\alpha]^{22}_D$ = -3.8° (C=1.30, CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 3510 br (OH), 1724 s (C=O), 1664 (C=C); δ_H (400 MHz, $CDCl_3$) 5.38-5.30 (1H, m, H_α-C(3)), 5.16-5.07

(1H, m, H_E-C(1)), 4.85 (1H, dq, $J_{20,21}$ 6.3, $J_{20,17}$ 10.5 Hz, H_α-C(20)), 4.12-4.05 (1H, m, H_β-C(5)), 2.07 (3H, s, H_3CCO), 2.02 (3H, s, H_3CCO), 1.76 (3H, s, H_3C(19)), 1.15 (3H, s, $J_{21,20}$ 5.9 Hz, H_3C(21)), 0.66 (3H, s, H_3C(18)); δ_C (270 MHz, $CDCl_3$) 170.53 (C=O), 170.40 (C=O), 123.78 (C-10), 117.48 (C-1), 72.70 (C-3), 71.03 (C-20), 67.08(C-5); *m/z* (C.I.) 421 ([M^++1], 1%), 403 (1), 361 (5), 343 (9), 301 (22), 283 (25); Found C, 70.3; H, 9.53. $C_{25}H_{40}O_5.{}^1/_3H_2O$ requires C, 70.4; H, 9.55%.

Attempted elimination of C-5 hydroxyl of (E)-3β,20β-diacetoxy-5,10-secopreg-n-1(10)-en-5α-ol (**87**)

Method 1

A solution of *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5α-ol* (**87**) (100mg, 0.24mM) and tosyl chloride (55mg, 0.29mM) in pyridine (5 cm^3) was stirred at 0°C for 2h and a further 48h at room temperature under nitrogen. Water (10 cm^3) was added and the resulting white solid filtered and washed with dil. HCl (5 cm^3), followed by water (2 x 20 cm^3) and then dissolved in methylene chloride (15 cm^3). The organic solution was washed with water (2 x 5 cm^3), dried over magnesium sulphate, and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:19) gave *3β,20β-diacetoxy-5(10→1βH)abeopregn-10(19)-ene* (**88**) (95mg, 92%) as a colourless oil, (R_f = 0.75, ethyl acetate-petrol (3:7)); υ_{max} (FILM)/cm^{-1} 1732 s (C=O), 1727 s (C=O), 1632 (C=C), 1243 (C-O); δ_H (270 MHz, $CDCl_3$) 5.19-5.10 (1H, m, H_α-C(3)), 4.92-4.75 (1H, m, H-C(20)), 4.82 (1H, s, H-C(19)), 4.65 (1H, s, H-C(19)), 2.02 (3H, s, H_3CCO), 1.98 (3H, s, H_3CCO), 1.08 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 0.64 (3H, s, H_3C(18)); *m/z* (C.I.) 403 ([M^++1], 1%), 342 (22), 325 (2), 299 (1), 283 (100), 267 (3), 253 (2); Found : C, 74.4; H, 9.64. $C_{25}H_{38}O_4$ requires C, 74.6; H, 9.45%.

Method 2

In the same way, *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5α-ol* (**87**) (50mg,

0.12mM) and mesyl chloride (0.01 cm^3, 0.14mM) in a 1:1 mixture of DMF : pyridine (2 cm^3) gave *3β,20β-diacetoxy-5(10→1βH)abeopregn-10(19)-ene* (88) (38mg, 79%), purified by column chromatography, eluting with ethyl acetate-petrol (1:19), as a colourless oil, (R_f = 0.75, ethyl acetate-petrol (3:7)).

Method 3

A solution of *(E)-3β,20β-diacetoxy-5,10-secopregnan-5α-ol* (87) (20mg, 0.05mM) sodium iodide (29mg, 0.19mM), and trimethylsilyl chloride (0.012 cm^3, 0.09mM) in acetonitrile (5 cm^3) and stirred at 0-10°C under nitrogen for 1h. After warming to room temperature, water (5 cm^3) was added and the mixture extracted with methylene chloride (2 x 10 cm^3). The combined organic extracts were dried over magnesium sulphate, evaporated under reduced pressure, and purified by column chromatography, eluting with ethyl acetate-petrol (1:19) to give *3β,20β-diacetoxy-5(10→1βH)abeopregn-10(19)-ene* (88) (10mg, 52%) as a colourless oil, (R_f = 0.75, ethyl acetate-petrol (3:7)).

Attempted deoxygenation of (E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5α-ol (87)

Method 1

Pentafluorophenyl chlorothionoformate (0.046 cm^3, 0.29mM) was added to a stirred solution of *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5α-ol* (87) (100mg, 0.24mM), 3N-hydroxysuccinimide (6mg, 0.05mM) and pyridine (0.02 cm^3, 0.25mM) in toluene (10 cm^3). The reaction mixture was refluxed for 1h, and after cooling diluted with water (10 cm^3). The mixture was extracted with methylene chloride (2 x 10 cm^3). The combined organic extracts were dried over magnesium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:19) to give *3β,20β-diacetoxy-5(10→1βH)abeopregn-10(19)-ene* (88) (81mg, 85%) as a colourless oil, (R_f = 0.75, ethyl acetate-petrol (3:7)).

Method 2

In a similar way, the above reaction mixture was stirred at -78 - -40°C for 4h. Water (5 cm^3) was added and the mixture allowed to warm to room temperature. The solution was extracted with methylene chloride (2 x 10 cm^3), and the combined organic extracts dried over magnesium sulphate, and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:19) gave *3β,20β-diacetoxy-5(10→1βH)abeopregn-10(19)-ene* (88) (76mg, 79%) as a colourless oil, (R_f = 0.75, ethyl acetate-petrol (3:7)).

1β,10α-Epoxy-3β,20β-diacetoxy-5,10-secopregnan-5-one (90)

60% m-Chloroperoxybenzoic acid (690mg, 2.39mM) was added to a stirred solution of *(E)-3β,20β-diacetoxy-5,10-secopregn-1(10)-en-5-one* (39) (1.0g, 2.39mM) in methylene chloride (40 cm^3). The reaction mixture was stirred at room temperature for 2h. Then 25% sodium sulphite solution and 25% sodium bicarbonate solution were added to quench, until the starch-iodide test gave a negative result. The layers were separated and the organic layer washed with aq. sodium bicarbonate (2 x 20 cm^3) and water (2 x 20 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate-petrol (3:17) to give the *1β,10α-epoxide* (90) (966mg, 93%) as a white solid, m.p. 172-173°C (R_f = 0.42, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 1732 s (C=O), 1708 s (C=O), 1241 (C-O); δ_H (270 MHz, $CDCl_3$) 5.41-5.33 (1H, m, H_α-C(3)), 4.86-4.80 (1H, m, H-C(20)), 2.71 (2H, d, $J_{4,3}$ 8.1 Hz, H-C(4)), 2.50 (1H, d, $J_{1,2}$ 9.2 Hz H_α-C(1)), 2.04 (3H, s, H_3CCO), 2.01 (3H, s, H_3CCO), 1.35 (3H, s, H_3C(19)) 1.14 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 0.67 (3H, s, H_3C(18)); *m/z* (C.I.) 435 ([M^++1], 6%), 417 (4) 391 (10), 375 (100), 357 (16), 331 (3), 315 (71), 297 (42), 279 (10); Found : C, 68.0; H, 9.04. $C_{25}H_{38}O_6.{}^1/_3H_2O$ requires C, 68.2; H, 8.79%.

3α-Methoxy-20β-acetoxy-1(10→1βH), 4(5→4βH)-abeopregnan-10α-ol-5-one (**92**) Potassium carbonate (4.0g, 29.0mM) was added to a stirred solution of *1β,10α-epoxy-3β,20β-diacetoxy-5,10-secopregnan-5-one* (90) (4.0g, 9.22mM) in methanol (200 cm^3). The reaction mixture was stirred at room temperature for 25 min, after which water (50 cm^3) was added. The mixture was extracted with methylene chloride (2 x 100cm^3), and the combined organic extracts were washed with dil. HCl (40 cm^3) and water (2 x 40 cm^3). The organic layer was dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (2:3) to give *1β,10α-epoxy-20β-acetoxy-5,10-secopregn-3(4)-en-5-one* (**91**) (732mg, 21%) as a white solid, m.p. 213-214°C (R_f = 0.46, ethyl acetate-petrol (1:1)); υ_{max} (NUJOL)/cm^{-1} 1732 s (C=O), 1655 s (C=O), 1632 (C=C), 1245 (C-O); δ_H (270 MHz, $CDCl_3$) 7.04 (1H, qd, $J_{3,2'}$ 2.2, $J_{3,2''}$ 10.0 Hz, H-C(3)), 6.00 (1H, dd, *J* 2.5, $J_{4,3}$ 10.0 Hz, H-C(4)), 4.84 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.3 Hz, H-C(20)), 2.66 (1H, dt, *J* 5.2, *J* 18.5 Hz, H-C(1)), 2.02 (3H, s, H_3CCO), 1.15 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 1.14 (3H, s, $H_3C(19)$), 0.65 (3H, s, $H_3C(18)$); *m/z* (C.I.) 375 ([M^++1], 100%), 357 (11), 315 (72), 297 (42), 279 (3), 247 (3), 205 (4); Found : C, 73.5; H, 9.39. $C_{23}H_{34}O_4$ requires C, 73.8; H, 9.09%.

Further elutes gave *3α-methoxy-20β-acetoxy-1(10→1βH),4(5→4βH)-abeopregnan-10α- ol-5-one* (**92**) (2.84g, 76%) as a white solid, m.p. 179-180°C (R_f = 0.33, ethyl acetate-petrol (1:1)); $[\alpha]^{22}_D$ = + 4.7° (C = 1.07, CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 3522 br (OH), 1733 s (C=O), 1698 s (C=O), 1247 (C-O); δ_H (270 MHz, $CDCl_3$) 4.83 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.3 Hz, H-C(20)), 4.01-3.95 (1H, m, H_β-C(3)), 3.29 (3H, s, H_3CO), 2.02 (3H, s, H_3CCO), 1.15 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 1.12 (3H, s, $H_3C(19)$), 0.63 (3H, s, $H_3C(18)$); *m/z* (C.I.) 407 ([M^+ + 1], 5%), 389 (11), 375 (50), 347 (71), 329 (94), 315 (100), 297 (32), 279 (6); Found : C, 70.5; H, 9.62. $C_{24}H_{38}O_5$. $^1/_8H_2O$ requires C, 70.5; H, 9.37%.

1β,10α-Epoxy-3β,20β-diacetoxy-5,10-secopregnan-5α-ol (93)

Sodium borohydride (20mg, 0.53mM) was added to a stirred solution of *1β,10α-epoxy-3β,20β-diacetoxy-5,10-secopregnan-5-one* (90) (200mg, 0.46mM) in ethanol (10 cm^3) at 0°C under nitrogen. The reaction mixture was stirred at 0-10°C for 1h and room temperature for further 45 min. Water (5 cm^3) was added to quench and the mixture extracted with methylene chloride (2 x 15 cm^3). The combined organic extracts were dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (3:7) to give the *5α-hydroxy epoxide* (**93**) (104mg, 52%) as a white solid, m.p. 195-196°C (R_f = 0.17, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3455 br (OH), 1727 s (C=O), 1246 (C-O); δ_H (270 MHz, $CDCl_3$) 5.11-5.00 (1H, m, H_α-C(3), 4.91-4.78 (1H, m, H-C(20)), 3.88-3.78 (1H, m, H_β-C(5)), 3.48-3.40 (1H, m, H-OC(5)), 2.80 (1H, d, $J_{1,2}$ 10.1 Hz, H-C(1)), 2.22 (1H, dd, *J* 3.8, *J* 13.5 Hz, H_α-C(4)), 2.08 (3H, s, H_3CCO), 2.02 (3H, s, H_3CCO), 1.25 (3H, s, H_3C(19)), 1.14 (3H, d, $J_{21,20}$ 6.2 Hz, H_3C(21)), 0.67 (3H, s, H_3C(18)); *m/z* (C.I.) 437 ([M^++1], 10%), 419 (10), 391 (14), 377 (47), 359 (71), 317 (53), 299 (100), 281 (43), 271 (10); Found : C, 67.9; H, 9.25. $C_{25}H_{40}O_6.{}^1/_3H_2O$ requires, C, 67.9; H, 9.20%.

1β,10α-Epoxy-3β,20β-diacetoxy-5α-tosyl-5,10-secopregnane (94)

To a stirred solution of tosyl chloride (29mg, 0.15mM) and pyridine (0.01 cm^3, 0.14mM) in methylene chloride (2 cm^3), at 0°C, was added the *5α-hydroxy epoxide* (**93**) (60mg, 0.14mM) in methylene chloride (1 cm^3). The reaction mixture was stirred at room temperature for 72h. Dil. HCl (1 cm^3) was added dropwise and the mixture extracted with methylene chloride (2 x 5 cm^3). The combined organic extracts were washed with water (2 x 5 cm^3), dried over magnesium sulphate, and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:4) to give the *5α-tosylate* (94) (70mg, 86%) as a white solid, m.p. 125-126°C (R_f = 0.37, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 1729 s (C=O), 1244 (C-O), 1180 (S=O), 1176 (S=O), 907 (C-H, Ar); δ_H (270 MHz,

$CDCl_3$) 7.79 (2H, d, *J* 8.2 Hz, Ar-H_{ortho}), 7.35 (2H, d, *J* 8.3 Hz, Ar-H_{meta}), 5.25-5.14 (1H, m, H_α-C(3)), 4.90-4.74 (2H, m, H_β-C(5)) + H-C(20)), 2.72 (1H, d, $J_{1,2}$ 9.0 Hz, H-C(1)), 2.45 (3H, s, $\underline{H_3}$CPh), 2.02 (3H, s, H_3CCO), 1.97 (3H, s, H_3CCO), 1.20 (3H, s, H_3C(19)), 1.14 (3H,d, $J_{21,20}$ 6.1 Hz, H_3C(21)), 0.65 (3H, s, H_3C(18)). *m/z* ((+)FAB in NBA), 591 ([M^++1], 1%), 580 (44), 568 (39), 549 (49), 531 (80), 516 (47), 505 (56), 493 (55), 359 ([M^++1-ArOH-TsOH], 13%), 341 (4), 331 (4), 317 (11), 299 (39), 281 (22), 173 (100); Found : C, 64.5; H, 7.89. $C_{32}H_{46}SO_8.{}^1/_4H_2O$ requires C, 64.6; H, 7.82%.

1β,10α-Epoxy-3β,20β-diacetoxy-5,10-secopregnane (95)

Method 1

Tributyl tin hydride (0.92 cm^3, 3.17mM) was added dropwise, under nitrogen to a stirred solution of the *5α-thionoformate* (97) (1.40g, 2.11mM) and azobisisobutyronitrile (410mg, 2.50mM) in toluene (50 cm^3). The reaction mixture was stirred at 110°C for 15 min under nitrogen. After cooling water (20 cm^3) was added, the organic layer separated, and the aqueous layer was extracted with diethyl ether (2 x 20 cm^3). The combined organic extracts were washed with water (2 x 20 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography on silcia gel, eluting with ethyl acetate-petrol (1:39) to give *1β,10α-epoxy-3β,20β-diacetoxy-5,10-secopregnane* (95) (844mg, 95%) as a white solid, m.p. 117-119°C (R_f = 0.69, ethyl acetate-petrol (3:17)); υ_{max} (NUJOL)/cm^{-1} 1730 s (C=O), 1244 s (C-O); δ_H (400 MHz, $CDCl_3$) 5.02-4.95 (1H, m, H_α-C(3)), 4.84 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 12.2 Hz, H-C(20)), 2.87 (1H,d, $J_{1,2}$ 9.2 Hz, H-C(1)), 2.04 (3H, s, H_3CCO), 2.02 (3H, s, H_3CCO), 1.26 (3H, s, H_3C(19)), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, H_3C(21)), 0.67 (3H, s, H_3C(18)); *m/z* (C.I.) 421 ([M^++1], 2%), 401 (2), 377 (1), 361 (100), 343 (6), 301 (100), 283 (61), 271 (5), 243 (3), 215 (5), 177 (12).

Method 2

1β,10α-Epoxy-3β,20β-diacetoxy-5,10-secopregnan-5-one (**90**) (250mg, 0.58mM) in DMF (2 cm^3) was added to a stirred solution of p-tosyl hydrazide (130mg, 0.70mM), p-TSA (25mg, 10% w/w) and sodium cyanoborohydride (145mg, 2.30mM) in sulpholane (2 cm^3). The reaction mixture was refluxed for 2.5h under nitrogen. After cooling the reaction mixture was poured into saturated brine (15 cm^3) and the resulting precipitate filtered off. The white solid was washed with water (30 cm^3) and dissolved in methylene chloride (20 cm^3). The organic solution was washed with water (2 x 5 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:39) to give *1β,10α-epoxy-3β,20β-diacetoxy-5,10-secopregnane* (**95**) (34mg, 14%) as a white solid, m.p. 117-119°C (R_f = 0.69, ethyl acetate-petrol (3:17)).

Method 3

A solution of *1β,10α-epoxy-3β,20β-diacetoxy-5-tosylhydrazone-5,10-secopregnane* (**96**) (150mg, 0.25mM), sodium cyanborohydride (63mg, 1.00mM) and p-TSA (15mg, 10% w/w) in a 1:1 mixture of DMF and sulfolane (4 cm^3) was refluxed for 2h. After cooling, water (10 cm^3) was added and the resulting white solid filtered off. The solid was washed with water (20 cm^3) and dissolved in methylene chloride (20 cm^3). The organic solution was washed with water (2 x 10 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:39) to give *1β,10α-epoxy-3β,20β-diacetoxy-5,10-secopregnane* (**95**) (28mg, 27%) as a white solid, m.p. 117-119°C (R_f = 0.69, ethyl acetate-petrol (3:17)).

1β,10α-Epoxy-3β,20β-diacetoxy-5-tosylhydrazone-5,10-secopregnane (**96**)

1α,10α-Epoxy-3β,20β-diacetoxy-5,10-secopregnan-5-one (**90**) (1.0g,. 2.30mM) was added to a stirred solution of p-tosylhydrazide (472mg, 2.54 mM) in methylene chloride (20 cm^3). The reaction mixture was stirred at room temperature under

nitrogen for 5 days. The mixture was diluted with methylene chloride (20 cm^3) and washed with water (2 x 10 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:4) to give the *5-tosyl hydrazone* (96) (472mg, 34%) as a white solid, m.p. 103-104°C (R_f = 0.44, ethyl acetate-petrol (2:3)); υ_{max} (NUJOL)/cm^{-1} 3180 br (NH), 1723 s (C=O), 1631 (C=N), 1597 s (C-C, Ar), 1376 s (S=O), 1244 (C-O), 1167 (S=O), 815 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 9.27 (1H, s, NH), 7.85 (1H, d, *J* 8.3 Hz, Ar-H), 7.77 (1H, d, *J* 8.1 Hz, Ar-H), 7.33-7.26 (2H, s, Ar), 5.52-5.41 (1H, m, H_α-C(3)), 4.85 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 9.7 Hz, H-C(20)), 2.72 (1H, d, $J_{1,2}$ 13.2 Hz, H-C(1)), 2.43 (3H, s, $\underline{H}_3CPh$), 2.03 (3H, s, H_3CCO), 2.01 (3H, s, H_3CCO), 1.25 (3H, s, $H_3C(19)$), 1.16 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.66 (3H, s, $H_3C(18)$); *m/z* (C.I.) 603 ([M^+ + 1] missing), 391 ([M^+-$TsNHNCCH_3$], 8%), 377 (1), 359 (4), 299 (4), 279 (3), 227 (4), 187 (8), 172 (4), 157 (29); Found : C, 62.6; H, 7.62; N, 5.03. $C_{32}H_{46}SN_2O_7.{}^2/_3H_2O$ requires C, 62.5; H, 7.71; N, 4.56%.

1β,10α-Epoxy-3β,20β-diacetoxy-5α-pentafluorophenylthionoformoxy-5,10-secopregnane (97)

Pentaflurorophenyl chlorothionoformate (1.3 cm^3, 8.08mM) was added dropwise to a solution of *1β,10α-epoxy-3β,20β-diacetoxy-5,10-secopregnan-5α-ol* (93) (2.30g, 5.28mM), N-hydroxysuccinimide (152mg, 1.32mM) and pyridine (0.64 cm^3, 7.92mM) in toluene (50 cm^3), and the mixture refluxed at 80°C for 90 min under nitrogen. After cooling, water (20 cm^3) was added and the mixture extracted with methylene chloride (2 x 100 cm^3). The combined organic layers were washed with water (2 x 20 cm^3), dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate-petrol (1:19) to give the *5α-thionoformate* (97) (2.37g, 68%) as a colourless oil, (R_f = 0.44, ethyl acetate-petrol (3:17)); υ_{max} (FILM)/cm^{-1} 1727 s (C=O), 1651 (C=S), 1523 s (C-C, Ar), 1300 (C-F, Ar), 1240 (C-O); δ_H (400 MHz, $CDCl_3$) 5.44-5.30 (2H, m, H_α-C(3) + H_β-C(5)), 4.85 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$

10.4 Hz, H-C(20)), 2.85 (1H, d, $J_{1,2}$ 9.2 Hz, H-C(1)), 2.07 (3H, s, H_3CCO), 2.03 (3H, s, H_3CCO), 1.31 (3H, s, $H_3C(19)$), 1.15 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.69 (3H, s, $H_3C(18)$); δ_F ($CDCl_3$) - 152.74 (2F, d, *J* 17.3 Hz, F_{ortho}), -157.11 (2F, t, *J* 22.0 Hz, F_{meta}), -162.44 (1F, q, *J* 17.9, J 21.4 Hz, F_{para}); *m/z* ((+) FAB in NBA) 622 (M^+, 2%), 603 (6), 543 (4), 525 (2), 419 (33), 359 (100), 299 (61), 281 (37), 121 (61), 93 (78).

3β,20β-Diacetoxy-5,10-secopregnane (**99**)

A solution of *3β,20β-diacetoxy-5,10-secopregn-1(10)-ene* (86) (147mg, 0.36mM) and 10% palladium on activated carbon (15mg, 10% w/w) in ethanol (20 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was stirred under an atmosphere of hydrogen, at atmospheric pressure for 2 weeks. The mixture was filtered, the filtrate concentrated under reduced pressure and the residue dissolved in methylene chloride (15 cm^3). The organic solution was washed with water (2 x 5 cm^3), dried over magnesium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:39) gave *3β,20β-diacetoxy-5,10-secopregnane* (**99**) (51mg, 35%) as a colourless oil, (R_f = 0.59, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 1732 s (C=O), 1244 s (C-O); δ_H (270 MHz, $CDCl_3$) 5.24-5.16 (1H, m, H_α-C(3)), 4.84 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.3 Hz, H-C(20)), 2.03 (3H, s, H_3CCO), 2.02 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.86 (3H, d, $J_{19,10}$ 7.0 Hz, $H_3C(19)$), 0.66 (3H, s, $H_3C(18)$).

20β-Acetoxy-5,10-secopregnan-3β-ol (**100**)

Method 1

Potassium carbonate (20mg, 0.14mM) was added to a stirred solution of *3β,20β-diacetoxy-5,10-secopregnane* (**99**) (13mg, 0.03mM) in methanol (1 cm^3). The reaction mixture was stirred at room tempterature for 24h, after which water (3 cm^3) was added. The mixture was extracted with methylene chloride (2 x 5 cm^3), and the combined organic extracts washed with dil. HCl (2 cm^3) and water (2 x 3 cm^3). The

organic layer was dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:4) to give *20β-acetoxy-5,10-secopregnan-3β-ol* (**100**) (7mg, 60%) as a colourless oil, (R_f = 0.12, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 3378 br (OH), 1731 s (C=O), 1243 (C-O); δ_H (270 MHz, $CDCl_3$) 4.83 (1H, dq, $J_{20,21}$ 6.2, $J_{20,17}$ 10.1 Hz, H-C(20)), 4.14-4.03 (1H, m, H_α-C(3)), 2.02 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.87 (3H, d, $J_{19,10}$ 7.0 Hz, $H_3C(19)$), 0.65 (3H, s, $H_3C(18)$).

Method 2

A solution of *20β-acetoxy-5,10-secopregn-10(19)-en-3β-ol* (**101**) (50g, 0.14mM) and 10% palladium on activated carbon (5mg, 10% w/w) in ethanol (10 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was stirred under an atmosphere of hydrogen at atmospheric pressure for 24h. After filtration, the filtrate was concentrated under reduced pressure and dissolved in methylene chloride (10 cm^3), washed with water (2 x 5 cm^3) and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:4) gave *20β-acetoxy-5,10-secopregnan-3β-ol* (**100**) (36mg, 72%) as a colourless oil, (R_f = 0.12, ethyl acetate-petrol (1:9)).

20β-Acetoxy-5,10-secopregn-10(19)-en-3β-ol (**101**)

Pentafluorophenyl chlorothionoformate (0.11 cm^3, 0.68mM) was added to a stirred solution of N-hydroxysuccinimide (14mg, 0.12mM), pyridine (0.04 cm^3, 0.50mM) and *3β,20β-diacetoxy-5,10-secopregn-10(19)-en-1α-ol* (98) (200mg, 0.48mM) in toluene (5 cm^3). The reaction mixture was refluxed for 2h. After cooling, the mixture was filtered to remove the pyridine hydrochloride. To the filtrate was added azobisisobutyronitrile (60mg, 0.37mM) and tributyltin hydride (0.15 cm^3, 0.56mM), and the mixture was refluxed at 110°C for 30 min, under nitrogen. After cooling water (5 cm^3) was added and the mixutre extracted with methylene chloride (2 x 10 cm^3). The combined organic extracts were dried over magnesium sulphate and

concentrated *in vacuo* to give crude *3β,20β-diacetoxy-5,10-secopregn-10(19)-ene* (182mg). The crude *3β,20β-diacetate* (182mg) was dissolved in ethanol (5 cm^3) and potassium carbonate (200mg, 1.45mM) added. The reaction mixture was stirred at room temperature for 24h, after which it was diluted with water (5 cm^3). The mixture was extracted with methylene chloride (2 x 15 cm^3). The combined organic extracts were washed with dil. HCl (5 cm^3) and water (2 x 5 cm^3), dried over anhydrous sodium sulphate and evaprated at reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give *20β-acetoxy-5,10-secopregn-10(19)-en-3β-ol* (**101**) (67mg, 39%) as a colourless oil, (R_f = 0.23, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 3430 br (OH), 1723 s (C=O), 1628 (C=C), 1242 s (C-O); δ_H (270 MHz, $CDCl_3$) 5.12 (1H, s, H-C(19)), 5.03 (1H, s, H-C(19)), 4.86 (1H, dq, $J_{20,21}$ 6.2, $J_{20,17}$ 10.3 Hz, H-C(20)), 3.88-3.79 (1H, m, H_α-C(3)), 2.02 (3H, s, H_3CCO), 1.15 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.68 (3H, s, $H_3C(18)$); *m/z* (C.I.) 363 ([M^++1], 4%), 345 (2), 303 (53), 285 (100), 245 (4), 217 (6), 206 (18), 121 (16).

20β-Acetoxy-5,10-secopregnan-3α-ol (**103**)

To a stirred solution of *20β-acetoxy-5,10-secopregnan-3β-ol* (**100**) (60mg, 0.16mM), triphenyl phosphine (86mg, 0.33mM) and formic acid (0.01 cm^3, 0.27mM) in THF (6 cm^3) was added diethylazodicarboxylate (0.04 cm^3, 0.25mM). The reaction mixture was stirred at room temperature for 4h, after which half of the solvent was removed *in vacuo*. Water (5 cm^3) was added and the mixture extracted with methylene chloride (2 x 10 cm^3). The combined organic extracts were dried over magnesium sulphate, concentrated under reduced pressure, and purified by column chromatography, eluting with ethyl acetate-petrol (1:39) to give the *3α-formyl ester* (**102**) (40mg, 62%) as a colourless oil, (R_f = 0.62, ethyl acetate-petrol (3:17). Potassium carbonate (40mg, 0.29mM) was added to a stirred solution of *3α-formoxy-20β-acetoxy-5,10-secopregnane* (**102**) (40mg, 0.10mM) in a 1:1 mixture of methanol and methylene chloride (4 cm^3). The reaction mixture was stirred at room temperature for 24h, after which

the solvent was removed under reduced pressure. The residue was dissolved in methylene chloride (10 cm^3), washed with dil. HCl (2 cm^3) and water (2 x 3 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromtography, eluting with ethyl acetate-petrol (1:4) to give *20β-acetoxy-5,10-secopregnan-3α-ol* (**103**) (16mg, 43%) as a colourless oil,(R_f = 0.20, ethyl acetate-petrol (3:17)), υ_{max} (FILM)/cm^{-1} 3366 br (OH), 1728 s (C=O), 1243 s (C-O); δ_H (270 MHz, $CDCl_3$) 4.84 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 10.3 Hz, H-C(20)), 3.72-3.63 (1H, m, H_β-C(3)), 2.02 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.86 (3H, d, $J_{19,10}$ 7.0 Hz, $H_3C(19)$), 0.68 (3H, s, $H_3C(18)$).

Preparation of 5,10-secopregnan-3α-ol-20-one (107)

3α-Tertbutyldimethylsiloxy-20β-acetoxy-5,10-secopregnane (104)

A solution of *20β-acetoxy-5,10-secopregnan-3α-ol* (103) (15mg, 41.μM), tertbutyldimethylsilyl chloride (10mg, 66.2μM) and imidazole (5mg, 74μM) in DMF (1 cm^3) was stirred at room temperature under nitrogen for 24h. Silica was added, the solvent removed *in vacuo*, and purification by column chromatography, eluting with ethyl acetate-petrol (1:49) gave the *3α-silyl ether* (104) (19mg, 96%) as a colourless oil, (R_f = 0.87, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 1733 s (C=O), 1247 s (C-O), 1073 (O-Si).

3α-Tertbutyldimethylsiloxy-5,10-secopregnan-20β-ol (105)

Potassium carbonate (20mg, 145 μM) was added to a stirred solution of *3α-tertbutyldimethylsiloxy-20β-acetoxy-5,10-secopregnane* (104) (19mg, 39.6μM) in methanol (2 cm^3). The reaction mixture was refluxed for 48h. After cooling the mixture was diluted with water (2 cm^3) and extracted with methylene chloride (2 x 5 cm^3). The combined organic extracts were washed with dil. HCl (2 cm^3) and water (2 x 3 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol

(1:19) to give the *20β-hydroxy silyl ether* (**105**) (9mg, 52%) as a colourless oil, (R_f = 0.49, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 3389 br (OH), 1243 (C-O), 1073 (O-Si).

3α-Tertbutyldimethylsiloxy-5,10-secopregnan-20-one (**106**)

A solution of *3α-tertbutyldimethylsiloxy-5,10-secopregnan-20β-ol* (**105**) (8mg, 18.3μM) and pyridinium chlorochromate (6mg, 27.8μM) in methylene chloride (1 cm^3) was stirred at room temperature for 2h. Silica gel was added, the solvent removed under reduced pressure, and purification by column chromatography, eluting with ethyl acetate-petrol (1:39) gave *3α-tertbutyldimethylsiloxy-5,10-secopregnan-20-one* (**106**) (3mg, 38%) as a colourless oil, (R_f = 0.66, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 1701 s (C=O), 1279 (C-O), 1073 (O-Si); δ_H (270 MHz, $CDCl_3$) 4.34-4.25 (1H, m, H_β-C(3)), 2.18 (3H, s, H_3CCO), 0.89 (9H, s, $(H_3C)_3CSi$), 0.87 (3H, d, $J_{19,10}$ 6.8 Hz, $H_3C(19)$), 0.70 (3H, s, $H_3C(18)$), 0.05 (6H, s, $(H_3C)_2Si$).

5,10-Secopregnan-3α-ol-20-one (**107**)

1M TBAF in THF (3.0μl, 10.3μM) was added to a stirred solution of *3α-tertbutyldimethylsiloxy-5,10-secopregnan-20-one* (**106**) (2mg, 4.5μM) in THF (0.5 cm^3). The reaction mixture was stirred at room temperature for 24h. The solvent was removed *in vacuo*, and the residue dissolved in methylene chloride (3 cm^3), washed with dil. HCl (1 cm^3) and water (2 x 1 cm^3). The organic layer was dried over anhydrous sodium sulphate, concentrated *in vacuo* and purified by column chromatography, eluting with ethyl acetate-petrol (1:4) to give *5,10-secopregnan-3α-ol-20-one* (**107**) (0.5mg, 35%) as a colourless oil, (R_f = 0.11, ethyl acetate-petrol (3:17)); υ_{max} (FILM)/cm^{-1} 3321 br (OH), 1699 s (C=O), 1244 (C-O); *m/z* (C.I.) 321 ([M^++1], 3%), 303 (2), 275 (6), 257 (100).

3β,20β-Diacetoxy-5(10→1βH)abeopregn-10(19)-en-5α-ol (**109**)

Ceric ammonium nitrate (16.3g, 29.7mM) in water (15 cm^3) was added to a stirred solution of *3β,20β-diacetoxypregnan-5α-ol* (**38**) (5.0g, 11.9mM) in acetonitrile (50 cm^3) at 80°C. The reaction mixture was stirred at 80°C for 5 min. Then the mixture was poured into ice/water (200 cm^3) and extracted with methylene chloride (3 x 100 cm^3). The combined organic extracts were dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography eluting with ethyl acetate-petrol (1:4) to give *3β,20β-diacetoxy-5(10→1βH)abeopregn-10(9)-en-5α-ol* (**109**) (3.88g, 78%) as a white solid, m.p. 94-95°C (R_f = 0.32, ethyl acetate-petrol (1:4)); $[\alpha]^{22}_D$ = + 43.9° (C=0.66, CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 3509 sh (OH), 1727 s (C=O), 1628 (C=C), 1240 (C-O); δ_H (270 MHz, $CDCl_3$) 5.27-5.19 (1H, m, H_α-C(3)), 5.16 (1H, s, H-C(19)), 5.03 (1H, s, H-C(19)), 4.92-4.80 (1H, m, H-C(20)), 2.05 (3H, s, H_3CCO), 2.02 (3H, s, H_3CCO), 1.15 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.70 (3H, s, $H_3C(18)$); *m/z* (C.I.) 419 ([M^++1], 2%), 401 (3), 358 (31), 341 (49), 299 (100), 281 (85), 271 (5), 253 (3), 149 (18); Found : C, 72.1; H, 9.61. $C_{25}H_{38}O_5$ requires C, 71.8; H, 9.09%.

20β-Acetoxy-5(10→1βH)abeopregn-10(19)-ene-3β,5α-diol (**110**)

Potassium carbonate (10.0g, 72.4mM) was added to a stirred solution of *3β,20β-diacetoxy-5(10→1βH)abeopregn-10(19)en-5α-ol* (**109**) (10.0g, 23.8mM) in methanol (200 cm^3). The reaction mixture was stirred at room temperature for 30 min. Water (50 cm^3) was added and the mixture extracted with methylene chloride (2 x 200 cm^3). The combined organic extracts were washed with dil. HCl (50 cm^3) and water (2 x 50 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (3:7) gave the *3β,5α diol* (**110**) (8.21g, 92%) as a colourless oil (R_f = 0.31, ethyl acetate-petrol (2:3)); $[\alpha]^{22}_D$ = +38.4° (C=1.25, CH_2Cl_2); υ_{max} (FILM)/cm^{-1} 3519 br (OH), 3447 br (OH), 1731 s (C=O), 1628 (C=C), 1243 (C-O); δ_H (400 MHz, $CDCl_3$) 5.13 (1H, s, H-C(19)), 4.99 (1H, s, H-C(19)), 4.85 (1H, dq, $J_{20,21}$ 6.4, $J_{20,17}$ 10.3 Hz, H-C(20)),

4.47-4.42 (1H, m, H_α-C(3)), 2.44 (1H, dd, $J_{1,2'}$ 6.8 $J_{1,2''}$ 14.7 Hz, H-C(1)), 2.05 (3H, s, H_3CCO), 1.15 (3H, d, $J_{21,20}$ 6.4 Hz, $H_3C(21)$), 0.68 (3H, s, $H_3C(18)$); δ_C ($CDCl_3$) 170.36 (C=O), 151.36 (C-10), 113.76 (C-19), 78.50 (C-5), 72.63 (C-20), 70.40 (C-3); *m/z* (C.I.) 377 ([M^++1], 4%), 359 (8), 341 (6), 317 (11), 299 (100), 281 (21), 271 (4), 255 (2), 230 (2); Found : C, 71.3; H, 10.1. $C_{23}H_{36}O_4.\ ^2/_3H_2O$ requires C, 71.1; H, 9.6%.

3α-Formoxy-20β-acetoxy-5(10→1βH)abeopregn-10(19)-en-5α-ol (**111**)

Diethylazodicarboxylate (3.0 cm^3, 19.1mM) was added dropwise to a stirred solution of *20β-acetoxy-5(10→1βH)abeopregn-10(19)-ene-3β-5α-diol* (**110**) (4.80g, 12.8mM), triphenyl phosphine (6.65g, 25.4mM) and formic acid (0.6 cm^3, 15.9mM) in THF (150 cm^3) under nitrogen at 0°C. The reaction mixture was stirred under nitrogen at 0°C for 2h and at room temperature for a further 2h. The solvent was removed *in vacuo* and the residue was diluted with methylene chloride (100 cm^3). The organic solution was washed with water (2 x 30 cm^3), dried over anhydrous sodium sulphate, and purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give the *3α-formate* (**111**) (4.11g,80%) as a colourless oil, (R_f = 0.80, ethyl acetate-petrol (2:3)); υ_{max} (FILM)/cm^{-1} 3531 sh (OH), 1725 br (C=O), 1630 (C=C), 1245 (C-O), 1185 (C-O); δ_H (270 MHz, $CDCl_3$) 8.04 (1H, s, HCOO), 5.34-5.23 (1H, m, H_β-C(3)), 5.19 (1H, s, H-C(19)), 5.07 (1H, s, H-C(19)), 4.91-4.79 (1H, m, H-C(20)), 2.74 (1H, dd, $J_{1,2'}$ 8.6, $J_{1,2''}$ 11.0 Hz, H-C(1)), 2.02 (3H, s, H_3CCO), 1.15 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.68 (3H, s, $H_3C(18)$); *m/z* (C.I.) 405 ([M^++1], 20%), 287 (4), 358 (26), 345 (59), 327 (51), 299 (78), 281 (100), 272 (8), 233 (5); Found : C, 70.6; H, 9.26. $C_{24}H_{36}O_5.^1/_4H_2O$ requires C, 70.5; H, 8.94%.

20β-Acetoxy-5(10→1βH)abeopregn-10(19)-ene-3α-5α-diol (**112**)

Potassium carbonate (4.0g, 29.0mM) was added to a stirred solution of *3α-formoxy-20β-acetoxy-5(10→1βH)abeopregn-10(19)-en-5α-ol* (**111**) (3.50g, 8.66mM) in methanol (150 cm^3). The reaction mixture was stirred at room

temperature for 30 min, after which water (50 cm^3) was added and the mixture extracted with methylene chloride (2 x 150 cm^3). The combined organic extracts were washed with dil. HCl (50 cm^3) and water (2 x 50 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (3:7) to give the *3α,5α diol* (**112**) (3.17g, 97%) as a colourless oil, (R_f = 0.31, ethyl acetate-petrol (2:3); $[\alpha]^{22}_D$ = +44.4° (C=1.42, CH_2Cl_2); υ_{max} (FILM)/cm^{-1} 3512 br (OH), 3438 br (OH), 1729 s (C=O), 1630 (C=C), 1245 (C-O); δ_H (270 MHz, $CDCl_3$) 5.21 (1H, s, H-C(19)), 5.15 (1H, s, H-C(19)), 4.91-4.79 (1H, m, H-C(20)), 4.29-4.17 (1H, m, H_β-C(3)), 3.17 (1H, d, *J* 9.3 Hz, H-COC(3)), 2.81 (1H, t, $J_{1,2}$ 9.7 Hz, H-C(1)), 2.55 (1H, s, H-OC(5)), 2.02 (3H, s, H_3CCO), 1.15 (3H, d, $J_{21,20}$ 6.1 Hz, H_3C(21)), 0.67 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 170.37 (C=O), 151.67 (C-10), 114.04 (C-19), 79.42 (C-5), 72.56 (C-20), 71.86 (C-3); *m/z* (C.I.) ([M^++1], 28%), 359 (13), 341 (10), 317 (36), 299 (100), 281 (57), 269 (3), 255 (2); Found : C, 72.6; H, 10.0. $C_{23}H_{36}O_4.{}^1/_4H_2O$ requires C, 72.5; H, 9.59%.

3α-Tertbutyldimethylsiloxy-20β-acetoxy-5(10→1βH)abeopregn- 10(19)-en-5α-ol (**113**)

To a stirred solution of tertbutyldimethylsilyl chloride (1.33g, 8.81mM) and imidazole (600mg, 8.82mM) in DMF (50 cm^3) was added *20β-acetoxy-5(10→1βH)abeopregn-10(19)-ene-3α,5α-diol* (**112**) (3.0g, 7.98mM). The reaction mixture was stirred at room temperature under nitrogen for 1h. DMF was removed *in vacuo* and the residue diluted with methylene chloride (50 cm^3). The solution was filtered and the filtrate washed with water (2 x 20 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:39) gave the *3α-silyl ether* (**113**) (3.19g, 82%) as a colourless oil, (R_f = 0.56, ethyl acetate-petrol (1:19)); υ_{max} (FILM)/cm^{-1} 3532 sh (OH), 1730 s (C=O), 1629 (C=C), 1244 (C-O), 1097 (O-Si); δ_H (270 MHz, $CDCl_3$) 5.10 (1H, s, H-C(19)), 5.03 (1H, s, H-C(19)), 4.90-4.78 (1H, m, H-C(20)), 4.38-4.30 (1H, m, H_β-C(3)), 2.71 (1H, s, H-OC(5)), 2.56 (1H, dd, $J_{1,2'}$ 7.5, $J_{1,2''}$ 11.9 Hz, H-C(1)), 2.01 (3H, s, H_3CCO),

1.14 (3H, d, $J_{21,20}$ 6.2 Hz, $H_3C(21)$), 0.88 (9H, s, $(H_3C)_3CSi$), 0.66 (3H, s, $H_3C(18)$), 0.05 (3H, s, H_3CSi), 0.04 (3H, s, H_3CSi); δ_C ($CDCl_3$) 175.22 ((C=O), 156.10(C-10), 118.41 (C-19), 83.15 (C-5), 77.42 (C-3), 76.68 (C-20); *m/z* (C.I.) 491 ([M^++1], 22%), 473 (8), 431 (39), 413 (28), 373 (13), 341 (21), 323 (2), 281 (100), 159 (13); Found : C, 70.4; H, 10.3. $C_{29}H_{50}SiO_4.{}^1/_4H_2O$ requires C, 70.4; H, 10.1%.

3α-Tertbutyldimethylsiloxy-5(10→1βH)abeopregn-10(19)-ene-5 α,20β-diol (**114**)
A stirred solution of potassium carbonate (3.0g, 21.7mM) and *3α-tertbutyldimethylsiloxy-20β-acetoxy-5(10→1βH)abeopregn- 10(19)-en-5α-ol* (**113**) (3.0g, 6.12mM) in methanol (100 cm^3) was refluxed for 24h. After cooling water (40 cm^3) was added and the mixture extracted with methylene chloride (2 x 100 cm^3). The combined organic extracts were washed with dil.HCl (30 cm^3) and water (2 x 30 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:19) to give the *5α,20α-diol* (**114**) (2.39g, 87%) as a colourless oil, (R_f = 0.23, ethyl acetate-petrol (1:19)); $[\alpha]^{22}_D$ = + 18.5° (C=0.92, CH_2Cl_2); υ_{max} (FILM)/cm^{-1} 3524 br (OH), 3470 br (OH), 1628 (C=C), 1251 (C-O), 1097 (O-Si); δ_H (400 MHz, $CDCl_3$) 5.12 (1H, s, H-C(19)), 5.04 (1H, s, H-C(19)), 4.35-4.27 (1H, m, H_β-C(3)), 3.73 (1H, dq, $J_{20,21}$ 6.1, $J_{20,17}$ 9.8 Hz, H-C(20)), 2.68 (1H, dd, $J_{1,2'}$ 7.0, $J_{1,2''}$ 11.6 Hz, H-C(1)), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.89 (9H, s, $(H_3C)_3CSi$), 0.79 (3H, s, $H_3C(18)$), 0.06 (3H, s, H_3CSi), 0.05 (3H, s, H_3CSi); δ_C ($CDCl_3$) 151.33 (C-10), 113.64 (C-19), 78.33 (C-5), 71.86 (C-20), 70.45 (C-3); *m/z* (C.I.) 499 ([M^++1], 4%), 431 (50), 413 (21), 391 (11), 373 (10), 281 (100), 255 (40), 159 (20); Found : C, 71.3; H, 10.9. $C_{27}H_{48}SiO_3.{}^1/_3H_2O$ requires C, 71.4; H, 10.7%.

3α-Tertbutyldimethylsiloxy-5(10→1βH)abeopregn-10(19)-en-5α -ol-20-one (**115**)
Pyridinium chlorochromate (900mg, 4.18mM) was added to a stirred solution of *3α-tertbutyldimethylsiloxy-5(10→1βH)abeopregn-10(19)ene-5α ,20β-diol* (**114**) (1.25g, 2.79mM) in methylene chloride (40 cm^3). The reaction mixture was stirred at

room temperature under nitrogen for 3h. Silica gel was added directly to the reaction mixture and the solvent removed *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:9) gave the *20-ketone* (**115**) (1.17g, 94%) as a colourless oil, (R_f = 0.27 ethyl acetate-petrol (1:9)); $[\alpha]^{22}_D$ = + 73.5° (C=1.13, CH_2Cl_2); υ_{max} (FILM)/cm^{-1} 3527 sh (OH), 1703 s (C=O), 1628 (C=C), 1252 (C-O), 1097 (O-Si); δ_H (400 MHz, $CDCl_3$) 5.13 (1H, s, H-C(19)), 5.06 (1H, s, H-C(19)), 4.35-4.27 (1H, m, H_β-C(3)), 2.76 (1H, s, H-OC(5)), 2.67 (1H, dd, $J_{1,2'}$ 7.3, $J_{1,2''}$ 11.3 Hz, H-C(1)), 2.56 (1H, t, $J_{17,16}$ 9.2 Hz, H-C(17)), 2.12 (3H, s, H_3C(21)), 0.88 (9H, s, $(H_3C)_3CSi$), 0.66 (3H, s, H_3C(18)), 0.06 (3H, s, H_3CSi), 0.05 (3H, s, H_3CSi); *m/z* (C.I.) 447 ([M^++1], 46%), 429 (22), 411 (3), 389 (7), 371 (2), 315 (6), 297 (100), 279 (11), 253 (5); Found : C, 70.7; H, 10.8. $C_{27}H_{46}SiO_3.{}^2/_3H_2O$ requires C, 70.7; H, 10.3%.

3α,5α-Dihydroxy-5(10→1βH)abeopregn-10(19)-en-20-one (**116**)

1M Tetrabutylammonium fluoride in THF (1.1 cm^3, 1.08mM) was added dropwise to a stirred solution of the *3α-silyl ether* (**115**) (400mg, 0.90mM) in THF (20 cm^3). The reaction mixture was stirred at room temperature for 4h, after which it was diluted with methylene chloride (50 cm^3) and washed with dil. HCl (20 cm^3) and water (2 x 20 cm^3). The organic layer was dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:1) to give the *3α,5α diol* (**116**) (260mg, 87%) as a white solid, m.p. 159-160°C (R_f = 0.18, ethyl acetate-petrol (3:7)); $[\alpha]^{22}_D$ = + 84.0° (C=1.00, CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 3519 br (OH), 3393 br (OH), 1700 s (C=O), 1635 (C=C); δ_H (400 MHz, $CDCl_3$) 5.23 (1H, s, H-C(19)), 5.18 (1H, s, H-C(19)), 4.28-4.20 (1H, m, H_β-C(3)), 2.80 (1H, t, $J_{1,2}$ 9.8 Hz, H-C(1))), 2.57 (1H, t, $J_{17,16}$ 9.2 Hz, H-C(17)), 2.12 (3H, s, H_3C(21)), 0.66 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 209.38 (C=O), 151.33 (C-10), 114.41 (C-19), 79.52 (C-5), 71.95 (C-3); *m/z* (C.I.) 333 ([M^++1], 48%), 315 (50), 297 (100), 279 (11), 253 (5), 215 (4), 177 (4); Found : C, 74.1; H, 9.86. $C_{21}H_{32}O_3.{}^1/_2H_2O$ requires C, 73.9; H, 9.94%.

3α,5α-Dihydroxy-5(10→1βH)abeopregnan-20-one (**117**)

Method 1

A stirred solution of 10% palladium on activated carbon (19mg, 10% w/w) and *3α,5α-dihydroxy-5(10→1βH)abeopregn-10(19)-en-20-one* (**116**) (190 mg, 0.57mM) in ethanol (15 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was stirred under an atmosphere of hydrogen at atmospheric pressure for 15h. The mixture was filtered and the filtrate concentrated *in vacuo*. The residue was dissolved in methylene chloride (25 cm^3), washed with water (2 x 10 cm^3), dried over anhydrous sodium sulphate and evaporated under reduced pressure. Purification by column chromatography, eluting with ethyl acetate-petrol (2:3) gave *3α,5α-dihydroxy-5(10→1βH)abeopregnan-20-one* (**117**) (166mg, 87%) as a white solid, m.p. 152-153°C (R_f = 0.38, ethyl acetate-petrol (1:1)); $[\alpha]^{22}_D$ = + 12.8° (C=1.02, CH_2Cl_2); υ_{max} (NUJOL)/cm^{-1} 3296 br (OH), 3237 br (OH), 1700 s (C=O); δ_H (400 MHz, $CDCl_3$) 4.33-4.27 (1H, m, H_β-C(3)), 2.57-2.53 (1H, m, H-C(1)), 2.12 (3H, s, H_3C(21)), 1.24 (3H, d, $J_{19,10}$ 7.3 Hz, H_3C(19)), 0.63 (3H, s, H_3C(18)); δ_C ($CDCl_3$) 209.72 (C-20), 84.46 (C-5), 72.57 (C-3); *m/z* (low eV E.I.) 334 (M^+, 69%), 316 (100), 298 (62), 246 (27), 232 (42), 128 (44), 111 (34), 71 (28); Found : C, 74.9; H, 10.5. $C_{21}H_{34}O_3.{}^1/_8H_2O$ requires C, 74.9; H, 10.2%.

Method 2

A solution of *3α,5α-dibenzoxy-5(10→1βH)abeopregn-10(19)-en-20-one* (**121**) (100mg, 0.20mM) and 10% palladium on activated carbon (20mg, 20% w/w) in ethanol (20 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was stirred under an atmosphere of hydrogen at atmospheric pressure for 24h. The mixture was filtered and the filtrate evaporated under reduced pressure. The residue was dissolved in methylene chloride (30 cm^3), washed with water (2 x 10 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified

by column chromatography, eluting with ethyl acetate-petrol (2:3) to give *3α,5α-dihydroxy-5(10→1βH)abeopregnan-20-one* (**117**) (25mg, 39%) as a white solid, m.p. 152-153°C (R_f = 0.38, ethyl acetate-petrol (1:1)).

3α,5α-Dibenzoxy-20β-acetoxy-5(10→1βH)abeopregn-10(19)-ene (**119**)

20β-Acetoxy-5,10-secopregn-1(10)-en-3α-ol-5-one (56) (1.50g, 3.99mM) in THF (10 cm^3) was added dropwise to a stirred suspension of sodium hydride (300 mg, 12.5mM), previously washed with petrol, in THF (40 cm^3) at room temperature. The mixture was stirred for 15 min after which the evolution of hydrogen ceased and it was cooled to 0°C. Benzyl bromide (0.95 cm^3, 7.97mM) was added dropwise and the reaction mixture stirred at 0°C under nitrogen for 4h, and a further 24h at room temperature. Water (10 cm^3) was added to quench and the mixture was extracted with methylene chloride (2 x 50 cm^3). The combined organic extracts were dried over magnesium sulphate, evaporated under reduced pressure, and purified by column chromatography, eluting with ethyl acetate-petrol (1:19) to give the *dibenzyl ether* (**119**) (1.26g, 68%) as a white solid, m.p. 135-137°C (R_f = 0.67, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 1725 s (C=O), 1651 (C=C), 1629 s (C-C, Ar), 1602 s (C-C, Ar), 1244 (C-O), 754 (C-H, Ar), 732 (C-H, Ar), 701 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 9.52 (1H, s, H-C(19)), 7.34-7.11 (10H, m, Ar), 5.27 (1H, s, H-C(19)), 4.89-4.75 (1H, m, H-C(20)), 3.48-3.40 (1H, m, H_β-C(3)), 3.23-2.81 (4H, m, $PhC\underline{H}_2O$ x 2), 2.05 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.65 (3H, s, $H_3C(18)$); *m/z* (C.I.) 557 ([M^++1], 2%), 539 (2), 497 (3), 479 (4), 405 (1), 357 (11), 211 (51), 123 (66), 107 (100); Found : C, 79.6; H, 8.70. $C_{37}H_{48}O_4$ requires C, 79.8; H, 8.63%.

3α,5α-Dibenzoxy-5(10→1βH)abeopregn-10(19)-en-20β-ol (**120**)

Potassium carbonate (1.0g, 7.25mM) was added to a stirred solution of *3α,5α-dibenzoxy-20β-acetoxy-5(10→1βH)abeopregn-10(19)-ene* (**119**) (1.0g, 1.80mM) in methanol (50 cm^3). The reaction mixture was refluxed for 24h. After

cooling the mixture was diluted with water (30 cm^3) and extracted with methylene chloride (2 x 50 cm^3). The combined organic extracts were washed with dil. HCl (20 cm^3) and water (2 x 20 cm^3), dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:4) to give the *20β-hydroxyl* (**120**) (665mg, 72%) as a white solid, m.p. 121-122°C (R_f = 0.40, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3483 br (OH), 1635 (C=C), 1611 s (C-C, Ar), 1596 s (C-C, Ar), 732 (C-H, Ar), 697 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 9.51 (1H, s, H-C(19)), 7.32-7.14 (10H, m, Ar), 5.28 (1H, s, H-C(19)), 3.77-3.65 (1H, m, H-C(20)), 3.48-3.40 (1H, m, H_β-C(3)), 3.23-2.83 (4H, m, $PhC\underline{H}_2O$ x 2), 1.14 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.75 (3H, s, $H_3C(18)$); *m/z* (C.I.) 515 ([M^++1], 18%), 497 (25), 479 (17), 453 (16), 423 (21), 395 (16), 211 (42), 123 (58), 107 (78), 89 (100); Found : C, 81.7; H, 9.07. $C_{35}H_{46}O_3$ requires C, 81.7; H, 8.95%.

3α,5α-Dibenzoxy-5(10→1βH)abeopregn-10(19)-en-20-one (**121**)

Pyridinium chlorochromate, PCC, (90mg, 0.42mM) was added to a stirred solution of *3α,5α-dibenzoxy-5(10→1βH)abeopregn-10(19)-en-20β-ol* (**120**) (140mg, 0.27mM) in methylene chloride (3 cm^3). The reaction mixture was stirred at room temperature for 3h, after which silica gel was added and the reaction mixture evaporated *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:9) gave the *20-ketone* (**121**) (138mg, 99%) as a white solid, m.p. 80-81°C (R_f = 0.47, ethyl acetate-petrol (1:4)); υ_{max} (NUJOL)/cm^{-1} 1700 s (C=O), 1643 (C=C), 1621 (C-C, Ar), 1597 s (C-C, Ar), 1165 (C-O), 752 (C-H, Ar), 701 (C-H, Ar); δ_H (270 MHz, $CDCl_3$) 7.34-7.13 (10H, m, Ar), 5.13 (1H, s, H-C(19)), 4.98 (1H, s, H-C(19)), 3.72-3.66 (4H, m, $PhC\underline{H}_2O$ x 2), 2.13 (3H, s, $H_3C(21)$), 0.68 (3H, s, $H_3C(18)$); *m/z* (C.I.) 512 ([M^++1] missing), 421 ([M^+ - $PhCH_3$], 1%), 409 (1), 391 (1), 333 (2), 307 (4), 293 (10), 275 (10), 211 (100), 123 (80), 107 (19); Found : C, 81.4; H, 8.87. $C_{35}H_{44}O_3.{}^1/_4H_2O$ requires C, 81.3; H, 8.62%.

(E)-3α-Tertbutyldimethylsiloxy-20β-acetoxy-5,10-secopregn-1(10)-en-5α-ol (**123**)
Sodium borohyride (100mg, 2.63mM) was added portionwise to a stirred solution of the *siloxy ketone* (**57**) (1.0g, 2.04mM) in ethanol (50 cm^3). The reaction mixture was stirred at room temperature under nitrogen for 48h. Water (20 cm^3) was added to quench and the solution extracted with methylene chloride (2 x 50 cm^3). The combined organic extracts were dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography to give the *siloxy-5α-ol* (**123**) (773mg, 77%) as a colourless oil (R_f = 0.16, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 3441 br (OH), 1733 s (C=O), 1661 (C=C), 1242 (C-O), 1073 (O-Si); δ_H (270 MHz, $CDCl_3$) 5.44-5.41 (1H, m, H_E-C(1)), 4.90-4.80 (1H, m, H-C(20)), 4.28-4.10 (2H, m, H_β-C(3) + H_β-C(5)), 2.02 (3H, s, H_3CCO), 1.67 (3H, s, H_3C(19)), 1.15 (3H, d, $J_{21,20}$ 6.1 Hz, H_3C(21)), 0.92 (9H, s, $(CH_3)_3Si$), 0.72 (3H, s, H_3C(18)), 0.12 (3H, s, H_3CSi), 0.10 (3H, s, H_3CSi); *m/z* (C.I.) 493 ([M^++1], 4%), 475 (3), 433 (14), 415 (30), 343 (22), 283 (100), 257 (5); Found : C, 70.5; H, 10.7. $C_{29}H_{52}SiO_4$ requires C, 70.7; H, 10.6%.

3α-Tertbutyldimethylsiloxy-20β-acetoxy-5(10→1βH)abeopregn- 10(19)-ene (**124**)

Method 1

20β-Acetoxy-5(10→1βH)abeopregn-10(19)-en-3α-ol (**131**) (90mg, 0.,25mM) was added to a stirred solution of *tert*butyldimethylsilyl chloride (45mg, 0.30mM) and imidazole (21mg, 0.30mM) in DMF (1 cm^3). The reaction mixture was stirred at room temperature for 1h. The mixture was preabsorbed onto silica gel and purification by column chromatography, eluting with ethyl acetate-petrol (1:49) gave the *3α-silyl ether* (**124**) (115mg, 97%) as a white solid, m.p. 76-77°C (R_f = 0.86, ethyl acetate-petrol (1:9)); υ_{max} (NUJOL)/cm^{-1} 1733 s (C=O), 1634 (C=C), 1244 (C-O), 1061 (O-Si); δ_H (270 MHz, $CDCl_3$) 4.92-4.78 (1H, m, H-C(20)), 4.82 (1H, s, H-C(19)), 4.15-4.03 (1H, m, H_β-C(3)), 2.82 (1H, q, $J_{1,2'}$ 10.9, $J_{1,2''}$ 18.4 Hz, H-C(1)), 2.02 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.0 Hz, H_3C(21)), 0.90 (9H, s, $(H_3C)_3CSi$),

0.66 (3H, s, $H_3C(18)$), 0.06 (6H, s, $(H_3C)_2Si$); *m/z* (C.I.) 475 ([M^++1], 3%), 459 (4), 415 (42), 357 (18), 343 (8), 283 (100), 149 (20); Found : C, 73.0; H, 10.8. $C_{29}H_{50}SiO_3$ requires C, 73.4; H, 10.5%.

Method 2

To a stirred solution of tosyl chloride (407mg, 2.13mM) and pyridine (1.15 cm^3, 14.2mM) in methylene chloride (10 cm^3) was added a solution of *(E)-3α-tertbutyldimethylsiloxy-20β-acetoxy-5,10-secopregn- 1(10)-en-5α-ol* (**123**) (700mg, 1.42mM) in methylene chloride (10 cm^3). The reaction mixture was stirred at 50°C for 48h. Water (10 cm^3) was added, the organic layer was separated, and the aqueous layer extracted with methylene chloride (2 x 15 cm^3). The combined organic extracts were dried over magnesium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:49) to give *3α-tertbutyldimethylsiloxy-20β-acetoxy-5(10→-1βH)abeopregn -10(19)-ene* (**124**) (647mg, 96%) as a white solid, m.p. 76-77°C (R_f = 0.86, ethyl acetate-petrol (1:9)).

3α-Tertbutyldimethylsiloxy-5(10→1βH)abeopregn-10(19)-en-20 β-*ol* (**125**)

Potassium carbonate (200mg, 1.45mM) and the *20β-acetate* (**124**) (200mg, 0.42mM) were refluxed together in methanol (10 cm^3) for 24h. After cooling water (5 cm^3) was added and the mixture extracted with methylene chloride (2 x 20 cm^3). The combined organic extracts were washed with dil. HCl (5 cm^3) and water (2 x 10 cm^3), dried over anhydrous sodium sulphate, and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol (1:19) to give the *20β-hydroxyl silyl ether* (**125**) (166mg, 91%) as a colourless oil, (R_f = 0.60, ethyl acetate-petrol (1:9); υ_{max} (FILM)/cm^{-1} 3384 br (OH), 1632 (C=C), 1068 (O-Si); δ_H (270 MHz, $CDCl_3$) 4.79 (1H, s, H-C(19)), 4.63 (1H, s, H-C(19)), 4.27-4.22 (1H, m, H_β-C(3)), 3.74-3.63 (1H, m, H-C(20)), 3.06 (1H, q, $J_{1,2'}$ 8.8, $J_{1,2''}$ 18.0 Hz, H-C(1)), 1.16 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.84 (9H, s, $(H_3C)_3CSi$), 0.83 (3H, s, $H_3C(18)$), 0.05 (6H, s, $(H_3C)_2Si$); *m/z* (C.I.) 433 ([M^++1], 4%), 415 (21), 375 (26), 357 (13), 283

(100), 260 (10); Found : C, 74.8; H, 11.4. $C_{27}H_{48}SiO_2$ requires C, 75.0; H, 11.1%.

3α-Tertbutyldimethylsiloxy-5(10→1βH)abeopregn-10(19)-en-20-one (**126**)

To a stirred solution of *3α-tertbutyldimethylsiloxy-5(10→1βH)abeopregn-10(19)-en-20 β-ol* (**125**) (150mg, 0.35mM) in methylene chloride (10 cm^3) was added pyridinium chlorochromate (112mg, 0.52mM). The reaction mixture was stirred at room temperature under nitrogen for 3h. Silica gel was added to the reaction mixture and the solvent removed *in vacuo*. Purification by column chromatography, eluting with ethyl acetate-petrol (1:49) gave the *20-ketone* (**126**) (142mg, 95%) as a colourless oil, (R_f = 0.74, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 1706 s (C=O), 1635 (C=C), 1061 (O-Si); δ_H (270 MHz, $CDCl_3$) 4.80 (1H, s, H-C(19)), 4.65 (1H, s, H-C(19)), 6.78-6.72 (1H, m, H_β-C(3)), 3.05 (1H, q, $J_{1,2'}$ 9.0, $J_{1,2''}$ 17.6 Hz, H-C(1)), 2.07 (3H, s, H_3C(21)), 0.84 (9H, s, $(H_3C)_3CSi$), 0.60 (3H, s, H_3C(19)), 0.05 (6H, s, $(H_3C)_2Si$); *m/z* (C.I.) 430 (M^+, 2%), 373 (19), 297 (26), 281 (5), 258 (4), 149 (16), 75 (100); Found : M^+, 430.32671. $C_{27}H_{46}SiO_2$ requires M, 430.32434, 5%.

5(10→1βH)Abeopregn-10(19)-en-3α-ol-20-one (**127**)

1M TBAF in THF (0.28 cm^3, 0.28mM) was added dropwise to a stirred solution of *3α-tertbutyldimethylsiloxy-5(10→1βH)abeopregn-10(19)-en-20 -one* (**126**) (100mg, 0.23mM) in THF (5 cm^3). The reaction mixture was stirred at room temperature for 2h. Methylene chloride (15 cm^3) was added and the solution was washed with dil. HCl (5 cm^3) and water (2 x 10 cm^3). The combined organic extracts were dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:9) to give *5(10→1βH)abeopregn-10(19)-en-3α-ol-20-one* (**127**) (72mg, 98%) as a colourless oil, (R_f = 0.33, ethyl acetate-petrol (3:7)); υ_{max} (FILM)/cm^{-1} 3392 br (OH), 1704 s (C=O), 1634 (C=C); δ_H (270 MHz, $CDCl_3$) 4.89 (1H, s, H-C(19)), 4.73 (1H, s, H-C(19)), 4.42-4.37 (1H, m, H_β-C(3)), 3.17 (1H, q, $J_{1,2'}$ 8.8, $J_{1,2''}$ 18.1 Hz, H-C(1)), 2.12 (3H, s, H_3C(21)), 0.67 (3H, s, H_3C(18)); *m/z* (C.I.) 317 ([M^++1], 92%), 299 (100), 281 (28), 271 (4), 232 (5),

161 (9), 99 (22), 85 (53); (E.I.) Found : M^+, 316.24023. $C_{21}H_{32}O_2$ requires M, 316.24125, 77%.

5(10→-1βH)Abeopregnan-3α-ol-20-one (**128**)

A solution of *5(10→1βH)abeopregn-10(19)-en-3α-ol-20-one* (**127**) (200mg, 0.63mM) and 10% palladium on activated carbon (20mg, 10% w/w) in ethanol (20 cm^3) was degassed under nitrogen for 15 min. The reaction mixture was stirred under an atmosphere of hydrogen at atmospheric pressure for 12h. After this the mixture was filtered and the filtrated washed with water (2 x 10 cm^3), after diluting with methylene chloride (30 cm^3). The organic layer was dried over anhydrous sodium sulphate and purified by column chromatography, eluting with ethyl acetate-petrol (3:17) to give *5(10→1βH)abeopregnan-3α-ol-20-one* (**128**) (178mg, 88%) as a white solid, recrystallised from petrol, m.p. 113-114°C (R_f = 0.36, ethyl acetate-petrol (3:7)); υ_{max} (NUJOL)/cm^{-1} 3388 br (OH), 1703 s (C=O); δ_H (270 MHz, $CDCl_3$) 4.34-4.31 (1H, m, H_β-C(3)), 2.53 (2H, t, $J_{1,2}$ 9.2 Hz, H-C(1)), 2.11 (3H, s, H_3C(21)), 0.99 (3H, d, $J_{19,10}$ 7.3 Hz, H_3C(19)), 0.64 (3H, s, H_3C(18)); *m/z* (C.I.) 319 ([M^++1], 85%), 301 (100), 283 (37), 257 (8), 233 (4), 215 (5), 149 (8); Found : C, 78.7; H, 10.9. $C_{21}H_{34}O_2$. $^1/_9H_2O$ requires C, 78.8; H, 10.7%.

20β-Acetoxy-5(10→1βH)abeopregn-10(19)-en-3β-ol (**129**)

Potassium carbonate (500mg, 3.62mM) was added to a stirred solution of *3β,20β-diacetoxy-5(10→1βH)abeopregn-10(19)-ene* (**88**) (350mg, 0.87mM) in methanol (20 cm^3). The reaction mixture was stirred at room temeprature for 2.5h. Water (10 cm^3) was added and the mixture extracted with methylene chloride (2 x 25 cm^3). The combined organic extracts were washed with dil. HCl (10 cm^3) and water (2 x 10 cm^3), dried over anhydrous sodium sulphate, and concentrated under reduced pressure. Purification by column chromatography, eluting with ethyl acetate-petrol (3:17) gave the *3β-hydroxyl-20β-acetate* (**129**) (262mg, 84%) as a colourless ol, (R_f = 0.11 ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 3394 br (OH), 1732 s (C=O), 1633

(C=C), 1243 (C-O); δ_H (270 MHz, $CDCl_3$) 4.86 (1H, s, H-C(19)), 4.82-4.78 (1H, m, H-C(20)), 4.70 (1H, s, H-C(19)), 4.42-4.36 (1H, m, H_α-C(3)), 3.17 (1H, q, $J_{1,2}$ 9.3 Hz, H-C(1)), 2.02 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 6.1 Hz, $H_3C(21)$), 0.68 (3H, s, $H_3C(18)$); *m/z* (E.I.) 360 (M, 5%), 342 (10), 300 (6), 285 (5), 243 (7), 163 (12), 84 (42); (Found : M^+, 360.26645. $C_{23}H_{36}O_3$ requires M, 360.26952, 41%.)

3α-Formoxy-20β-acetoxy-5(10→1βH)abeopregn-10(19)-ene (**130**)

Diethylazodicarboxylate (0.16ml, 1.04mM) was added dropwise to a stirred solution of *20β-acetoxy-5(10→1βH)abeopregn-10(19)-en-3β-ol* (**129**) (250mg, 0.69mM), triphenylphosphine (364mg, 1.39mM) and formic acid (0.05 cm^3, 1.39mM) in THF (20 cm^3) under nitrogen. The reaction mixture was stirred at room temperature under nitrogen for 7h. Water (20 cm^3) was added and the mixture extracted with methylene chloride (2 x 30 cm^3). The combined organic extracts were dried over anhydrous sodium sulphate, concentrated *in vacuo*, and purified by column chromatography, eluting with ethyl acetate-petrol (1:19) to give the *3α-formate* (**130**) (197mg, 73%) as a colourless oil, (R_f = 0.56, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 1727 s (C=O), 1711 s (C=O), 1636 (C=C), 1245 (C-O); δ_H (270 MHz, $CDCl_3$) 8.05 (1H, s, HCO), 5.17-5.08 (1H, m, H_β-C(3)), 4.91 (1H, s, H-C(19)), 4.87-4.80 (1H, m, H-C(20)), 4.80 (1H, s, H-C(19)), 2.92 (1H, q, $J_{1,2'}$ 9.9, $J_{1,2''}$ 18.1 Hz, H-C(1)), 2.02 (3H, s, H_3CCO), 1.15 (3H, d, $J_{21,20}$ 6.0 Hz, $H_3C(21)$), 0.68 (3H, s, $H_3C(18)$); *m/z* (E.I.) 388 (M^+, 2%), 342 (13), 328 (8), 313 (4), 282 (7), 149 (17), 59 (38); (Found : M^+, 388.26136. $C_{24}H_{36}O_4$ requires M, 388.26125, 8%).

20β-Acetoxy-5(10→1βH)abeopregn-10(19)-en-3α-ol (**131**)

Potassium carbonate(110mg, 0.80mM) and the *3α-formate* (**130**) (110mg, 0.28mM) were stirred together in methanol (10 cm^3) at room temperature for 1h. Water (5 cm^3) was added to the reaction mixture and then it was extracted with methylene chloride (2 x 20 cm^3), dried over anhydrous sodium sulphate, and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with ethyl acetate-petrol

(1:9) to give the *3α-hydroxyl* (**131**) (96mg, 94%) as a colourless oil, (R_f = 0.11, ethyl acetate-petrol (1:9)); υ_{max} (FILM)/cm^{-1} 3364 br (OH), 1730 s (C=O), 1633 (C=C), 1244 (C-O); δ_H (270 MHz, $CDCl_3$) 4.92-4.78 (1H, m, H-C(20)), 4.86 (1H, s, H-C(19)), 4.70 (1H, s, H-C(19)), 4.42-4.37 (1H, m, H_β-C(3)), 3.17 (1H, q, $J_{1,2'}$ 9.0, $J_{1,2''}$ 18.6 Hz, H-C(1)), 2.02 (3H, s, H_3CCO), 1.14 (3H, d, $J_{21,20}$ 5.9 Hz, $H_3C(21)$), 0.68 (3H, s, $H_3C(19)$); *m/z* (E.I.) 360 (M^+, 7%), 342 (26), 300 (16), 285 (15), 267 (15), 243 (28), 173 (16), 121 (39), 43 (100).

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Appendix 1 Review of steroidal anaesthetics

In 1927, Cashin and Moravek[1] reported that high doses of cholesterol induced 'anaesthesia'. Cholesterol was found to potentiate the action of anaesthetic agents such as ether, chloroform or sodium pentobarbitone in the rabbit[2] and other animals.[3]

In 1941, Hans Selye[4] was the first to report the finding of reversible unconsciousness in rats, following the intraperitoneal injection of large quantities of several steroidal hormones. Selye noted that it was difficult to produce acute overdosage phenomena, even when giving enormous doses, whilst looking at the physiology of steroidal hormones. He discovered that various steroid hormones, especially desoxycorticosterone acetate (DCA) and progesterone (Figure 1) produced deep anaesthesia in rats and mice if injected into the peritoneum, where they could be rapidly absorbed. After recovery from the anaesthesia such animals showed no ill effects. Partially hepatectomized rats were found to be much more sensitive to the anaesthetic effect of the steroids than intact controls and also nephrectomized rats. Thus Selye concluded that the liver played an important role in the detoxification of these compounds, and also that males are less sensitive than females of the same size.

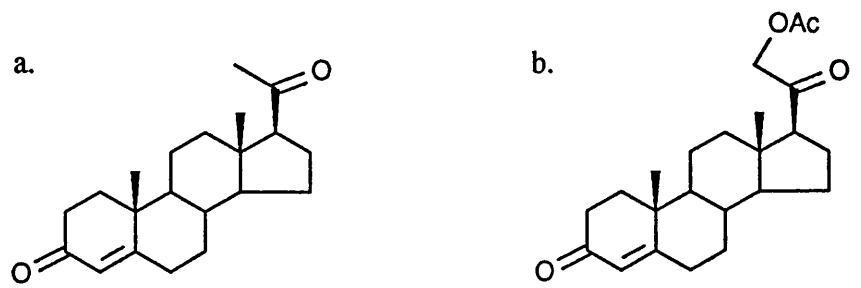


Figure 1. The first reported steroidal anaesthetics, a) progesterone, b) DCA.

Both progesterone and DCA were added to the rats in peanut oil, as they were practically insoluble in water. The anaesthesia produced was found to be so deep that Selye was able to perform prolonged abdominal operations in rats anaesthetized with DCA or progesterone. If lethal doses were administered, death was apparently due to anaesthesia of the respiratory centres.

Selye[4] attempted to obtain some information concerning the correlation between chemical structure and anaesthetic effect. His report included a table comparing the chemical structure of some fourteen steroids with their anaesthetic action. However, it was noted that only hormonally active steroids showed any anaesthetic effect, even though the activity was not limited to compounds with any one specific type of hormonal action.

Shortly after this publication, Selye published a paper on the acquired adaptation to the anaesthetic effect of steroid hormones.[5] Selye reported that steroid hormones caused deep anaesthesia in various experimental animals, such as rats, if they were administered intraabdominally or intravenously in sufficiently large doses.[6] This type of anaesthesia was similar to that obtained with other injected anaesthetics.[7]

It was also found that the anaesthesia produced by progesterone and DCA was usually not complicated by any untoward overdosage symptoms. On the other hand, anaesthetic doses of testosterone often caused convulsions and sometimes fatal pulmonary edema.

In the case of daily intraabdominal injections, a high degree of immunity to the anaesthetic action of such steroids was rapidly acquired. Of the two most active anaesthetic steroids, adaptation to progesterone was much more rapidly acquired than to DCA.

The prolonged treatment with hormones leads to the development of a resistance to their actions. This resistance is the result of the formation of hormone-inhibiting substances termed *antihormones*, which can be seen in the blood of the resistant animal.

Rats rendered resistant to the anaesthetic action of a certain steroid usually exhibited a high degree of resistance to other steroids as well, hence, the resistance was not strictly specific to the hormone with which the animal had been pretreated. Cholesterol, a steroid compound which does not produce anaesthesia, was unable to impart such resistance to anaesthetic steroids. The high degree of resistance to the anaesthetic action of steroid hormones (produced by prolonged pretreatment) was not accompanied by the appearance of demonstrable antihormones in the blood. Selye concluded that he had induced a resistance to a certain pharmacological action rather than to a certain chemical substance.[8] Experimental evidence was presented in the paper, involving various steroids. This furnished another example of an acquired hormone resistance without antihormone formation.

Also, in 1941, Selye published a more detailed paper on the anaesthetic action of steroid hormones,[9] where, based upon his observations, he came up with the *'fundamental law of steroid anaesthesia'*, namely that *'all compounds having a steroid hormone action are capable of producing anaesthesia, while no compound devoid of hormone action possesses this power'*.[4,5,10]

This was true even though the anaesthetic effect was not dependent upon any particular type of hormone activity. Progesterone and DCA were seen to be much more potent anaesthetics than the androgens, which in turn were more potent than the estrogens, but all these compounds possessed some measure of anaesthetic action, while even their closest chemical derivatives, if hormonally inactive, were

devoid of it.

Selye had also previously discovered that the anaesthetic effect of all steroid hormones was more marked in female than in male animals.[4] Spaying did not alter the sensitivity of females, but castration raised the responsiveness of males to the female level.[11]

In the aforementioned report,[9] Selye conducted experiments on rats, designed to test the validity of the 'fundamental law of steroid hormone anaesthesia' with a series of compounds which had not been studied so far from this point of view.

Two adrenal steroids were examined for possible anaesthetic effect; Reichstein's compound "J" (17-ethylandrostane-3,17,20-triol)[12], which was used in the form of its 3,20-diacetate, and Kendall's compound "A" (17-ethyl-Δ^4-androstene-21-ol-3,11,20-trione or dehydrocorticosterone)[12]. Also the vitamin ascorbic acid was tested, as Israel and Meranze claimed that this compound had 'progesterone-like' actions.[13]

The diacetate of Reichstein's compound "J", which was hormonally inactive, had no anaesthetic effect. Kendall's compound "A", which was an active cortical hormone, exhibited marked anaesthetic potency. Vitamin activity was found not to confer an anaesthetic action upon a compound, since even large doses of ascorbic acid, *dl*-α-tocopherol, and calciferol proved completely devoid of this effect.

The next compound to be tested was Δ^5-acetoxy pregnenolone. It was readily available *via* partial synthesis as it represents an intermediary step in the commercial manufacture of DCA. Selye observed that this compound had an anaesthetic action.[9] This appeared to, at first, represent an exception to the 'fundamental law of steroid hormone anaesthesia', since it was not known to

possess any hormonal action. However, on carrying out tests for other physiological effects, Selye found that it possessed definite adrenal cortical hormone activity.[9] Since this compound was not known to occur naturally, Selye reported that this may well be the first instance of an "artificial cortical hormone". Up to now 'the fundamental law of steroid anaesthesia' had been adhered to and, unless further research would reveal important exceptions, it appeared that a test of anaesthetic potency would represent a valuable short cut in the detection of hormonal activity. The only apparent exception was 17-ethinyl testosterone, a hormonally active compound with which Selye was unable to produce anaesthesia.[9] However the compound was so insoluble that its inactivity as an anaesthetic was most probably due to the fact that sufficient quantities could not be rapidly administered in solution.

In many instances simultaneous administration of two anaesthetic drugs elicits a more pronounced anaesthesia than could be expected by mere summation of their effect. However, combined simultaneous treatment with two anaesthetic steroids led to a summation, but not a potentiation, of their actions.[9] Selye showed this by experiments in which DCA and progesterone were given at the same time.[9] Selye also noted that in these experiments, progesterone was definitely more potent than DCA, while in previous experiments[4] the latter was found to be more active.

It became evident that in animals heavier than about 80 grams DCA produced more marked anaesthesia than progesterone. This difference became more evident as the weight of the animals rose. On the other hand small rats weighing 80 grams or less were definitely more sensitive to progesterone.

There appeared, however, to be a true potentiation of the actions of volatile anaesthetics, such as ether or chloroform, when administered simultaneously with a steroid anaesthetic, for instance progesterone.[9]

Adrenalectomy was seen to sensitize the organism to the anaesthetic action of DCA, estradiol, testosterone, and progesterone although it did not influence the anaesthesia caused by chloroform, ether, or magnesium chloride.[9] Hypophysectomy sensitized the organism to the anaesthetic action of DCA and progesterone in a similar fashion to adrenalectomy.[9] Thyroidectomy did not influence the course of the anaesthesia produced by progesterone or DCA administration.[9] Bilateral nephrectomy also did not change the course of anaesthesia caused by DCA and progesterone.[9] Thus Selye was able to draw certain conclusions with regards the mechanism of the steroid anaesthetics and their overdosage.[9] He concluded that the pituitary, adrenals, and the liver, were, in the same way involved in the 'detoxification of the steroids', and that in the absence of one of the aforementioned glands, the sensitivity to the anaesthetic effect increased, because the anaesthetic remained in an active form in the organism for a longer period of time.

During the course of Selye's studies[9] he found that shortly after an atropine injection the resistance of animals towards the anaesthetic effect of steroids increased, but prolonged pretreatment with atropine decreased resistance to the anaesthetic action of progesterone. This could be due to the well-known fact that, while the organism acquires adaptation to a certain stimulus, its resistance to other agents decreases. This was supported by experiments showing that rats which acquired a great deal of specific adaptation to toxic doses of formaldehyde, or forced muscular exercise, also became particularly sensitive to progesterone anaesthesia.[9]

Acetylcholine did not influence progesterone anaesthesia in any way.[9] Selye drew no notable conclusions regarding this.

It was known that steroid hormones produced anaesthesia when injected intraperitoneally or intravenously. However, if they were injected subcutaneously they did not exhibit any anaesthesia, probably because the rate of absorption was comparitively slow.[5]

Winter[11] reaffirmed that female rats were more sensitive to the anaesthetic effect of progesterone than males, but this sex difference only became apparent after maturity. Winter suspected that the endocrine secretion of the gonad influenced hormone sensitivity, as the relative resistance of the male developed only after puberty. A number of experiments were carried out to elucidate this phenomenon.[11]

Progesterone was administered to normal males and females, castrated males and females, and both males and females castrated and treated with the artificial testoid substance methyl testosterone. After carrying out the experiments Winter concluded that the normal endocrine activity of the testis was largely, if not entirely, responsible for the comparitive resistance of the males, since castration increased sensitivity in males, but was without effect in female rats.[11] Conversely, the resistance of castrated males and females was raised by the administration of methyl testosterone. Secretion of luteoids in the female did not confer some resistance towards progesterone anaesthesia, as while the secretion of testoids in the male is continuous, luteoid secretion in the female is intermittent and of relatively short duration.

Treatment with glucose and adrenalin tended to increase, while treatment with insulin or exposure to excessive heat or cold tended to decrease, the resistance of the rat to the anaesthetic effect of progesterone.[11]

In 1942 Selye investigated the antagonism between anaesthetic steroid hormones

and pentamethylenetetrazol (metrazol), using DCA and progesterone as examples.[14] Beecher has published a review[15] reporting that analeptic, convulsive drugs such as picrotoxin or metrazol, counteract the narcotic effect of certain anaesthetics, especially the barbiturates and tribromoethanol (avertin).

Steroid anaesthesia was suitable when long-lasting narcosis was required, but cannot be used satisfactorily in cases in which a deep anaesthesia of short duration was required, because a dose sufficient to produce deep anaesthesia, usually maintained the state of narcosis for several hours. Hence, Selye thought it would be of interest to establish whether metrazol could arouse animals from the hormone anaesthesia whenever desired.[14]

Selye performed experiments on albino rats,[14] which indicated that the anaesthesia produced by the intraperitoneal administration of steroid hormones, DCA or progesterone, could be interrupted at will by the administration of metrazol. It should be noted that this was not merely a prevention, but an actual interruption of the anaesthesia. Selye found that on administration of metrazol to the anaesthetized rats, they were on their feet after five minutes and showed only slight traces of narcosis. In this respect the anaesthesia produced by DCA and progesterone resembled that elicited by the barbiturates or tribromoethanol. Selye also discovered that fatal doses of metrazol were readily tolerated, without causing convulsions or lung edema, by rats receiving suitable doses of anaesthetic steroid hormones. Also, in this respect, the steroids again resembled the barbiturates. Thus, Selye concluded that the antagonism between DCA or progesterone and metrazole was mutual.

All of Selye's published results[14] are shown in Table 1.

Treatment	Number off animals	Average degree of anaesthesia	Deaths
DCA	7	+++	0
Metrazole and DCA	7	trace	1
Metrazole	7	0	7
Progesterone	10	+++	0
Metrazole and Progesterone	12	0	0
Metrazole	10	0	10

Table 1. Antagonism between DCA/progesterone and metrazole, +++ indicates a 100 percent result.

In order to measure the anaesthetic effect of steroids with some degree of accuracy, a sensitive bioassay method was designed using immature female, partially hepatectomized rats.[16] However, the applicability of this bioassay method was somewhat limited by the fact that steroids were only very sparingly soluble in non-toxic solvents, whilst it was required to administer them in very high concentrations.

Steroids, unlike most other anaesthetics, appeared to be most active in animals whose central nervous system was least developed. Selye reported that a few preliminary experiments in fish[17] proved this to be correct. He found that anaesthesia was readily produced if the steroids were added to the water in which the fish were swimming. However, the invertebrates examined proved completely resistant to the anaesthetic effect of steroids. It was evident that the most important factor for the production of steroid anaesthesia in fish, was to achieve an adequate concentration of the anaesthetic in the water. Although the size and sex of the fish played some role, they were comparatively less important. For various reasons, Selye devised a bioassay method using immature specimens of the redfin minnow *Notropis Cornutus*.[17]

This test had the advantage over the previously used rat assay in that it was simpler

and more sensitive.

Selye assayed forty-seven steroid derivatives using the fish and rat tests.[17] In general, it was found that the steroids most active in the rat were also most active in the fish. The rat bioassay is detailed in a previous report.[16]

In the fish assay, the steroids were taken up in a few drops of alcohol (ethanol) and injected directly into the water. The anaesthetic effect of the alcohol played no part in the narcosis obtained as it was not used in sufficient amounts.

Selyes' results were in agreement with 'the fundamental law of steroid hormone anaesthesia'. The fish assay further validated the view that intense anaesthetic activity was largely dependent upon the solubility of the compound in the solvent in which they were administered.

The five most active steroid anaesthetics are shown in Table 2. Note, that the previously untested steroid, pregnanedione, showed the most anaesthetic potency and was ten times as active as DCA and five times as active as progesterone in the fish assay.

A.F.U. is the *anaesthetic fish unit,* which is defined as the minimum amount of a compound (expressed in mg.), which must be added, per minnow and per 200 cc. of tap water in order to produce deep anaesthesia.

The following conclusions were drawn by Selye,[17] with regards the comparison between chemical structure and anaesthetic effect from the fish assay, on the basis

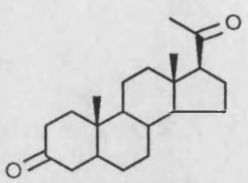
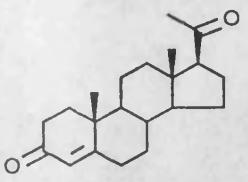
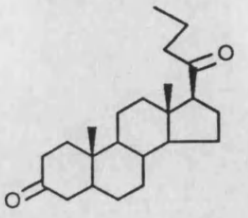
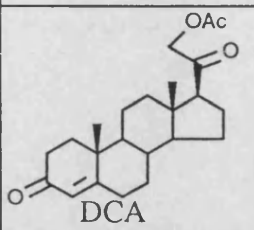
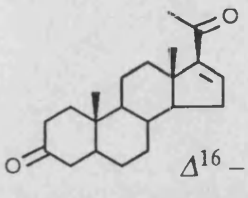
Steroid anaesthetic	 Pregnanedione	 Progesterone	 21 - Ethylprogesterone
A.F.U.	0.05	0.25	0.25
Steroid anaesthetic	 DCA	 Δ^{16} – Pregnenedione	
A.F.U.	0.50	0.50	

Table 2. Active steroid anaesthetics in Selye's fish assay.[17]

of compounds examined. No compound having a long C(17) side-chain possessed anaesthetic activity. A side chain, of 7 or more carbon atoms, was not compatible with anaesthetic activity, but at least 4 carbon atom side chains were compatible with marked anaesthetic potency.

Oxygenation at the two extreme poles of the molecule, *i.e.* C(3) and C(17) in the case of compounds possessing no side chain, and C(3) and C(20) or C(21) among those having a C(17) alkyl side chain, possessed the greatest anaesthetic activity. Further oxygenation was detrimental to anaesthetic potency. This was illustrated by the example of progesterone, which was more active than 6-hydroxyprogesterone, or the most active anaesthetic steroid, pregnanedione, which became practically inert upon further oxygenation, as shown by pregnanedionediol and pregnanetrionol. However, the presence of oxygen was indispensable for any type of hormonal or anaesthetic activity among the steroids.

The degree of unsaturation was also found to be important in determining

anaesthetic potency.[17] "One double bond does not appear to interfere seriously, with the anaesthetic effect, if it is situated in ring A or B, but two or more double bonds in these two rings, or one double bond in ring D are detrimental".[17] 16-Dehydro-progesterone was less active than progesterone, $\Delta^{5,6}$-pregnadienolone was inert, whereas pregnenolone exhibited a moderate narcotic potency, and the same decrease in activity was noted in pregnanedione following the introduction of a Δ^{16}-double bond.

It was also noted that, at least in an animal with such a primitive type of nervous system as that of the fish, the steroids compared favourably, in narcotic potency, with some of the most active anaesthetics known to pharmacologists, such as ether, alcohol, chloroform, morphine sulphate or magnesium chloride.[17]

Selye, as it has previously been stated, has shown that for the production of general anaesthesia in mammals, it was essential that the steroid should rapidly achieve a high concentration in the blood.[9] Selye[18] noted that a variety of steroid compounds (DCA, progesterone, and pregnane-3,17-dione) were absorbed into the gastrointestinal tract with sufficient speed to induce general anaesthesia in the rat, when administered orally. Under the conditions of Selye's experiments it appeared that the alkaline reaction, and the digestive enzymes of the intestinal secretions were unable to destroy any significant amount of the steroids during the comparatively short time required for their absorption. It must be noted that this was in the rat, and conditions vary from the rat to the human gastointestinal tract. It was also contradictory with respect to steroid hormones.

A factor which decreased the anaesthetic activity of orally administered steroids, with respect to intraperitoneally, subcutaneously, and intramuscularly administered steroids, was that their primary route is through the liver, which is known to take an active part in their detoxification.[10,19] Introduction of an ethyl or methyl group

at C(17) increased anaesthetic activity as it appeared that these compounds were less readily destroyed by the liver than their parent compounds.

This oral activity was achieved by the administration of finely ground steroid crystals in distilled water through a fine rubber tube introduced into the stomach. The comparative inactivity of orally administered hormones, as well as their 'great' anaesthetic activity when given *via* this route, was explained by a greater speed of absorption.

Selye[10,19] also confirmed the fact that, steroid sensitivity of the central nervous system is common to a large variety of animals, by use of the bird. He showed that intraperitoneal administration of progesterone in oil, caused deep and prolonged anaesthesia in young pigeons.[19]

In 1944 Selye and Stone[20] used their knowledge, that detoxification of steroid anaesthetics occurred in the liver, to gauge the speed with which the steroid was absorbed. In this manner it was possible to estimate the degree of hepatic detoxification by the decrease in the anaesthetically-effective dose condition by partial ablation of the liver. They also found that some steroid compounds had definite anaesthetic properties although they possessed no hormonal activity, contradictory to 'the fundamental law of steroid hormone anaesthesia'.

A report by Farson *et al*,[21] and also by Cashin and Moravek,[1] questioned the suitability of the word 'anaesthesia', when looking at the effects of the steroid hormones. Etymologically 'anaesthesia' means without sensation, but usage confined it to a description of a reversible process, unless accidently it became terminal. On studying 'Selye's generalisations', Farson *et al* initially used cholesterol.[21] After finding a high degree of fatality, occurring after loss of consciousness, various hormonal steroids were investigated, including

progesterone. Farson discovered that, neither in animals which died nor with those that recovered after administration of progesterone, was there a period in which the animal did not respond promptly to pain stimuli. He, thus, concluded that this syndrome cannot directly be referred to as anaesthesia, when the sensory pathways were not blocked. This was contradictory to earlier reports by Selye, outlined in this report. A better term would be the 'central depressant action' of the steroidal hormones.

Nearly ten years passed before the next communication on steroidal anaesthetics. Fourteen years after Selye first reported on the existence of steroidal anaesthetics, Laubach *et al*[22] reported on the first intravenous anaesthetic in man. Hydroxydione, 21-hydroxypregnane-3,20-dione sodium succinate (introduced by Pfizer as 'Viadril', and later by Schering as 'Presuren'), Figure 2, was found to be the most promising of a number of water-soluble steroids.

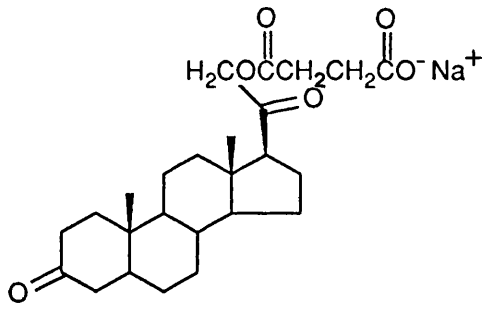


Figure 2. Viadril, the first successful intravenous anaesthetic in man.

Hydroxydione resembled pregnanedione, except that a sodium succinate group replaced a hydrogen atom in the 21-position of pregnanedione. This was therefore the sodium salt of a weak acid and, as such, the pH of its aqueous solution was well above neutral (8.5-9.8). It was studied in a number of patients, and was found to be a safe, convenient, and practical basal anaesthetic for surgical procedures in man.[22]

It must be noted that around this time a report appeared[23] in which it was claimed that sleep was induced in man by intravenous administration of progesterone.

In the mouse and rat, hydroxydione was found to have an anaesthetic potency equal to that of thiopental sodium, a known barbiturate anaesthetic, and a therapeutic index in excess of that of the thiobarbiturate. However, in cats, dogs, and monkeys hydroxydine was not as active (milligram for milligram) as thiopental sodium was, but was much less toxic than thiopental sodium. The advantages of the anaesthesia produced by hydroxydione were the relatively low degree of respiratory depression and rapid uncomplicated recovery with minimum post-anaesthetic depression.[22] Little or no endocrine activity was demonstrated.[22]

In England, Lerman (1956),[24] Taylor and Shearer (1956),[25] and Galley and Rooms (1956)[26] published reports of their work with hydroxydione.

Gordon *et al*[27] studied the effects of Viadril on the cerebral metabolism of humans and reported metabolic changes identical with those of barbiturate anaesthesia. These studies culminated in the successful trial of Viadril with nitrous oxide supplement, as the anaesthetic for a prefrontal lobotomy.[27] Encouraged by this, DeBon *et al*[28] gave Viadril a clinical trial as a basal anaesthetic for surgery at the University of California, School of Medicine. The technique is detailed in the report, with the appropriate doses of meperidene (Demerol) hydrochloride, and scopolamine or atropine used as premedication.

Viadril was used in 125 patients for basal anaesthesia in a variety of surgical procedures, and in 3 patients for sedation during regional anaesthesia. It was given by intravenous injection as a 2.5 percent solution, which was freshly prepared by means of a previously set dextrose drip infusion. The anaesthetic solution was injected into the tubing, connecting the drip with the patient, so that mixing and

dilution occurred before the solution reached the vein. In the lightly premedicated patient, Viadril produced sleep in 5 to 10 minutes after adminsitration, and lasted for a maximum period of approximately two hours, after which more Viadril, in smaller doses, could be added to prolong anaesthesia. The anaesthesia was a smooth, quiet process devoid of any excitement phase, and it was noted that Viadril had the ability to obtund reflexes, particularly those of the pharynx and the larynx, i.e., coughing. Viadril was seen to produce a definite analgesic state far greater than that produced by the barbiturates. The amounts of relaxant drugs used were considerably smaller than those found necessary when employing thiopental sodium and nitrous oxide anaesthesia, Table 3.

Compound	Animal species	Route of administration	No. of animals anaesthetized*/ dead	Onset min±S.E.	Duration min ±S.E.	T.I.
Hydroxy - dione	mice	I.V.	10 / 0	3.2±0.1	34.9±3.5	11.6
	rats	I.V.	10 / 1	3.9±0.3	37.3±2.0	7.8
	mice	oral	10 / 2	3.1±0.2	174.2±23.2	12.0
Thiopental sodium	mice	I.V.	10 / 0	immediate	4.5±0.8	4.6
	rats	I.V.	10 / 5	immediate	163.0±12.1	2.5
	rats	oral	10 / 9			1.6

Table 3. Comparative anaesthetic effect of Viadril and thiopental sodium in mice and rats. From *Postgrad. Med. J. Suppl.*, 1972, **48**, p.9, J. Sutton. *Dose employed was twice the AD_{50}.

The various characteristics associated with the anaesthesia produced by Viadril, namely control of pain and reflexes, and production of relaxation, classified it as a true anaesthetic agent best compared with tribromoethanol. It had a pleasing lack of postoperative depression, with the majority of patients being responsive at the end of the procedures. Bouts of coughing and straining, sometimes observed during light thiopental sodium-nitrous oxide-oxygen anaesthesia did not occur with

Viadril. Galley and Rooms[26] noted that while recovery was prolonged it was a relatively pleasant experience.

DeBon *et al*[28] noted no complications after the use of Viadril, except on three occasions when the administration of the drug was followed by thrombophlebitis in the injected vein. Some patients also complained of nausea and vomiting after operative procedures in which the drug was used. Hypotension was also noted in a number of cases soon after administration of Viadril, but this hypotension was easily controlled by the use of small doses of a vasopressor in the cases where the pressure dropped lower than was considered safe or where it failed to return spontaneously within a few minutes. DeBon concluded that Viadril was a true anaesthetic agent and that the more satisfactory results were obtained when administering the drug together with nitrous oxide (75%) and oxygen (25%). The administration of Viadril was reasonably safe, and it possessed a high therapeutic index.[28]

Further studies[29] revealed that, on injection of hydroxydione cases of post-anaesthetic thrombophlebitis were so common, that this was a severe drawback. The delayed onset of not only anaesthesia, but also any side-effects was a second drawback. These two disadvantages meant that hydroxydione (Viadril) was unsuitable for clinical use. Several possible ways of overcoming the problem of venous thrombosis were discussed by Robertson and Wynn-Williams,[29] but none were satisfactory. Thus the problem was never overcome and, as inhalation anaesthetics improved, so hydroxydione (Viadril) became obsolete.

Robertson and Wynn-Williams[29] suspected that some metabolites of hydroxydione could be responsible for much of its anaesthetic activity. There was particular interest in those which had a hydroxyl group at either end of the molecule in place of the ketone group and the succinate moiety of hydroxydione. Though they were

potent anaesthetics they were not water-soluble. If clinically, such hydrogenation and hydrolysis were necessary before an active compound appeared, this would explain the delayed onset of action of Viadril. It would also explain the relatively low potency of Viadril compared with later steroid anaesthetics, because it was unlikely that such hydrolysis would produce a 100 percent yield of active steroid.

After hydroxydione anaesthesia two pregnandiol-like metabolites (allopregnan-e-diol and pregnanediol) were seen in the urine. Pregnanediol was known to be the most important excretion product of progesterone in urine. Ylinen[30] examined the excretion of urinary pregnanediol in a series of climacteric and postclimacteric gynaecological patients before and after operations performed under steroid anaesthesia. The conditions of anaesthesia used were as reported by DeBon *et al.*[28] Urinary pregnanediol was determined in a series of gynaecological operations performed with one gram of hydroxydione as basal anaesthetic, according to Jensen's method.[31]

Twenty-four hour collections of urine were made before the operation, and similar collections were performed after the operation.

According to Klopper *et al*,[32] the average daily output of pregnanediol in males was 1.11mg, and post-menopausal women excreted an average of 0.60mg/24 hours. After intravenous administration of hydroxydione there was a marked rise in the urinary pregnanediol (approx. 20-85mg/24 hours). This increase was most prominent during the first four hours of the anaesthesia. After the second postoperative day, the pregnanediol excretion was not seen to increase as an effect of hydroxydione.

The pronounced rise in the excretion of pregnanediol after administration of hydroxydione was connected with the metabolism of the steroid. Considering the

central role which was known to be played by progesterone in the biosynthesis of the steroids, it was apparent that the increase in the pregnanediol excretion after administration of hydroxydione was connected with the increase of progesterone at some stage in the metabolism of hydroxydione. Klopper *et al* stated "all the biosynthetic pathways lead through progesterone".[32]

In corresponding control cases with other forms of anaesthesia, no such rise in urinary pregnanediol was present.[32]

Pauling[33] divided general anaesthetics into two classes; those capable of hydrogen bond formation, such as barbiturates, steroids and aliphatic alcohols, were regarded as specifically inhibiting the processes supplying energy for the maintenance of cerebral electrical activity. Whilst those incapable of hydrogen bonding, for example the simple gaseous anaesthetics, were postulated to depress the electrical oscillations of the brain through hydrated microcrystal formation.

Ahmad *et al*[34] reported that there was an absence of anaesthesia in alcohols (eg. terpenoid hemisuccinates) possessing molecular weights between those of simple aliphatic alcohols and those of the anaesthetically active steroids. The results supported the conclusions of Figdor *et al*,[35] that the steroidal general anaesthetics displayed a high degree of structural specificity.

Coupled with Pauling's observations,[33] these results suggested that anaesthetics capable of hydrogen bonding acted *via* a specific mechanism and that anaesthetic activity was a result of retention of affinity for the receptors, *i.e.* from a direct stimulant action.

In 1963, Brown and Sarett[36] prepared a number of functional derivatives of the anaesthetic steroids, pregnan-3α-ol-20-one and pregnan-3α-ol-11,20-dione. The

synthesis of two of the more interesting compounds, namely the 3-phosphate ester of pregnan-3α-ol-20-one and 21-carboxypregnan-3α-ol-11,20-dione is described.[36] It was thought these could conceivably be hydrolysed to the parent sterol *in vivo,* since phosphate esters of 21-oxy steroids were known to possess the antiinflammatory activity of the parent steroid,[37] and the solubilizing carboxy group would be removed *in vivo.*

The phosphate ester of pregnanolone was prepared by the addition of pregnanolone to phosphorous oxychloride, followed by hydrolysis in dilute acid. The 21-carboxy derivative of 11-ketopregnanolone was obtained *via* the oxalyl derivative.[36]

Intravenous administration of the 3-phosphate ester of pregnanolone to dogs at a dose of 10mg/kg, produced no anaesthesia. Similarly, administration of the sodium salt of 21-carboxy-11-ketopregnanolone, at a dose of 10mg/kg (11-ketopregnanolone equivalent) resulted in only a transient sedation with no measurable anaesthesia.

Grassi and Raimondi[38] reported that steroid anaestheia had no effect on the vaginal cytology of the newborn female child after operative delivery. This was confirmed by the detection of the urinary 17-ketosteroids, which also remained normal.

In 1964, Atkinson *et al*[39] reported on the results of studies on mice of the hypnotic (and other) effects on the central nervous system of 168 steroids (142 pregnane derivatives and 26 other steroids), 149 of which had not previously been tested in this way. Earlier Witzel had summarized similar results of 124 steroids.[40] The term 'hypnotic' was preferred to 'anaesthetic', because some steroids induced light sleep in mice, without reaching surgical anaesthesia.

Atkinson used male mice, which were injected intravenously with the steroids at 1 percent concentration either dissolved in water or suspended in saline.[39] When hypnosis resulted the times between the injection and loss of the righting reflex *'the induction time'*, and between the loss and recovery of the righting reflex *'the sleep time'*, were recorded. For some steroids the intravenous LD_{50} values were determined. It was also usual to determine the AD_{50} *i.e.* the dose which caused half the mice to lose their righting reflex. However Atkinson determined the dose that induced sleep for 25 minutes for the anaesthetically active steroids.[39]

The steroids mentioned within the report of Atksinson *et al*[39] were synthesized according to Cocker *et al.*[41]

Ten of the 63 5α-pregnanes and 52 of the 76 5β-pregnanes produced hypnosis. The three 5-unsaturated pregnane derivatives tested were inactive. Thirteen 5-epimeric pairs of pregnane derivatives were tested, but no hypnotic 5α-steroid was encountered with an inactive 5β-epimer.

The results of Atkinson *et al*[39] were as follows:

3-Hydroxypregnanone derivatives. The 3-hydroxy-5-pregnan-20-one series and their 11-oxo derivatives were tested. 3α-Hydroxy-5β-pregnan-20-one was found to be as potent as the most active steroid tested thus far, its 11-oxo derivative was only half as potent as the parent steroid. The high hypnotic activity of 3α-hydroxy-5β-pregnan-20-one has previously been reported by Witzel.[40] The 3-esters of hypnotic steroids were seen to be in general, less potent than the free alcohols, as previously stated by Figdor et al.[35] The 3α-hydroxyl steroids had short induction times, while esterification was seen to lengthen induction times. It was concluded that the most potent and rapidly acting compounds of this group were those with a free 3α-hydroxyl, whatever the configuration at C(5).

Substituents on the steroid nucleus found to result in a loss of hypnotic activity were 16-methyl, 12α-acetoxyl, 17α-hydroxyl or -acetoxyl, 17α-hydroperoxyl, 16α-hydroxyl, and the 16α,17α-epoxide.

The 20-ethylene ketal was almost as potent a hypnotic as its parent, although it had an increased induction time and was almost twice as toxic. A 9(11)-ethylenic bond appeared to introduce weak hypnotic activity, whilst 16,17-unsaturation abolished any hypnotic activity present in the parent steroid. Halogenation at C(21) either decreased or abolished hypnotic activity.

Pregnanedione derivatives. 5β-Pregnane-3,20-dione was a potent hypnotic, but it had a long induction time. Its 11-oxo and 11(12)-unsaturated derivatives were less potent. 6α-Methylation abolished any hypnotic activity.

5α-Pregnane-3,20-dione and its 11-oxo derivative caused convulsions, whilst its 6α-methyl and 1(2)-unsaturated derivatives were inactive.

21-Hydroxypregnanedione derivatives. 21-Hydroxy-5β-pregnane-3,20-dione had the same hypnotic activity as the most potent of the series, 3α-hydroxy-5β-pregn-an-20-one. Its induction time was lengthy. Although an unesterified 3-hydroxyl usually conferred rapid induction, this property was not shared by the 21-hydroxyl or 3-oxo derivatives.

Some of the hypnotic activity of 3-oxo steroids was thought to result from their metabolic transformation to active 3-hydroxy steroids.[35] It was shown that hydroxydione was metabolised in human subjects to 3α-hydroxy steroids, while 3β-hydroxy steroids were not found.

The phosphate ester of 21-hydroxy-5β-pregnan-3,20-dione was found to be almost as active and about half as toxic as hydroxydione. The 11-oxo derivative of 21-hydroxy-5β-pregnan-3,20-dione was found to be inactive, as was the 21-acetate, although the 21-succinate had a moderate potency.[39]

The succinate esters of the 17α-hydroxy derivatives of 21-hydroxypregnanedione and the analogous trione were found to be inactive.

3,21-Dihydroxypregnanone derivatives. Isomerism at C(3) and C(5) gives four possible structures for 3,21-dihydroxypregnan-20-one. Some of these and some of their 11-oxo derivatives, as both free alcohols and esters, were examined.[39]

3α,21-Dihydroxy-5β-pregnan-20-one was almost as active as the corresponding 3-oxo and 21-deoxy compounds, 21-hydroxy-5β-pregnan-3,20-dione and 3α-hydroxy-5β-pregnan-20-one. Its induction time was between theirs. The 21-acetate was only half as potent as the parent steroid, but its induction time was halved. The 3β-isomer was less hypnotic than the corresponding 3α-isomer.[39]

The 11-oxo derivative of 3α,21-dihydroxy-5β-pregnan-20-one was inactive, like its 3-oxo counterpart, whereas the 3-acetate was weakly active with a long induction time.[39] The 21-acetate of the 11-oxo series had a short induction time and was more potent than its 3-oxo analogue, but not as potent as its 11-deoxy derivative. The 3,21-diacetate of the 11-oxo series was too toxic for adequate testing, although it was clearly hypnotic. The 21-succinate and 21-phosphate were weakly hypnotic with long induction times. The more potent, water-soluble, 21-ester had a short induction time, indicating that it dissolved rapidly in the bloodstream. It was possible that the 11-oxo derivative was too polar to pass readily into the brain, and that its 21-succinate and -phosphate had little hypnotic activity because they too were too polar, or because they were readily hydrolysed in the bloodstream. The

less polar, less readily hydrolysed 21-esters may pass as such into the brain, there to act by virtue of their unesterified 3α-hydroxyl groups.[39]

11-Substitution. Of 26 pairs of steroids tested, in only three had the 11-oxo steroid a greater hypnotic activity than the 11-unsubstituted steroid. Induction times tended to be shorter with the 11-oxo steroids. The therapeutic indices (LD_{50}/25 min. sleep times) of 11-oxo steroids tended to be higher than their 11-deoxy analogues.[39]

11-Hydroxy or -acetoxy and 9,11- or 11,12-epoxy substitution diminished or abolished any hypnotic activity of the parent steroids. Water-soluble 11-hydroxy steroids were less toxic than their 11-deoxy counterparts.[39]

From the studies,[39] 3α-hydroxy-5β-pregnan-11,20-dione 3-phosphate disodium was considered promising as an intravenous anaesthetic, as it formed stable aqueous solutions, had a high therapeutic index and did not produce thrombophlebitis in experimental animals, as hydroxydione did. It was used in man as an intravenous anaesthetic, but produced an unpleasant paraesthesia in the neck and spinal area. Although this symptom ceased spontaneously after a few minutes, and did not recur with subsequent doses, it was considered sufficiently serious to preclude further use of the steroid.

Of the 26 other steroids that were tested some produced convulsions, but none had hypnotic activity.

It was well known that the activity of the enzyme systems located in liver microsomes, which oxidize drugs, is influenced by such factors as sex, age, species, and the administration of drugs and other foreign compounds.[42] It was also known that hepatic microsomal hydroxylation of steroid hormones is also

influenced in an identical manner by these factors.[43] The chronic administration of several drugs and insecticides, stimulated the activity of steroid hydroxylases in liver microsomes.

Kuntzman *et al*[44] showed that the chronic administration of drugs and insecticides which induced the synthesis of oxidative enzymes in liver microsomes, decreased the anaesthetic action of steroids by increasing the *in vitro* hydroxylation of steroids by liver microsomal enzymes.

Male rats were treated with drugs or insecticides such as phenobarbital, chlorcyclizine, phenylbutazone, chlordane or DDT (which had been shown to markedly stimulate liver microsomal enzyme activity[42,45]) for 4 days.[44] On the fifth day the rats received an intraperitoneal injection of the steroid (progesterone, androsterone, Δ^4-androstene-3,17-dione, and deoxycorticosterone were used) and the duration of the anaesthetic effect was determined, using the loss of righting reflex as a measure of anaesthetic action. None of the steroids caused a loss of righting reflex in animals pretreated with a drug or insecticide.

Kuntzman suggested that the decrease in effect of the administered anaesthetic steroid may have been due to its increased rate of metabolism *in vivo*, which would have resulted in lower steroid levels in the brains of the chronically treated rats.[44]

In 1967, Heuser[46] discovered that in high doses, some steroids caused convulsive activity in animals, which may cause behavioural abnormalities such as sedation, hallucination, and tranquillisation. Heuser looked closely at the sleep induced by the steroids progesterone and hydroxydione, and the barbiturate pentobarbital. One stage of normal sleep that could not be distinguished from wakefulness is named *paradoxical*. Features of paradoxical sleep in cats include; twitching of

whiskers, ears, paws, and tail; irregular respiration; irregular heart rate; REM; and an increase in brain temperature. Pentobarbital was found to significantly reduce, if not eliminate, paradoxical sleep. Oswald discovered that in man, upon withdrawl of the barbiturate, a 'rebound' occurred during which the subjects spent an excessive percentage of sleep in the paradoxical stage, which often led to nightmares.[47] This might be one explanation for the fact that patients resist withdrawal of barbiturates.

Heuser reported[46] that progesterone induced natural sleep with no complete reduction in the paradoxical phase, and with no 'rebound' after withdrawal. When administered in sub-anaesthetic doses, progesterone was found to facilitate the occurrence of natural sleep. Heuser suggested the term *"hypnaesthetic"* for progesterone, as it referred to both the hypnotic and anaesthetic activities of the steroid.[46]

Hydroxydione was also found to reduce the occurrence of paradoxical sleep, but was less striking than that seen after pentobarbital, when administered in water intraperitoneally and intravenously. When anaesthetic doses were given paradoxical sleep was eliminated altogether.[46]

Heuser suggested that a steroid which induced natural sleep (without reduction of paradoxical sleep during, and without 'rebound' after treatment) would be a therapeutic agent in all situations of uncomplicated sleeplessness.[46]

Spironolactone[48] and norbolethone,[49] an anabolic steroid, have been shown to possess antianaesthetic properties in inhibiting the anaesthetic and sedative effects of pentobarbital, methylprylon and various steroid hormones or hormone derivatives.

In 1970, Selye[50] carried out comparative experiments with many steroids to determine their influence upon anaesthesia produced by progesterone or pentobarbital. Rats were pretreated with various steroids (detailed in the paper[50]) twice daily for four days prior to, and on the day of the anaesthesia test. Pretreatment with various steroids was seen to protect the rat against the induction of anaesthesia by progesterone or pentobarbital.

There was no manifest relationship between the chemical structure and the antianaesthetic effects of the steroids tested. Among the active compounds were anabolic androgens, androstane, pregnane, gonane, and estrane derivatives, with or without ring unsaturation. There was also no clear-cut relationship between the antianaesthetic and other pharmacological actions of the compounds tested.[50]

Many of the compounds given as a pretreatment to inhibit anaesthesia were themselves anaesthetics, if administered for the first time. Thus it appeared that the anti-anaesthetic effect was an independent action of steroids, not referable to any of their other known hormonal actions.[50]

The mechanism of the antianaesthetic action was unclear. Almost thirty years ago Selye discovered that the anaesthetic effect of progesterone was more pronounced in female than male rats.[4] These findings suggested that even the physiological amounts of anabolic androgens secreted by the testis, could significantly influence resistance to steroid anaesthesia. Hence, Selye showed that the resistance of females could be raised by a variety of anabolic steroids.[51] Several days of pretreatment was necessary to obtain any of the antianaesthetic effects. Recent work performed by Selye[51] showed that pretreatment *in vivo* with the steroids increased the oxidation of pentobarbital by isolated hepatic microsomes *in vitro*. Hence, it was thought that at least some protective effects of steroids were due to activation of hepatic microsomal enzymes.

After the demise of hydroxydione and its analogue, 3α-hydroxy-5β-pregna-n-11,20-dione 3-phosphate disodium, Glaxo left the topic of steroidal anaesthetics for several years. They returned to it late in 1965, with the objectives of finding a water-soluble steroid with the wide margin of safety, and pleasant induction and recovery, found with hydroxydione. Such an anaesthetic should combine the advantages of hydroxydione and the barbiturates, without their disadvantages. The screening programme centred on steroids with a free 3α-hydroxy group present.

Of the many compounds tested by intravenous injection into mice, the most potent was 3α-hydroxy-5α-pregnan-11,20-dione, *alphaxalone.* The anaesthetic potency of alphaxalone was high and induction rapid.[55] Mice receiving a dose as low as 1 to 2mg/kg lost their righting reflex within a few seconds of injection. The safety margin was wide and there was no evidence of thrombophlebitis. Alphaxalone, being insoluble in water, was best administered in the biologically-acceptable media, *cremophor EL* (polyoxyethylated castor oil), the non-ionic surfactant previously employed in the non-steroid intravenous anaesthetic, propanidid. The solubility of alphaxalone in 20 percent cremophor EL, though one hundred times its solubility in water, was still only 3mg/kg, and this was not considered sufficient. Fortunately, the addition of a small amount of 21-acetoxy-3α-hydrox-y-5α-pregnan-11,20-dione, *alphadolone acetate,* increased the solubility of alphaxalone in cremophor EL more than threefold. Alphadolone acetate had anaesthetic properties similar to alphaxalone, although it was only half as potent, and simply additive with alphaxalone.[55]

Althesin was formulated by Glaxo as a 3:1 mixture of alphaxalone and alphadolone acetate respectively, dissolved in 20 percent cremophor EL and made isotonic with blood using sodium chloride.

The pharmacological properties of althesin in animals were reported by Child *et al*[57] in 1971.

Davis and Pearce carried out experiments to show the similarities and differences between althesin, hydroxydione, thiopentone, methohexitone, propanidid and ketamine in animals.[53]

The anaesthetic dose 50 (AD_{50}) and lethal dose 50 (LD_{50}) for althesin and other intravenous anaesthetics were determined and shown in Table 4.

Anaesthetic agent	AD_{50} mg / kg	LD_{50} mg / kg	Therapeutic index (LD_{50} / AD_{50})
Thiopentone sodium	13.2	90.5	6.9
Methohexitone sodium	5.4	39.4	7.4
Propanidid	22.9	185.0	8.1
Ketamine hydrochloride	12.7	108.0	8.5
Hydroxydione	18.0	311.0	17.3
Althesin	1.8	54.7	30.4

Table 4. Anaesthetic activity and lethal dose of intravenous anaesthetics in mice.

From *Postgrad. Med. J. Suppl.*, 1972, **48**, p.13, Davis and Pearce.

The safety margin with steroidal anaesthetics was found to be much wider than with other intravenous anaesthetics, as was confirmed by althesin and hydroxydione.[53]

After studying the effects of repeated doses it was discovered that althesin had a desirable freedom from a cumulative effect, whereas thiopentone was very cumulative and ketamine moderately so. This was confirmed using cats, in which althesin was infused at a constant rate for several hours to maintain stable anaesthesia.[53]

The effects of acute liver damage on anaesthesia were studied after dosing with thioacetamide.[53] The duration of althesin anaesthesia was slightly increased by pretreatment with thioacetamide. The metabolism of normal doses of althesin was well within the capacity of the liver enzymes and was little affected by liver damage.

Local vascular effects were also studied *via* the appearance of mice tail veins.[53] Twenty-four hours after injection the tails of mice given hydroxydione were swollen and blue-black over their entire length. The tails of mice given althesin were indistinguishable from those given saline. They appeared normal except for slight bruising at the site of injection. Hence, Althesin was free of thrombophlebitis and paraesthesia.

Any problems encountered with the use of althesin were most likely due to cremophor EL. Cremophor EL was unsuitable for use in dogs as it caused the release of histamine or histamine-like substances in this species.[54]

Surgical procedures were performed in the cat under althesin anaesthesia alone.[10] Althesin could also be used as an induction anaesthetic followed by conventional inhalational anaesthetics, and used in conjunction with pre- and post-operative medicaments. Clinical trials in man and other animals confirmed the authors optimism in althesin.[53] However, althesin was found to be highly toxic to newborn animals.[54]

A conference on the 'Steroid Anaesthesia of Althesin' covered its pharmacology, clinical pharmacology, and clinical assessment (Postgraduate Medical Journal, June 1972, Supplement 2, Volume 48). Sutton[55] described a brief history of steroid anaesthesia before althesin, in 1972.

At the beginning of the 1970's Glaxo intensified their research in the area of novel steroidal anaesthetics. They set about identifying steroids more active, less toxic, and giving even better quality of anaesthesia than althesin. More than a thousand steroids were prepared and tested, *via* the intravenous route, in male mice, given as solutions or suspensions in 20 percent cremophor EL, unless they were water-soluble.

The findings were described by Phillipps,[56] in 1974, in an excellent report on the structure-activity relationship (SAR) in steroidal anaesthetics. Phillipps reported on analogues of 3α-hydroxy-5α-pregnan-11,20-dione (alphaxalone). His findings are briefly reported hereafter.

Unlike the 5β-series, the 3-keto-5α-steroids were generally inactive. Introduction of a 3β-methyl group into alphaxalone converted the compound from a secondary into a tertiary alcohol; thus preventing oxidation to the 3-ketone *in vitro* and also slowing the rate of formation of conjugates of the 3α-hydroxy group and therefore, inactivation. However, the activity and duration were virtually unaffected.[56]

Introduction of extra hydroxyl groups at the 2α, 4β, 7β, 9α, 16α, 16β, or 17α-positions produced inactive compounds, and at the 2β- or 21-positions less active compounds. Compounds lacking the 3α-hydroxyl, but with a 2α, 2β, or 3β-hydroxyl were inactive, and introduction of extra oxo groups at the 7 or 16-positions also destroyed activity.[56]

11-Desoxy compounds were in general, poorer in activity than the 11-ketones. Introduction of oxo groups at the 6- and 12-positions removed activity completely, and at the 7-position considerably reduced it. Potency was also lessened by 11α, 11β, or 21-hydroxyl groups and eliminated by a 19-hydroxyl.[56]

Phillipps included tables showing the relative activities of the steroids tested.[56]

It was thought that the effect of substitution at the 2-position was of particular interest because in the rat, alphaxalone was metabolized in part to the inactive 2α-hydroxy analogue[57]. However, a 2α-methyl group which may block 2α-hydroxylation did not affect activity, but the introduction of a 2α-bromine atom reduced activity. The 2β-morpholino compound, previously described by workers at the Organon Laboratories,[58] was considerably less active than alphaxalone with about the same toxicity. A fluorine substituent also had a detrimental effect on activity, although the larger halogens, chlorine, bromine, and iodine, or a thiocyanato group, all gave products with good activity. The chloro compound being active at lower concentrations than alphaxalone. Although substitution with a 2β-hydroxyl gave a weakly active-compound, esterification of the 2β-hydroxyl as the acetate or propionate, almost completely regenerated the activity, indicating that the esters were acting as esters and were not readily hydrolysed. The 2β-ethers were particularly active, and gave longer sleep times at 3.1 mg/kg than alphaxalone. The best compounds were the ethoxy and *n*-butoxy derivatives. The *i*-propoxy compound was less toxic than the *n*-propoxy compound and activity was even retained in the presence of the bulky *t*-butoxy substituent. The 2β-methyl derivative was twice as active as alphaxalone, and the 2β-*n*-butyl derivative gave even longer sleep times, but was more toxic.

Substitution at the 4β-position with a wide variety of groups, gave compounds almost devoid of activity.[56]

Introduction of double bonds into ring A reduced toxicity and gave useful activity with 3α-hydroxy steroids. The introduction of a 5,6-double bond was less satisfactory, an 8,9-double bond reduced activity somewhat, a 14,15-double bond more so and a 16,17-double bond completely.[56]

Phillipps speculated that the conformation, that iis shape, of ring A may be of importance.[56] In particular, the shape of the ring would determine the manner in which the 3-hydroxyl group, with its essential oxygen atom, projected from the molecule. Phillipps surmized that ring A in the 'α-boat' conformation was an active conformation, and when it is in the 'β-boat' conformation it is inactive. He included an excellent discussion on the various possible conformations of ring A. The 2β,19-oxide was prepared, in order to hold ring A in the chair form. As he forecasted, this compound was inactive.[56]

Removal of the angular methyl group from C(10) in alphaxalone gave the corresponding 19-nor compound, which was just as active as alphaxalone but less toxic. Therefore, Phillipps prepared the 2β-ethoxy-19-nor steroid,[56] in which there could be no 1,3-diaxial repulsion between the substituent on C(2) in the absence of the angular methyl on C(10). As predicted there was no gain in activity, instead there was a loss. The 2α,5α-oxide in the 11-desoxy series was inactive,[56] perhaps because the oxide bridge projected on the α-face of the molecule, thus blocking it.

Turning to the other end of the molecule, substitution at the 21-position was rewarding.[56] The 21-hydroxy compound and its acetate (alphadolone acetate) both retained moderate activity. The 21-thiol was only active at toxic doses, whereas the acetylthio derivative was more active than alphaxalone, and no more toxic. 21-Alkoxy compounds showed reasonable activity, as did compounds with an extended alkyl side chain. The 21,21-ethylidene derivative was as active as alphaxalone, but less toxic. Fluorination at the 21-position had little effect on activity, but did enhance solubility in 20 percent cremophor EL.

A 21-cyano substituent reduced the activity and the compound was weakly acidic. However, it was sufficiently acidic to give a sodium enolate, soluble in water with

fair stability. The aqueous solution of the sodium salt had virtually the same activity as did the parent compound in 20 percent cremophor EL.[56]

A series of compounds with a basic nitrogen atom, to give water-soluble salts within the 2β- or 21-substituent were prepared.[56] The 2β-piperidinoacetate compound showed instantaneous induction of anaesthesia but was much less active than the corresponding 2β-acetoxy compound.[56] Its citrate was water-soluble and showed similar potency. The 2β-morpholinoethoxy compound was less active than the 2β-ethoxy analogue. The 21-morpholinoacetylthio derivative proved to be the first potentially water-soluble compound with activity comparable to that of alphaxalone. It showed similar activity in aqueous solution as its hydrochloride.[56]

Direct substitution at the 21-position with various amines gave a more stable group of compounds.[56] Those with a free NH group were inactive. The most promising compounds were those substituted by heterocyclic amines, for example, morpholine or thiomorpholine.[56] Substitution with a 2β-ethoxy group on the morpholino compound doubled the activity.

All the compounds thus far had been pregnan-20-ones, *i.e.* they had a 17β-acetyl side chain. Compounds with a 17α-acetyl side chain showed, at best, weak activity.[56] Reduction of the 20-carbonyl to a 20α or 20β-hydroxyl group destroyed activity, and complete reduction to give the 17β-ethyl compound resulted in only weak activity.[56]

Insertion of an oxygen atom between C(17) and C(20) gave the inactive 17β-acetoxy compound, but insertion of an oxygen atom between C(20) and C(21) reduced potency only slightly.[56] This compound was the methyl ester of the 17β-carboxylic acid, the acid itself was inactive. Other esters and thiols also showed activity.[56] The dialkylamides of C(17) were active but monoalkylamides

were less so, and the amide was inactive. Dehydration of the amide group gave the nitrile, which was very close in activity to alphaxalone.[56] The esters of the carboxylic acid at C(17) also provided water-soluble derivatives with good stability in aqueous solutions. The morpholino-ethyl ester showed fair activity which was maintained in compounds with larger alkyl chains in the ester group. Further introduction of a 2β-ethoxy group improved the activity. This was retained in aqueous solution of the citrate and other salts, such as the hydrochloride lactate, methanesulphate, ascorbate, or phosphate. The ester of morpholino-ethanethiol also gave an improved activity.[56]

Phillipps[56] confirmed the essential nature of an oxygen at the 3α-position, and also the importance of the exact manner it projected from ring A. He also concluded that it was still difficult to forecast activity with any accuracy even after testing many combinations of groups. The effects of the substituents on the steroid nucleus were not simply additive.[56] For example, a substituent in the 2β-position, which was normally favourable, produced a very poor activity in the presence of a particular side chain in the 17-position. Hence, Phillipps was unable to predict activity from structure, outside small groups of related compounds.

All of the previous work was carried out without much knowledge of the mechanism of steroidal anaesthesia. Thus, various research groups attempted to explain this particular action of anaesthesia.

It was generally accepted that liposomes provided realistic and valuable models for biological membranes. Connor *et al* demonstrated the membrane disordering effect of a series of anaesthetics related to, and including alphaxalone, by measuring the increases in the release rate of sequestered sodium ions from sonicated egg lecithin liposomes.[59] Flame photometry was used to measure the release rates of sodium. Steroid was added both as a solid and in ethanolic solution, in the sodium release

experiments.

Connor *et al* showed that steroids which possessed high anaesthetic activity, as measured intravenously in mice, generally showed the largest effect on the liposomes in terms of increased sodium release (20-35% enhancement).[59] Little or no effect was shown by anaesthetically inactive steroids or steroids of low activity, whilst steroids of intermediate anaesthetic potency had corresponding intermediate effects on the liposomes. The steroid 5α-pregnan-3,11,20-trione, which was not an anaesthetic but a convulsant, showed inhibition (2-3%) rather than enhancement of sodium release.[59]

Thus Connor *et al* concluded that membrane labilization was an essential step in the operations of steroidal anaesthetics.[59]

The introduction of althesin in England prompted a review of steroidal anaesthetics by Gyermek and Soyka,[60] in which pregnanolone and althesin were discussed in detail.

Steroid anaesthetics and short-acting barbiturates appeared to have the same or very similar electrical effects in the central nervous system, although electrophysiologic and neuropharmacologic evaluations of steroid anaesthetics had been far less extensive than those of the barbiturates or inhalational anaesthetics. Rosner and Clark summarized the neurophysiological effect of anaesthetics.[61]

A unique side effect of 5β-steroid hypnotics in contrast to the 5α-analogues, was thermogenesis, with the effect only being apparent in man. This was a major obstacle to the clinical use of many potent 5β-steroids.[61]

Althesin had been found to have no adverse effects in cats premedicated with a

variety of drugs frequently given to patients, including parasympathetic blocking agents, opiates, tranquilizers, and neuromuscular blocking agents.[62] But it was found to be highly toxic to the foetus, when given to pregnant mice in labor, and also caused increased toxicity to newborn rats.[62] Small doses (1-2 mg/kg) produced minor changes in the blood pressure of animals, followed by a moderate pressor effect on emergence from anaesthesia.

The metabolism of alphaxalone, the major active ingredient of althesin, was investigated in rats.[57] The compound had a very short half-life in plasma following intravenous administration. About 60-70 % of an injected dose was excreted within five days, mostly in the form of metabolites in the faeces. Urinary excretion accounted for the remainder. Glucuronidation was an important step in the metabolism of alphaxalone. Radioautographic studies in the rat indicated that alphaxalone was widely distributed in the tissues one minute after intravenous injection.[63] No selective uptake in the brain occurred. Soon after administration radioactivity was present predominantly in the excretory organs, *eg*. liver, and after an hour the bulk of the steroid (or its metabolites) was found in the gut and bladder. In pregnant rats relatively small amounts reached the foetus.[57]

When studied in the horse and sheep althesin was shown to elicit excitatory symptoms.[64] Althesin anaesthesia in Landrace pigs, which were susceptible to hyperthermia under general anaesthesia, gave no hyperthermic effect and was seen to prevent muscle rigidity and hyperthermia.[64] Thus the author suggested that althesin might be a useful anaesthetic for patients known to be liable to develop hyperthermic reactions.[64]

Gyermek and Soyka[60] also discussed the results of studies of althesin in man. The minimal effective dose was 0.6-0.7 mg/kg of the two active ingredients, which was equivalent to 4 mg/kg thiopental, and produced sleep in less than 60 seconds with a

duration of 1.5 to 13 minutes.[65] The cardiovascular effects of althesin in man usually consisted of a moderate increase in pulse rate and a decrease in systolic and diastolic pressure.[66] Cardiac output was either slightly increased or remained unchanged.[67]

Prys-Roberts *et al*[68] did not consider althesin advantageous for hypertensive patients. DeCailar[69] reported that althesin produced smaller decreases of blood pressure than other intravenous anaesthetics. An additional advantage of this agent for the cardiovascular system was its antiarrhythmic effect, which was demonstrated in man.[70]

Althesin has since been used successfully for neurosurgical procedures.[71] Following intravenous administration, althesin usually produced brief respiratory depression.[65,72] Additional potential advantages of althesin in clinical anaesthesia included a transient decrease of intraocular pressure in man following its administration,[73] which made it suitable for intraocular procedures. Althesin had no analgesic potency and was thought to share the same type of antianalgesic action as the barbiturates.[65,74]

Other side effects of althesin could be placed in two categories; those that occur on first administration and those that usually manifest following previous administration, and are of an allergic nature. The common clinical side-effects were involuntary muscle movements, twitchings and tremors, hiccupping, and respiratory and circulatory depression with large doses.[75] Hypotension, erythematous rash, anaphylactoid reactions with bronchospasm and sometimes severe cardiorespiratory collapse may occur in sensitized patients.[76] Studies indicated that cremophor EL was the offending sensitizing agent.

In general, althesin compared favourably with other inducing agents with respect

to such side-effects as nausea, vomiting, and even local irritation.

The advantages of althesin-like steroidal anaesthetics were deemed to be; 1) minor cardiovascular and respiratory depression, 2) wide therapeutic index, 3) laryngeal relaxation, 4) minimal cumulative effect on repeated or continuous administration, 5) less chance of serious occlusive phenomena in the case of accidental intraarterial administration and, 6) the different metabolic handling of steroidal anaesthetics suggested that their use might be applicable in the presence of agents that can interact adversely with barbiturates, or in diseases where abnormal sensitivity to barbiturates may exist.

As previously stated, in rats male sex hormones or chronic treatment with massive doses of steroids, conferred resistance to the anaesthetic actions of steroids.[8,9] In 1975 Taché *et al*[77] examined the influence upon steroidal anaesthesia of various catatoxic steroids. Catatoxic steroids were characterized by their ability to protect against numerous intoxications independently of their specific hormonal actions.[78]

Taché *et al* found[77] that in rats, the sleeping time induced by overdosage with any of the eight steroid anaesthetics; althesin, 3-(3-oxo-17β-hydroxy-19-nor-4-androsten-17α-yl)-propioinic acid-lactone, 21-hydroxy-5α-pregnan-3,20-dione, 4-pregnan-3,11,20-trione, 17-hydroxy-3-oxo-4-androstene-17α-propionic acid-δ-lactone, 3α-hydroxy-5β-pregnan-11,20-dione, 5β-pregnan-3,11,20-trione and hydroxydione; was abolished or considerably reduced by a variety of catatoxic compounds, particularly 3β-hydroxy-20-oxo-5α-pregnan-16α-carbonitrile (PCN), 9α-fluoro-11β,17-dihydroxy-3-oxo-4-androstene-17α-propio nic acid potassium salt, prednisolone, ethylestrenol, and spironolactone. The non-steroidal microsomal enzyme inducers, phenobarbital and diphenylhydantoin, were also eminently efficacious. Of the two glucocorticoids trested, prednisolone unlike triamcinolone significantly reduced steroid anaesthesia. A possible explanation for

this could be that prednisolone, unlike triamcinolone, also exerted some catatoxic effects.[78] In contrast, estradiol, progesterone, desoxycorticosterone, and hydroxydione, which exerted little or no catatoxic activity, were devoid of antianaesthetic activity, and in some cases, even prolonged sleep times.

The above tests were carried out on female rats. To obtain an optimal catatoxic effect it was important to allow four days of pretreatment. The catatoxic compounds were administered twice daily at a dose level of 10 mg in 1 ml water and the sleeping times were calculated in minutes, as the period between the loss and return of the righting reflex.

Increased biliary flow[79] and hepatic microsomal drug-metabolizing enzyme induction[80] by catatoxic compounds was thought to accelerate the destruction and elimination of steroidal anaesthetics and probably accounted entirely, or at least predominantly, for the absence, or short duration of their action.

In the New Zealand Veterinary Journal, Evans[81] quoted *"saffan"* (Glaxo trade name for althesin) as a good anaesthetic for cats. An appropriate dose of saffan, administered intravenously, produced relaxation in about 9 seconds, and surgical anaesthesia in about 25 seconds, lasting for about 10 minutes. The anaesthesia could be deepened or lengthened, as requested, by supplementary doses of saffan, or by the use of inhalational anaesthetics. There was a good relaxation of abdominal muscles and respiration was well maintained. There were no lapses to deeper unconsciousness during recovery and, once recovered, the cat was alert and would usually eat shortly afterwards.

Results varied when saffan was given *via* intramuscular routes, but this could be minimized by ensuring that the drug was given by deep intramuscular injection, after which the anaesthetic was effective. Trials and extensive usage of saffan are

discussed in the literature.[64,82]

The side-effects encountered under clinical conditions were listed by Stock[83] as urination, defecation, muscle tremor, and salivation. These effects only occurred infrequently and were not considered to be of practical importance. Another side-effect observed, was hyperaesthesia, or edema, of the paws, ears and muzzle, in some cats following intravenous administration. It lasted for less than 2 hours usually, and was of no clinical significance nor cause for concern. Evans reported that the incidence of this effect could be dramatically reduced by the administration of an antihistamine 15 minutes before induction.[81] An appropriate dose of an antihistamine could also be used to shorten the duration of edema once it had occurred.

The use of premedicants such as the phenothiazine derivatives, slightly lengthened the duration of anaesthesia and recovery time.[81] Evans also stated that saffan must not be given after the administration of barbiturates.[81]

Holzbauer reported[84] on the possibility that several steroids secreted by the ovary which possessed strong anaesthetic potencies, fulfilled a physiological role as modulators of the activity of certain brain regions. When injected intravenously these steroids entered the brain rapidly. Pregnanolone, the most potent among them and a metabolite of progesterone, caused immediate anaesthesia, and reached its peak concentrations in the brain after 3 minutes. Thirty minutes later, at a time when anaesthesia was still deep, the brain concentrations were only about 2% of the peak concentrations. It appeared that on entering the brain some process was triggered off, which maintained the state of anaesthesia long after the steroid had been metabolized and removed.[84]

The effect of steroids on the electrical activity of the brain has been studied on

many occasions.[85] When pregnanedione was injected intravenously, into cats, an inhibition of the electrocortical arousal reaction, elicited by stimulating the mid-brain reticular formation, occurred. The actions of progesterone on the brain have been reviewed by Kramer, Damrosch, and Klink.[86] It was suggested that progesterone exerted its effect on neuronal activity indirectly through an increase in blood pressure.

The translocation, into the rat brain, of intravenously injected progesterone, pregnanolone, and pregnanedione in propylene glycol was studied by Raisinghani *et al.*[87] It appeared that the depth of sleep was not directly related to the concentration of pregnanolone, a metabolite of the injected progesterone responsible for its hypnotic effect, in the brain. The possibility of an interaction between the steroid originally injected and the pregnanolone formed was considered.[87]

The presence of endogenously secreted steroids in the central nervous system was first demonstrated by Henkin *et al.*[88]

The translocation of a steroid from the blood into the brain could be influenced by a number of factors. Bidder[89] found that oestradiol or phenobarbitone, when infused into one carotoid artery of the ether anaesthetized rat, significantly decreased the entry into the brain of simultaneously infused progesterone. It was difficult to assess the potency of a given steroid when it was secreted endogenously into the blood stream, as the vehicle was seen to affect the hypnotic potency of the steroids considerably. For example, after the intravenous injection of a suspension of 3α-hydroxy-5α-pregnan-20-one in 0.5% Tween 80 at a dose of 24 mg/kg, a mean sleeping time of 20 minutes was obtained.[89] When the steroid was dissolved in cremophor EL, the same sleeping time was obtained with a dose of only 6 mg/kg.[89]

Metabolism of steroid hormones in the liver to ring A reduced compounds which did not possess any conventional hormonal activities has long been known.[90] However, in the liver the metabolites were also esterified, which decreased their ability to penetrate the brain.[89] Hence it was unlikely that the steroid metabolites formed in the liver would effect the activity of the central nervous system considerably, unless they were present in large quantities. There are at least two other sites in the body in which ring A reduced pregnane derivatives, with oxygen functions at carbons 3 and 20, were formed and secreted, and from where they could reach the brain before being esterified in the liver, namely the ovaries and the adrenal glands.[89]

The effect of different pituitary hormones on the concentrations of pregnane derivatives in the ovarian venous blood of rats, has been studied.[91] Luteinising hormone (LH) caused a linear rise in the secretion of progesterone and allopregnanedione. No significant responses to LH were shown by any steroid with a 20α-hydroxyl group.[91] Human chorionic gonadotrophin (HCG) and pregnant mare serum (PMS) caused a considerable rise in the secretion of the 20-keto pregnane derivatives and also an increase of 20-dihydroprogesterone secretion.[91] Mason observed that ovaries from rats stimulated with PMS formed allopregnanediol as the major product from [^{14}C] progesterone.[92] The activity of the enzyme 5α-reductase was high in ovaries in the follicular phase and low in ovaries in the luteal phase.[92]

From observations made,[92] the secretion of allopregnanolone and allopregnanedione, like that of progesterone, is under the control of the pitiutary gland. The fact that the secretion of allopregnanedione and allopregnanolone fell, during pregnancy,[92] was probably due to the decreased production of LH at this time. The ovary, like the adrenal gland, does not store any appreciable quantities

of its secretion product.

As described,[84] Holzbauer showed that the ovary of the rat secreted considerable amounts of several steroids (which were known to possess high anaesthetic potencies) during the oestrous cycle, which may contribute to variations in behaviour. In the metoestrus, the quantities of anaesthetic steroids secreted within 24 hours was large enough to cause deep anaesthesia if injected intravenously, as a single dose, to a rat. Whether steroids with anaesthetic properties are secreted by the human ovary has yet to be investigated.

The enzyme 5α-steroid reductase, was also found in the adrenal gland of the rat,[93] the chick, and the guinea-pig.[94] It was observed that in *in vitro* experiments [^{14}C] pregnenolone, [^{14}C] progesterone, and [^{14}C] deoxycorticosterone when added to the incubation medium of rat adrenal section, were not only converted to corticosterone, 18-hydroxycorticosterone and aldosterone respectively but also to allotertrahydrocorticosterone, a ring A reduced metabolite of corticosterone.[93] The anaesthetic dose of the 5α-reduced metabolite of deoxycorticosterone was equal to that of thiopentone sodium, the 5β-metabolite was three times more active than the barbiturate.[93]

The occurrence of a 5α-steroid reductase, in the rat hypothalamus,[95] and the *in vitro* conversion of progesterone to pregnanolone by this tissue,[96] and the anterior pituitary gland[97] have been reported.

It is purely of interest that some water beetles (the German water-beetle and the Indian water-beetle) make use of the anaesthetic effect of steroids in their defence reactions. These beetles when attacked by fish release a milky fluid from their postoracic glands, which contains various steroids possessing anaesthetic potency. When added to water these steroids cause a reversible paralysis in goldfish.[98]

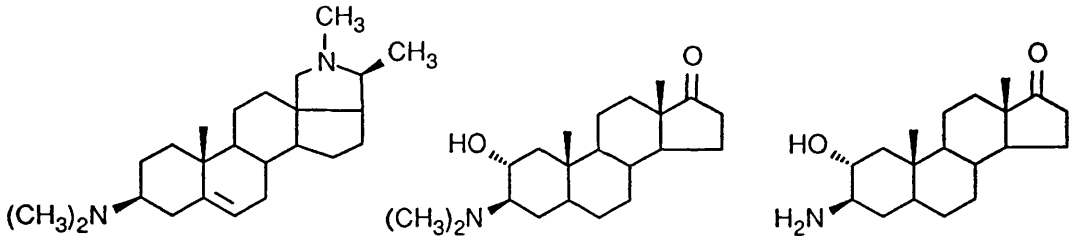
In 1976, Lee[99] presented a model for the mode of operation of local anaesthetics.[99] It was postulated that the sodium channel of the nerve was surrounded by lipid in a rigid state, with the rigidity keeping the sodium channel open.[99] Addition of local anaesthetics to the membrane, after binding to the lipid component of the membrane, lowered the transition temperature for the annular lipid, thus transforming it into a fluid state. When the lipid was in this fluid state, the sodium channel could collapse inwards, closing the pore and reducing the sodium ion conductance, and producing anaesthesia.[99]

Hydrophobic interactions between local anaesthetics and their sites of action were reported to be sterically undemanding.[100] The interaction was also reported to be undemanding with respect to charge, since neutral, positively and negatively charged molecules were all known to act as local anaesthetics.[100]

The model presented by Lee[99] sought a correlation between the concentrations of compound that produced a significant drop (3-4°C) in lipid transition temperature and the concentration that blocked the sodium conductance in the nerve. The compounds tested included alcohols, amines, barbiturates, chlorpromazine, β-blockers, trihexylphenidyl, and benztropine.[99]

In 1979, Lee extended his studies to the steroid molecules hydroxydione sodium succinate, conessine, alphaxalone, and the amino steroids, Org. NA13 and Org. 6001 (Figure 3) of which some showed local anaesthetic activity.[101]

Samples were prepared by dissolving lipid plus chlorophyll A and anaesthetic in chloroform (if water insoluble). The samples were evaporated to dryness, buffer



Conessine ORG. NA13 ORG. 6001

Figure 3. Some steroidal local anaesthetics.

added (plus steroid if water soluble) and then shaken. Fluorescence measurements were made and temperature continuously monitored. A theoretical model involving Langmuir adsorption isotherms for the binding of the drug were used to find the effect of the drugs on phase transition temperatures.[101]

It has been shown[102] that changes in the fluorescence of chlorophyll A incorporated into liposomes could be used to measure the temperature of lipid phase transitions.

The results showed that the amino steroids, Org. NA13 and Org. 6001, interacted with the lipid, dipalmitoyl phosphatidylcholine, and produced a decrease in the phase transition temperature.[101] A build-up of positive charge on the liposomes tended to limit the binding of the drug, and incorporation of negatively charged lipid increased the effect of the drugs, contrary to the suggestion of Ueda *et al.*[103] Org. 6001 caused a large decrease in transition temperature, at pH values at which it is predominantly in the charged form, thus both charged and uncharged forms of a drug were able to bind to a membrane.[104] In the cases of both the amino steroids, the concentration required to produce a 3 degree drop in transition temperature was about 1mM. These results fitted the annular transition model for local anaesthesia.[99]

Alphaxalone had no effect on the transition temperature of dipalmitoyl phosphatidylcholine,[101] in contrast with the results of Conner *et al.*[59] This lack of effect suggested that either alphaxalone was not a local anaesthetic, or that if it was

then its mechanism of action must be different to that of other local anaesthetics.[101] In fact, Rao and Wang reported[105] that alphaxalone showed no local anaesthetic action.

The general anaesthetic action of alphaxalone remained unexplained, but could be due to either a decrease in the amount of transmitter released by a nerve impulse at a synapse, or due to a decrease in the sensitivity of the post-synaptic membrane to the transmitter. Alphaxalone seemed to have some effect on membranes containing cholesterol and brought about a decrease in the upper transition temperature in this system, whereas Δ^{16}-alphaxalone had no general anaesthetic effect and no effect on the bilayers containing cholesterol.[101]

Lee[101] also showed that hydrocortisone had no effect on the phase transition temperature of dipalmitoyl phosphatidylcholine. Munck[106] suggested that hydrocortisone lies *"on edge"* at a water-heptane interface, in order to permit entry into the water phase of all its polar groups, including the C(11) hydroxyl. Adopting a similar orientation in the lipid-water interface would mean little interaction with the lipid fatty acyl chains, and thus have little effect on the phase transition temperature, as was observed.[101] A similar orientation could be expected for alphaxalone since the hydrophobic oxygen containing groups are spaced along one side/edge of the molecule.

Of the other steroids tested[101] pregnanedione and pregnenolone, also had no effect on the temperature of lipid phase transitions. Although these compounds have been reported to possess general anaesthetic properties[4] no studies have been made of any local anaesthetic activity. The lack of effect of these steroids on phase transition temperatures was not due to a failure to incorporate into the bilayers since they abolished the lipid pre-transition.[101]

Hempelmann and Schaps reported on yet further observations on althesin.[107] Since, in man the liver is the major site for the metabolism and the kidneys for the excretion of althesin, reduced doses were necessary in patients with severe liver damage and impaired renal function.[108] Althesin proved highly toxic when given to pregnant mice during labour,[60] however Hollmèn *et al*[109] could not find any significant difference in the clinical condition of newborns delivered from mothers receiving either thiopentone or althesin for caesarian section.

Althesin should also be handled with caution in patients suffering from thrombotic disease and hypercoagulability, as Gaszynski *et al*[110] found that althesin brought about a selective action of platelet release from systemic stores.

Carbohydrate and fat metabolism in man was not significantly influenced by althesin and Mehta and Burton[111] suggested that it may be a useful alternative in diabetic subjects. Also Duval and Gaudy[112] reported that althesin was probably not the drug of choice in patients with a history of allergy and in those with asthma.

The effects of the steroid anaesthetic on cerebral circulation have been investigated by Pickerodt *et al.*[113] In man there was a fall in intracranial pressure, which was interpreted as a result of a reduction in cerebral blood flow and cerebral blood volume.[114]

Hempelmann and Schaps[107] reported on the effects of althesin on the cardiovascular system. Child and co-workers[115] reported on a non-dose related transient fall in blood pressure and a slight tachycardia in cats following injection with althesin. According to the results of Kettler and Sonntag[116] the haemodynamic effects of althesin were characterized by a peripheral vasodilation and a moderate fall in blood pressure. A subsequent increase in heart rate was

interpreted as a reflectory mechanism, causing an increase in cardiac index. Althesin, as well as propanidid and thiopental, induced severe haemodynamic changes in patients with cardiac disease. From the haemodynamic point of view, methohexital seemed to be the drug of choice for the induction of anaesthesia.[117]

Hempelmann and Schaps also briefly discussed[107] the administration and dosage of althesin as well as the side-effects.

Al-Khawashki *et al* studied the effects of althesin and its steroidal components on neuromuscular transmission and skeletal muscle of three different species of laboratory animals (cat, toad, and rat).[118] Certain well defined discrepancies and differences in the pattern of neuromuscular responses to althesin were revealed, largely determined by the nature of the components of althesin and their possible metabolic products in the body, besides the species of experimental animal. Reference, in this respect, was made to the depressant effect of the intravenous anaesthetic dose of althesin on the monosynaptic spinal knee jerk reflex in chloralosed cats, an action which was exhibited predominantly by alphaxalone but not by the other two components in the mixture, alphadolone acetate and cremophor EL.[118] This type of activity was attributed to a possible depolarizing influence of alphaxalone.[119] Such a depolarizing effect by alphaxalone could account for the motor excitatory manifestations, evidenced by jerky movements and muscular twitches of the limbs occurring during the induction phase of intrvenous althesin anaesthesia.

The isolated rats phrenic nerve diaphragm preparation seemed peculiar for its lack of neuromuscular responses to althesin and the two steroidal components.[119]

Bowman indicated[120] that among common laboratory animals, cats closely resembled human beings in their sensitivity to a variety of drugs causing

neuromuscular block by acting on the motor end plate. This conformed with the lack of any appreciable influence by the two steroidal althesin components on the tone of the isolated toad *rectus abdominis* muscle, and its contraction responses to acetylcholine.

In 1979, Phillipps *et al* reviewed water-soluble steroidal anaesthetics,[121] with special reference to minaxolone citrate, Figure 4.

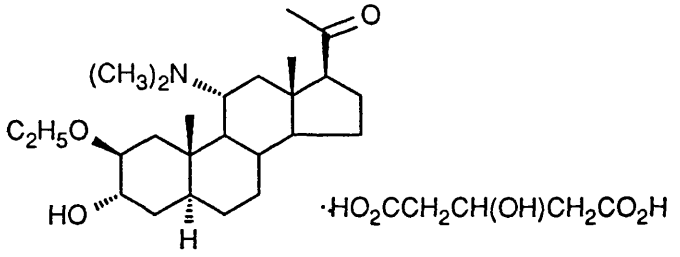


Figure 4. Minaxolone citrate.

In 1980, Fukada and Fukada published a review on althesin as an intravenous anaesthetic.[122]

General anaesthesia can be induced by such a wide variety of molecules that attempts to formulate on all-embracing hypothesis concerning their mode of action was unlikely to be successful.

The potency of anaesthetics has been correlated with some of their general physical properties, primarily their lipid solubility.[123] However, this did not hold true for steroid anaesthetics. As seen earlier, small changes in steroid structure led to large differences in anaesthetic activity.[39]

In 1983, Makriyannis and Fesik postulated[124] that the considerable difference in activity exhibited by alphaxalone and Δ^{16}-alphaxalone may be due to differences in their interactions with cellular membranes. Makriyannis and Fesik investigated the

actions of those two steroids with phosphatidylcholine bilayer vesicles, used as model membranes, using NMR experiments (^{13}C, ^{1}H and ^{2}H).[125] The results obtained suggested that alphaxalone was considerably more mobile in the phospholipid bilayer than Δ^{16}-alphaxalone. The data also suggested that the acetyl group moved faster than the steroid nucleus.[124]

The observed differences in mobility between the two steroids in the bilayer was explained by assuming that the variation in steroid geometry led to different phospholipid-steroid interactions.[124] Thus it was postulated that the inactive Δ^{16}-alphaxalone interacted with the fatty acid chains of phosphatidylcholine bilayer in such a manner that the most stable bilayer geometry was maintained, while the interacting steroid was partially immobilized.[124] This interaction may be similar to the interaction of cholesterol with phospholipids.[124] In contrast the biologically active alphaxalone, because of its slightly different stereochemical features, interacted with the bilayer differently. This interaction was thought to result in a disruption of the bilayer geometry, which was evidenced by the higher mobility of the incorporated steroid.[124] The perturbation produced by the anaesthetic molecule in the lipid region of the membrane could be transmitted to the membrane-associated proteins, resulting in a modification of their functions.[126] The mobility of alphaxalone in the bilayer decreased at lower temperatures.[124]

The results could be used to explain the different biological activities of alphaxalone and its Δ^{16} analog as anaesthetics. Makriyannis and Fesik tested the phospholipid system on a number of steroids structurally related to alphaxalone, possessing widely differing anaesthetic activities.[124] The results were consistent with the hypothesis that steroid anaesthetic activity depended on the ability of the drug to perturb the membrane lipids.

Harrison and Simmonds[127] offered another explanation for the mechanism of

action of steroid anaesthetics; modulation of the GABA receptor complex. It was postulated[127] that the remarkable potency of alphaxalone, together with the inactivity of the corresponding β-hydroxy isomer, suggested receptor sites may be involved in its actions. The possibility of a molecular interaction between alphaxalone and the GABA receptor-ionophore complex was investigated using electrophysiological and radioligand binding techniques.

The results of the experiments suggested that alphaxalone was a potent modulator of the GABA receptor complex.[127] This action appeared to be specific for the GABA system. Furthermore, the enhancing effect of the steroid anaesthetic on $GABA_A$ receptor binding was not reproduced when radioligands for other brain receptors were substituted for [^{3}H] muscimol.[127]

There were considerable similarities between the actions of pentobarbitone, a barbiturate anaesthetic, and alphaxalone at the GABA receptor complex.[127] Like pentobarbitone, alphaxalone enhanced [^{3}H] GABA[128] and [^{3}H] muscimol binding in the presence of chloride ions. Treatment with the detergent Triton abolished the enhancing effect of alphaxalone on [^{3}H] muscimol binding, possibily by altering the conformation of the membrane protein(s) which constitute the GABA receptor complex, and "uncoupling" the site at which alphaxalone acted from the muscimol binding site, as was suggested for the Triton-sensitivity of the pentobarbitone effect.[129] All these observations suggested that pentobarbitone and alphaxalone potentiated the actions of GABA *via* a common site or mechanism. Since pentobarbitone prolonged the average open-channel lifetime of the chloride channels operated by GABA,[130,131] it was of interest to know whether alphaxalone shared this property.

The actions of alphaxalone described occurred independently of "*classical*" intracellular steroid receptors, as shown by the radioligand binding experiments.[127]

The concentrations of alphaxalone arising in the brain during surgical anaesthesia, in man, was likely to be in the low micromolar range.[132] This supported the hypothesis of Scholfield[133] that alphaxalone may produce anaesthesia by enhancement of inhibition. The modulation of the GABA receptor complex provided a molecular basis for the prolongation by alphaxalone of inhibitory events in the mammalian CNS. This lent further support to the idea that the neurotransmitter receptors may be important in the mechanism of action of some general anaesthetics.[134]

Banks and Peace demonstrated that a group of naturally occurring steroids, formed by the reduction of progesterone, differed in their ability to inhibit a soluble enzyme with a hydrophobic substrate-binding site and also investigated their anaesthetic potencies.[135] It was shown that the potent anaesthetic, 5β-prengn-3α-ol-20-one, was able to inhibit bacterial luciferase competitively, whilst the corresponding, non-anaesthetic, diol (5β-pregna-3α-20α-diol) did not. It was also demonstrated that 5α-pregnan derivatives, which were anaesthetics, failed to inhibit luciferase, whilst the anaesthetic 5β-derivatives were inhibitors.[135] The diols, which were inactive as anaesthetics, also failed to inhibit the enzyme.[135] The activity of the compounds was measured by inhibition of the light production by bacterial luciferase. Thus some anaesthetic steroids were able to act as inhibitors of luciferase, others were not, and some closely related molecules were inactive in both respects.[135]

As stated earlier,[124] attention has been focussed on interactions between anaesthetics and the lipid components of membranes. However, experiments on cell-free extracts and purified luciferase showed that the ability of simple anaesthetics to extinguish the light produced by cultures of luminous bacteria[136] could not be ascribed to an interaction with the cell membrane, but resulted from a

direct effect on the enzyme concerned.[137] Due to the fact that relatively small changes in structure caused marked changes in anaesthetic potency, Banks and Peace suggested that such molecules could engage in specific interactions with proteins and that by inference, suitable steroids could bind specifically to membrane-bound enzymes, receptors or transporters to induce anaesthesia.[135] Such interactions need not be at the active site as with luciferase, but could be at any site on a protein, the occupancy of which leads to a functional change.[138]

Franks and Lieb provided evidence[139] that a range of anaesthetics were able to act competitively against the substrate luciferin, to inhibit luciferase from the firefly *Photinus pyralis*. The authors concluded that general anaesthetics, despite their chemical and structural diversity, acted by competing with endogenous ligands for binding to specific receptors.[139]

These views substantiated the view of Harrison and Simmonds,[127] whereby the steroid anaesthetics potentiated the action of GABA *via* interaction with a receptor site.

In 1988, File and Simmonds hypothesised that the ability of a compound to reduce the potency of the muscimol antagonist picrotoxin, as a antagonist at the $GABA_A$ receptor complex would correlate with anticonvulsant properties.[140] Three steroids were chosen that were distinctly different from each other in terms of their interactions with the $GABA_A$ receptor complex (alphaxalone, pregnanolone and 5β-alphaxalone) together with the veterinary anaesthetic saffan, to study as potential anticonvulsants.[140] In order to carry this out, experiments were designed to study the incidence of myoclunus following intravenous administration of the steroids in question. The potencies of the steroids, as potentiators of muscimol, were alphaxalone> pregnanolone> 5β-alphaxalone.[141] Alphaxalone was also administered intraperitoneally to investigate the importance of route of

administration.[140]

After administration of the steroids, to respective male mice, the incidence of myoclonic jerks were recorded.[140]

All three of the steroids, as well as saffan, caused myoclonic jerks in doses that were insufficient to cause a loss of righting reflex.[140] This effect of alphaxalone was seen following either intravenous or intraperitoneal injection. There was little difference between the threshold doses of these steroids for the myoclonic activity although at the higher doses tested, the duration was greater for pregnanolone than for alphaxalone or 5β-alphaxalone.[140]

The findings did not support the initial hypothesis of File and Simmonds.[140] There was no evidence that the relative potencies of the three steroids, either as potentiators of muscimol, or as attenuators of picrotoxin, corresponded in any way with their potencies at inducing seizures. However, it was possible that the seizure activity was related to actions at sites outside the $GABA_A$ receptor complex.

Seeman reported[123] that local anaesthetics induced conformational change of the sodium channels, that resulted in blockage of nerve conduction. They also inhibited mast cell secretion, since they inhibited calcium influx into mast cells by altering the calcium channels, as they altered the sodium channels in nerve membranes.[142]

Suzuki *et al*[143] investigated the effects of alphaxalone, alphadolone acetate, a mixture of the two (molar ratio 1:1), and betaxalone (3β-alphaxalone) on histamine release from rat mast cells. Purified mast cells were obtained from the peritoneal cavity of male rats, and the histamine release from the cells was stimulated by treatment with concanavalin A. The released histamine was determined by the

method of Shore *et al.*[144]

Alphaxalone inhibited histamine release from rat mast cells induced by concanavalin A.[143] Alphadolone acetate also inhibited the release induced by concanavalin A. These inhibitory effects were not due to the anaesthetic properties, or configurations, of the compounds, since betaxalone, an isomer of alphaxalone with no anaesthetic activity, was also inhibitory. Alphaxalone and alphadolone acetate also inhibited calcium ion uptake induced by concanavalin A, but did not influence ^{125}I-concanavalin A binding to mast cells, indicating that they inhibited histamine release by blocking calcium channels of mast cells.[143] It was also suggested that these drugs interfered with steps in the release cascade that occurred after the opening of calcium channels.

The steroidal structure appeared unlikely to be sufficient alone for inhibition of histamine release, since progesterone, β-estradiol, testosterone, and tetrahydrocortisone did not inhibit histamine release.[145] Althesin was well known to cause marked histamine release in humans.[145] But the intravenous administration of cremophor EL did not elicit histamine release into the blood plasma, although the effect of cremophor EL seemed to counteract the inhibitory effects of alphaxalone and alphadolone acetate.[146]

In 1987, Harrison *et al*[147] established structure-activity relationships for steroid action on the GABA receptor complex.[147] The addition of a hydroxyl group at C-21 induced approximately a three-fold decrease in the potency of the molecule in modulating the GABA receptor complex. Another pharmacological action of alphaxalone was the inhibition of acetylcholine evoked currents.[148] These results indicated the involvement of the GABA system in mediating alphaxalone depressant actions at the level of the central nervous system.

Recently, Benoit *et al*[149] investigated the effects of alphaxalone on potassium and sodium currents of myelinated nerve fibres.[149] The results were interpreted as evidence that the steroid anaesthetic produced a 'local-anaesthetic-like' action on the peripheral nervous system by specifically interacting with potassium and sodium channel gating systems. If this was correct, it was thought the responses evoked by a structurally related analogue of alphaxalone, which had a different anaesthetic potency, should not be the same. In order to test this theory, Benoit *et al*[150] examined the effects of alphadolone acetate on the voltage-clamped node of Ranvier of the frog isolated myelinated nerve fibres from the sciatic nerve of the frog. Differences were found not only in the potency of the two steroids, alphaxalone and alphadolone acetate, but also in their mechanism of action on potassium and sodium channels of the model membrane.[150]

Benoit *et al*[150] showed that external application of 0.05 to 0.5mM alphadolone acetate depressed both potassium and sodium currents in the frog node of Ranvier.[150] Alphadolone acetate was 3 to 9 times less effective than alphaxalone. However, there may be a direct one-to-one relationship between alphadolone acetate molecules, and both sodium and potassium channels. This was not found for alphaxalone.[150] It was also noted that the decrease in sodium current was less pronounced than that in potassium current at corresponding concentrations of alphadolone acetate, as previously described for alphaxalone.[149]

Alphadolone acetate had no marked effect upon the time course or voltage dependence of the model potassium current.[150] Similar results were obtained for the general anaesthetic, ketamine.[151] This suggested that the potassium channels were indifferently blocked in their resting or open state. On the other hand, alphaxalone preferentially blocked open potassium channels.[149]

Hille suggested that anaesthetic molecules were preferentially bound to

inactivated sodium channels.[152] The effects of alphaxalone have already been interpreted in these terms.[149] That alphadolone acetate evoked a similar negative shift of the steady-state sodium inactivation-voltage curve provided further support for this hypothesis.[152]

That the anaesthetic effects of alphaxalone and alphadolone acetate were strictly additive,[153] when mixtures were used, could be explained by their having different sites, by virtue of their differing chemical constitutions.

Although there were similarities between the effects of alphaxalone and alphadolone acetate upon the nodal sodium current, their effects upon the nodal potassium current differed markedly.[149,150] These results suggested that the receptor sites for the anaesthetic molecules, associated with potassium channels, were different from those associated with sodium channels. The simplest explanation of these results was that the anaesthetic recetor sites formed parts of the channel proteins, since several types of receptor sites through which anaesthetics exert their different actions, may exist upon potassium and sodium channels.[154]

Returning to the membrane perturbation theory, in 1990 Makriyannis *et al*[155] studied the interactions of the anaesthetic steroid, alphaxalone and its inactive isomer, Δ^{16}-alphaxalone, with model membrane bilayers, using differential scanning calorimetry, small angle X-ray diffraction and solid state NMR. Studies were carried out using aqueous multiamellar dispersions of dipalmitoylphosphatidylcholine (DPPC) with specific ^{13}C and ^{2}H labels and also multiammellar bilayer dispersions of dimyristoylphosphatidylcholine with pre-deuterated acyl chains (DMPC-d_{54}) as model membranes. Makriyannis *et al* also studied the interactions of a series of structurally related pregnane analogues possessing a wide range of anaesthetic potencies.[155] Results showed a good correlation between

the motional properties and anaesthetic properties of the steroids.

Differential scanning calorimetry was used to study the effects of the drugs on the phase properties of membranes,[156] small angle X-ray diffraction to identify the exact location of the drug in the bilayer,[157] and solid state NMR to identify changes in membrane conformation and dynamics produced by the drug,[158] and thus to determine the orientation and conformation of the drug in the membrane.

It was shown that the anaesthetic steroid broadened the membrane phase transition and increased the ratio of *gauche* to *trans* conformers in the membrane.[155] Δ^{16}-Alphaxalone had only small effects on the membrane and incorporated, to a limited degree, in the bilayer.[155] The inactivity of Δ^{16}-alphaxalone was attributed to a combination of a favourable packing, which led to insufficient lipid perturbation and the inability to incorporate substantially in the membrane.[155]

The anaesthetic steroid alphaxalone, was located near the membrane interface[155] (the junction of the polar and hydrophobic regions of the phospholipids forming the bilayer). It acquired an orientation which placed its long axis parallel to the chains of the lipid membranes and its 3α-hydroxyl group near the *Sn*-2 carbonyl of the lipid membranes. The carbonyl at C(20) was probably buried in the membrane bilayer, Figure 5.

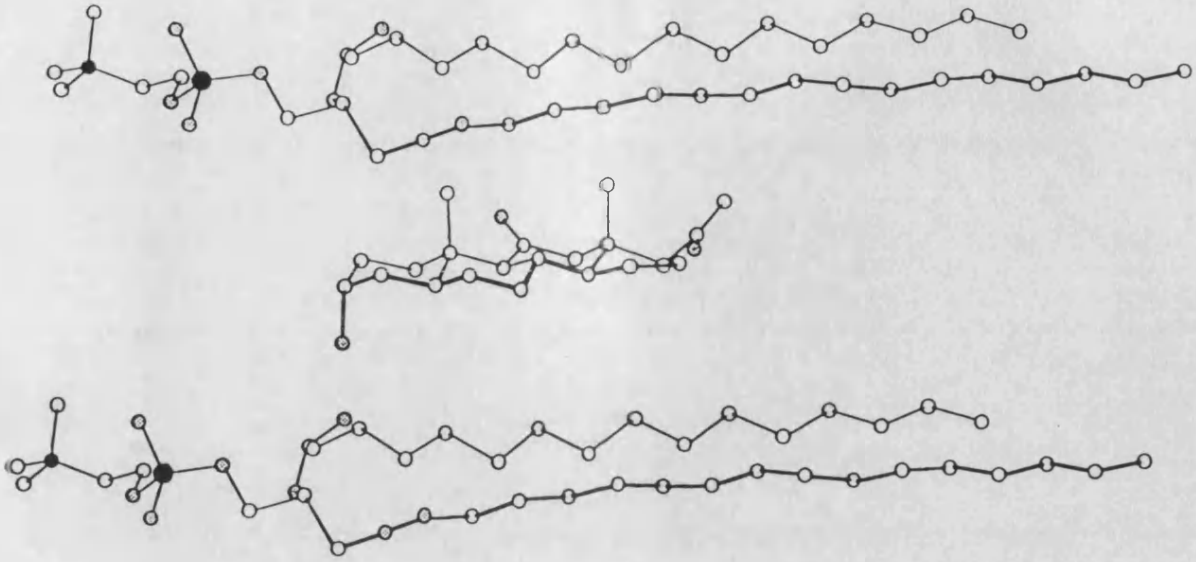


Figure 5. Representation of a model showing the position and orientation of alphaxalone with respect to the DPPC layer. Alphaxalone anchors at the membrane interface and may form a hydrogen bond between its 3α-hydroxyl group and the *Sn*-2 carbonyl group of DPPC. From Makriyannis *et al.*, *Ciba Found. Symp.*, 1990, **153**, 172.

Anchoring of the steroid at the membrane interface and imperfect packing with the bilayer chains may be involved in membrane perturbation and eventually lead to anaesthesia.[155] Thus, the active steroid required more space[155] in order to be accommodated between the phospholipid chains. The ability of a steroid to induce such perturbations was related to its stereochemical characteristics which were responsible for an imperfect steroid-bilayer packing.[155]

Experiments showed[155] that steroids possessing anaesthetic activity caused an increase in the fluidity of the model membrane, whereas structurally related inactive analogues produced much less disorder. The two steroids alphaxalone and Δ^{16}-alphaxalone, were seen to have very different motional properties in the lipid bilayer. Alphaxalone was more mobile than Δ^{16}-alphaxalone, thus the biologically

active steroid perturbed the phospholipid bilayer more effectively than its inactive analogue.[155]

In the same journal, Bäckström *et al*[159] published a report on "steroids in relation to epilepsy and anaesthesia". Clinical and experimental data was discussed which pointed to the effects of progesterone and its metabolites in anaesthesia and epilepsy. Injection of 5β-pregnan-3α-ol-20-one (produced by the human corpus luteum from 5β-pregnan-3,20-dione) caused no fever,[159] but did so in a paper by Kappas *et al*,[160] in which it was injected in cremophor EL. The side-effects were attributed to the vehicle, cremophor EL.

The reduced progesterone metabolite, 5α-pregnan-3α-ol-20-one and its 5β-analogue decreased the epileptic activity resulting from a penicillin-induced cortical focus in cats.[159]

5α-Pregnan-3α-ol-20-one was found to be eight times more potent than methohexitone (the most potent anaesthetic barbiturate).[161] The 5β-isomer was less active than its 5α-analogue but was still more potent than methohexitone. Hence the 5α- and 5β-pregnanolones were not equipotent or interchangeable.

It has not been definately established that the GABA-potentiating effect of the steroid anaesthetics is primarily responsible for causing anaesthesia in organisms. For instance, it has also been proposed that the steroids may act *via* a non-specific lipid bilayer mediated mechanism.[162] To distinguish between these two possibilities Oliver *et al*[162] tested 21 aquatic species for susceptibility to anaesthesia by pregnanolone, and also to diethyl ether and short chain alkanols as positive controls. This served as a critical test of the bilayer mediated mechanism by which all organisms would be expected to respond to the steroid.

Clinical concentrations of anaesthetic were added directly to water in which the test organisms were acclimatised.[162] In the case of the steroid anaesthetic, pregnanolone was dissolved in a small physiologically, insignificant volume of ethanol, then injected into the water. In most cases, lack of righting reflex or escape response was taken as indication of anaesthesia.[17] At onset of anaesthesia organisms were immediately transferred to clean water and their recoveries monitored. Inability of an organism to achieve full recovery was considered to be an indication of toxicity, rather than anaesthesia. Drug-naive animals were used in most cases.

Oliver *et al*[162] found that all organisms tested were anaesthetized by diethyl ether, ethanol and *n*-butanol, but only the chordates responded to pregnanolone.[162] The behavioural anaesthetic effect caused by pregnanolone in vertebrates was consistent with two neurobiological findings about steroid anaesthetics. Firstly, structure-activity studies have shown that the hydroxyl group at C-3 must be in the α position in order for the compound to cause the dramatic potentiation of GABA.[147] The 3β-isomer of pregnanolone had no anaesthetic effect.[39] Secondly, the behavioural response to picrotoxin was consistent with the neurobiological data. The convulsant, picrotoxin is an antagonist to the $GABA_A$ receptor. It appeared to bind to the channel and block chloride ion flux,[163] thereby inhibiting the anaesthetic effect. In the presence of picrotoxin the average time required for the guppy to recover from anaesthesia was reduced by approximately half. The steroid anaesthetic was also seen to protect against, and reverse convulsions caused by picrotoxin.[162] Thus, the behavioural anaesthetic response to pregnanolone was stereospecific and picrotoxin-sensitive, both which are consistant with a $GABA_A$-receptor site for steroid anaesthetic action.

Direct delivery of pregnanolone to the invertebrate neuron appeared not to affect a known GABA receptor.[162] Preliminary data[162] suggested that inadequate delivery

was not the reason for invertebrate insensitivity to the steroid anaesthetic. Since pregnanolone appeared to act by binding to the $GABA_A$ ionophore complex, and also since GABA receptors occurred in both invertebrate and vertebrate species,[164] it was suggested that a binding site at which steroid binding caused organismal anaesthesia developed on the GABA receptor early in chordate evolution.[162] Evolutionary development of a membrane protein binding site has been previously documented.[165] Finally, because lipid bilayer membranes are universal components of animal cells, the insensitivity of invertebrates to pregnanolone appeared to exclude a bilayer site for steroid anaesthetics.

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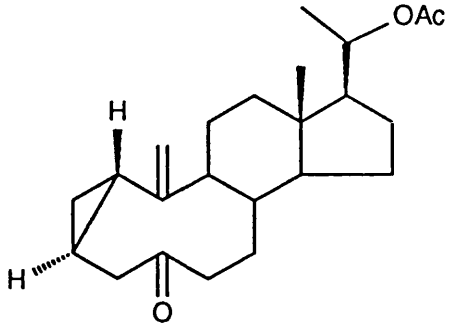
APPENDIX 2

X-ray crystallographic data for 55



55

Compound **55** was crystallised from ethyl acetate.

A crystal of approximate dimensions 0.2 x 0.2 x 0.5 mm was used for data collection.

Crystal data: $C_{23}H_{34}O_3$, M = 358.5 orthorhombic, a = 7.925(1), b = 13.703(3), c = 19.147(4)Å, U = 2079.3 Å^3, space group $P2_12_12_1$, Z = 4, D_c = 1.15 gcm^{-3}, μ(Mo-K_α) = 0.70 cm^{-1}, F(000) = 784. Data were measured at room temperature on a CAD4 automatic four-circle diffractometer in the range $2 \leq \theta \leq 24^\circ$. 1899 reflections were collected of which 955 were unique with $I \geq 2\sigma(I)$. Data were corrected for Lorentz and polarization but not for absorption. The structure was solved by Direct methods and refined using the SHELX[1,2] suite of programs. In the final least squares cycles all atoms were allowed to vibrate anisotropically. Hydrogen atoms were included at calculated positions except in the instance of H31, H51, H191 and H192 where the protons were located in an advanced Difference Fourier and refined at a distance of 1.00 Å from the relevant parent atoms. Final residuals after 12 cycles of least squares were R = 0.0463, R_w = 0.0394, for a weighting scheme of $w = 2.5234/[\sigma^2(F) + 0.000193(F)^2]$. Max. final shift/esd was 0.000. The max. and min. residual densities were 0.07 and -0.06 eÅ^{-3} respectively. Final fractional atomic coordinates and isotropic thermal parameters, bond distances and angles are given in Tables 5, 9, and 10 respectively. Tables of anisotropic temperature factors are available as supplementary data. The asymmetric unit is shown in Fig. 6, along with the labelling scheme used.

1. Sheldrick G.M., SHELX86, a computer program for crystal structure determination, University of Göttingen, 1986.

2. Sheldrick G.M., SHELX76, a computer program for crystal structure determination, University of Cambridge, 1976.

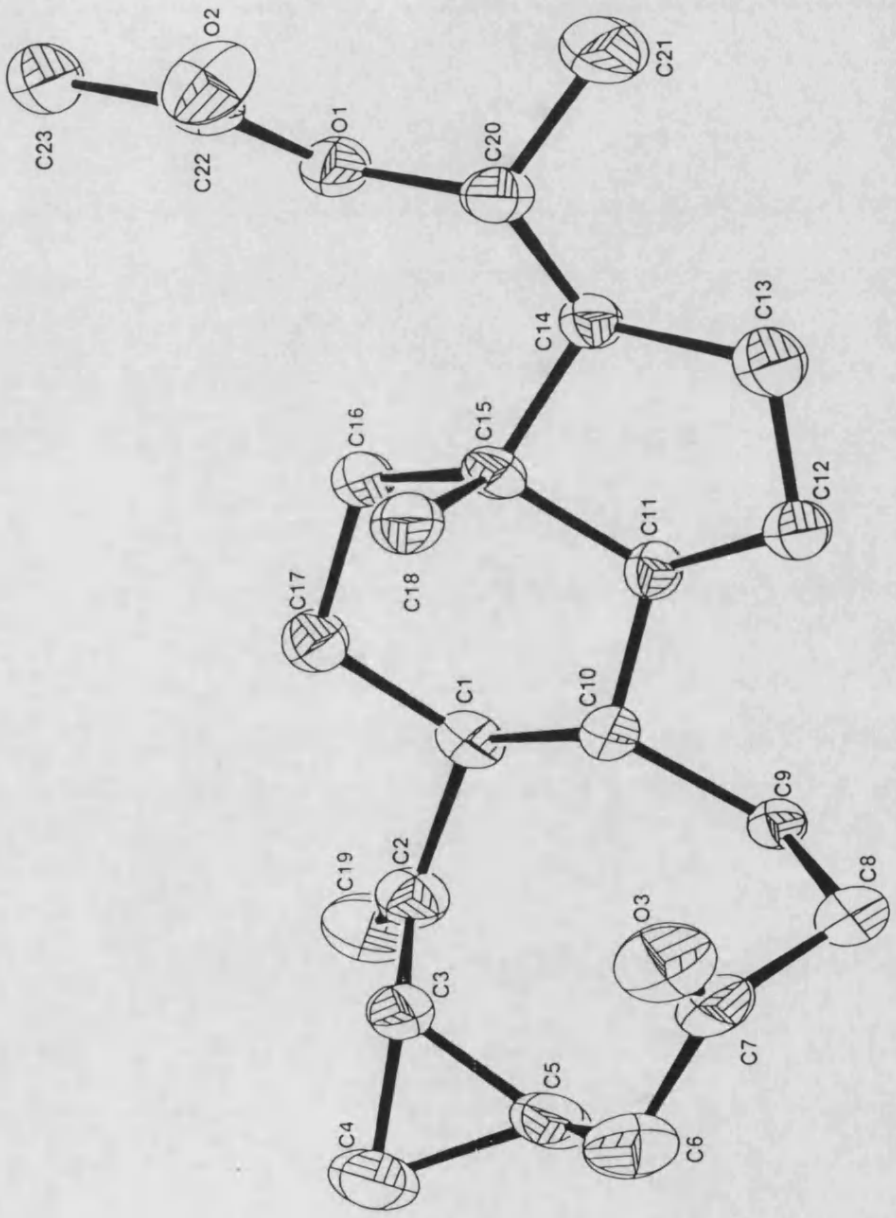


Figure 6. X-ray structure of 20β-acetoxy-1,3(10→1βH,5→3αH)*abeo*pregn-10(19)-en-5-one (**55**).

Table 5 Fractional atomic co-ordinates ($x10^4$) and equivalent isotropic temperature factors ($Å^2$ $x10^3$) for **55**

Atom	x	y	z	U
O(1)	-689(5)	-7185(3)	-2450(2)	60(2)
O(2)	-884(6)	-5564(3)	-2354(3)	90(2)
O(3)	493(7)	-8992(4)	1360(3)	100(2)
C(1)	-1952(8)	-10289(4)	-613(3)	52(2)
C(2)	-3088(8)	-10834(5)	-108(3)	60(3)
C(3)	-3139(9)	-10480(5)	618(3)	59(2)
C(4)	-3959(10)	-11013(5)	1222(4)	89(3)
C(5)	-2087(10)	-11008(5)	1162(4)	70(3)
C(6)	-974(10)	-10439(6)	1647(4)	96(4)
C(7)	404(10)	-9863(6)	1271(4)	77(3)
C(8)	1553(8)	-10375(6)	774(3)	76(3)
C(9)	746(8)	-10678(4)	67(3)	64(3)
C(10)	-303(7)	-9878(4)	-285(3)	46(2)
C(11)	666(7)	-9359(4)	-856(3)	47(2)
C(12)	2412(7)	-8927(5)	-692(3)	64(3)
C(13)	2779(8)	-8243(5)	-1311(4)	75(3)
C(14)	1098(7)	-8182(4)	-1736(3)	49(2)
C(15)	-272(7)	-8508(4)	-1215(3)	45(2)
C(16)	-1876(7)	-8922(4)	-1535(3)	55(2)
C(17)	-2922(7)	-9485(5)	-995(3)	64(3)
C(18)	-697(8)	-7673(4)	-702(3)	64(2)
C(19)	-4019(11)	-11563(6)	-327(4)	88(4)
C(20)	931(7)	-7191(5)	-2086(3)	58(2)
C(21)	2305(8)	-7000(5)	-2610(4)	88(3)
C(22)	-1400(8)	-6315(5)	-2555(3)	58(3)
C(23)	-3011(8)	-6428(5)	-2962(3)	76(3)

Table 6 Fractional atomic co-ordinates ($x10^4$)

	x	y	z
O(1)	-689(5)	-7185(3)	-2450(2)
O(2)	-884(6)	-5564(3)	-2354(3)
O(3)	493(7)	-8992(4)	1360(3)
C(1)	-1952(8)	-10289(4)	-613(3)
C(2)	-3088(8)	-10834(5)	-108(3)
C(3)	-3139(9)	-10480(5)	618(3)
C(4)	-3959(10)	-11013(5)	1222(4)
C(5)	-2087(10)	-11008(5)	1162(4)
C(6)	-974(10)	-10439(6)	1647(4)
C(7)	404(10)	-9863(6)	1271(4)
C(8)	1553(8)	-10375(6)	774(3)
C(9)	746(8)	-10678(4)	67(3)
C(10)	-303(7)	-9878(4)	-285(3)
C(11)	666(7)	-9359(4)	-856(3)
C(12)	2412(7)	-8927(5)	-692(3)
C(13)	2779(8)	-8243(5)	-1311(4)
C(14)	1098(7)	-8182(4)	-1736(3)
C(15)	-272(7)	-8508(4)	-1215(3)
C(16)	-1876(7)	-8922(4)	-1535(3)
C(17)	-2922(7)	-9485(5)	-995(3)
C(18)	-697(8)	-7673(4)	-702(3)
C(19)	-4019(11)	-11563(6)	-327(4)
C(20)	931(7)	-7191(5)	-2086(3)
C(21)	2305(8)	-7000(5)	-2610(4)
C(22)	-1400(8)	-6315(5)	-2555(3)
C(23)	-3011(8)	-6428(5)	-2962(3)

Table 7 Anisotropic temperature factors (Å x10^3)

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	57(3)	59(3)	63(3)	10(2)	-2(3)	-3(3)
O(2)	105(4)	54(3)	109(4)	5(3)	-30(4)	-12(3)
O(3)	119(4)	93(3)	86(4)	-7(3)	-8(4)	-1(4)
C(1)	50(4)	51(4)	56(4)	-4(3)	7(4)	-6(4)
C(2)	59(5)	53(4)	67(5)	3(4)	7(4)	-7(4)
C(3)	62(4)	55(4)	58(4)	13(4)	12(4)	6(4)
C(4)	94(6)	91(5)	82(5)	18(5)	32(5)	4(5)
C(5)	76(6)	73(5)	61(5)	5(4)	25(5)	4(5)
C(6)	110(7)	119(6)	59(4)	13(5)	8(6)	12(6)
C(7)	72(5)	97(6)	61(5)	22(5)	-16(5)	4(6)
C(8)	68(5)	100(5)	61(5)	24(5)	-3(4)	11(5)
C(9)	63(5)	63(4)	65(4)	8(4)	15(4)	12(4)
C(10)	45(4)	45(4)	46(4)	0(3)	-3(3)	3(3)
C(11)	36(4)	49(4)	55(4)	0(3)	-7(3)	-1(4)
C(12)	44(4)	80(5)	68(4)	12(4)	-3(4)	-1(4)
C(13)	53(4)	88(5)	86(5)	12(4)	-1(4)	-7(4)
C(14)	37(4)	65(4)	46(3)	3(3)	-2(3)	-2(3)
C(15)	42(4)	52(4)	40(3)	-9(3)	3(3)	-3(3)
C(16)	43(4)	66(4)	57(4)	14(3)	-3(3)	-4(4)
C(17)	44(4)	72(4)	77(5)	14(4)	-4(4)	-10(4)
C(18)	71(4)	58(4)	63(4)	1(3)	2(4)	8(4)
C(19)	98(6)	81(6)	85(6)	-1(5)	18(6)	-21(6)
C(20)	50(5)	72(4)	50(4)	8(3)	-7(4)	-17(4)
C(21)	69(5)	105(6)	91(5)	22(5)	1(5)	-19(5)
C(22)	66(5)	54(4)	54(4)	8(4)	4(4)	-8(4)
C(23)	53(5)	91(5)	84(5)	10(4)	-1(4)	-5(4)

Table 8 Hydrogen fractional atomic co-ordinates ($x10^4$) and isotropic temperature factors ($Å^2$ $x10^3$)

	x	y	z	U
H(11)	-1593(8)	-10772(4)	-945(3)	73(4)
H(41)	-4572(10)	-11606(5)	1138(4)	73(4)
H(41)	-4503(10)	-10650(5)	1588(4)	73(4)
H(61)	-1664(10)	-9989(6)	1904(4)	73(4)
H(62)	-446(10)	-10886(6)	1965(4)	73(4)
H(81)	2486(8)	-9950(6)	677(3)	73(4)
H(82)	1959(8)	-10957(6)	998(3)	73(4)
H(91)	24(8)	-11229(4)	149(3)	73(4)
H(92)	1636(8)	-10862(4)	-246(3)	73(4)
H(101)	-575(7)	-9426(4)	82(3)	73(4)
H(111)	792(7)	-9925(4)	-1148(3)	73(4)
H(121)	2391(7)	-8569(5)	-261(3)	73(4)
H(122)	3245(7)	-9434(5)	-663(3)	73(4)
H(131)	3102(8)	-7609(5)	-1146(4)	73(4)
H(132)	3664(8)	-8506(5)	-1597(4)	73(4)
H(141)	1020(7)	-8603(4)	-2136(3)	73(4)
H(161)	-2542(7)	-8394(4)	-1716(3)	73(4)
H(162)	-1576(7)	-9356(4)	-1908(3)	73(4)
H(171)	-3863(7)	-9778(5)	-1232(3)	73(4)
H(172)	-3329(7)	-9029(5)	-654(3)	73(4)
H(181)	322(8)	-7417(4)	-503(3)	73(4)
H(182)	-1410(8)	-7920(4)	-337(3)	73(4)
H(183)	-1277(8)	-7164(4)	-949(3)	73(4)
H(201)	1011(7)	-6693(5)	-1735(3)	73(4)
H(211)	3379(8)	-7003(5)	-2378(4)	73(4)

H(212)	2126(8)	-6376(5)	-2825(4)	73(4)
H(213)	2287(8)	-7501(5)	-2961(4)	73(4)
H(231)	-3513(8)	-5799(5)	-3034(3)	73(4)
H(232)	-3780(8)	-6837(5)	-2709(3)	73(4)
H(233)	-2764(8)	-6720(5)	-3406(3)	73(4)
H(31)	-3169(73)	-9753(15)	645(28)	73(4)
H(51)	-1657(65)	-11637(22)	968(25)	73(4)
H(191)	-3832(75)	-11831(35)	-806(15)	73(4)
H(192)	-4775(56)	-11919(33)	-4(22)	73(4)

Table 9 Bond lengths (Å)

C(20)-O(1)	1.462(7)	C(22)-O(1)	1.334(8)
C(22)-O(2)	1.172(7)	C(7)-O(3)	1.208(8)
C(2)-C(1)	1.517(9)	C(10)-C(1)	1.556(9)
C(17)-C(1)	1.529(9)	C(3)-C(2)	1.473(9)
C(19)-C(2)	1.310(10)	C(4)-C(3)	1.515(10)
C(5)-C(3)	1.518(11)	C(5)-C(4)	1.488(11)
C(6)-C(5)	1.500(11)	C(7)-C(6)	1.527(11)
C(8)-C(7)	1.492(10)	C(9)-C(8)	1.554(10)
C(10)-C(9)	1.532(9)	C(11)-C(10)	1.514(9)
C(12)-C(11)	1.538(10)	C(15)-C(11)	1.544(9)
C(13)-C(12)	1.540(10)	C(14)-C(13)	1.563(9)
C(15)-C(14)	1.541(9)	C(20)-C(14)	1.520(9)
C(16)-C(15)	1.521(9)	C(18)-C(15)	1.545(9)
C(17)-C(16)	1.533(9)	C(21)-C(20)	1.504(10)
C(23)-C(22)	1.504(10)		

Table 10 Bond angles (deg.)

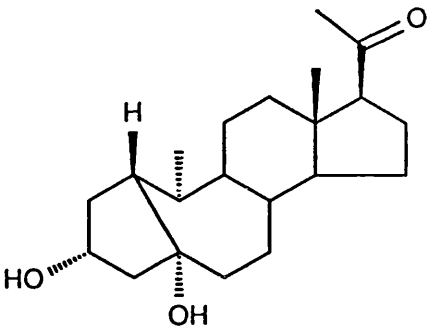
C(22)-O(1)-C(20)	116.6(6)	C(10)-C(1)-C(2)	114.7(6)
C(17)-C(1)-C(2)	111.1(6)	C(17)-C(1)-C(10)	110.7(5)
C(3)-C(2)-C(1)	117.1(7)	C(19)-C(2)-C(1)	120.3(7)
C(19)-C(2)-C(3)	122.5(7)	C(4)-C(3)-C(2)	125.0(7)
C(5)-C(3)-C(2)	118.4(7)	C(5)-C(3)-C(4)	58.8(5)
C(5)-C(4)-C(3)	60.7(6)	C(4)-C(5)-C(3)	60.5(6)
C(6)-C(5)-C(3)	120.0(7)	C(6)-C(5)-C(4)	122.7(8)
C(7)-C(6)-C(5)	113.4(7)	C(6)-C(7)-O(3)	119.1(9)
C(8)-C(7)-O(3)	121.3(9)	C(8)-C(7)-C(6)	119.6(8)
C(9)-C(8)-C(7)	115.5(7)	C(10)-C(9)-C(8)	114.5(6)
C(9)-C(10)-C(1)	112.0(5)	C(11)-C(10)-C(1)	107.7(5)
C(11)-C(10)-C(9)	112.2(6)	C(12)-C(11)-C(10)	119.3(6)
C(15)-C(11)-C(10)	115.6(5)	C(15)-C(11)-C(12)	103.5(5)
C(13)-C(12)-C(11)	104.3(6)	C(14)-C(13)-C(12)	105.8(5)
C(15)-C(14)-C(13)	104.3(5)	C(20)-C(14)-C(13)	110.6(6)
C(20)-C(14)-C(15)	118.9(6)	C(14)-C(15)-C(11)	99.7(5)
C(16)-C(15)-C(11)	107.5(5)	C(16)-C(15)-C(14)	115.9(5)
C(18)-C(15)-C(11)	112.4(5)	C(18)-C(15)-C(14)	110.5(5)
C(18)-C(15)-C(16)	110.5(6)	C(17)-C(16)-C(15)	111.6(6)
C(16)-C(17)-C(1)	114.4(6)	C(14)-C(20)-O(1)	107.0(5)
C(21)-C(20)-O(1)	108.4(5)	C(21)-C(20)-C(14)	112.7(7)
O(2)-C(22)-O(1)	126.0(7)	C(23)-C(22)-O(1)	110.1(7)
C(23)-C(22)-O(2)	123.9(8)		

Table 11 Selected non-bonded distances (Å)

Intramolecular:

O(2)-O(1)	2.235	C(14)-O(1)	2.396

X-ray crystallographic data for **117**



117

Compound **117** was crystallised from ethyl acetate.

A crystal of approximate dimensions 0.3 x 0.3 x 0.3 mm was used for data collection.

Crystal data: $C_{21}H_{34}O_3$, $M = 334.5$ orthorhombic, $a = 6.6262(7)$, $b = 12.554(1)$, $c = 22.968(2)$Å, $U = 1910.6$ Å^3, space group $P2_12_12_1$, $Z = 4$, $D_c = 1.16$ gcm^{-3}, μ(Mo-K_α) = 0.70 cm^{-1}, $F(000) = 736$. Data were measured at room temperature on a CAD4 automatic four-circle diffractometer in the range $2 \leq \theta \leq 24^\circ$. 1776 reflections were collected of which 1195 were unique with $I \geq 2\sigma(I)$. Data were corrected for Lorentz and polarization but not for absorption. The structure was solved by Direct methods and refined using the SHELX[1,2] suite of programs. In the final least squares cycles all atoms were allowed to vibrate anisotropically. Hydrogen atoms were included at calculated positions except in the instance of H1 and H2, (attached to O1 and O2 respectively) where the protons were located in an advanced Difference Fourier and refined at a distance of 0.96 Å from the relevant parent atoms.

Examination of the gross molecular packing revealed that the lattice was dominated by intermolecular hydrogen bonds through the hydroxyl groups. Typically, H2 of the molecule as presented interacts with O1 of the lattice neighbour generated via the operator 0.5+*x*, 0.5-*y*, 2-*z* (H2 -O1, 1.863 Å), while O2 bonds to H1 of the molecule generated via the operator 1+*x*,*y*,*z* (O2 -H1, 1.813 Å). The ultimate consequence of these intermolecular contacts is an array of infinite herringbone polymers parallel to the *a* axis. The backbone of each herringbone is composed of two 1-dimensional polymers, each containing molecules removed from each other by one unit cell translation along *a* and linked through O2 -H1

bonds. These polymers (related by the symmetry element 0.5+x, 0.5-y, -z) are, in turn, cross-linked by the H2-O1 bonds.

Final residuals after 12 cycles of least squares were $R = 0.0458$, $R_w = 0.0460$, for a weighting scheme of $w = 1.7976/[\sigma^2(F) + 0.000606(F)^2]$. Max. final shift/esd was 0.000. The max. and min. residual densities were 0.08 and -0.08 eÅ^{-3} respectively. Final fractional atomic coordinates and isotropic thermal parameters, bond distances and angles are given in Tables 12, 16, and 17 respectively. Tables of anisotropic temperature factors are available as supplementary data. The asymmetric unit is shown in Fig. 7, along with the labelling scheme used.

1. Sheldrick G.M., SHELX86, a computer program for crystal structure determination, University of Göttingen, 1986.
2. Sheldrick G.M., SHELX76, a computer program for crystal structure determination, University of Cambridge, 1976.

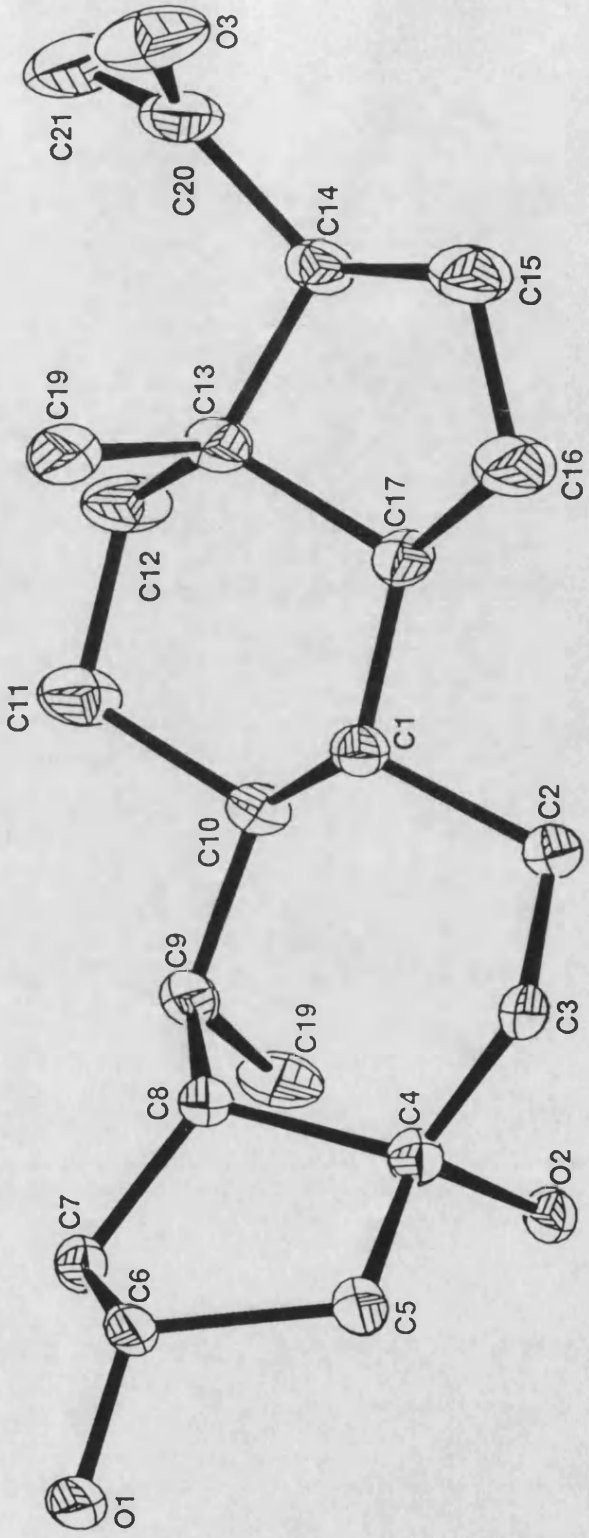


Figure 7. X-ray structure of 3α,5α-dihydroxy-5(10→1βH)*abeo*pregnan-20-one (**117**).

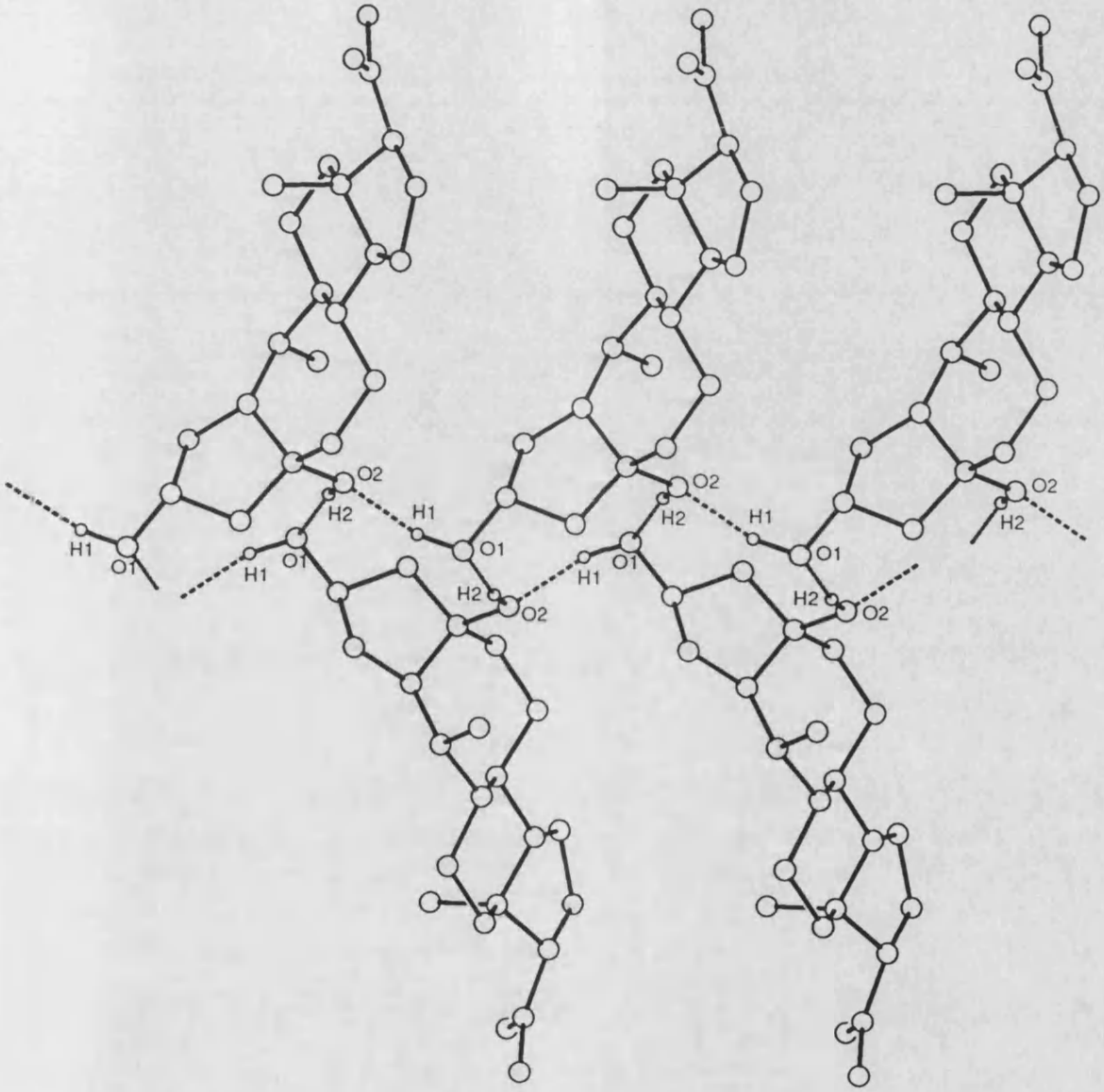


Figure 8. Solid state molecular packing of **117**.

Table 12 Fractional atomic co-ordinates ($x10^4$) and equivalent isotropic temperature factors ($Å^2$ $x10^3$) for **117**

	x	y	z	U
O(1)	592(5)	2580(3)	9412(1)	40(1)
O(2)	6973(5)	1536(3)	9543(1)	39(1)
O(3)	7105(7)	-5097(3)	7374(2)	79(2)
C(1)	6690(7)	-1134(3)	8665(2)	32(1)
C(2)	7875(7)	-87(3)	8619(2)	40(2)
C(3)	6604(8)	923(3)	8578(2)	37(2)
C(4)	5463(7)	1227(4)	9130(2)	35(1)
C(5)	3934(7)	2153(4)	9034(2)	41(2)
C(6)	1856(7)	1732(4)	9210(2)	34(2)
C(7)	2356(7)	877(3)	9650(2)	35(2)
C(8)	4093(7)	305(3)	9347(2)	31(1)
C(9)	5003(7)	-643(3)	9681(2)	35(2)
C(10)	6257(7)	-1446(3)	9310(2)	37(2)
C(11)	5274(10)	-2545(4)	9358(2)	54(2)
C(12)	6402(10)	-3436(4)	9039(2)	55(2)
C(13)	6760(8)	-3151(4)	8404(2)	38(2)
C(14)	8314(8)	-3838(4)	8075(2)	41(2)
C(15)	9026(9)	-3123(4)	7574(2)	55(2)
C(16)	8583(10)	-1962(4)	7759(2)	58(2)
C(17)	7848(8)	-2067(3)	8386(2)	36(2)
C(18)	6148(8)	-377(4)	10244(2)	48(2)
C(19)	4761(8)	-3123(4)	8064(2)	56(2)
C(20)	7592(9)	-4915(4)	7873(3)	53(2)
C(21)	7504(13)	-5781(4)	8312(3)	89(3)

Table 13 Fractional atomic co-ordinates ($x10^4$)

	x	y	z
O(1)	592(5)	2580(3)	9412(1)
O(2)	6973(5)	1536(3)	9543(1)
O(3)	7105(7)	-5097(3)	7374(2)
C(1)	6690(7)	-1134(3)	8665(2)
C(2)	7875(7)	-87(3)	8619(2)
C(3)	6604(8)	923(3)	8578(2)
C(4)	5463(7)	1227(4)	9130(2)
C(5)	3934(7)	2153(4)	9034(2)
C(6)	1856(7)	1732(4)	9210(2)
C(7)	2356(7)	877(3)	9650(2)
C(8)	4093(7)	305(3)	9347(2)
C(9)	5003(7)	-643(3)	9681(2)
C(10)	6257(7)	-1446(3)	9310(2)
C(11)	5274(10)	-2545(4)	9358(2)
C(12)	6402(10)	-3436(4)	9039(2)
C(13)	6760(8)	-3151(4)	8404(2)
C(14)	8314(8)	-3838(4)	8075(2)
C(15)	9026(9)	-3123(4)	7574(2)
C(16)	8583(10)	-1962(4)	7759(2)
C(17)	7848(8)	-2067(3)	8386(2)
C(18)	6148(8)	-377(4)	10244(2)
C(19)	4761(8)	-3123(4)	8064(2)
C(20)	7592(9)	-4915(4)	7873(3)
C(21)	7504(13)	-5781(4)	8312(3)

Table 14 Anisotropic temperature factors (Å x10^3)

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	27(2)	42(2)	51(2)	-11(2)	0(2)	1(2)
O(2)	24(2)	50(2)	43(2)	-16(2)	1(2)	-10(2)
O(3)	99(3)	59(2)	79(3)	-23(2)	-35(3)	11(3)
C(1)	28(2)	35(2)	34(2)	-1(2)	-2(2)	1(2)
C(2)	37(3)	40(3)	44(3)	-5(2)	10(3)	-7(3)
C(3)	32(3)	33(3)	45(3)	1(2)	9(2)	-11(2)
C(4)	28(2)	41(3)	36(3)	0(2)	0(2)	-5(2)
C(5)	29(3)	41(3)	53(3)	2(2)	8(3)	-4(3)
C(6)	24(2)	42(3)	37(3)	-8(2)	3(2)	-2(2)
C(7)	26(3)	42(3)	36(3)	-2(2)	1(2)	-6(2)
C(8)	27(2)	32(2)	34(2)	-3(2)	2(2)	-3(2)
C(9)	31(3)	42(3)	31(2)	2(2)	4(2)	-1(2)
C(10)	36(3)	39(3)	36(2)	-3(2)	5(2)	1(2)
C(11)	73(4)	46(3)	42(3)	5(3)	22(3)	3(3)
C(12)	82(4)	37(3)	46(3)	5(2)	17(3)	9(3)
C(13)	37(3)	42(3)	35(3)	-2(2)	0(2)	3(3)
C(14)	34(3)	43(3)	46(3)	-3(2)	-2(2)	8(3)
C(15)	62(4)	56(3)	46(3)	-10(3)	9(3)	4(3)
C(16)	72(4)	52(3)	51(3)	-6(3)	20(3)	-2(3)
C(17)	40(3)	39(2)	31(2)	-2(2)	1(2)	-7(3)
C(18)	55(3)	54(3)	35(3)	-4(2)	-1(3)	15(3)
C(19)	46(3)	50(3)	70(4)	-13(3)	-2(3)	-1(3)
C(20)	51(3)	45(3)	63(4)	-14(3)	-2(3)	11(3)
C(21)	131(7)	46(4)	89(5)	-13(3)	30(5)	-13(5)

Table 15 Hydrogen fractional atomic co-ordinates ($x10^4$) and isotropic temperature factors ($Å^2$ $x10^3$)

	x	y	z	U
H(1)	-729(47)	2311(46)	9477(27)	103(24)
H(2)	6462(73)	1772(35)	9903(12)	57(15)
H(11)	5445(7)	-1011(3)	8462(2)	60(3)
H(21)	8707(7)	-125(3)	8277(2)	60(3)
H(22)	8717(7)	-29(3)	8958(2)	60(3)
H(31)	5635(8)	826(3)	8272(2)	60(3)
H(32)	7488(8)	1502(3)	8481(2)	60(3)
H(51)	3926(7)	2363(4)	8632(2)	60(3)
H(52)	4293(7)	2752(4)	9272(2)	60(3)
H(61)	1066(7)	1436(4)	8899(2)	60(3)
H(71)	2774(7)	1178(3)	10015(2)	60(3)
H(72)	1232(7)	408(3)	9713(2)	60(3)
H(81)	3697(7)	-114(3)	9017(2)	60(3)
H(91)	3778(7)	-996(3)	9794(2)	60(3)
H(101)	7589(7)	-1441(3)	9474(2)	60(3)
H(111)	5194(10)	-2731(4)	9762(2)	60(3)
H(112)	3937(10)	-2499(4)	9198(2)	60(3)
H(121)	5615(10)	-4078(4)	9058(2)	60(3)
H(122)	7679(10)	-3551(4)	9226(2)	60(3)
H(141)	9384(8)	-4043(4)	8333(2)	60(3)
H(151)	10445(9)	-3218(4)	7508(2)	60(3)
H(152)	8298(9)	-3293(4)	7224(2)	60(3)
H(161)	7559(10)	-1652(4)	7516(2)	60(3)
H(162)	9784(10)	-1536(4)	7739(2)	60(3)
H(171)	8993(8)	-2033(3)	8641(2)	60(3)
H(181)	5371(8)	117(4)	10471(2)	60(3)

H(182)	7426(8)	-63(4)	10148(2)	60(3)
H(183)	6364(8)	-1017(4)	10464(2)	60(3)
H(191)	3799(8)	-2695(4)	8271(2)	60(3)
H(192)	4248(8)	-3835(4)	8024(2)	60(3)
H(193)	4989(8)	-2824(4)	7685(2)	60(3)
H(211)	7935(13)	-5512(4)	8683(3)	60(3)
H(212)	8375(13)	-6353(4)	8194(3)	60(3)
H(213)	6144(13)	-6039(4)	8342(3)	60(3)

Table 16 Bond lengths (Å)

C(6)-O(1)	1.432(6)	C(4)-O(2)	1.433(6)
C(20)-O(3)	1.212(7)	C(2)-C(1)	1.535(8)
C(10)-C(1)	1.558(8)	C(17)-C(1)	1.540(8)
C(3)-C(2)	1.525(8)	C(4)-C(3)	1.523(8)
C(5)-C(4)	1.557(8)	C(8)-C(4)	1.553(8)
C(6)-C(5)	1.528(8)	C(7)-C(6)	1.513(8)
C(8)-C(7)	1.524(8)	C(9)-C(8)	1.539(8)
C(10)-C(9)	1.560(8)	C(18)-C(9)	1.537(8)
C(11)-C(10)	1.530(8)	C(12)-C(11)	1.532(8)
C(13)-C(12)	1.520(8)	C(14)-C(13)	1.541(8)
C(17)-C(13)	1.541(8)	C(19)-C(13)	1.538(9)
C(15)-C(14)	1.534(8)	C(20)-C(14)	1.508(9)
C(16)-C(15)	1.546(9)	C(17)-C(16)	1.526(8)
C(21)-C(20)	1.483(8)	H(1)-O(1)	0.950(20)
H(2)-O(2)	0.941(20)	H(11)-C(1)	0.960
H(21)-C(2)	0.960	H(22)-C(2)	0.960
H(31)-C(3)	0.960	H(32)-C(3)	0.960
H(51)-C(5)	0.960	H(52)-C(5)	0.960
H(61)-C(6)	0.960	H(71)-C(7)	0.960
H(72)-C(7)	0.960	H(81)-C(8)	0.960
H(91)-C(9)	0.960	H(101)-C(10)	0.960
H(111)-C(11)	0.960	H(112)-C(11)	0.960
H(121)-C(12)	0.960	H(122)-C(12)	0.960
H(141)-C(14)	0.960	H(151)-C(15)	0.960
H(152)-C(15)	0.960	H(161)-C(16)	0.960
H(162)-C(16)	0.960	H(171)-C(17)	0.960
H(181)-C(18)	0.960	H(182)-C(18)	0.960

H(183)-C(18)	0.960	H(191)-C(19)	0.960
H(192)-C(19)	0.960	H(193)-C(19)	0.960
H(211)-C(21)	0.960	H(212)-C(21)	0.960
H(213)-C(21)	0.960		

Table 17 Bond angles (deg.)

C(10)-C(1)-C(2)	112.0(4)	C(17)-C(1)-C(2)	111.6(5)
C(17)-C(1)-C(10)	107.2(4)	C(3)-C(2)-C(1)	115.7(5)
C(4)-C(3)-C(2)	115.6(5)	C(3)-C(4)-O(2)	105.8(5)
C(5)-C(4)-O(2)	110.2(5)	C(5)-C(4)-C(3)	113.1(5)
C(8)-C(4)-O(2)	113.4(4)	C(8)-C(4)-C(3)	111.8(5)
C(8)-C(4)-C(5)	102.8(4)	C(6)-C(5)-C(4)	106.9(4)
C(5)-C(6)-O(1)	110.9(4)	C(7)-C(6)-O(1)	116.0(4)
C(7)-C(6)-C(5)	103.0(5)	C(8)-C(7)-C(6)	101.2(4)
C(7)-C(8)-C(4)	103.7(4)	C(9)-C(8)-C(4)	120.5(5)
C(9)-C(8)-C(7)	115.6(5)	C(10)-C(9)-C(8)	115.9(4)
C(18)-C(9)-C(8)	116.4(5)	C(18)-C(9)-C(10)	109.7(5)
C(9)-C(10)-C(1)	117.1(4)	C(11)-C(10)-C(1)	111.9(4)
C(11)-C(10)-C(9)	108.4(5)	C(12)-C(11)-C(10)	114.6(5)
C(13)-C(12)-C(11)	111.2(5)	C(14)-C(13)-C(12)	116.3(5)
C(17)-C(13)-C(12)	107.9(5)	C(17)-C(13)-C(14)	99.7(5)
C(19)-C(13)-C(12)	111.0(6)	C(19)-C(13)-C(14)	109.8(5)
C(19)-C(13)-C(17)	111.7(5)	C(15)-C(14)-C(13)	104.3(5)
C(20)-C(14)-C(13)	116.1(5)	C(20)-C(14)-C(15)	113.1(5)
C(16)-C(15)-C(14)	106.7(5)	C(17)-C(16)-C(15)	103.8(5)
C(13)-C(17)-C(1)	115.3(5)	C(16)-C(17)-C(1)	119.1(5)
C(16)-C(17)-C(13)	104.6(5)	C(14)-C(20)-O(3)	123.0(6)
C(21)-C(20)-O(3)	119.6(6)	C(21)-C(20)-C(14)	117.5(6)
C(6)-O(1)-H(1)	109.0(39)	C(4)-O(2)-H(2)	114.6(33)
C(2)-C(1)-H(11)	105.6(3)	C(10)-C(1)-H(11)	110.0(4)
C(17)-C(1)-H(11)	110.4(3)	H(21)-C(2)-C(1)	107.9(3)
H(22)-C(2)-C(1)	107.8(3)	H(22)-C(2)-H(21)	109.5
C(3)-C(2)-H(21)	107.9(3)	C(3)-C(2)-H(22)	107.9(3)

H(31)-C(3)-C(2)	108.0(3)	H(32)-C(3)-C(2)	107.8(3)
H(32)-C(3)-H(31)	109.5	C(4)-C(3)-H(31)	108.0(3)
C(4)-C(3)-H(32)	107.9(3)	H(51)-C(5)-C(4)	110.1(3)
H(52)-C(5)-C(4)	110.1(3)	H(52)-C(5)-H(51)	109.5
C(6)-C(5)-H(51)	110.1(3)	C(6)-C(5)-H(52)	110.1(3)
H(61)-C(6)-O(1)	102.1(3)	H(61)-C(6)-C(5)	115.4(4)
C(7)-C(6)-H(61)	110.0(3)	H(71)-C(7)-C(6)	111.5(3)
H(72)-C(7)-C(6)	111.5(3)	H(72)-C(7)-H(71)	109.5
C(8)-C(7)-H(71)	111.5(3)	C(8)-C(7)-H(72)	111.5(3)
H(81)-C(8)-C(4)	108.3(3)	H(81)-C(8)-C(7)	114.4(3)
C(9)-C(8)-H(81)	94.3(3)	H(91)-C(9)-C(8)	99.2(3)
C(10)-C(9)-H(91)	107.5(3)	C(18)-C(9)-H(91)	106.8(4)
H(101)-C(10)-C(1)	101.7(4)	H(101)-C(10)-C(9)	105.8(3)
C(11)-C(10)-H(101)	111.6(4)	H(111)-C(11)-C(10)	108.2(3)
H(112)-C(11)-C(10)	108.1(4)	H(112)-C(11)-H(111)	109.5
C(12)-C(11)-H(111)	108.2(3)	C(12)-C(11)-H(112)	108.2(4)
H(121)-C(12)-C(11)	109.0(4)	H(122)-C(12)-C(11)	109.0(4)
H(122)-C(12)-H(121)	109.5	C(13)-C(12)-H(121)	109.0(4)
C(13)-C(12)-H(122)	109.1(4)	H(141)-C(14)-C(13)	109.9(3)
C(15)-C(14)-H(141)	113.2(4)	C(20)-C(14)-H(141)	100.6(4)
H(151)-C(15)-C(14)	110.2(4)	H(152)-C(15)-C(14)	110.1(4)
H(152)-C(15)-H(151)	109.5	C(16)-C(15)-H(151)	110.3(4)
C(16)-C(15)-H(152)	110.1(4)	H(161)-C(16)-C(15)	111.0(4)
H(162)-C(16)-C(15)	110.8(4)	H(162)-C(16)-H(161)	109.5
C(17)-C(16)-H(161)	110.9(4)	C(17)-C(16)-H(162)	110.9(4)
H(171)-C(17)-C(1)	96.0(3)	H(171)-C(17)-C(13)	113.1(3)
H(171)-C(17)-C(16)	108.7(4)	H(181)-C(18)-C(9)	109.5(3)
H(182)-C(18)-C(9)	109.4(4)	H(182)-C(18)-H(181)	109.5
H(183)-C(18)-C(9)	109.6(3)	H(183)-C(18)-H(181)	109.5

H(183)-C(18)-H(182)	109.5	H(191)-C(19)-C(13)	109.5(4)
H(192)-C(19)-C(13)	109.5(4)	H(192)-C(19)-H(191)	109.5
H(193)-C(19)-C(13)	109.5(4)	H(193)-C(19)-H(191)	109.5
H(193)-C(19)-H(192)	109.5	H(211)-C(21)-C(20)	109.4(4)
H(212)-C(21)-C(20)	109.5(4)	H(212)-C(21)-H(211)	109.5
H(213)-C(21)-C(20)	109.4(5)	H(213)-C(21)-H(211)	109.5
H(213)-C(21)-H(212)	109.5		

Table 18 Selected non-bonded distances (Å)

Intramolecular:

C(5)-O(1)	2.438	H(51)-O(1)	2.857
H(52)-O(1)	2.483	H(61)-O(1)	1.884
C(7)-O(1)	2.498	H(71)-O(1)	2.666
H(72)-O(1)	2.845	C(6)-H(1)	1.959
H(61)-H(1)	2.093	C(7)-H(1)	2.753
C(2)-O(2)	3.003	H(22)-O(2)	2.647
C(3)-O(2)	2.358	H(32)-O(2)	2.464
C(5)-O(2)	2.454	H(52)-O(2)	2.423
C(7)-O(2)	3.179	C(8)-O(2)	2.496
C(9)-O(2)	3.048	C(18)-O(2)	2.942
H(181)-O(2)	2.975	H(182)-O(2)	2.460
C(4)-H(2)	2.015	C(5)-H(2)	2.650
H(52)-H(2)	2.384	C(7)-H(2)	3.000
H(71)-H(2)	2.568	C(8)-H(2)	2.736
C(18)-H(2)	2.817	H(181)-H(2)	2.559
H(182)-H(2)	2.456	C(13)-O(3)	3.408
C(14)-O(3)	2.394	H(141)-O(3)	2.981
C(15)-O(3)	2.823	H(152)-O(3)	2.424
C(19)-O(3)	3.326	H(192)-O(3)	2.885
C(21)-O(3)	2.333	H(212)-O(3)	2.597
H(213)-O(3)	2.597	H(21)-C(1)	2.045
H(22)-C(1)	2.044	C(3)-C(1)	2.591
H(31)-C(1)	2.713	C(4)-C(1)	3.254
C(8)-C(1)	2.947	H(81)-C(1)	2.495
C(9)-C(1)	2.659	H(101)-C(1)	1.989
C(11)-C(1)	2.559	H(112)-C(1)	2.786

C(12)-C(1)	3.021	C(13)-C(1)	2.602
C(16)-C(1)	2.643	H(161)-C(1)	2.777
H(162)-C(1)	2.996	H(171)-C(1)	1.898
C(19)-C(1)	3.126	H(191)-C(1)	2.886
C(2)-H(11)	2.017	H(21)-H(11)	2.468
C(3)-H(11)	2.561	H(31)-H(11)	2.351
C(8)-H(11)	2.768	H(81)-H(11)	2.057
C(9)-H(11)	2.851	C(10)-H(11)	2.091
C(11)-H(11)	2.819	C(13)-H(11)	2.827
C(16)-H(11)	2.892	C(17)-H(11)	2.079
C(19)-H(11)	2.842	H(191)-H(11)	2.419
H(31)-C(2)	2.038	H(32)-C(2)	2.036
C(4)-C(2)	2.579	C(8)-C(2)	3.053
H(81)-C(2)	2.916	C(9)-C(2)	3.171
C(10)-C(2)	2.564	H(101)-C(2)	2.604
C(16)-C(2)	3.109	H(162)-C(2)	2.998
C(17)-C(2)	2.542	H(171)-C(2)	2.552
H(22)-H(21)	1.568	C(3)-H(21)	2.037
H(31)-H(21)	2.359	H(32)-H(21)	2.245
C(16)-H(21)	2.597	H(162)-H(21)	2.275
C(17)-H(21)	2.516	H(171)-H(21)	2.544
C(3)-H(22)	2.037	H(32)-H(22)	2.357
C(4)-H(22)	2.700	C(9)-H(22)	3.067
C(10)-H(22)	2.544	H(101)-H(22)	2.260
C(17)-H(22)	2.933	H(171)-H(22)	2.624
C(5)-C(3)	2.570	H(51)-C(3)	2.536
C(8)-C(3)	2.547	H(81)-C(3)	2.534
C(9)-C(3)	3.377	C(10)-C(3)	3.423
H(32)-H(31)	1.568	C(4)-H(31)	2.036

C(5)-H(31)	2.665	H(51)-H(31)	2.385
C(8)-H(31)	2.751	H(81)-H(31)	2.443
C(4)-H(32)	2.035	C(5)-H(32)	2.798
H(51)-H(32)	2.619	H(51)-C(4)	2.092
H(52)-C(4)	2.091	C(6)-C(4)	2.480
H(61)-C(4)	2.973	C(7)-C(4)	2.421
H(71)-C(4)	2.705	H(81)-C(4)	2.067
C(9)-C(4)	2.685	C(10)-C(4)	3.422
C(18)-C(4)	3.288	H(61)-C(5)	2.125
C(7)-C(5)	2.380	H(71)-C(5)	2.677
C(8)-C(5)	2.431	H(81)-C(5)	2.850
H(52)-H(51)	1.568	C(6)-H(51)	2.066
H(61)-H(51)	2.307	C(8)-H(51)	3.063
C(6)-H(52)	2.065	C(7)-H(52)	2.819
C(8)-H(52)	3.079	H(71)-C(6)	2.068
H(72)-C(6)	2.067	C(8)-C(6)	2.347
H(81)-C(6)	2.657	C(7)-H(61)	2.050
H(72)-H(61)	2.275	C(8)-H(61)	2.664
H(81)-H(61)	2.627	H(81)-C(7)	2.110
C(9)-C(7)	2.593	H(91)-C(7)	2.554
C(18)-C(7)	3.263	H(181)-C(7)	2.908
H(72)-H(71)	1.568	C(8)-H(71)	2.078
C(9)-H(71)	2.829	C(18)-H(71)	3.014
H(181)-H(71)	2.416	C(8)-H(72)	2.078
H(81)-H(72)	2.379	C(9)-H(72)	2.827
H(91)-H(72)	2.447	H(91)-C(8)	1.940
C(10)-C(8)	2.626	C(18)-C(8)	2.613
H(181)-C(8)	2.727	H(182)-C(8)	2.911
C(9)-H(81)	1.874	H(91)-H(81)	2.101

C(10)-H(81)	2.475	H(101)-C(9)	2.041
C(11)-C(9)	2.506	H(111)-C(9)	2.630
H(112)-C(9)	2.675	H(181)-C(9)	2.066
H(182)-C(9)	2.064	H(183)-C(9)	2.067
C(10)-H(91)	2.063	C(11)-H(91)	2.402
H(111)-H(91)	2.373	H(112)-H(91)	2.334
C(18)-H(91)	2.034	H(181)-H(91)	2.342
H(183)-H(91)	2.303	H(111)-C(10)	2.045
H(112)-C(10)	2.044	C(12)-C(10)	2.577
H(122)-C(10)	2.812	C(13)-C(10)	3.002
C(17)-C(10)	2.494	H(171)-C(10)	2.487
C(18)-C(10)	2.532	H(182)-C(10)	2.706
H(183)-C(10)	2.707	C(11)-H(101)	2.084
H(111)-H(101)	2.362	C(12)-H(101)	2.809
C(17)-H(101)	2.625	H(171)-H(101)	2.252
C(18)-H(101)	2.414	H(182)-H(101)	2.325
H(183)-H(101)	2.473	H(121)-C(11)	2.056
H(122)-C(11)	2.056	C(13)-C(11)	2.519
C(17)-C(11)	2.873	H(171)-C(11)	3.032
C(18)-C(11)	3.448	C(19)-C(11)	3.077
H(191)-C(11)	2.687	H(112)-H(111)	1.568
C(12)-H(111)	2.046	H(121)-H(111)	2.358
H(122)-H(111)	2.300	C(12)-H(112)	2.046
H(121)-H(112)	2.295	C(13)-H(112)	2.737
C(19)-H(112)	2.774	H(191)-H(112)	2.146
C(14)-C(12)	2.600	H(141)-C(12)	2.667
C(17)-C(12)	2.475	H(171)-C(12)	2.624
C(19)-C(12)	2.520	H(191)-C(12)	2.637
H(192)-C(12)	2.779	C(20)-C(12)	3.352

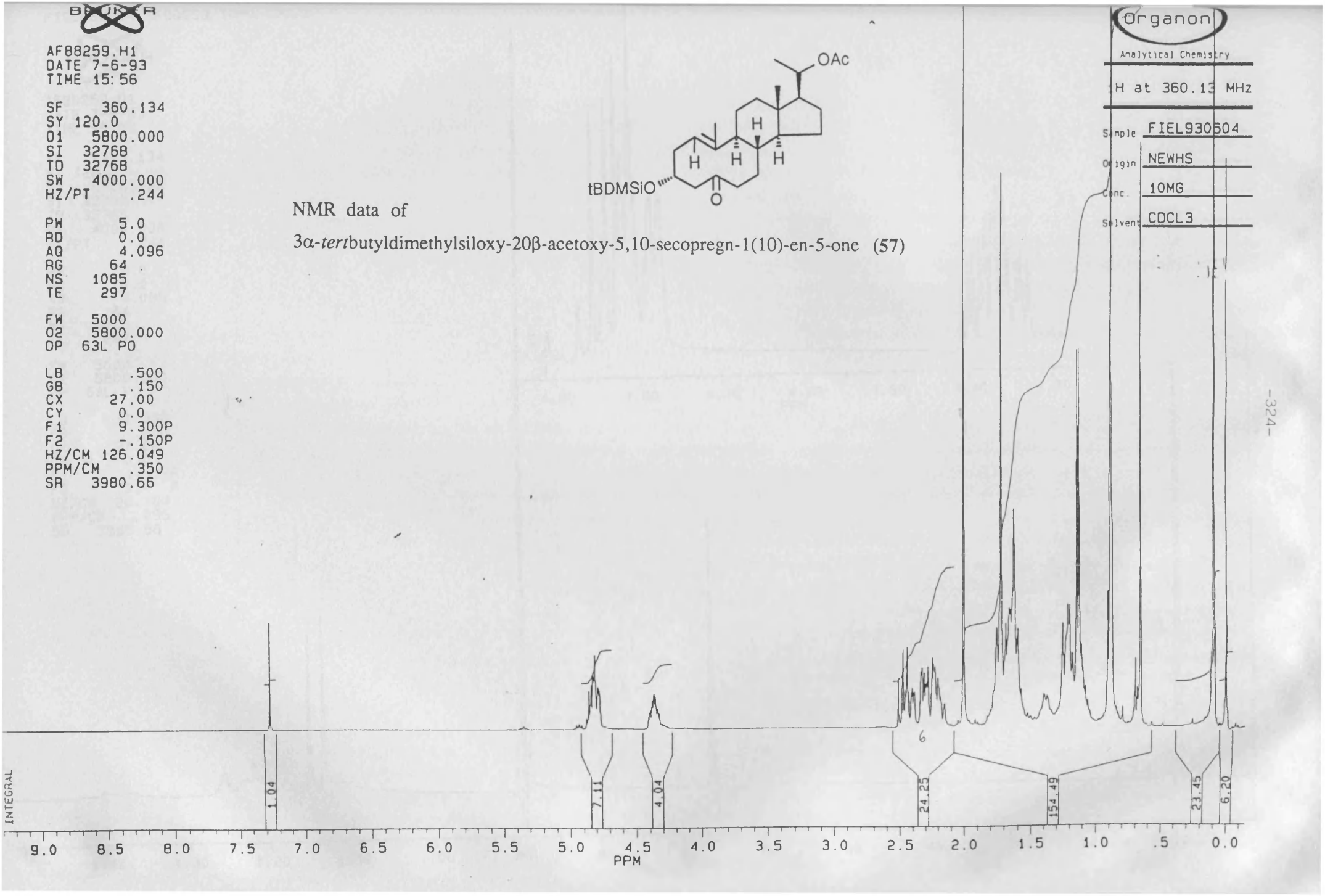
C(21)-C(12)	3.462	H(211)-C(12)	2.914
H(122)-H(121)	1.568	C(13)-H(121)	2.045
C(14)-H(121)	2.895	C(19)-H(121)	2.639
H(192)-H(121)	2.560	C(21)-H(121)	3.012
H(211)-H(121)	2.519	C(13)-H(122)	2.045
C(14)-H(122)	2.701	H(141)-H(122)	2.421
C(17)-H(122)	2.684	H(171)-H(122)	2.488
H(141)-C(13)	2.075	C(15)-C(13)	2.428
H(152)-C(13)	2.901	C(16)-C(13)	2.426
H(161)-C(13)	2.825	H(171)-C(13)	2.111
H(191)-C(13)	2.067	H(192)-C(13)	2.067
H(193)-C(13)	2.067	C(20)-C(13)	2.588
C(21)-C(13)	3.345	H(151)-C(14)	2.072
H(152)-C(14)	2.070	C(16)-C(14)	2.471
H(161)-C(14)	3.071	C(17)-C(14)	2.355
H(171)-C(14)	2.651	C(19)-C(14)	2.519
H(192)-C(14)	2.697	H(193)-C(14)	2.697
C(21)-C(14)	2.557	H(211)-C(14)	2.536
C(15)-H(141)	2.106	H(151)-H(141)	2.271
C(16)-H(141)	2.975	C(17)-H(141)	2.684
H(171)-H(141)	2.634	C(20)-H(141)	1.931
C(21)-H(141)	2.514	H(211)-H(141)	2.229
H(161)-C(15)	2.091	H(162)-C(15)	2.089
C(17)-C(15)	2.418	H(171)-C(15)	2.808
C(19)-C(15)	3.042	H(193)-C(15)	2.713
C(20)-C(15)	2.537	H(152)-H(151)	1.568
C(16)-H(151)	2.083	H(162)-H(151)	2.221
C(17)-H(151)	3.019	C(20)-H(151)	2.969
C(16)-H(152)	2.081	H(161)-H(152)	2.221

C(19)-H(152)	3.043	H(193)-H(152)	2.505
C(20)-H(152)	2.567	H(171)-C(16)	2.048
C(19)-C(16)	3.005	H(193)-C(16)	2.620
H(162)-H(161)	1.568	C(17)-H(161)	2.073
C(19)-H(161)	2.904	H(193)-H(161)	2.283
C(17)-H(162)	2.072	H(171)-H(162)	2.226
C(19)-C(17)	2.547	H(191)-C(17)	2.809
H(193)-C(17)	2.661	H(182)-H(181)	1.568
H(183)-H(181)	1.568	H(183)-H(182)	1.568
C(20)-C(19)	2.961	H(192)-H(191)	1.568
H(193)-H(191)	1.568	H(193)-H(192)	1.568
C(20)-H(192)	2.621	H(211)-C(20)	2.017
H(212)-C(20)	2.018	H(213)-C(20)	2.017
H(212)-H(211)	1.568	H(213)-H(211)	1.568
H(213)-H(212)	1.568		

Intermolecular:

O(2)-O(1a)	2.749	O(2)-O(1b)	2.798
H(2)-O(1b)	1.863	H(71)-O(1b)	2.766
H(181)-O(1b)	2.908	O(2)-H(1a)	1.813
H(2)-H(1a)	2.209	C(4)-H(1a)	2.976
H(2)-H(1b)	2.337	H(71)-H(1b)	2.438
C(6)-O(2c)	3.334	C(6)-H(2d)	2.784
H(11)-O(3e)	2.804	H(31)-O(3e)	2.616
H(162)-O(3f)	2.754	H(61)-C(2c)	2.922
H(61)-H(22c)	2.413	H(72)-H(22c)	2.469
H(61)-H(32c)	2.559	H(213)-H(51g)	2.575
H(152)-H(51h)	2.591	H(152)-H(61h)	2.637
H(122)-H(91i)	2.434	H(183)-H(112i)	2.642

C(18)-H(122j)	2.929	H(183)-H(122j)	2.601
H(212)-C(16k)	3.072	C(20)-H(162k)	3.024
H(212)-H(162k)	2.478	H(211)-H(181i)	2.575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Analytical Chemistry
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Organon
Analytical Chemistry

1H at 360.13 MHz

Sample FIEL930604
Origin NEWHS
Conc. 10MG
Solvent CDCL3

AF88259, FIEL930604, 10MG, C6D6

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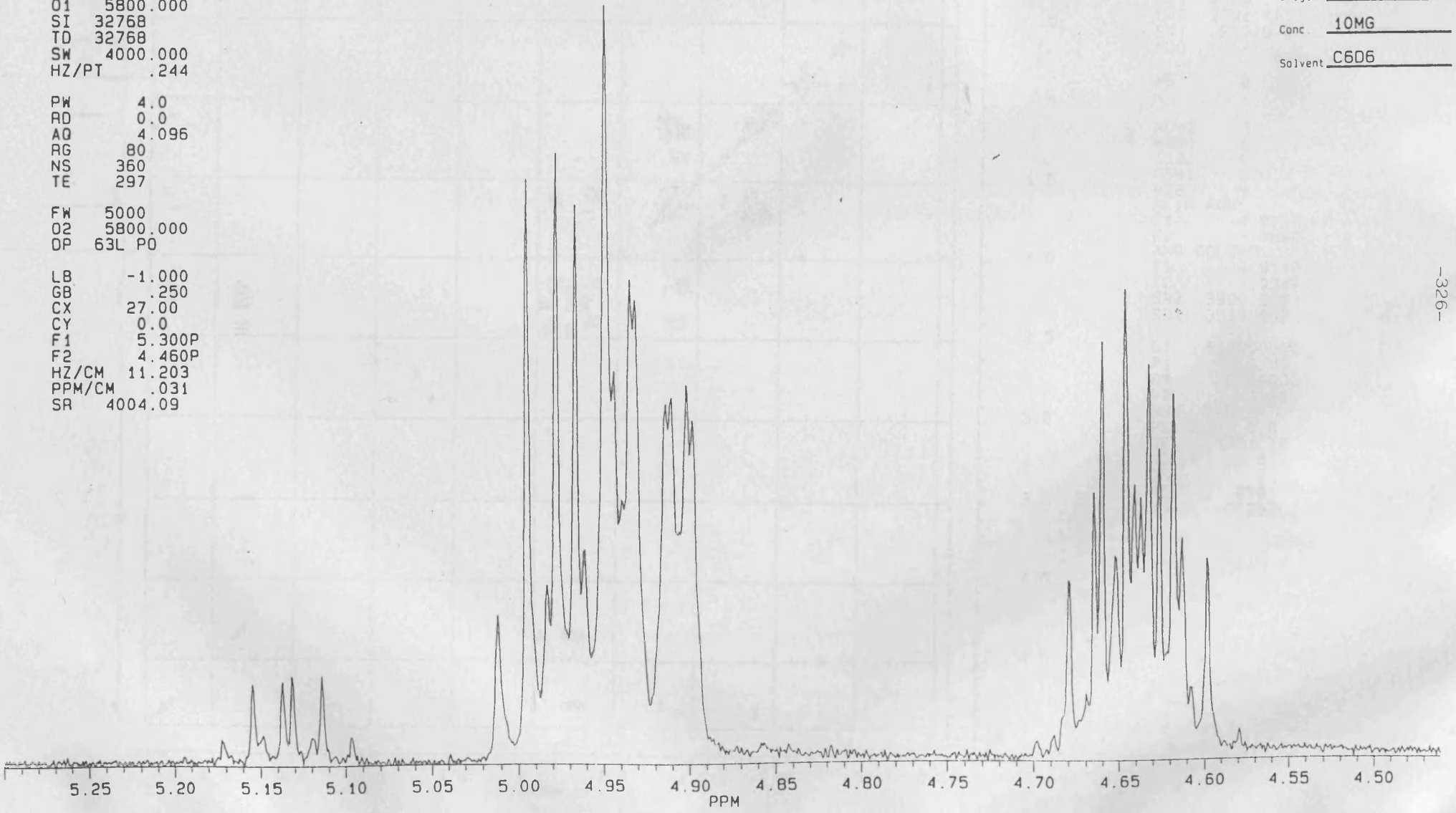
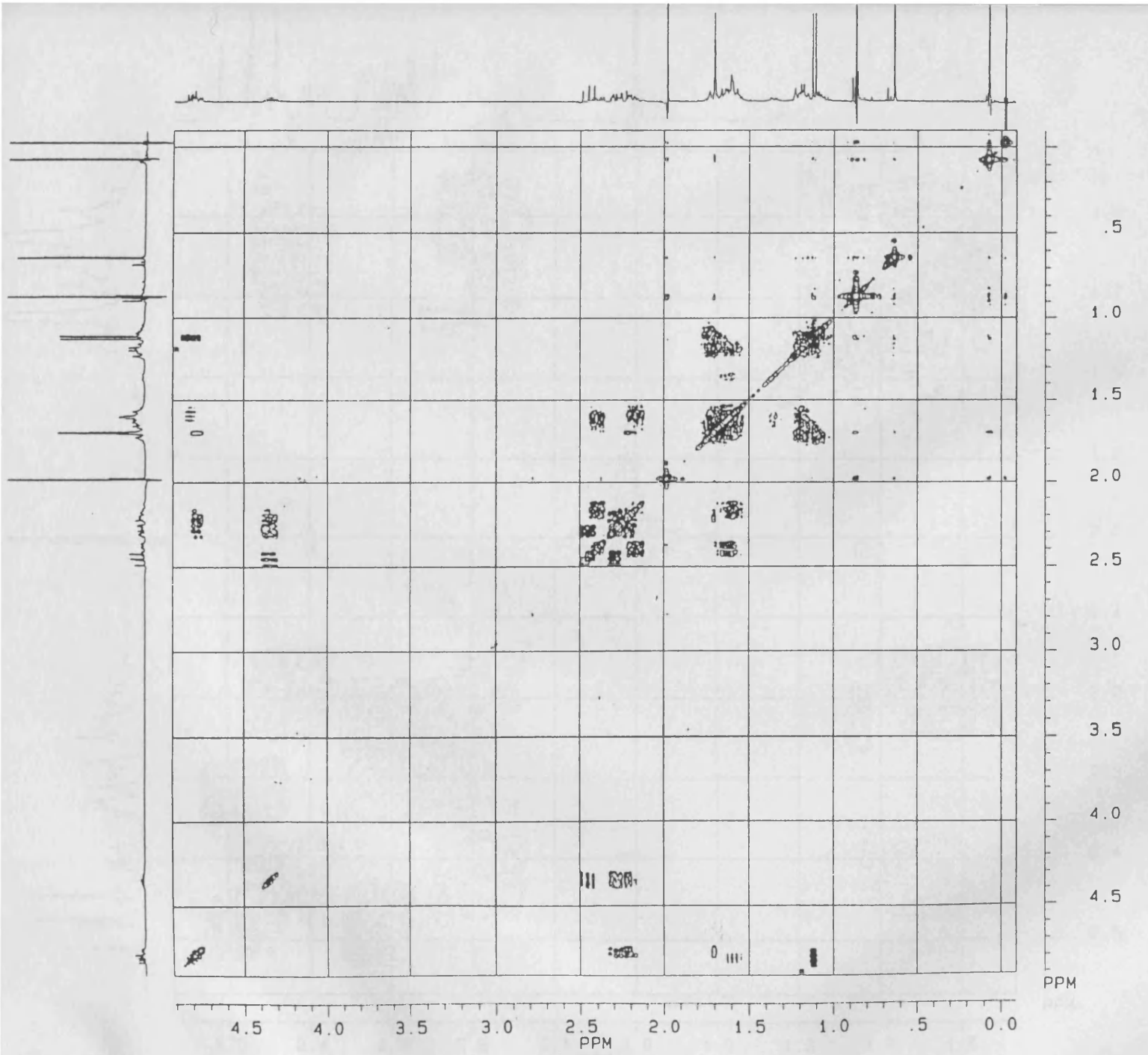
CX 27.00
CY 0.0
F1 5.300P
F2 4.460P
HZ/CM 11.203
PPM/CM .031
SR 4004.09

Organon
Analytical Chemistry

1H at 360.13 MHz

Sample FIEL930604
Origin NEWHS
Conc 10MG
Solvent C6D6





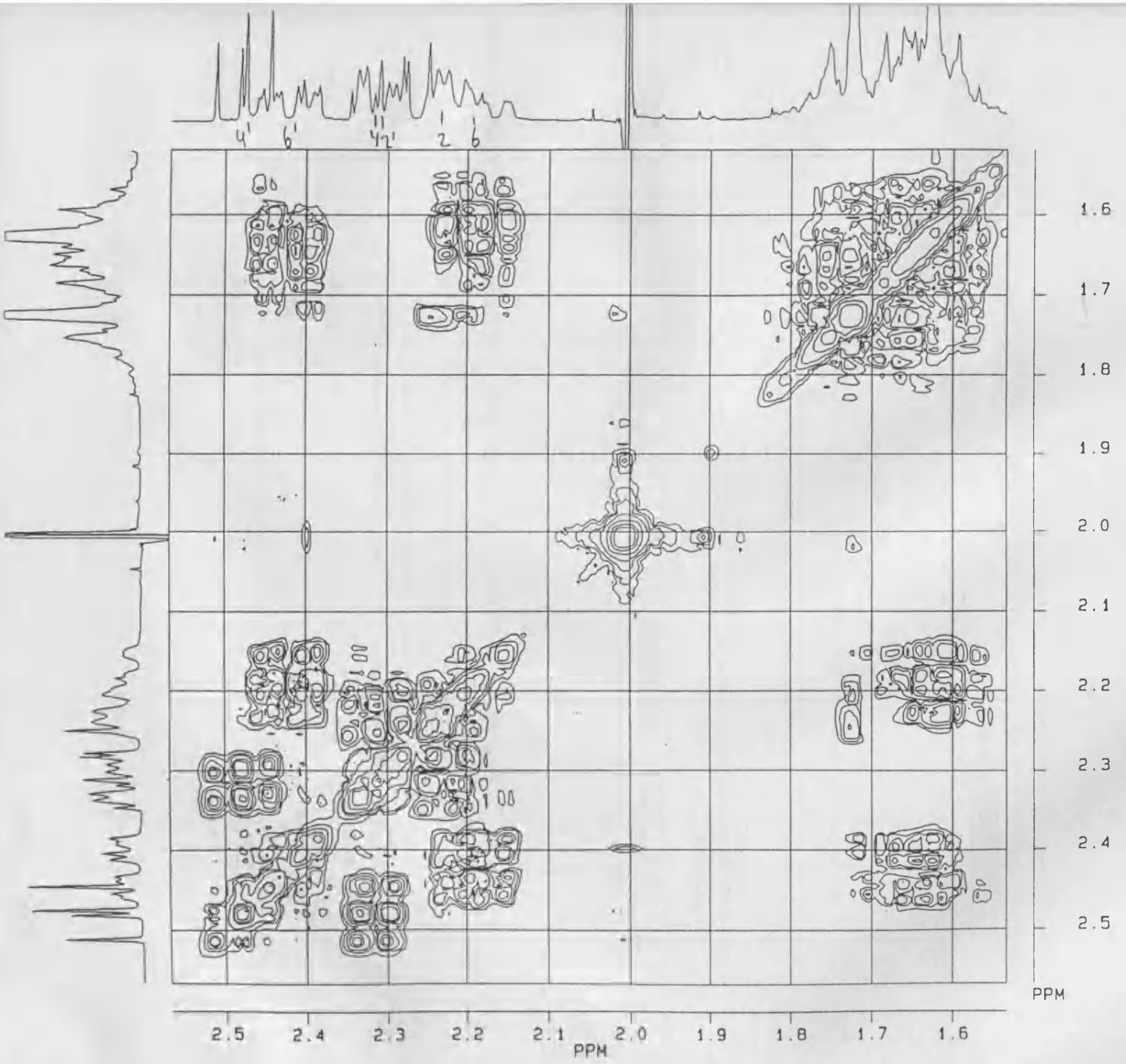
Organon
Analytical Chemistry

CF88259.SMX
F1 PROJ:
SPECFIEL
F2 PROJ:
SPECFIEL
AU PROG:
COSY.AU
DATE 11-6-93

1H at 360.13 MHz

Sample FIEL930604
Origin NEWH
Conc. 10MG
Solvent COCL3

SI2	2048
SI1	1024
SW2	1901.141
SW1	950.570
NDO	1
NS	8
WDW2	S
WDW1	S
SSB2	0
SSB1	0
MC2	M
PLIM ROW:	
F1	4.911P
F2	-.089P
AND COLUMN:	
F1	4.911P
F2	-.094P
SR2	3990.992
SR1	3990.992
D1	1.0000000
P1	6.80
DO	.0000030
P2	3.40
RD	0.0
PW	0.0
DE	331.30
NS	8
DS	2
NE	256
IN	.0005260



Organon

Analytical Chemistry

GW88259.SMX
F1 PROJ:
SPEC1H
F2 PROJ:
SPEC1H
AU PROG:
COSY.AU
DATE 11-6-93

1H at 360.13 MHz

Sample FIEL930604
Origin NEWHOUSE
Spec
Solvent CDCL3

SI2	2048
SI1	1024
SW2	1901.141
SW1	950.570
NDO	1
NS	8
WDW2	S
WDW1	S
SSB2	0
SSB1	0
MC2	M
PLIM ROW:	
F1	2.567P
F2	1.531P
AND COLUMN:	
F1	2.567P
F2	1.521P
SR2	3981.161
SR1	3981.161
D1	1.0000000
P1	6.80
DO	.0000030
P2	3.40
RD	0.0
PW	0.0
DE	331.30
NS	8
DS	2
NE	512
IN	.0005260