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SYNTHESIS OF POTENTIALLY CYTOTOXIC STEROIDAL LACTONES

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A DOCTORAL THESIS

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SUMMARY

A review of the biological properties and the synthesis of α-methylene-γ- and δ-lactones is presented.

Cholesterol (201) was converted into 4-oxa-3-oxo-5α-cholestane (203) and the α-methylene moiety was introduced by α-hydroxymethylenation, diethylamination and elimination of diethylamine after hydrogenation to give 2-methylene-4-oxa-3-oxo-5α-cholestane (225). The same sequence of reactions was employed to prepare 17β-hydroxy-2-methylene-4-oxa-3-oxo-5α-androstane (238) and its 17-yl acetate (239) from androst-5-en-3β-ol-17-one (228). Reactions of the above α-methylenelactones with L-cysteine gave the cysteine-lactone adducts in a Michael-type addition. Reaction of the lactone (203) with phenylmagnesium bromide gave 4-oxa-3-phenyl-5α-cholest-2-ene (278). On epoxidation this compound gave an unusual rearranged product, 3-hydroxy-4-oxa-3-phenyl-5α-cholestan-2-one (281) which on treatment with ethanol/hydrochloric acid formed 3-ethoxy-4-oxa-3-phenyl-5α-cholestan-2-one (282). Oxidation of the rearranged product (281) with lead tetra acetate gave 5-benzoyloxy-2,3-seco-5α-cholestan-2-oic acid (299) which on esterification gave the methyl ester (300).

The oxidised product (299), after hydrolysis, was cyclised to give A-nor-3-oxa-5α-cholestan-2-one (274).

Reaction of the lactone (203) with 4-methoxy phenyl magnesium bromide gave three major products, 3-(4-methoxyphenyl)-4-oxa-5α-cholest-2-ene (304), 3,3-di (4-methoxyphenyl)-4-oxa-5α-cholestane (305) and 3,3-di (4-methoxy phenyl)-5β-hydroxy-3,4-seco-5α-cholest-2-ene (306).

Hydrogenolysis of (305) gave a saturated alcohol, 3,3-di (4-methoxy phenyl) -5β-hydroxy-3,4-seco-5α-cholestane (314) and a ketone, 3,3-di (4-methoxy phenyl)-3,4-seco-cholestan-5-one (315). The structure of the saturated alcohol (314) was confirmed by hydrogenation of the unsaturated alcohol (306).

Oxidation of the saturated alcohol (314) with Jones' reagent gave a ketone identical with the ketone (315) obtained during hydrogenolysis. Treatment of the unsaturated alcohol (306) with a catalytic amount of toluene-p-sulphonic acid in benzene afforded a ketone, by a 1,5-hydride shift, identical to the ketone (315) obtained by successive hydrogenolysis and Jones' oxidation.

Manganic acetate-catalysed addition of acetic acid to 5α-cholest-2-ene (328) gave the γ-lactone (329).

Androst-5-en-3β-ol-17-one (241) was converted to 3β-hydroxy-17α-oxa-17-oxo-D-homo-5α-androstane (251). The D-ring lactone (251) on hydroxymethylation, gave 3β-hydroxy-16-hydroxymethyl-17α-oxa-17-oxo-D-homo-5α-androstane (256) and on mesylation resulted in a dimesylate (258) which, on base-catalysed elimination, gave an α-methylene-lactone. (259)

An α-methylene-D-ring lactone (255) was obtained from the D-ring lactone (251) directly by the Mannich reaction with paraformaldehyde/diethylamine/diethylamine hydrochloride in dioxane and an aldol-type condensation with paraformaldehyde/potassium hydroxide in ethanol.

The cytotoxic activity, (LD_{50}), determined by a tissue culture technique on KB cells was as follows; 2-methylene-4-oxa-3-oxo-5α-cholestane (0.80 μg/ml), 17β-acetoxy-2-methylene-4-oxa-3-oxo-5α-androstane (0.72 μg/ml), 17β-hydroxy-2-methylene-4-oxa-3-oxo-5α-androstane (0.80 μg/ml), 16-methylene-17α-oxa-17-oxo-D-homo-5α-androst-2-ene (0.92 μg/ml) and 3β-hydroxy-16-methylene-17α-oxa-17-oxo-D-homo-5α-androstane (1.0 μg/ml).

INTRODUCTION

INTRODUCTION

BIOLOGICAL PROPERTIES OF SESQUITERPENE LACTONES.

(i) CANCER CHEMOTHERAPY.

There has been a great deal of confusion in the medical literature about the growth of cancer. Cancer is widely regarded as a condition in which cells multiply without restraint and at a very rapid rate. It will be useful to scrutinize the concept that the cancer cells multiply rapidly. However, it is easy to show that several kinds of normal cells, essential to life, multiply far more rapidly than cancer cells. In the normal human being, cells of the bone marrow multiply rapidly enough to produce 2.5×10^6 red blood cells each second for a life-time. After haemorrhage this rate can go up seven-fold. The cells lining the intestine multiply at least as rapidly and there is some evidence to suggest that they may multiply even more rapidly than the bone marrow cells. Even the cells lining the mouth, which are not as important to life, multiply at a faster rate than cancer cells. Since normal cells, essential to life, multiply more rapidly than most cancer cells, it follows that a broad-spectrum inhibitor, whether synthetic or naturally occurring, which effects all rapidly multiplying cells will affect the bone-marrow and intestinal lining at least as much as the cancer cells. The likelihood is that such a material might temporarily slow tumour growth, but before stopping it completely, would kill the patient. On the other hand, a narrow-spectrum inhibitor, which affects one kind of cell much more than others, offers considerable promise. Theoretically , it is possible to find among such inhibitors one which will affect a particular cancer more than normal cells.

Cancer chemotherapy is a comparatively young science, and clinically useful agents have evolved only during the past thirty years. During this period, many hundreds of variants of known classes of chemotherapeutic agents have been synthesised and there is a good reason to expect that modification of known drugs will continue. However, some pessimism is apparent among workers, because of the selectively small improvements over the prototype drugs which have resulted from the extensive synthetic efforts to date. There is a need for new prototypes or templates for the synthetic organic chemist to use in the design of potentially superior chemotherapeutic agents. As a corollary statement, there is a need for elucidation of new biochemical mechanisms of growth regulation which may be more amenable to selective regulation. Recent studies in the isolation and structural elucidation of tumour inhibitors are yielding a fascinating array of novel types of growth-inhibitory compounds. There appears to be a reason for confidence that this approach may point the way to useful templates for new synthetic approaches to cancer chemotherapy.

The discovery of the anti leukaemic activity of nitrogen mustard in the 1940's[1] is considered by many to have been the start of modern cancer chemotherapy. An enormous amount of work has followed on the synthesis and evaluation of biological alkylating agents.[2,3] Although the modification of presently known alkylating agents continues, some pessimism is evident due to the high degree of reactivity of simple alkylating agents leading to indiscriminate reaction with many cell constituents and consequently narrow therapeutic indices.

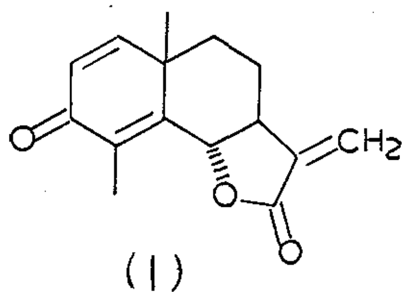
The recent discovery and structure elucidation of approximately 500 new sesquiterpene lactones, during a systematic study of Artemisia and other genera of the family of Compositae, provided the opportunity for further investigations on the potential of these sesquiterpene lactones as new therapeutic agents.[4,5] Although lactones (eudesmanolides) have been

reported from the roots of Liriodendron tulipifera(Magnoliaceae)[6] and from the bark of Brazilian species of Eremanthus(Compositae)[7], they are rarely found in stem and roots and are mostly concentrated in the leaves and flowering heads (phyllaries)[8].

(ii) ANTI-TUMOUR AND CYTOTOXIC ACTIVITY

Much of the earlier study on sesquiterpene lactones for the medicinal purposes has concentrated mainly on santonin (1), a santonolide, and its derivatives, which were known as important anthelmintic and ascaricidal agents[9]. The α-methylene-γ-butyrolactone moiety fused on various skeletons characterises a rapidly expanding group of sesquiterpene lactones, comprising to date more than 400 members[10].

Many sesquiterpene lactones bearing an α-methylene-γ- butyrolactone grouping exhibit significant growth-inhibitory activity in vivo against animal tumour systems and in vitro against cells derived from human carcinoma of the nasopharynx (KB)[11]. This activity appears to be associated with their ability to act as alkylating agents by virtue of conjugate addition of biological nucleophiles to the α-methylene lactone moiety. These include costunolide (2)[12] and tulipinolide (3),[6] the guanolides, gaillardin (4)[12]; elemanolides, vernolepin (5)[13] and vernomenin (6) [13]; the germacranolides, elephantopin (7A)[14], elephantin (7B)[14], helenalin (8)[15], euparotin acetate(9) and its companions [16] and the pseudoguaianolide, damsin (10)[17]. Unfortunately, active sesquiterpene lactones such as vernolepin (5)[13], elephantopin (7A)[14] and euparotin acetate (9)[16] have therapeutic indices which preclude their clinical use.



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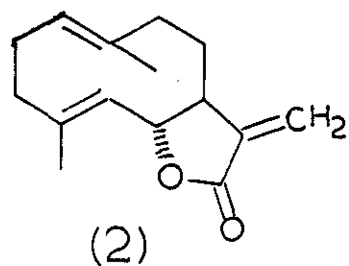

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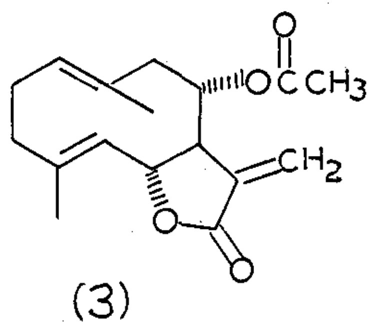

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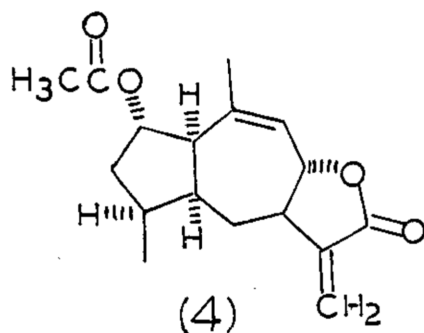

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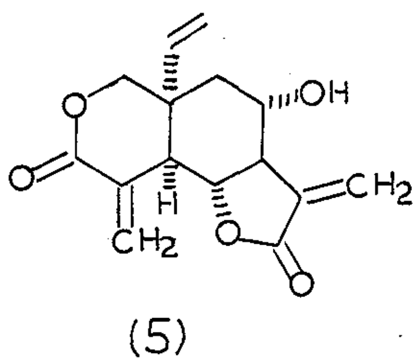

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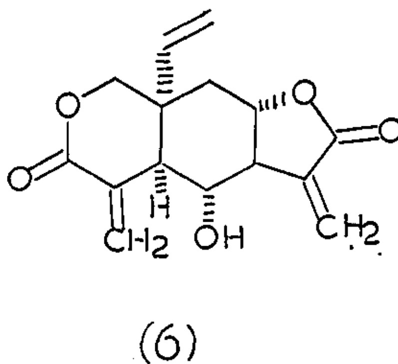

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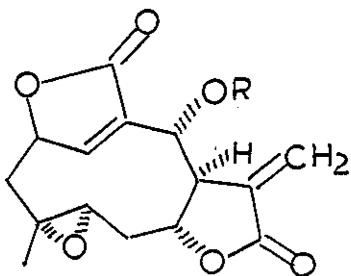

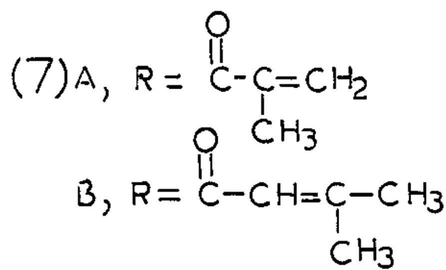

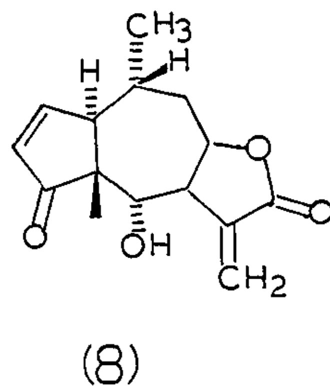

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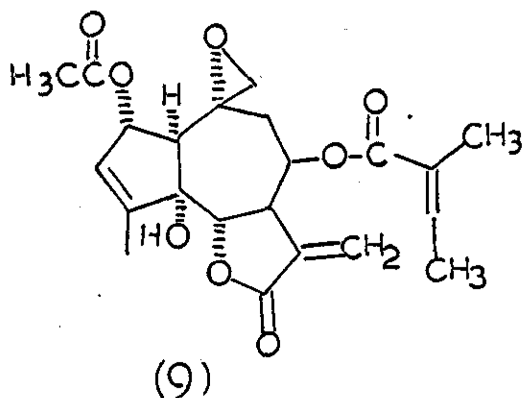
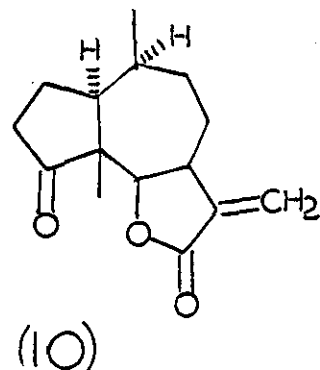




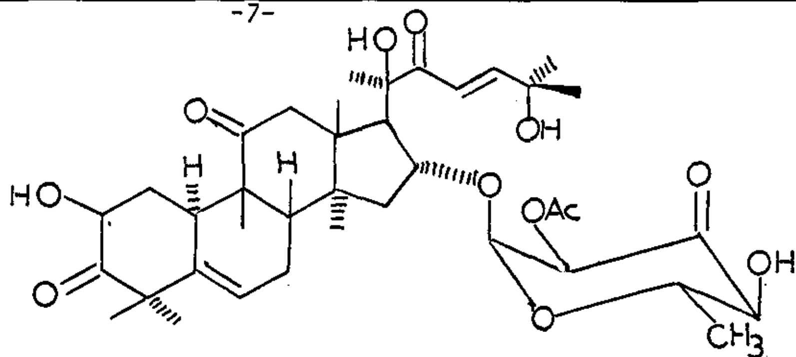
In an effort to obtain antineoplastic agents, over 500 sesquiterpenes from plants[18] were evaluated against numerous tumour models and it was found that all the known cytotoxic sesquiterpenes contained a lactone function, all but one of these was α,β-unsaturated and the α-ethylenic linkage was exocyclic in each case.

The most immediate and direct factor responsible for cytotoxicity of the compounds was the introduction of the O=C-CH= CH_2 system[19]. Compounds having endocyclic double bonds gave unstable cysteine adducts and thus were inactive[20]. However, sesquiterpene lactones which incorporated a cyclopentenone or α,β-unsaturated ketone (in addition to the α-methylene-γ -butyrolactone) appeared to have enhanced cytotoxicity whereas monofunctional sesquiterpenes containing only an α,β-unsaturated ester or cyclopentenone displayed non-significant activity.[11A, 21, 22.] Most bifunctional alkylating agents show a relationship between activity and distance between the alkylating sites [23,24]. In the series of open-chain α-methylene-γ- lactones, the distance between the two groups is critical and the activity increases as the distance betwen the groups decreases.[25] No enhancement of the cytotoxicity was observed in the bifunctional derivatives with a conjugated ester moiety.

Recently the α-methylene-γ-lactone moiety was reported to be highly toxic to normal human lymphoid cells as well as to CCRF-CEM, human lymphoblastic leukaemia cells in culture.[26] These cells have been characterised[27] as having an absolute nutritional requirement for exogenous L-cysteine. The use of CCRF-CEM cells to assay the cytotoxic properties was based on the expectation that α-methylene lactone systems would function as Michael acceptors,[20,28] and thus scavenge L-cysteine[29]. The cytotoxicity associated may arise from the pronounced (and indiscrimate) chemical reactivity of the α-methylene lactone moiety, whereas the anti tumour activity may arise from concurrent reactions of this moiety with biological nucleophiles of significance to tumour growth. These views are in accordance with the theory of tumour inhibition by selective alkylation of biological macromolecules. [11A, C, F, 28,30]

Modification of the chemical reactivity of the α-methylene lactone moiety, to the extent that anti tumour activity is maintained and toxicity is minimised, has not yet been established. In the natural compounds the β-carbon (alkylating site) of the α-methylene lactone is unsubstituted, thus substituent at this position should produce changes in physical properties and in chemical reactivity which could alter biological activity and therefore have important biological implications. Variation of substituents at the α-, and β-carbons of γ-crotonolactones has resulted in significant effects on anti tumour activity[31]. Attempts to modify the activity in simple synthetic α-methylene lactones by substitution of an alkyl and/or alkoxy group at the alkylation site resulted in compounds which were significantly toxic, [25,32] however, other factors such as changes in solubility, transport or metabolism may be involved.

The antitumour activity of cucurbitacins, datiscoside (11)[33], fabacein (12) and cucurbitacin B (13) [34,35] a group of highly oxygenated triterpenes, has been demonstrated[36,37].



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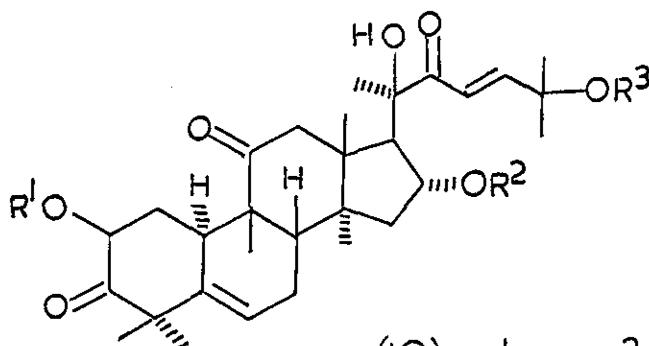
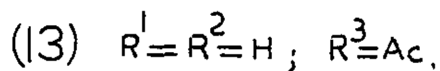
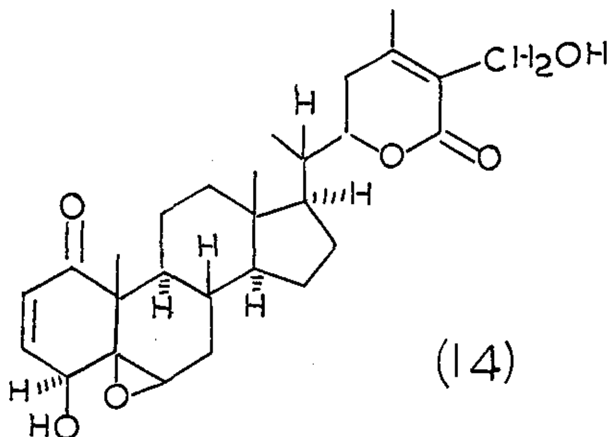
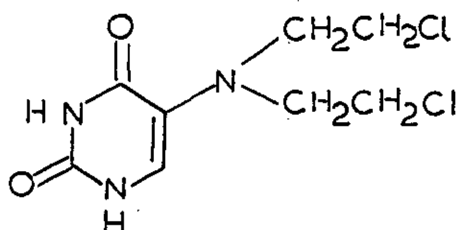




The action of cucurbitacins is more sensitive to the oxygen uptake than anaerolic glycolysis[36] as most cancer chemotherapeutic agents have immediate effects on one of the two primary energy-providing processes of cancer cell metabolism, namely glycolysis and respiration.[38] The isolation and structural elucidation of Withaferin A (14) from Withania somninfera L., a prototype of a novel class of polyfunctional steroid lactones, the withanolides was reported, at the same time but independently, by two groups.[39-42]



Lee _et al_[43] reported the anti tumour activity of α-methylene -γ- lactones of the nucleic acid bases, uracil, thymine and derivatives. They found that the introduction of the conjugated ester side chain enhanced this activity. The introduction of a carrier moiety into an alkylating centre has led to compounds, such as uracil mustard (15) of clinical interest, as anti cancer agents[44].



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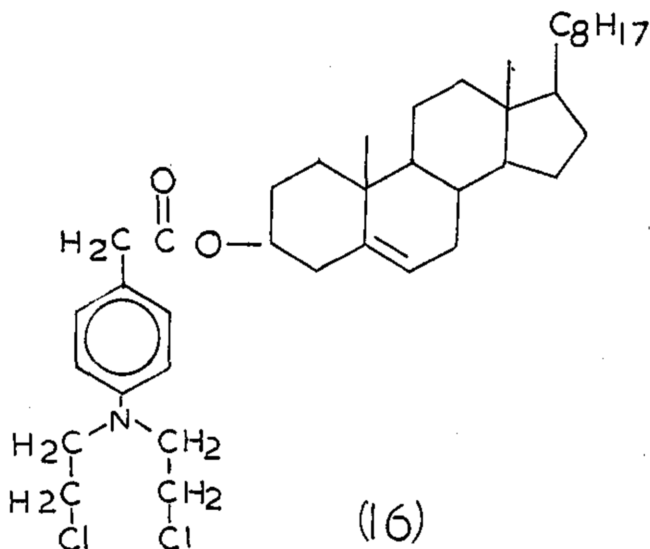
The work on the synthesis of compounds derived from the combination of an active alkylating agent and a carrier moiety, such as a steroidal hormone, has led to novel steroidal α-methylene-γ-lactone alkylating agents which are active against Walker 256 carcinosarcoma in rats[45]. These agents might be tumour specific (e.g. for breast or prostatic cancer) and thus clinically useful. Steroidal alkylating agents such as phenesterin (16),[46,47] oestradiol mustards (17)[48-50] and some homoaza steroid mustards (18)[51] of significant inhibitory activity on growth of various experimental tumours[52], are currently undergoing clinical trials.



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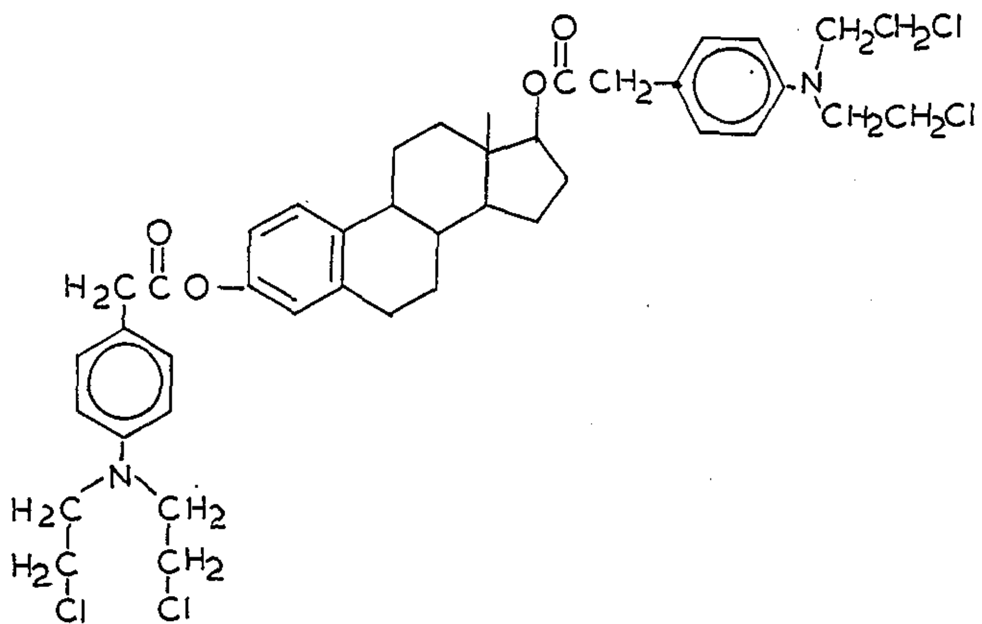

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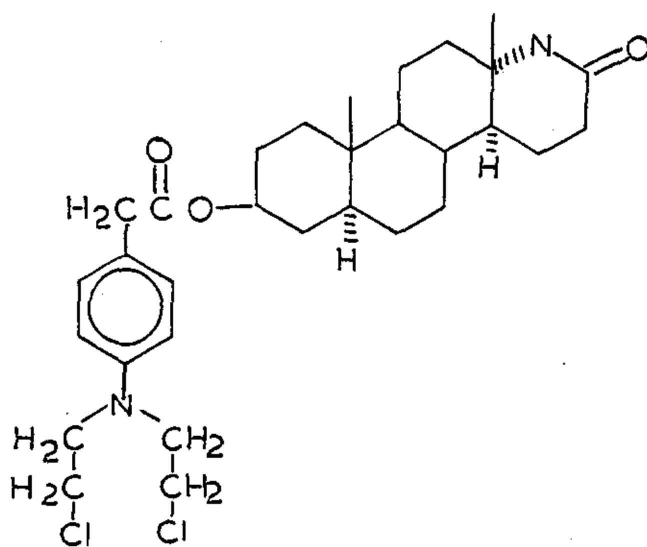

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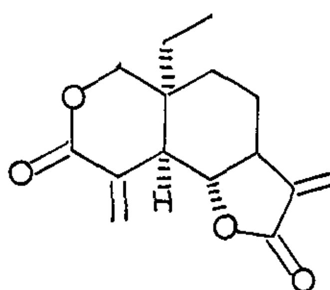
Grieco et al[53] reported that six-membered α-methylene-δ-valerolactone moiety is not necessarily less active than the five membered α-methylene-γ- butyrolactone moiety, despite its larger ring size. Also, vernolepin (5) derivatives, which lacked the hydroxy groups, were slightly more active than vernolepin. The enhanced activity of the deoxyvelnolepins (19) and (20) may be due to improved transport across the cell membrane because of increased lipophilicity or to sterically more favourable conditions for Michael addition of L-cysteine or other biological nucleophiles to the α,β-unsaturated lactone system.



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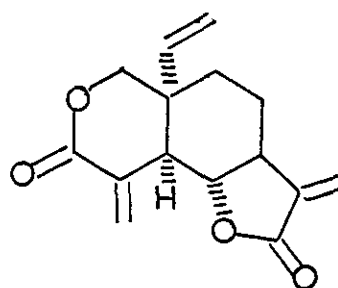

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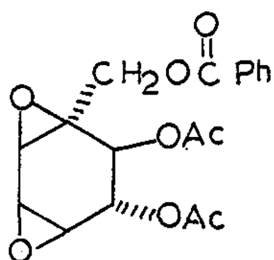
It is well known that certain classes of synthetic compounds[54], as well as naturally occuring substances, owe their antitumour or cytotoxic activity to the introduction of the diepoxide or triepoxide functionality, e.g. the cyclohexane diepoxide, crotoepoxide (21)[55], the sesquiterpene dilactone diepoxide, mikanolide (22)[18,56] and the diterpene triepoxides, triptolide (23) and tripdiolide (24)[57]. However, it was suggested[18], that cytotoxicity appears to be independent of the presence or absence of any epoxy group. Lee[58] indicated that the α-epoxy-ketonic moiety played a more important role than the α-epoxy-γ-lactonic moiety in the contribution and maintenance of the high level of cytotoxicity.



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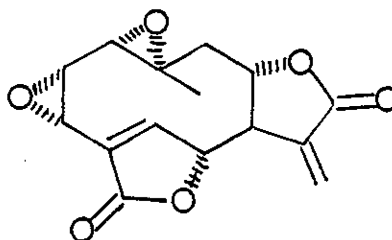

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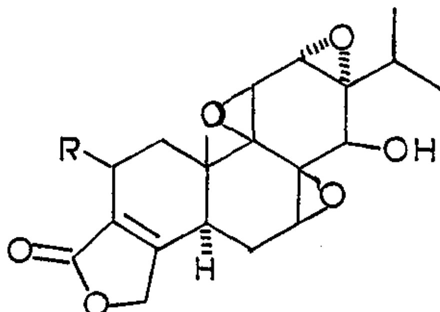


(23) R = H

(24) R = OH

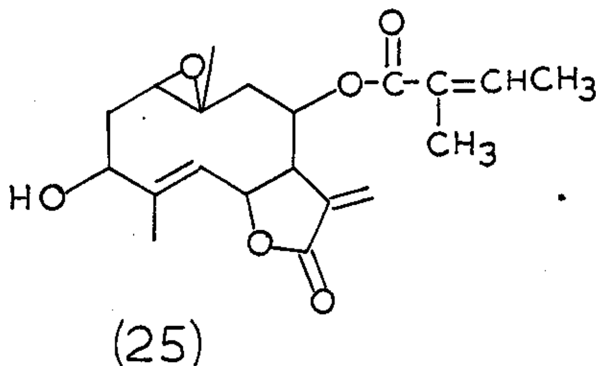
Ziegler *et al*[59] concluded that the presence of a lipophilic, conjugated ester or haloester situated homoallylically to the double bond of many naturally occuring α-methylene-γ - butyrolactones, enhances the cytotoxic activity.[11D, 15C]

ALLERGIC CONTACT DERMATITIS

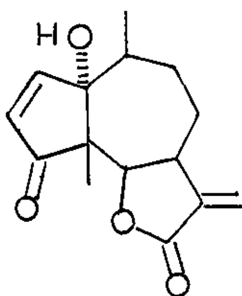
A major class of allergens causing allergic eczematous contact dermatitis in humans has been shown to be sesquiterpene lactones derived from species of the *Compositae*, *Lauraceae*, *Magnoliaceae* and from a liverwort *Frullania* (*Jubulaceae*).[60-69] The principle immunochemical requisite for the production of dermatitis was shown to be an α-methylene lactone moiety[62,70-72]. A "crude allergen", a glycoside causing an occupational disorder, "tulip fingers", was shown to consist of glucose and α-methylene-γ - butyrolactone[73], which on hydrogenation lacked allergenicity[62,73]. All the known allergenic lactones contain the α-methylene moiety which may conjugate with sulphydryl groups of proteins in cells by a Michael-type addition to form complete antigens capable of producing cell-mediated contact allergic reactions[71].

PLANT GROWTH ACTIVITY

Sesquiterpene lactones of different skeletal types have been reported to show the plant growth and antimitotic regulatory activity[74-81]. Yamaki et al[82] reported that the α-methylene-γ-lactone moiety was necessary for the suppressive activity of heliangine(25) in the Avena straight growth test. Also, heliangine(25), the major germacranolide of Helianthus tuberosus, inhibits elongation of Avena coleoptile sections but promotes the adventitious root formation of Phaseolus cuttings[83]. The effects of heliangine(25) were shown to be countered by thiols, the inhibition of elongation of root formation was reduced by L-cysteine[83A] and BAL (2,3-dimercapto-propan-1-ol)[83B].

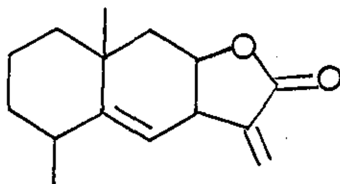


Several of the sesquiterpene dilactones (specifically, vernolepin (5), elephantopin (7A) and elephantin (7B) were found to be strong inhibitors of growth of wheat coleoptile[84]. If the inhibited sections are washed and subsequently treated with indole-3-acetic acid, the tissues responded to the auxin but the degree of elongation was determined by prior treatment with vernolepin(5). The inhibitory effect of vernolepin was reduced when, administering simultaneously, the amount of auxin was increased. The fact that vernolepin's plant-growth inhibitory activity is reversible suggests that the compound may have a natural function in the regulation of plant growth. Similar results were observed for parthenin (26) on Phaseolus vulgaris[85] and the crop plant, Eleusine caracana[86].



(26)

The sesquiterpene lactones were shown to inhibit lateral root growth but to stimulate respiration in Cucumis sativum[87]. Alantolactone (27) was reported to be a potent inhibitor of seed germination and of seedling growth. It was postulated that the lactone inhibits the enzymes associated with the degradation of starch (amylases) and of proteins (proteases), but there is no evidence to support this. It was further suggested that the presence of Inula and other sesquiterpene lactone-containing plants in the agricultural plots might reduce the percentage of crop seeds.[88]



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MICROBIAL GROWTH-INHIBITORS

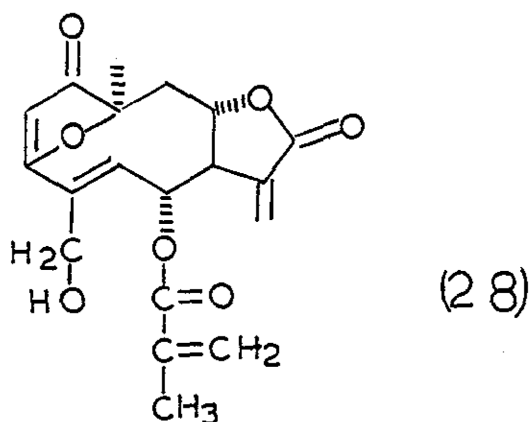
Some sesquiterpene lactones have been shown to possess anti-bacterial, anti-fungal or helminthic properties[89]. The germacranolides, mikanolide (22) and dihydromikanolide from Mikania monagasensis inhibit the growth in culture of a bacteria Staphylococcus aureus and also of a yeast Candida albicans[90]. Helenalin (8) exhibited activity against the human pathogenic fungi Trichophyton mentagrophytes, T. acriminatun and Epidermophyton sp.[91]

Parthenin (26) was reported to inhibit sporangial germination and zoospore mobility in Sclerospora graminicola but such activity against the conidial development of Aspergillus flavus was lacking.[92] Recently Vichkanova et al[93] studied 105 natural lactones for their anti-bacterial

and antifungal properties and reported that alantolactone (27) is active, particularly against Mycobacterium tuberculosis.

CHEMOPROPHYLAXIS BY LACTONES IN SCHISTOSOMIASES

Baker et al[7] have recently shown that some α-methylene-γ-lactones have antischistosomal activity. The sesquiterpene α-methylene-γ- lactones, obtained from wood-oils of some trees, inhibit the skin penetration by cercariae of the trematode Schistosoma mansoni. Recently, a noval germacranolide,goyazensolide (28), isolated from Eremanthus goyazensis, was shown to have schistosomicidal properties[94]. It was suggested that the activity of the schistosomicidal lactones may be related to inhibition of sulphydryl groups in cercarial enzymes.



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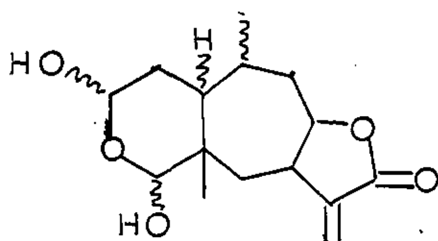
VERTEBRATE POISONING

The poisoning of the live stock foraging on the bitter tasting plants of the Compositae has been reported[95-98]. Hymenoxin (29), the major sesquiterpene lactone of Hymenoxys odorata from Texas, was recently shown to be the toxin involved in the death of sheep[97]. Similar poisoning (vomitting disease in sheep) has been noted among sheep grazing on South African species of Geigeria which contain vermeerin (30)[99]. It was suggested that the sesquiterpene lactone toxicant may alter the microbial composition of the rumen and thus affect vital metabolic functions.[97] The lactone tenulin (31), a constituent of Helenium amarum, imparted a

bitter taste to milk after oral administration to a lactating cow[98].



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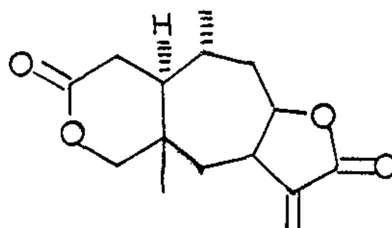

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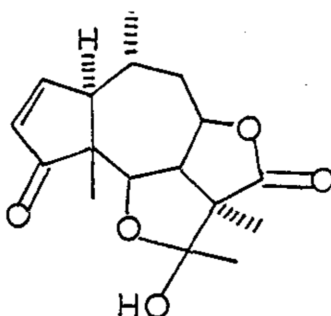

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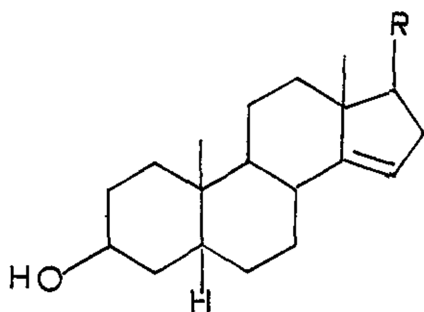
CARDIOTONIC ACTIVITY

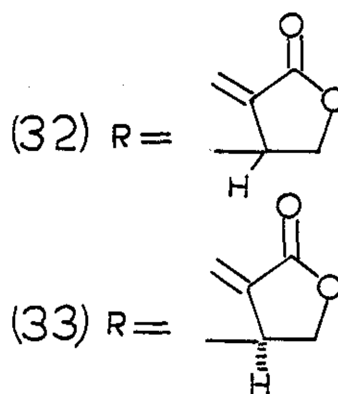
Cardiotonic steroids have been shown to possess strong cytotoxic activity against human carcinoma of the nasopharnyx carried in cell culture (K.B.)[100-103] It has been reported that the active transport of many amino acids is inhibited by cardenolides, possibly, because of an inter relationship between amino acid and sodium transport[104]. In as much as tumour cells are very active in accumulating amino acids[105], presumably due to their high amino acid requirement for growth, it is possible that the inhibition in growth of KB cells in culture by cardenolides is due to inhibition of amino acid accumulation. The fact that similar structural features are important for inhibition of transport of sodium-potassium adenosine triphosphatase (Na^{+}-K^{+} ATPase), KB cytotoxicity and arterial muscle inotropic activity refers to the view that the receptors for the cardenolides in the

above systems may be structurally very similar.

In vivo the activity of cardiotonic steroids have been correlated to the ability of the drug to be specific and reversible inhibitor of membrane-bound Na+-K+ ATPase, involved in the active transport of Na+ and K+ across the cell membrane against the electro chemical gradient[106-119] and thus the inhibition of transport of many essential amino acids.[104] Extensive studies[120-126] indicated that the α,β-unsaturated carbonyl of the lactone side chain is the active functional group which presumably binds to the hydroxyl group of phosphoric acid residue in the phosphorylated enzyme, resulting in an overall inhibition of the ATPase system[109, 125-127].

Recently, Fullerton *et al*[128] synthesised two cardenolides, with the α-methylene-γ- lactone group, from digitoxin and found that the 20 (R) isomer (32) in the Na^+ - K^+ ATPase inhibition studies, was twice as active as 14-dehydro-digitoxigenin, whereas the 20 (S) isomer (33) was significantly less active. This suggested that the cardenolide receptors are very sensitive to changes in geometry in the C_{17} side group and that the geometry may be more even more important than electronic factors.[122,129-132]

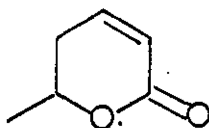




MODE OF ACTION OF SESQUITERPENE LACTONES.

Although the structural requirements for the antitumour or cytotoxic activity of some of the sesquiterpene lactones has been postulated[133] and attempts have been made to explain their mechanism of action,[11,133], there is still a need for further studies of other structural types of sesquiterpene lactones. Some recent observations have focussed attention on the importance of the conjugated α-methylene lactone function for the biological activity of the sesquiterpene lactones.

The reaction of α,β-unsaturated lactones with thiols has been suggested to play a key role in several biological growth regulatory phenomena. The ability of many α,β-unsaturated ketones to react with thiols[134] and that a compound capable of reacting with thiol groups should produce antibacterial effects is not surprising because of the ample evidence of the widespread significance of thiol groups in living systems[135]. The selective growth inhibitory action of S-hexenolactone(34) on certain animal tissues was shown to be antagonised by L-cysteine.[136]. Spectrophotometric and colorimetric studies showed a direct and reversible reaction took place between the lactone and the thiol grouping, and it was proposed that S-hexenolactone exerts its effect on cellular proliferation mainly through its reactivity with sulphydryl groups essential to enzyme function. Further studies led to similar proposals concerning their mode of action.[11,137,139].



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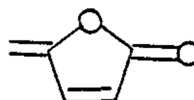

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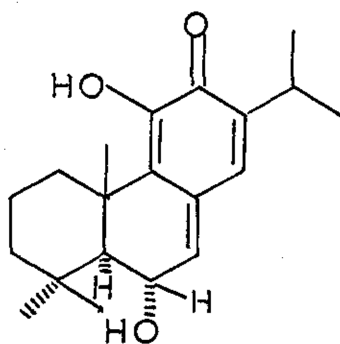
The inhibition of plant growth by protoanemonin (35),[40] heliangine (25),[83] and vernolepin (5) [84] is prevented by BAL and other sulphydryl compounds and has been attributed to the reaction of the inhibitors with sulphydryl enzymes. A study of the reactions of tumour-inhibitory α-methylene lactones with model biological nucleophiles revealed that thiols were the most reactive of the nucleophiles investigated and the successive thiol addition to bis-unsaturated lactones resulted in a marked diminution in the biological properties of the adducts.[28A] The inhibition of sulphydryl enzymes, phosphofunctokinase[28B] and glycogen synthetase[141] indicated that the inhibition resulted from the reaction with the sulphydryl groups of the enzyme.

The electrophilicity of the tumour inhibitory quinone methides taxodone (36) and taxodione (37) [142, 143] and their inhibition of phosphofructokinase upon reaction with selected sulphydryl groups suggested that these compounds may also act by alkylation of biologically important macromolecular thiols.[28] As the active lactones are all alkylating agents, their biological effect is probably due to an <u>in vivo</u> modification of nucleophilic groups either of proteins (including enzymes) or of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)[145].



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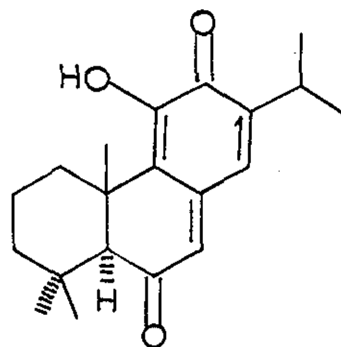

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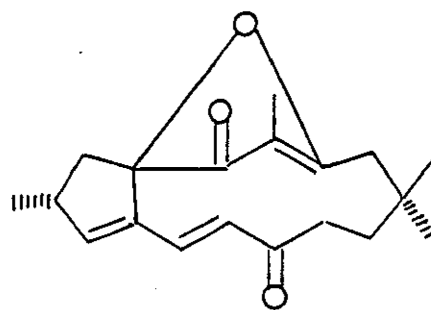
Hall <u>et al</u>,[146] in an attempt to propose a mechanism of inhibition of cancer growth, demonstrated that helenalin (8) and temulin (31)do not alkylate nucleophiles of purine bases but rather appear to be reactive with thiols of enzymes necessary for DNA replication, whereas the accepted mechanism of action of anti tumour agents, e.g., nitrogen mustards, is the alkylation at the N_7 nucleophile of guanosine and, to a lesser extent, the N_1 and N_3 position of adenosine[44]. Furthermore, they cause an increase in cyclic adenosine monophosphate (cAMP) levels of tumour cells which may be correlated with the suppression of chromatin protein phosphorylation necessary for cell replication and differentiation.

Jatrophone (38)[147], a novel macrocyclic tumour inhibitor reacts rapidly with simple thiols and with the macromolecular thiol, bovine serum albumin, and inhibits DNA-dependent-RNA plymerase from Escherichia coli with concurrent partial loss of enzyme sulphydryl groups.[148] Triptolide (23) and tripdiolide (24)[57] may mimic the inhibition of tumour growth via selective alkylation of thiols of key enzymes concerned with growth regulation.[149]

The presence of a free conjugated α-methylene lactone systems appears to be directly related to the cytotoxicity of sesquiterpene lactones. Modification of the unsaturated system results in diminution in cytotoxicity. The reaction of the α-methylene lactone system occurs by "Michael-type addition" with nucleophilic reagents such as the sulphydryl groups of cysteine.[28,72] (SCHEME 1)



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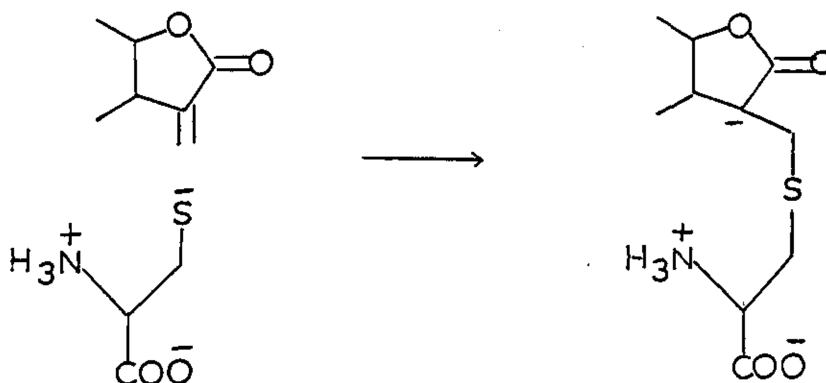
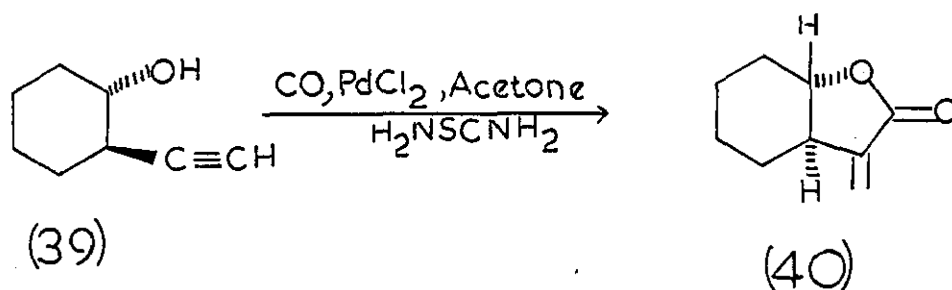


SCHEME I

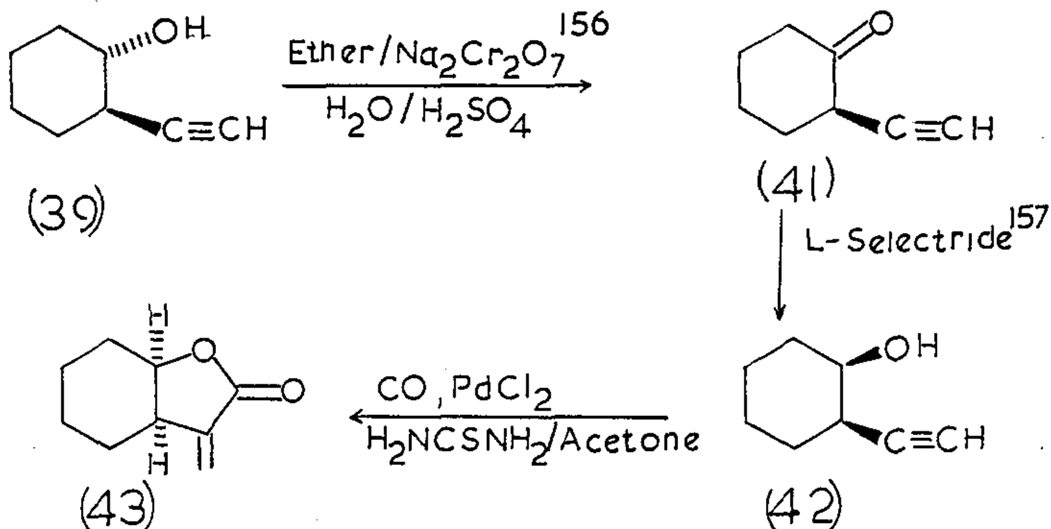
SYNTHESIS OF α-METHYLENE-LACTONES.

Since the early work of Jones,[150] considerable efforts to develop efficient and versatile synthesis of α-methylene-γ- and δ-lactones have been in progress due in large part to the intense interest in naturally occuring cytotoxic sesquiterpene lactones.[11,16,19,73,83,151,152] The general methods for the preparation of α-methylene lactones, up to 1975, have been extensively reviewed by Grieco[153] and Gammill[154].

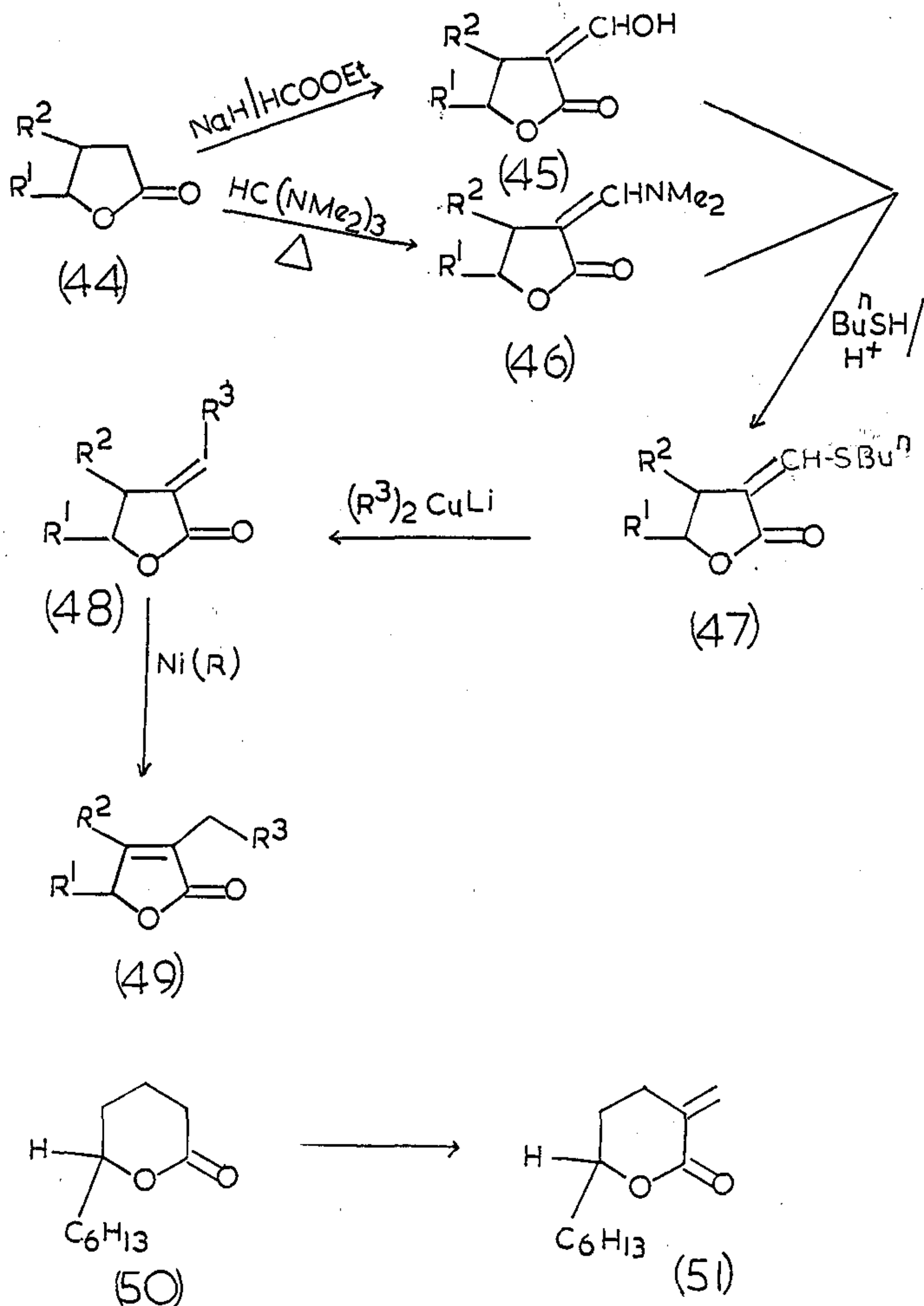
In a further extension of Jones[150] reaction, Norton[155A] and co-workers have achieved a high yield conversion of trans-2-ethynyl-cyclohexanol (39) to trans butyrolactone (40).



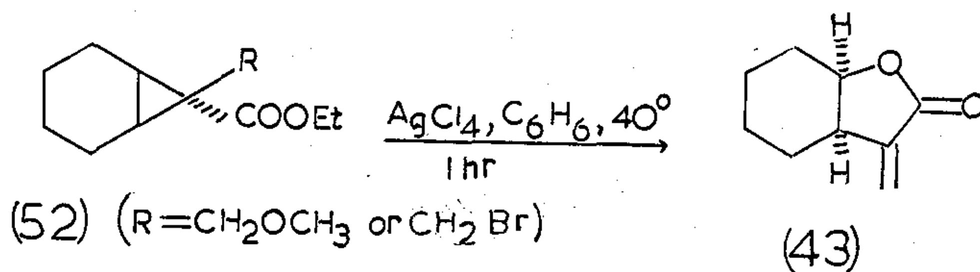
Due to the importance of cis-fused α-methylene lactones,[15,17,82,83,93,97,99] the trans-2-ethynyl cyclohexanol (39) was converted via (41) and (42) to give the cis-fused α-methylene lactone. (43)[155B]

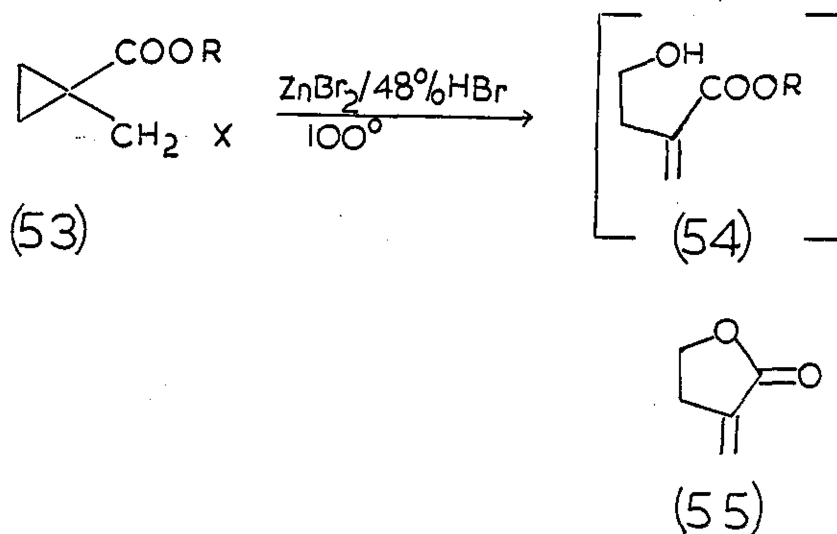


A new facile procedure for the efficient preparation of a number of α-alkylidene-γ-butyrolactones (48) and $\Delta^{\alpha,\beta}$-butenolides (49) from the readily available γ-lactone (44) was reported[158] which is comparable (intermediates (45) and (46) to the procedure of reductive-amination[159,160] of α-formyl lactones. Application of the reductive-amination procedure to the S-lactone (50) resulted in a 42% yield of the α-methylene-S-lactone (51).

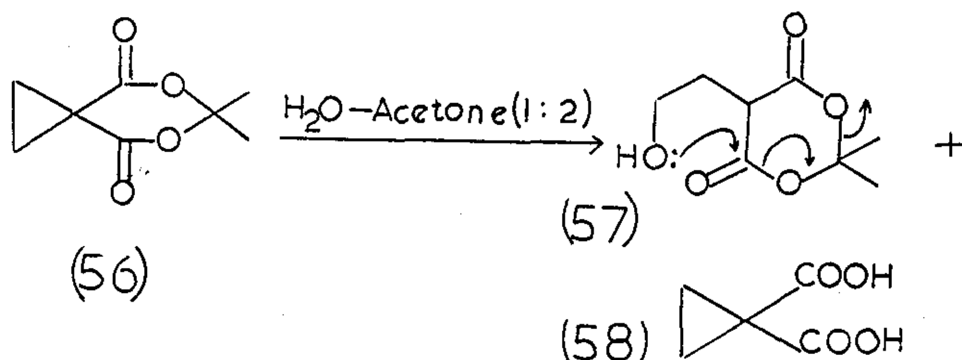


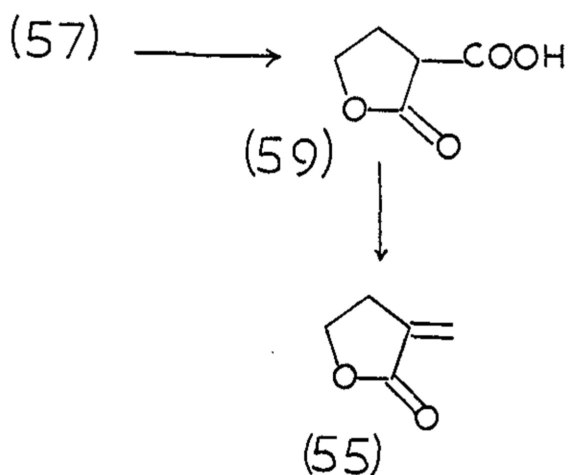
Silver perchlorate-catalysed rearrangement of the methyl ether or bromide (52) afforded the α-methylene-lactone (43). The method is analogous to the novel acid and metal ion promoted rearrangements of functionally substituted cyclopropanes (53) in the synthesis of α-methylene-γ- butyrolactone (55)[161].





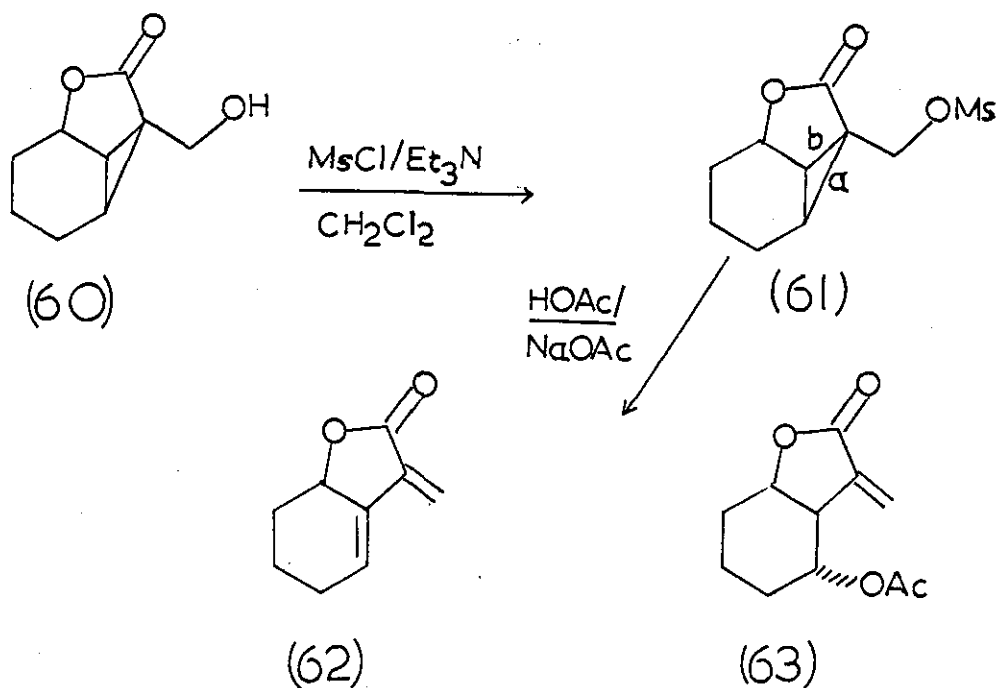
Danishefsky[162] has reported a method (Scheme 2) whereby suitably activated cyclopropanes (56) [163-165] can be converted into γ-lactones (59) with base.



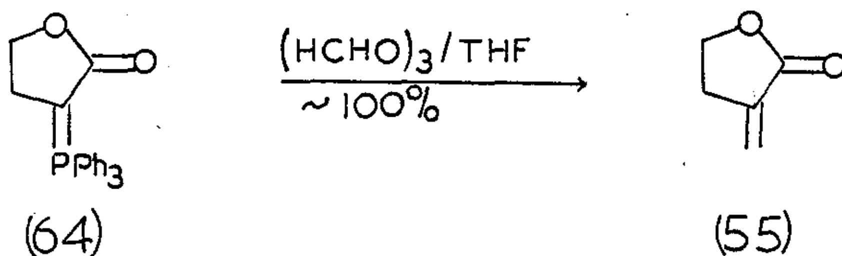


SCHEME 2

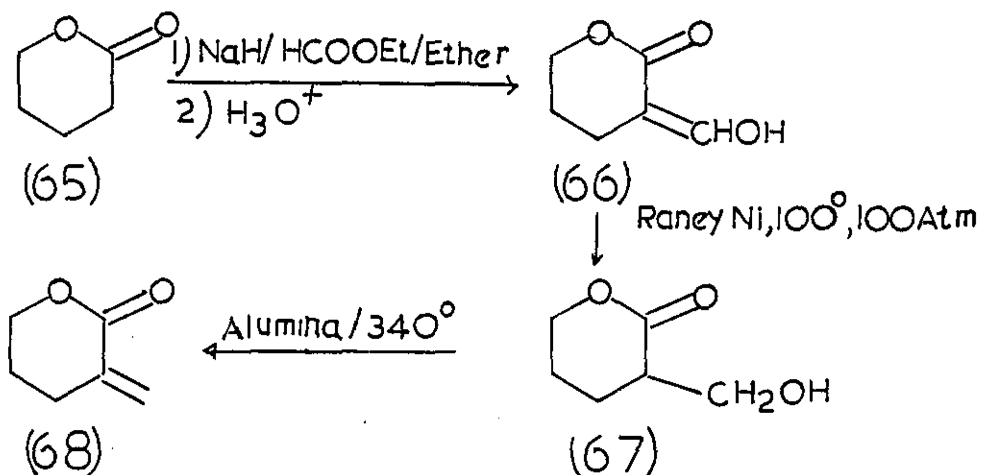
Ziegler[166] has recently reported a site-selective solvolysis of a cyclopropylcarbinyl methane sulphonate(61)resulting in the α-methylene lactones (62 and 63). Apparently, cleavage of bond a is favoured over bond b due to better overlap with the lactone carbonyl during solvolysis.

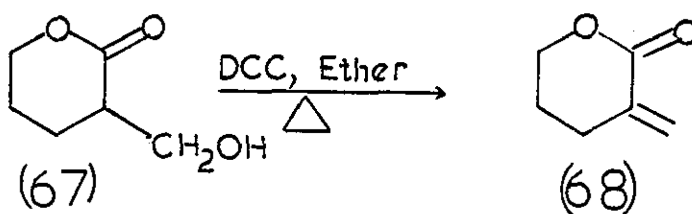


A direct and extremely efficient application of the Wittig reaction[32, 167] between formaldehyde and the ylid,γ-butyrolactonylidene-triphenylphosphorane (64), afforded α-methylene-γ- butyrolactone (55). The Wittig reaction between the ylid and aldehydes to obtain α-benzylidene-[168] and α-(2-furfurylidene) -γ- lactone[169] derivatives has been reported. Minami et al [170] reported that, in the reaction between α-phosphono-γ-butyrolactone carbanion and various carbonyls, the use of aldehydes with bulky groups gave only trans α-ylididene-γ- butyrolactone whereas a mixture of cis -and trans-α- ylididenes were obtained when aldehydes had rather small groups.

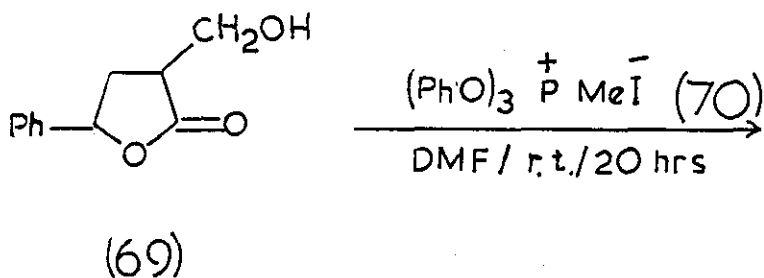


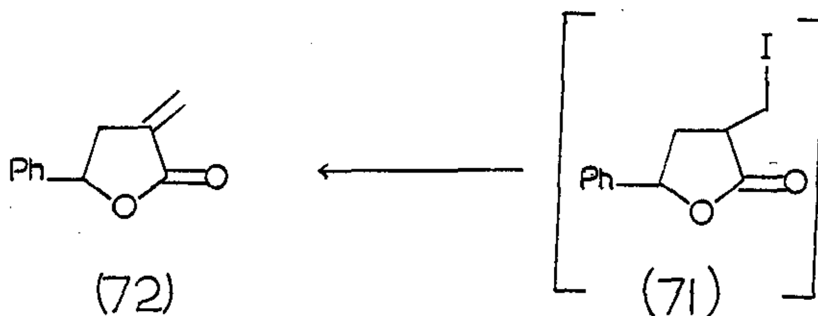
Dehydration over alumina [171] of α-hydroxymethyl-δ-valerolactone (67), obtained from δ-valerolactone (65) via α-hydroxymethylene-δ-valerolactone (66), gave the α-methylene-δ- valerolactone (68). The use of isocyanates and carbodimides as in situ dehydrating agents [172-174] was reported to be an improved mild procedure. The insolubility of dicyclohexylurea assures that the reaction is driven to completion.



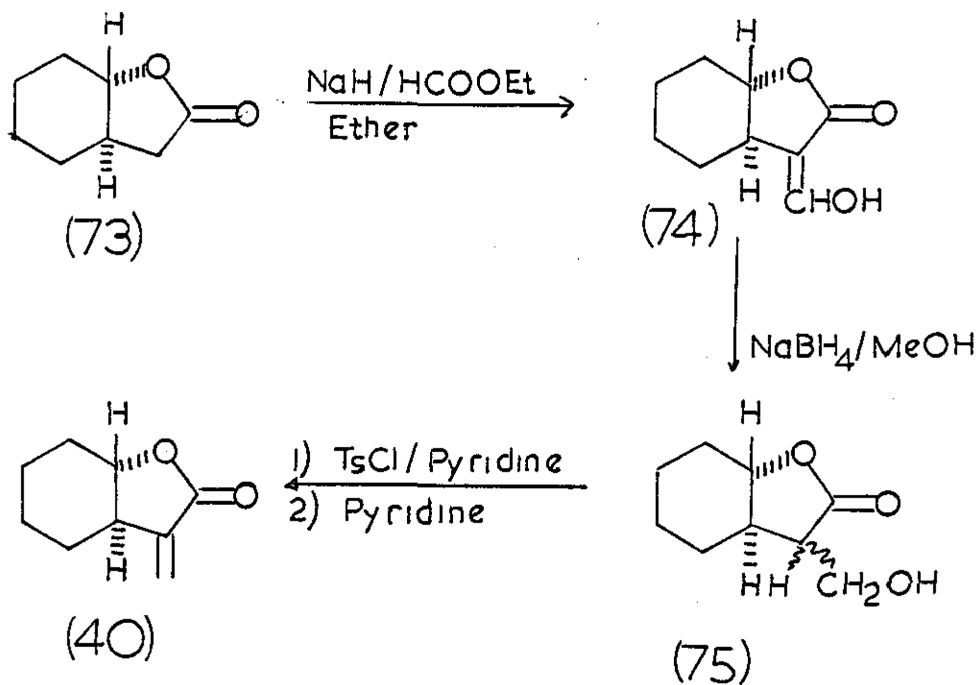


A similar dehydration of (69) with triphenylphosphite methiodide (70) has been effected in a quantitative yield[175]. It is presumed that this reagent forms the β-iodolactone (71) which undergoes elimination to afford the α-methylene-γ- lactone(72).

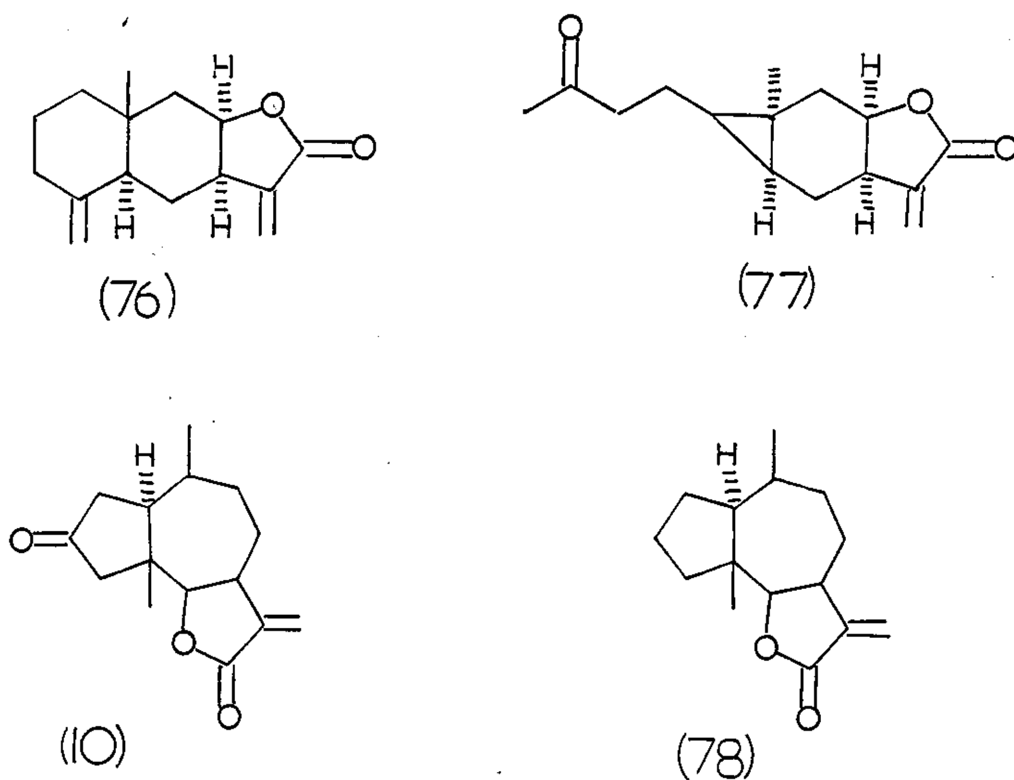




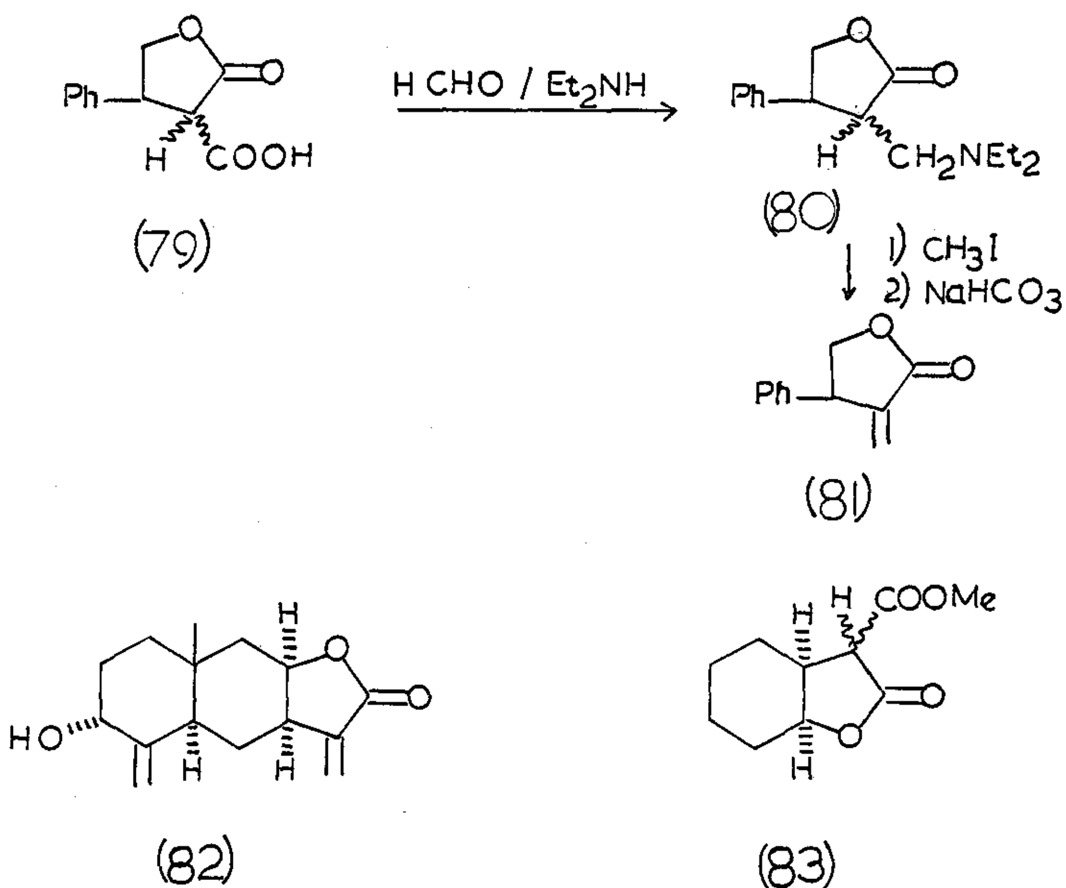
In a modification of McGraw's method, [171] Minato and Horibe[176] converted a trans-fused lactone (73) into a trans-fused α-methylene -γ - butyrolactone (40, 45-50%).



The above reduction-elimination procedure has been applied to the total synthesis of sesquiterpene lactones, iso alantolactone (76)[171,177], carabrone (77)[178], (±) damsin (10)[179] and (±) -4-deoxydamsin (78)[180].

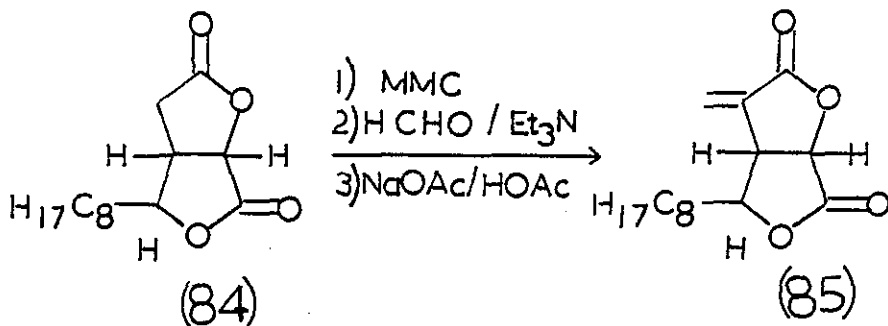


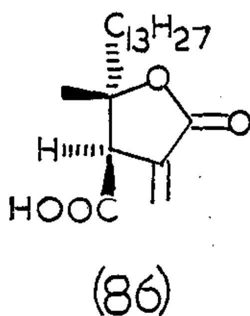
A potentially general and useful route for α-methylene lactone construction was demonstrated[181] when α-carboxy-β-phenyl-γ- butyrolactone (79), derived from diethylmalonate and styreneoxide, reacted with formaldehyde and diethylamine affording the Mannich product (80) which was converted into the α-methylene lactone (81) via base-catalysed elimination of tertiary amine from quarternary ammonium salt. Recently, isotelekin (82)[182] a sesquiterpene lactone, has been prepared employing a complementary synthetic route[183] from the methoxy-carbonylated lactone (83)[184].

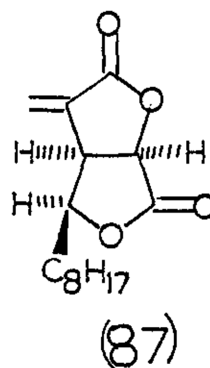


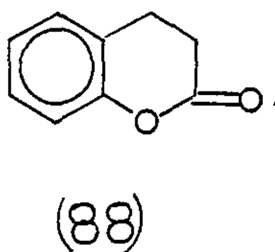
An alternative set of conditions and reagents have been offered to effect the same decarboxylative-elimination[185,186] where methyl methoxy-magnesium carbonate (MMC)[187], as a carboxylating agent, is employed. Extension of the above work provided total synthesis of (dl) -avenaciolide (85)[188], (dl)- protolichesterinic acid (86)[189] and (±) -4-isoavenaciolide (87)[190]. Unfortunately, the use of Stiles

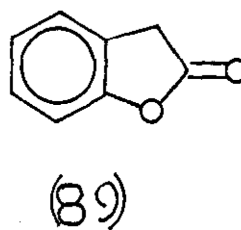
reagent (MMC) is limited, as compounds (88) and (89) either failed to carboxylate or provided limited amounts of unstable carboxylic acids which could not be converted to unsaturated lactonic material.



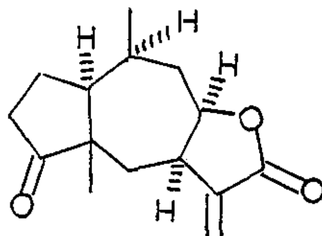








Diethyl-[191] and dimethyl-carbonate[192a] have been employed for carboethoxylation and carbomethoxylation of lactones respectively. The resultant products, α-carboxyesters, can be converted into α-methylene-lactones employing the above procedure. Synthesis of $\Delta^{\alpha\beta}$-butenolides [192b] employing diethylcarbonate and the total synthesis of (±) isotelekin (82) [182] and Confertin (90)[192] utilising dimethylcarbonate have been reported.



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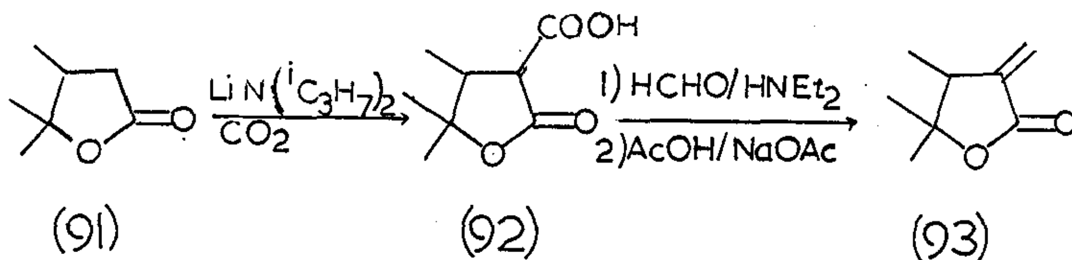
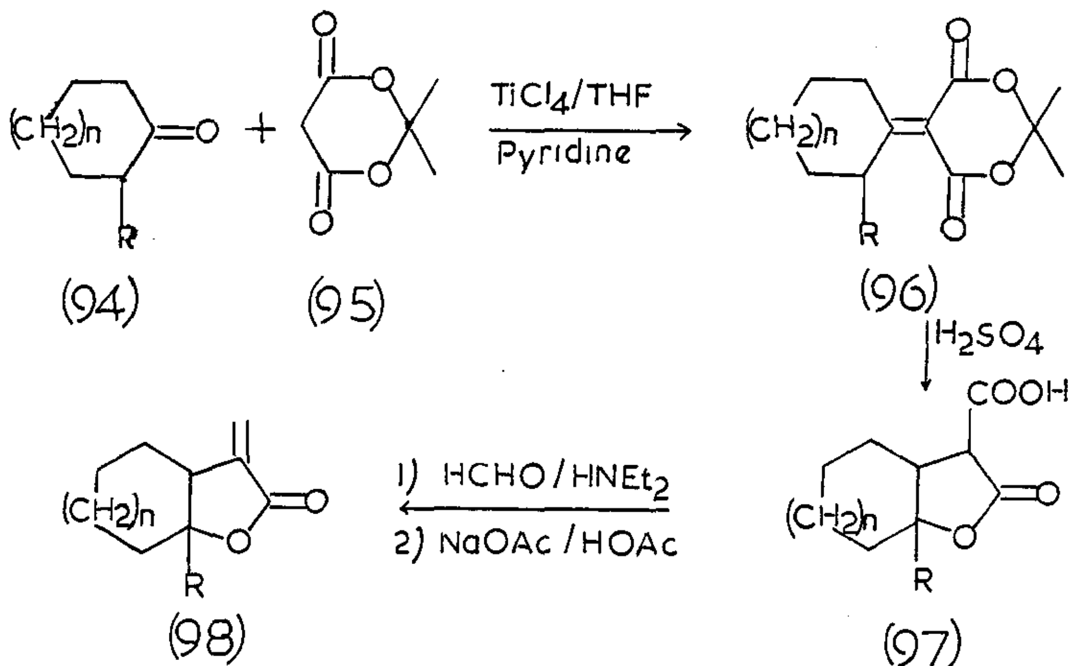
An efficient α-carboxylation of lactones (91) permitting a direct synthesis of an α-methylene lactone (93) has been reported.[194]

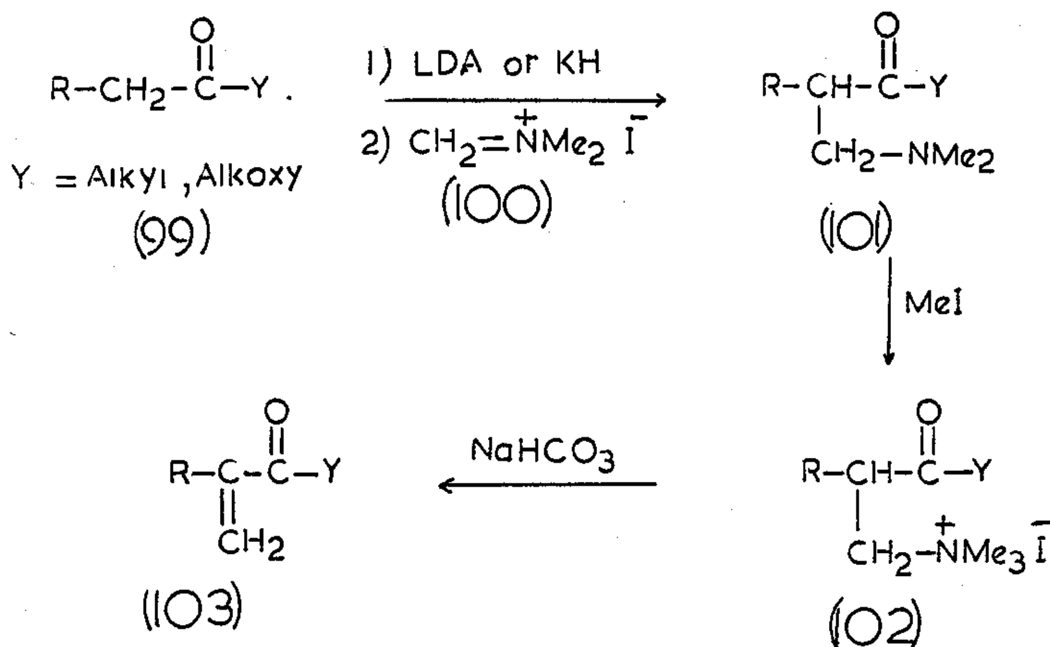


The use of Meldrum's acid, (cyclic isopropylidene ester of malonic acid) (95) has been reported,[195] to give the α-carboxy-γ-lactone (97) via an intermediate (96) and hence led to the α-methylene-γ-lactone (98).



α-Carboxy-γ-lactones, obtained via the opening of epoxides with sodium diethylmalonate, have been converted into α-methylene -γ- lactones[196].

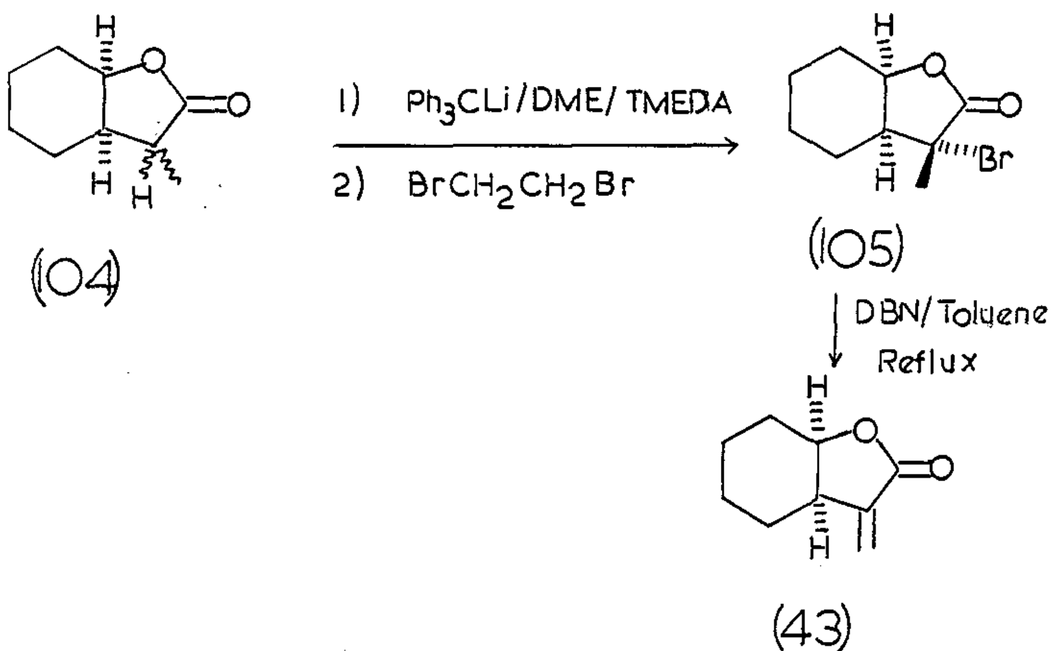
Recently,[197] the use of Eschenmoser's salt (dimethyl (methylene) ammonium iodide) (100)[198] proved to be an excellent method for introducing the dimethylaminomethylene group[199] which can be converted into an α-methylene moiety (Scheme 3).



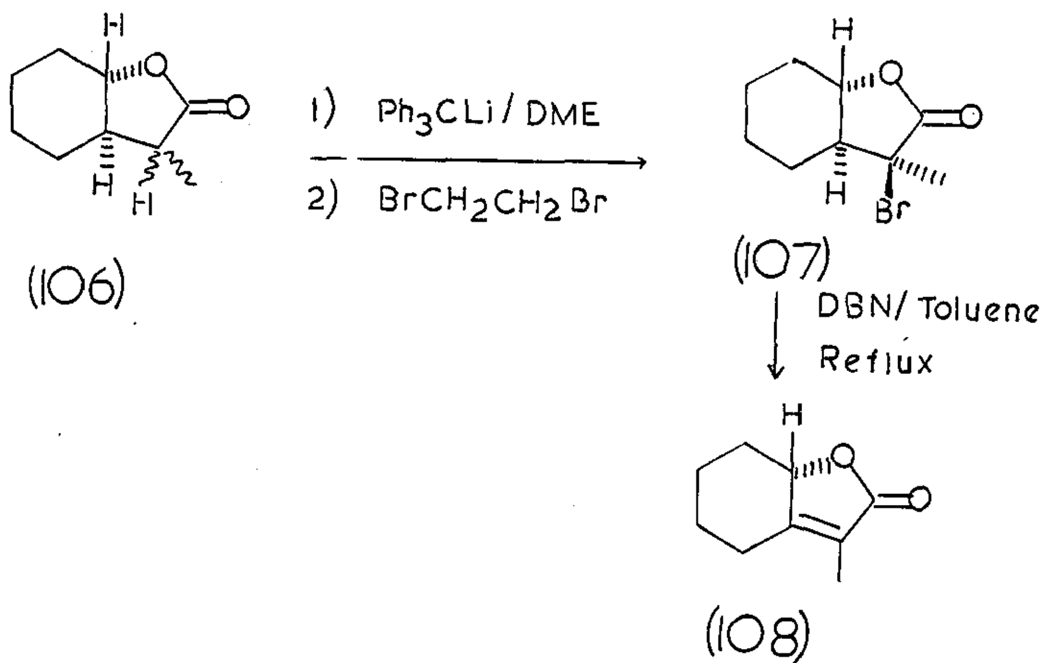
SCHEME 3

The introduction of the α-methylene lactone moiety in vernolepin (5) and vernomenin (6) employing the Eschenmoser salt (100) has been reported[200]. The reaction of Eschenmoser's salt (100) directly with silyl enol ethers[200] and enol borinates[201] contributes to the synthetic utility of the reaction[202] where the regiospecific generation of ester enolates is not possible. This approach is analogous to carbanion trapping with formaldehyde[203] which has been recently applied to regiospecifically generated anions[204].

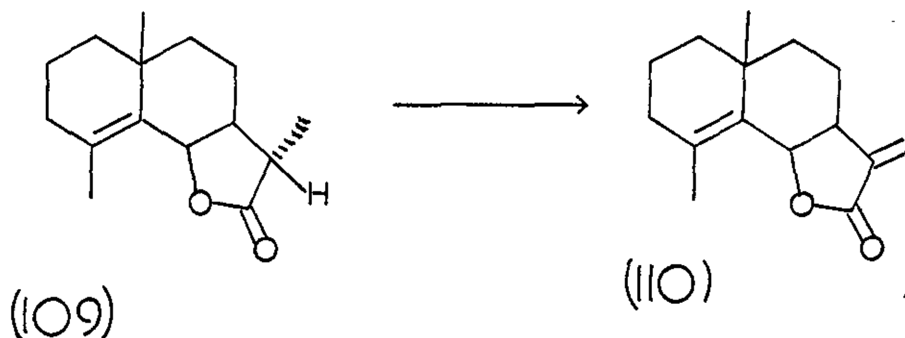
A synthetic sequence [205] has been reported that permits construction of the cis-fused α-methylene lactones from the corresponding α-methyl lactones, available either via direct methylation of lactone enolates[206-9] derived from natural[10] or synthetic[210] lactones. Thus the α-methyl lactone (104) is converted via (105) into (43).



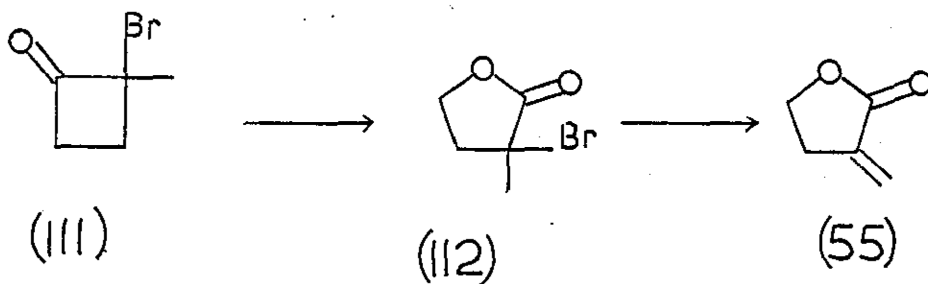
The product of cis-elimination, the endocyclic olefin (108) was detected in small quantity, whereas the α-bromo-trans-lactone (107) gave exclusively, the endocyclic lactone (108), under a variety of elimination conditions.[211]



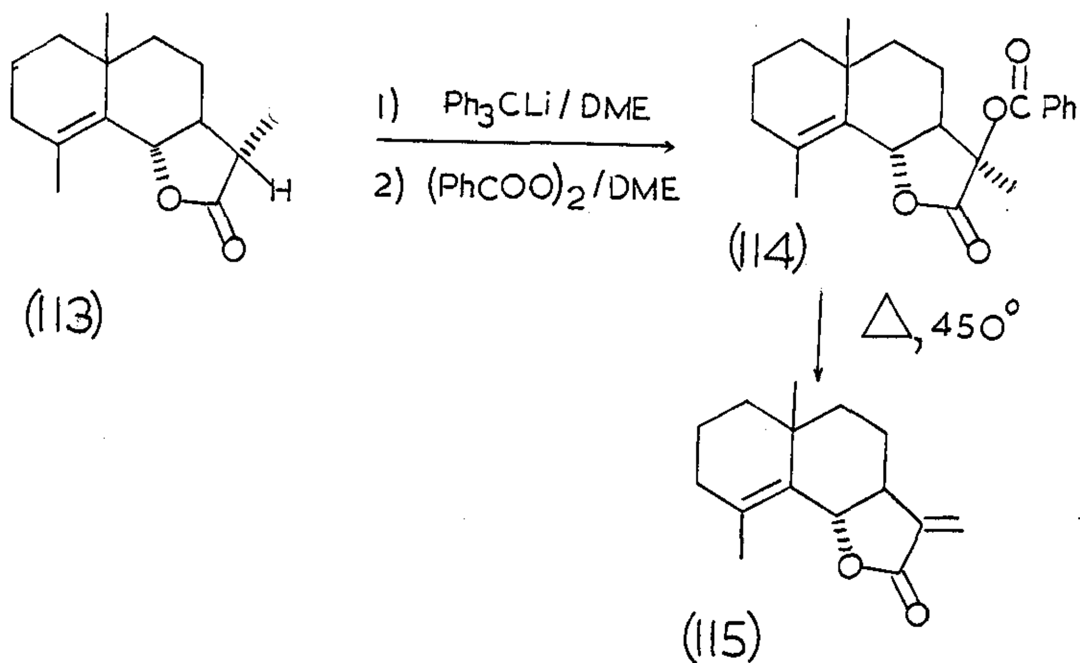
The utility of this general procedure was demonstrated in the synthesis of (+) - frullanolide (110), an allergenically active sesquiterpene lactone, from 1,2-dihydro-6-episantonin (109)[212].



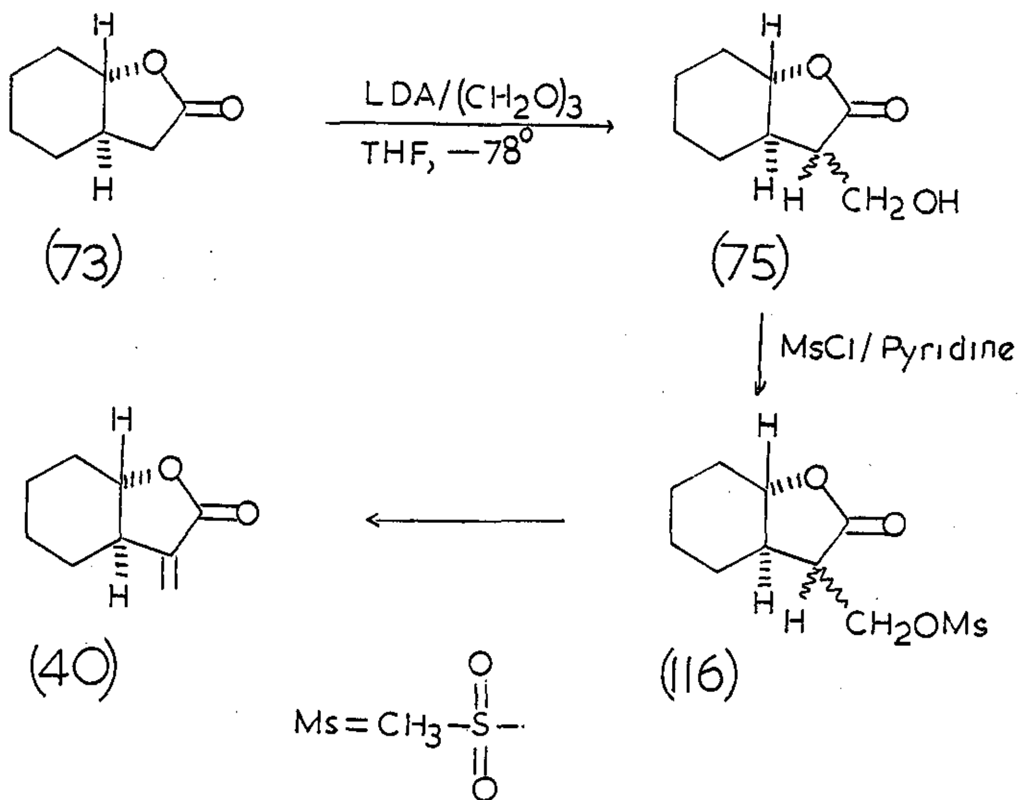
In a recently reported method[213] the required functionality is introduced at an early stage by the addition of an appropriately substituted ketone and olefin to give the exohalogeno cyclobutanene (111), preferentially[214], which on Baeyer-Villiger oxidation gave the γ-lactone (112)[215].



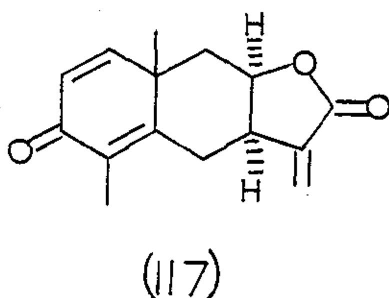
A complementary procedure for the construction of <u>trans</u>-fused α-methylene-γ - butyrolactones, based on a pyrolytic (cis) elimination, has been demonstrated [211,216] in the synthesis of (±) arbusculin B (115) but a mixture of endo-and exocyclic lactones was obtained.



Direct α-hydroxymethylation of lactone enolates followed by successive mesylation and heating in pyridine under reflux gave the α-methylene lactone [203a] in high yield and is exemplified by the conversion of the lactone (73) through the mesylate (116) to the α-methylenelactone (40).

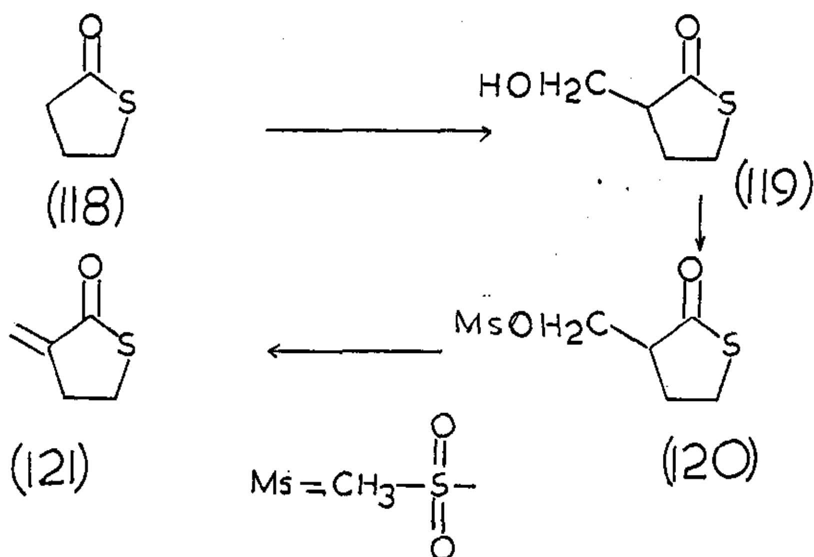


The utility of the above sequence has been demonstrated in the studies directed towards the synthesis of vernolepin (5) [13,217]. Since the isolation and structural elucidation of vernolepin (5) and vernomenin (6), a substantial amount of research [200,203, 217-221] has been performed aimed at the synthesis of these novel elemanolide dilactones. This has recently resulted in their total synthesis[200,218 D,E], in the synthesis of deoxyvernolepin (20)[218 F] and dl-yomogin (117)[222]

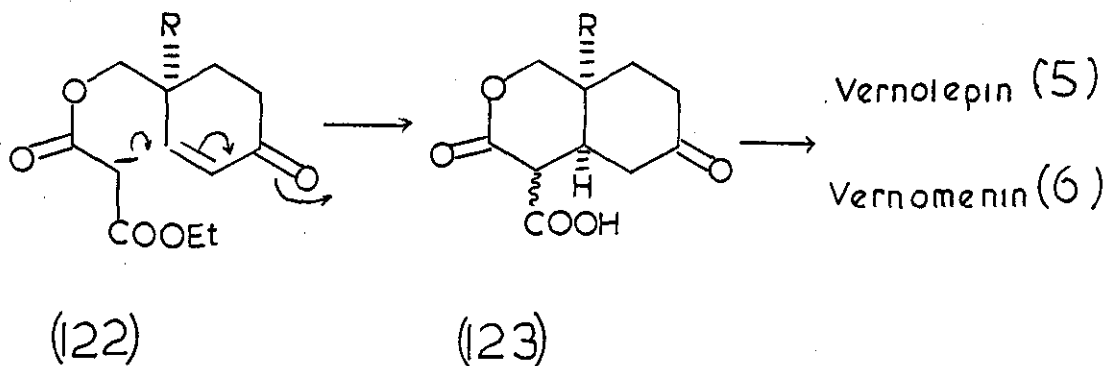


(117)

A sulphur analogue (121) of tulipalin A (55) was recently reported[223], employing the above sequence.



Recently a synthetic scheme[224] was reported in which the crucial step is a base-catalysed intra-molecular Michael-type cyclisation[225] of the enone-malonate (122) to produce a cis- fused A-ring in vernolepin (5) and vernomenin (6).



The fact that alkylation of a thioether, formed via a Michael-type addition, gives a sulphonium salt which on mild base treatment, generates the α-methylene group, suggests a novel route to α-methylene lactones [181A, 226]. This sequence is illustrated [227] on the α-carboxylactone (124) giving rise to the α-methylene-γ- lactone (40) through the thioether (126) and the sulphonium salt (127) intermediates.

H
H
COOH
(124)

1) LDA/THF, 78°
2) ICH_2SCH_3/HMPT/THF

H
H
HOOC CH_2SCH_3
(125)

Xylene, Reflux

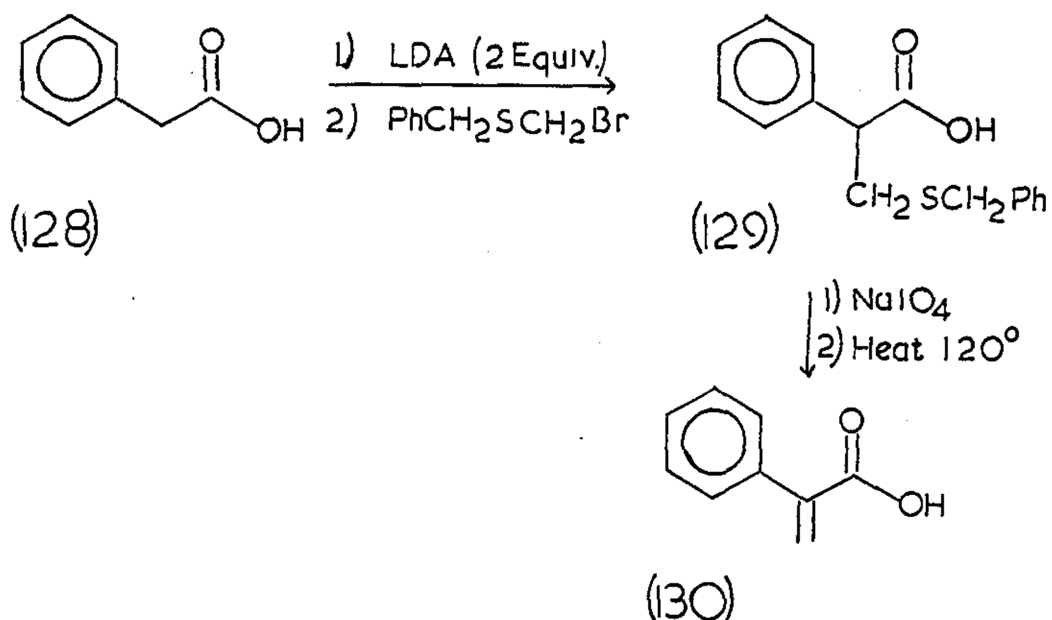
H
H
H CH_2SCH_3
(126)

CH_3I

H
H
H
$CH_2\overset{+}{S}CH_3$ $\bar{I}$
CH_3
(127)

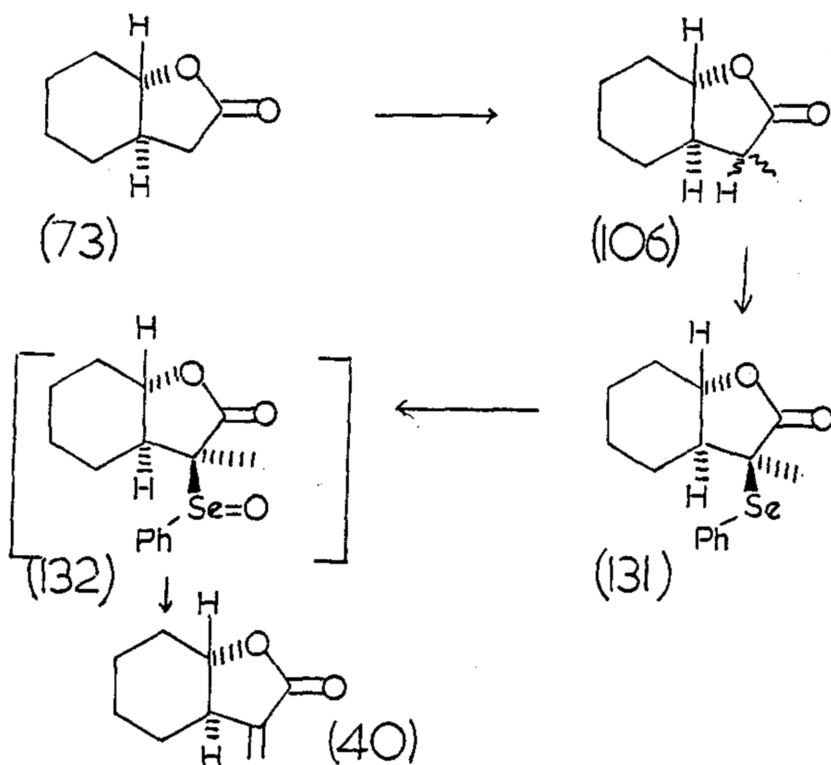
H
H
(40)

A similar method [228] involving alkylation of ketone and carboxylate enolates (128) with bromomethyl sulphide, followed by sulphoxide elimination, gives α-methylene ketone and acid (130.) However, ester and lactone enolates are not alkylated in useful yields.



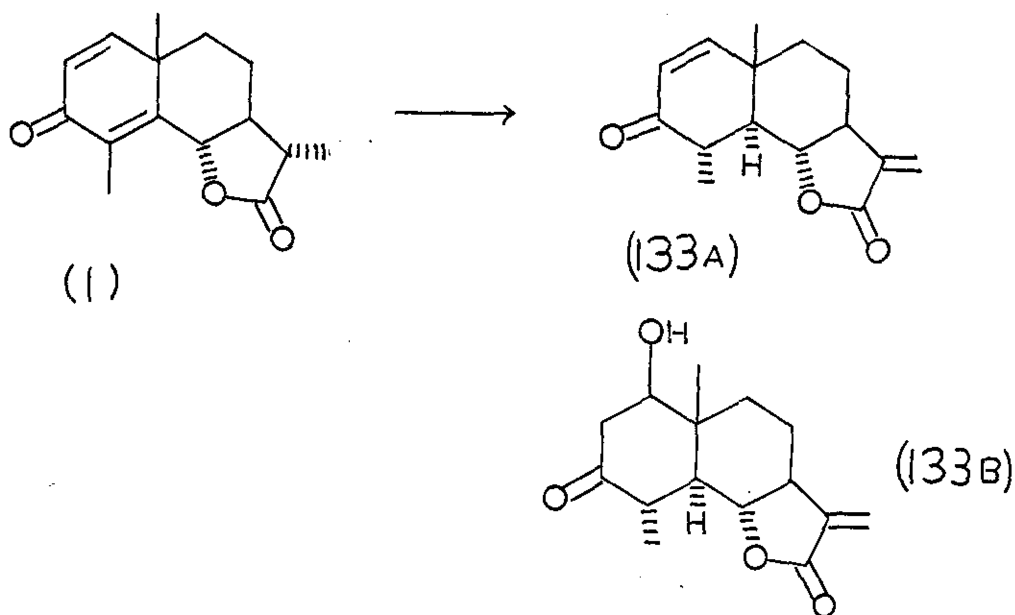
Bis (phenylthio) methyllithium ($(PhS)_2$ CH Li) and methoxy-phenylthiomethyllithium (PhS$\underset{CH}{\overset{OMe}{\diagdown}}$ Li) may be used to provide a convenient method for conversion of the readily available γ-butyrolactones into α-alkoxy-or α-methylene-γ- lactones[229]. A nitrogen species (e.g. piperidino) may be used instead of sulphur, as β-aminolactones undergo facile elimination with generation of α-methylene unit.[227]

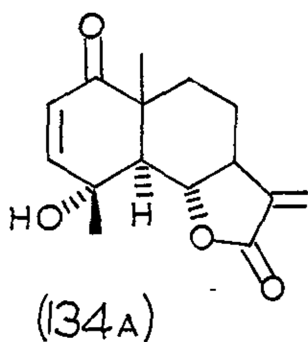
A similar, high yield " α-methylenation sequence" employing "alkylphenylselenoxides" has been developed [206] in which selenoxides undergo facile elimination at low temperature with exclusive formation of exocyclic olefin.

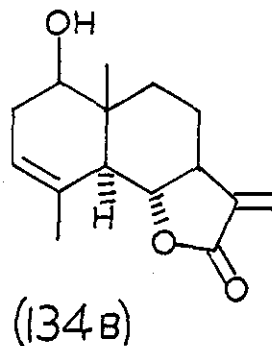


The formation of (40), with the complete exclusion of the endocyclic isomer (108) comes about as a result of stereospecific alkylation of the enolate of the lactone (106) with diphenyl-diselenide, which establishes the required anti- relationship between the α-phenyl seleno-substituent and the adjacent methine proton.

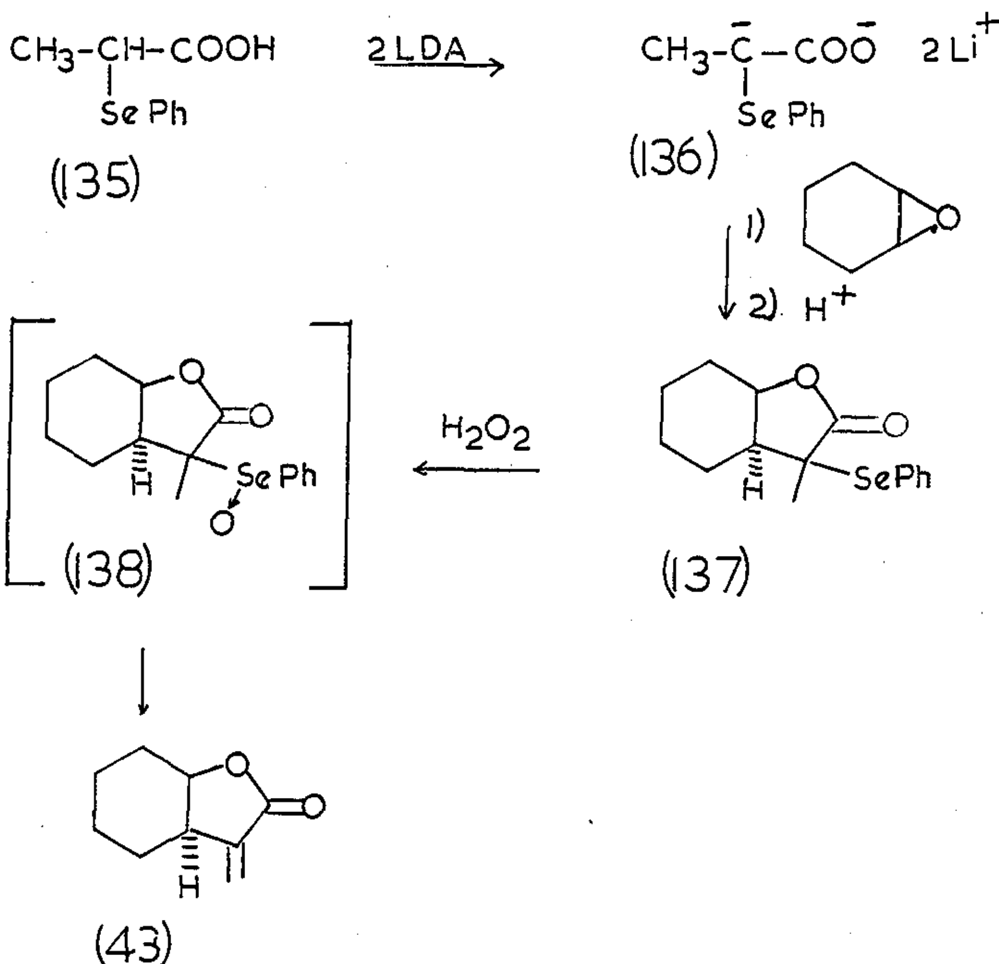
The usefulness of the above sequence was demonstrated [230] for the transformation of α-santonin (1) into tuberiferene (133A)[231] artecalin (133B)[230], anglanine (134A)[232] and santamarine (134B)[232]



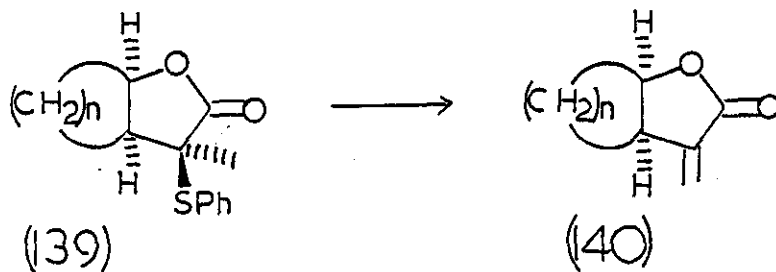


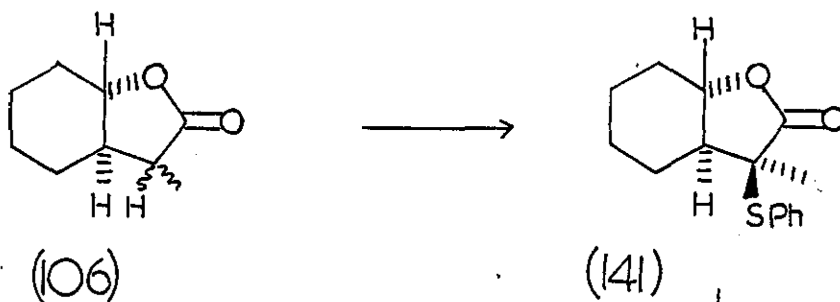


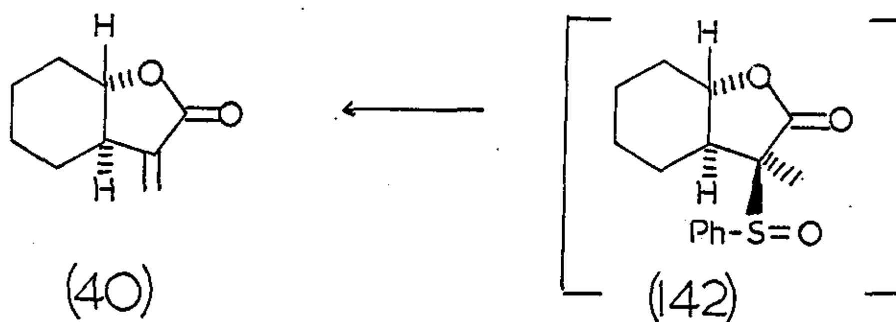
Recently a similar " α-phenylselenation sequence" has been reported[233] in which an epoxide is opened with 2-phenylseleno-propanoic acid (135), *giving rise to the α-methylene-γ-lactone* (43) *via the* elimination of phenylselenoxide (138).



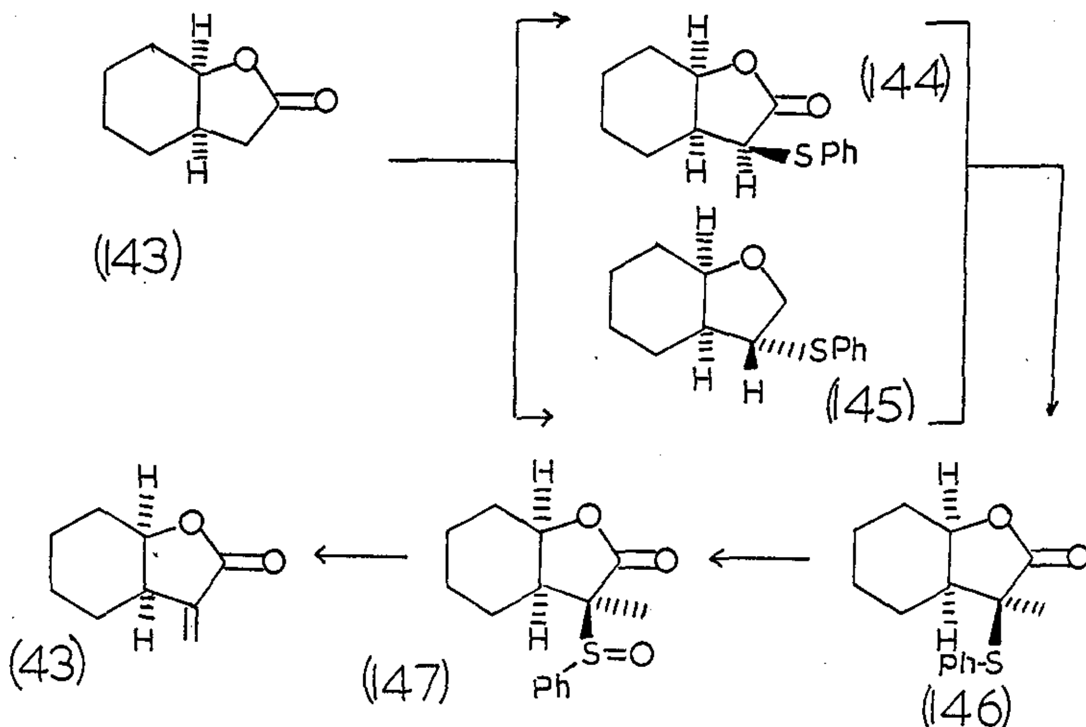
A complementary " α-methylenation sequence"[234] utilises (43) the ability of α-phenylsulphinyl ketones to undergo facile thermal <u>cis</u>-elimination where the α-phenylsulphinyl substituent and the adjacent methine proton possesses an <u>anti</u>-relationship (139- 140), as is illustrated by the conversion of (141) into (40)[235].



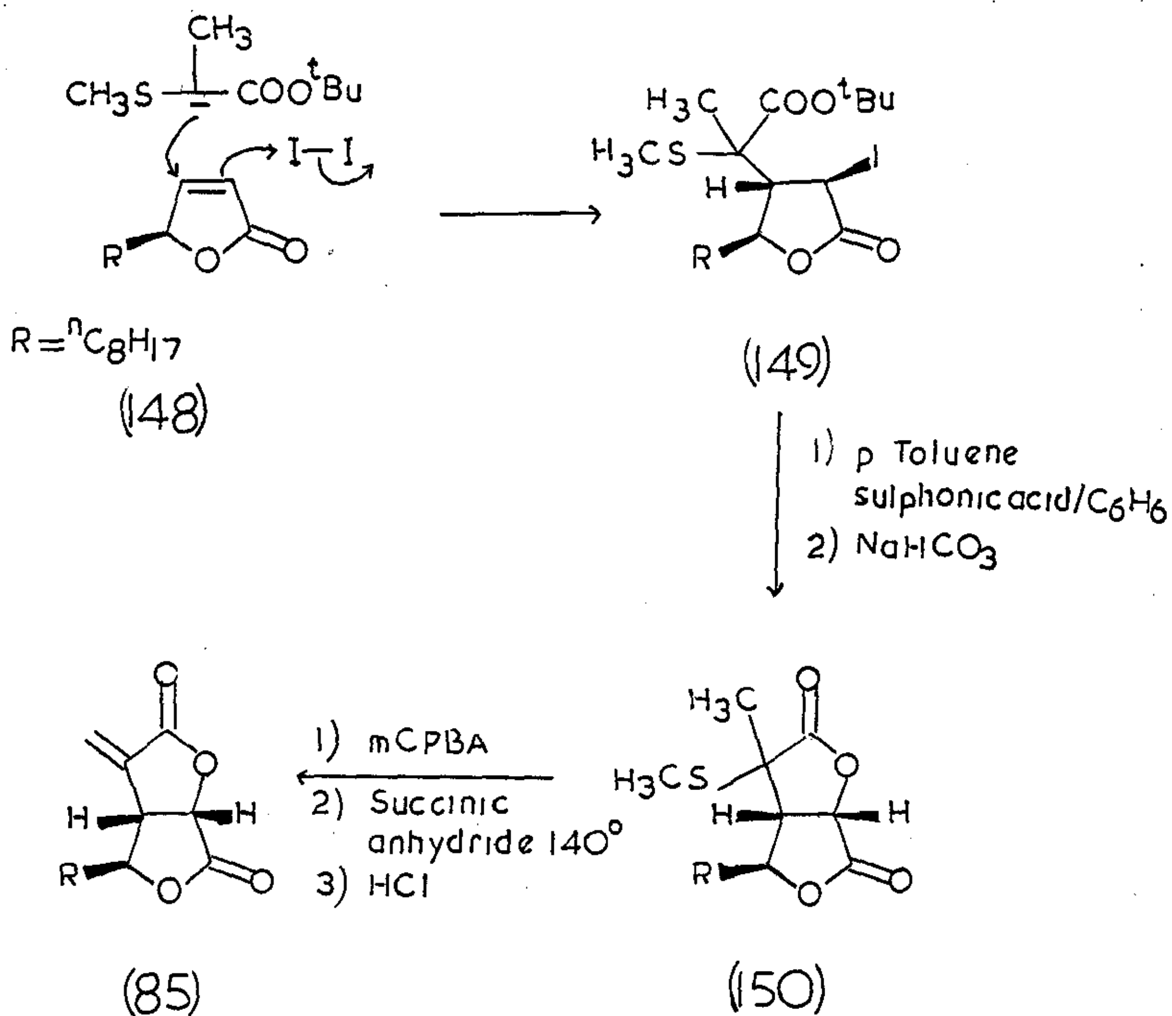




The successful application of the above method to the cis-fused lactone (143) was dependent upon the proper stereochemical relationship between the α-phenylsulphinyl substituent and the adjacent methine proton.



In an elegant synthesis of (dl) - avenaciolide (85), Schlessinger [236] has employed a "sulphoxide" sequence to obtain an α-methylene function. The stereoelectronic factors governing the α-sulphoxide elimination are analogous to those in the selenoxide cases. [206,234]



Trost and Leung [237] has reported a procedure in which opening of an epoxide with 2- (thiophenyl)-propionic acid] (151) leads to α-methylene-γ- lactone (40) via intermediates (152 - 154).

PhS$-\bar{C}-COO^-$ with CH_3

(151) (152)

1) CH_2N_2
2) mCPBA

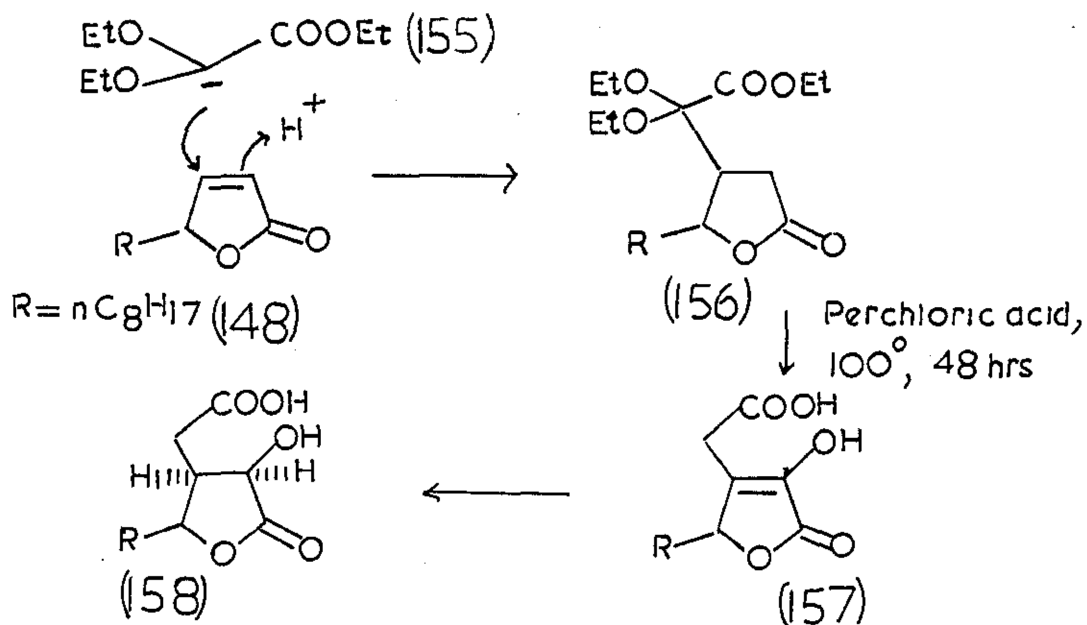
$(MeO)_3P/THF$
70°

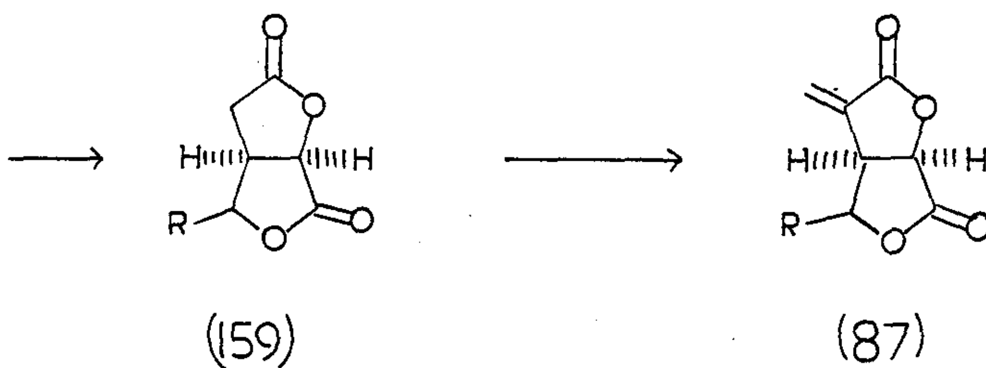
(154) (153)

C_6H_6 p-Toluene sulphonic acid

(40)

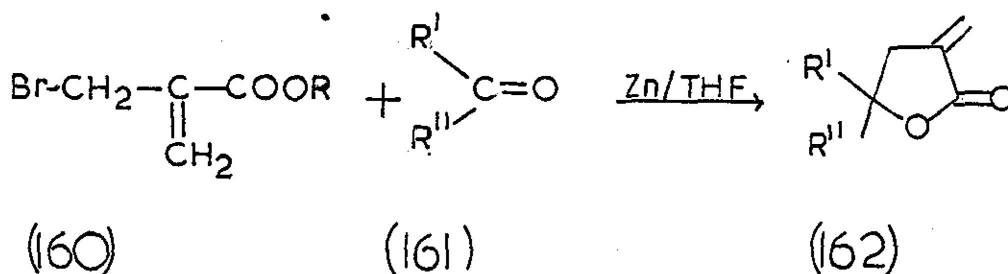
Recently, a total synthesis of 4-isoavenaciolide (87) in high yield, has been reported, in which the key step is the conjugate addition of the latent carbonyl anion (155) to the butenolide (148).





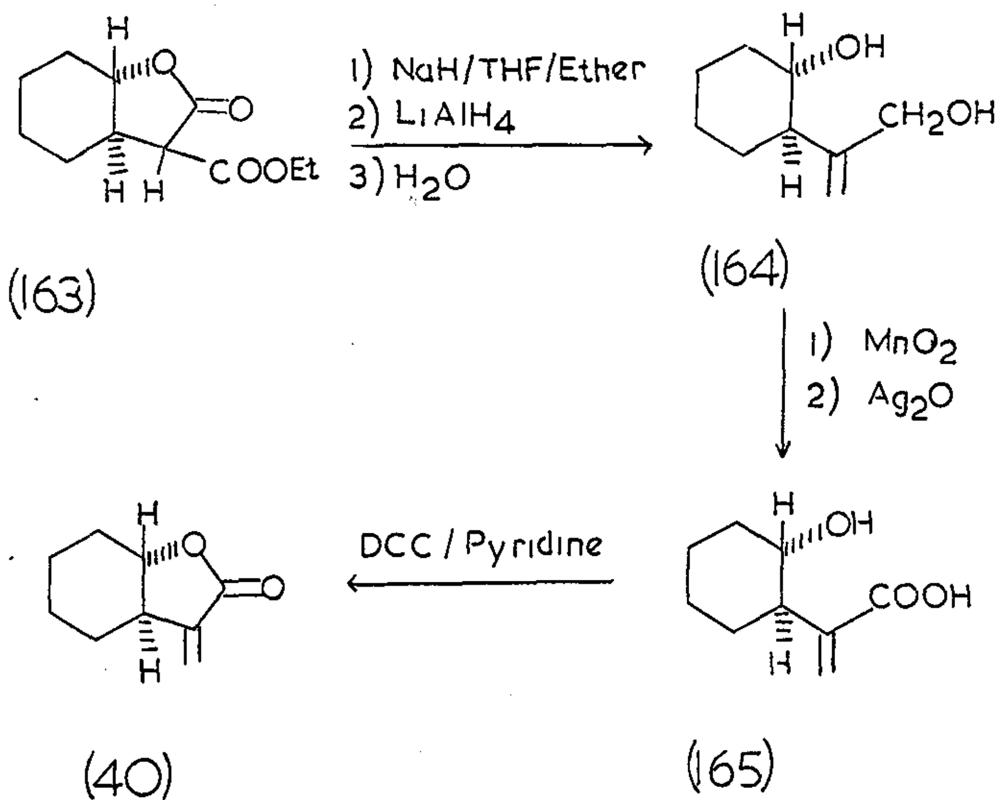
(159) (87)

An efficient route [186, 238, 239] has been reported involving treatment of the α-(bromomethyl) acrylic ester (160)[240] with zinc (Reformatsky Reaction) to form organozinc intermediates that react with a variety of aldehydes and ketones to provide α-methylene-γ-lactones (162).

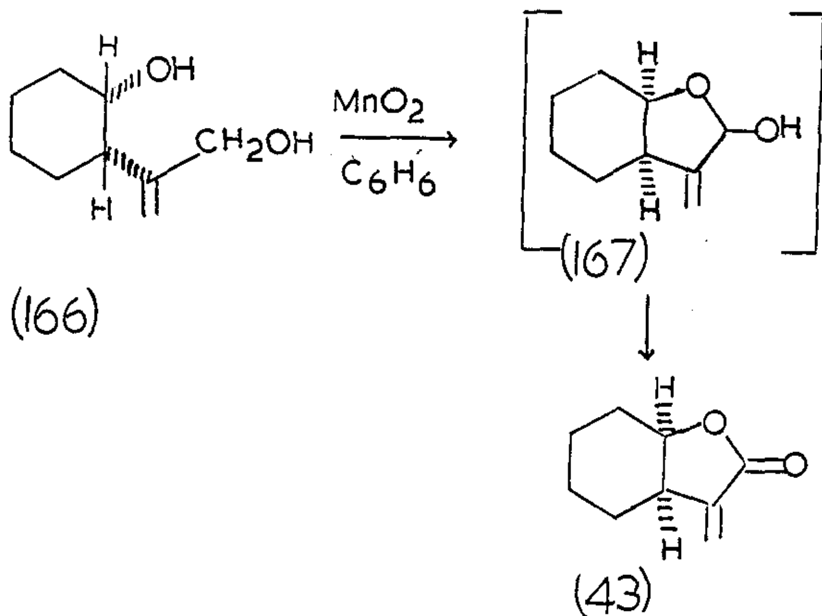


(160) (161) (162)

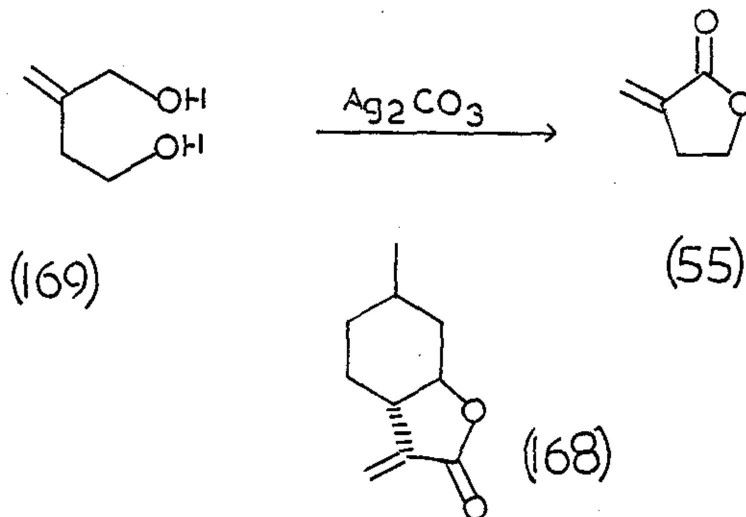
The observation that β-ketoesters, β-ketoaldehydes and β-diketones are reduced, via their enolates, to allylic alcohols by lithium aluminium hydride ($LiAlH_4$) led to the use of the malonic ester derivatives (163) in the synthesis of the α-methylenelactone (40)[241]



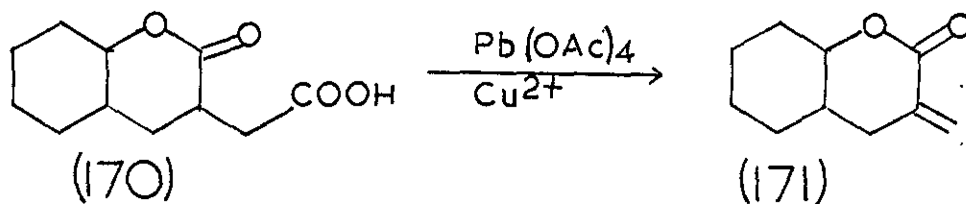
Similar treatment of the cis-methylene diol (166) with activated manganese dioxide afforded the cis-lactone (43) presumably via oxidation of the intermediate lactol (167).



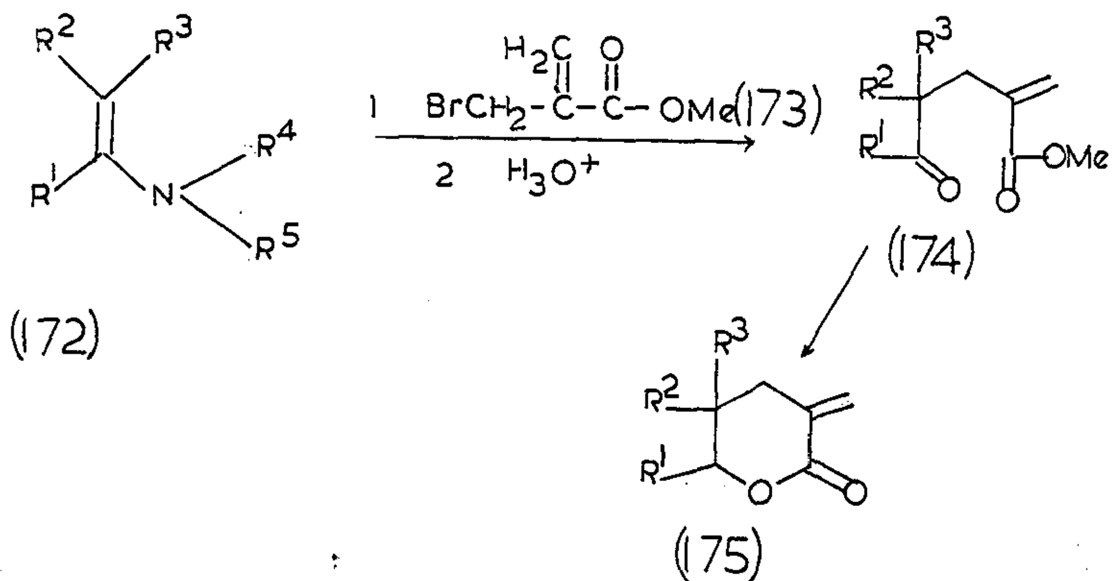
The above described reductive-elimination method [242,243] was applied to the synthesis of dl-alantolactone (27) and a trans-fused α-methylene-lactone (168) [244]. Analogous to the above described procedure is the preferential oxidation of allylic alcohol of diol (169), by silver carbonate, to the carboxylic acid, with subsequent formation of the lactone (55)[245].



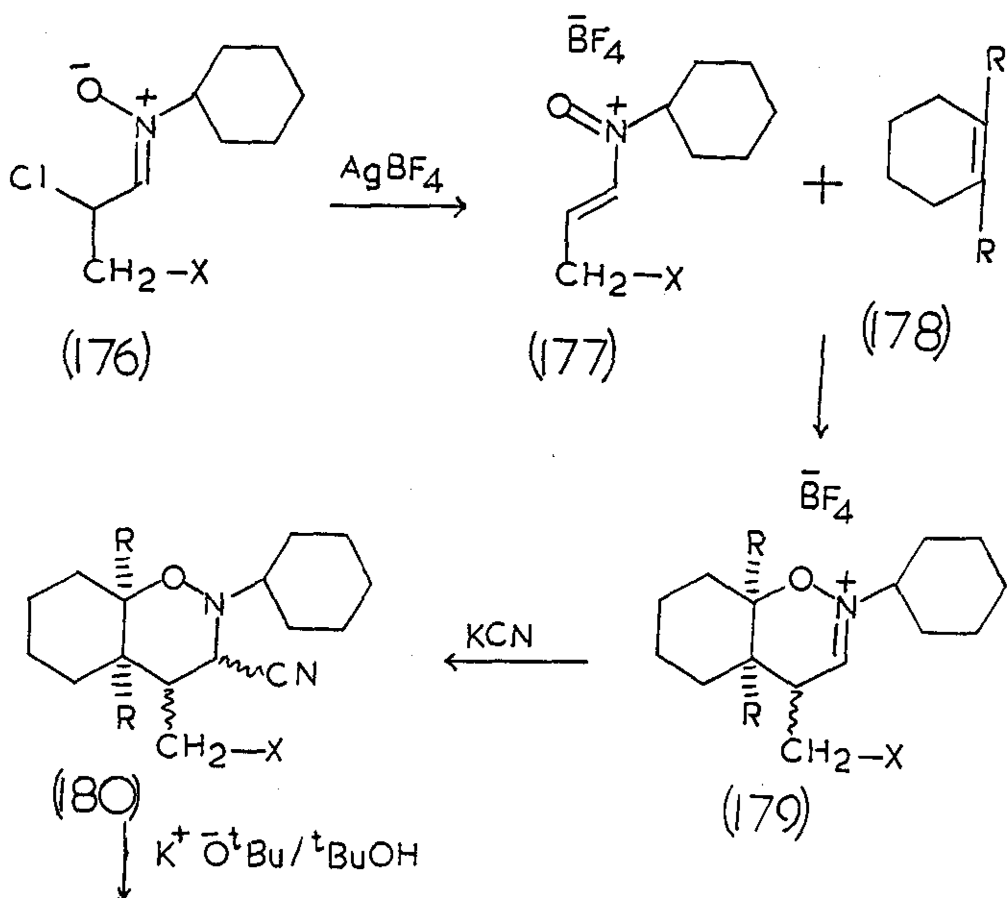
The observation,[246] that primary acids when treated with lead (IV) acetate in the presence of copper (II) acetate, undergo oxidative decarboxylation, resulted in Rao[247A] and Ho [247B] independently developing a route to the α-methylene lactones (171) from the lactonic acid (170).

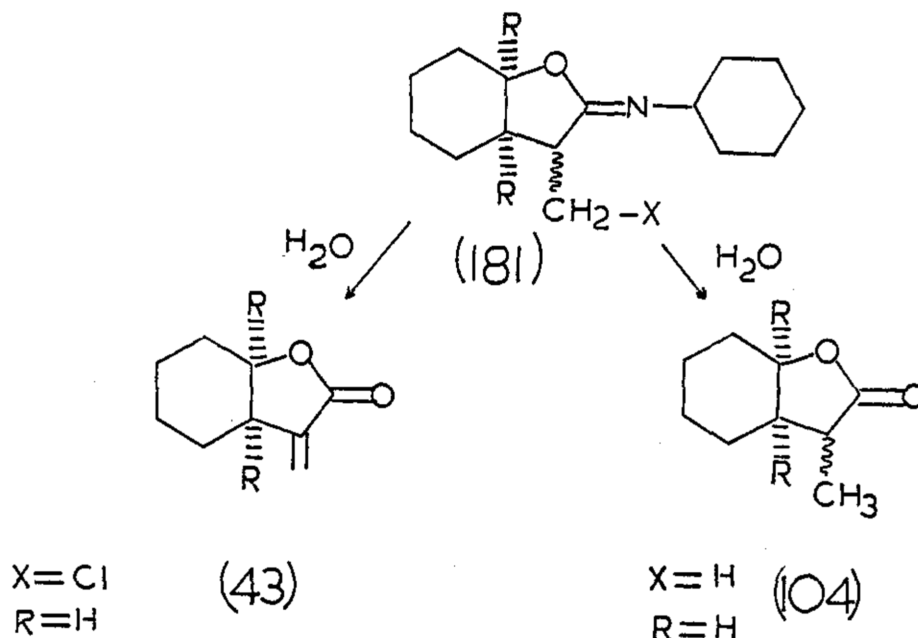


Interest in the α-methylene-δ-lactones has resulted in a new facile synthesis [248] involving alkylation of enamines (172) with methyl α-bromomethyl acrylate (173)[249] to form unsaturated keto esters (174) that can easily be converted into α-methylene-δ-lactones (175) via saponification, reduction and lactonisation.



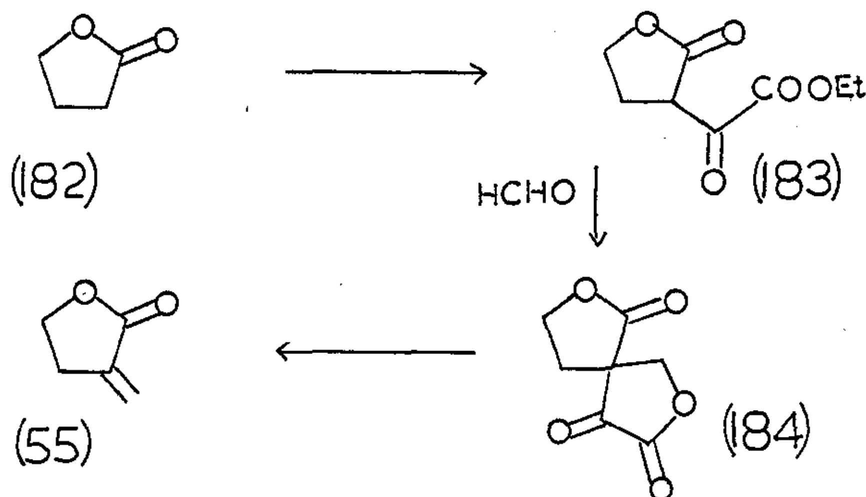
In an elegant use of a cycloaddition reaction, Eschenmoser[250] developed an excellent route to <u>cis</u>-α-methylene-γ- butyrolactones starting with simple olefins. Treatment of an α-chloronitrone (176) with silver tetrafluoroborate (Ag BF_4) yields an extremely powerful enophile (177) which undergoes a smooth 1,4-cycloaddition with unactivated olefin (178) and the resulting species has been subjected to a variety of transformations including conversion to γ-lactones and to α-methylene-γ- lactones (scheme 4).



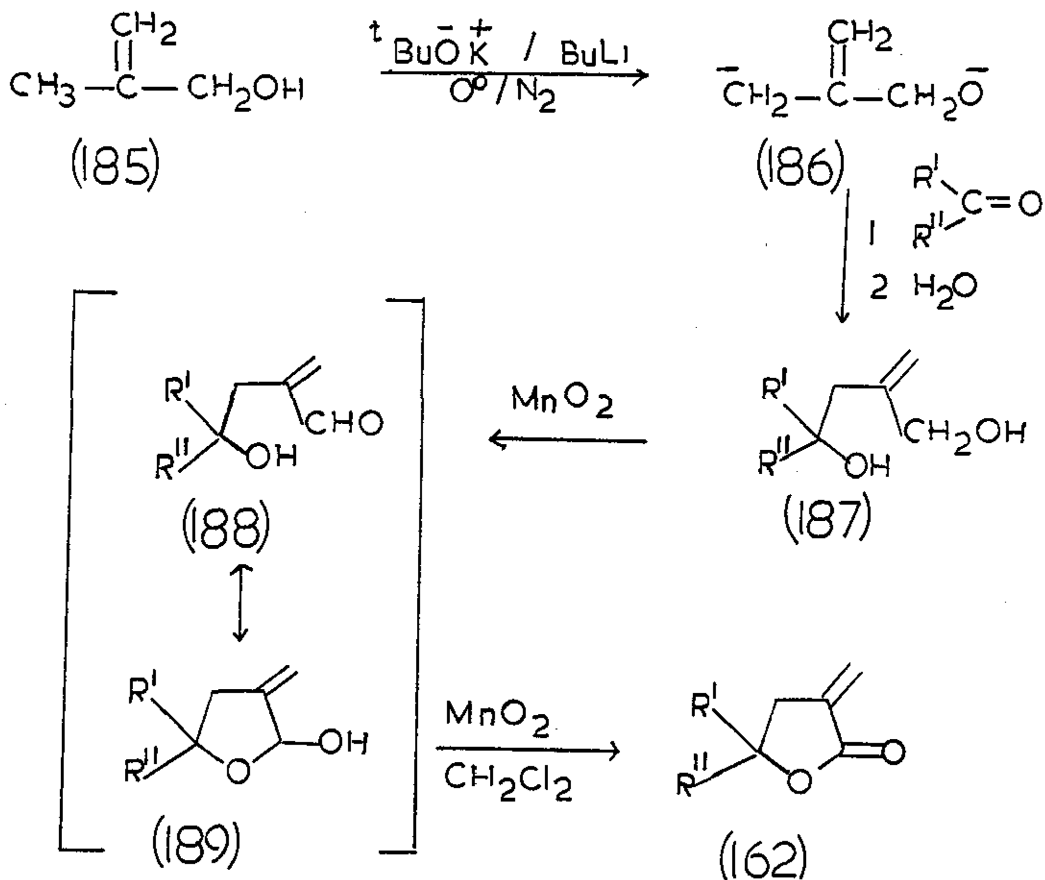


SCHEME 4

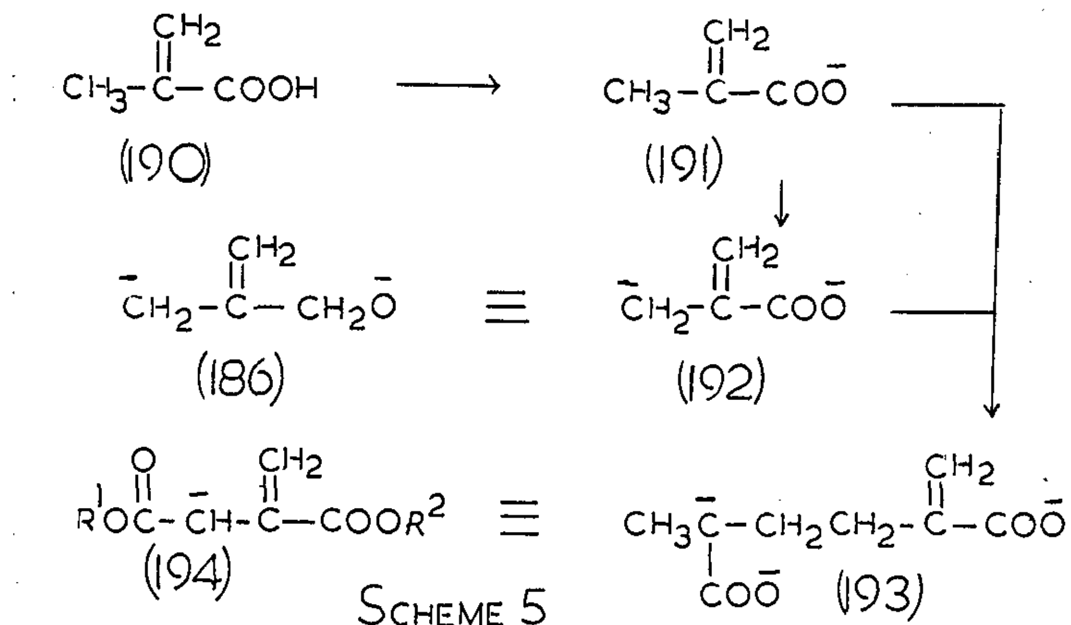
A further efficient method for α-methylene lactones, involving formation of an ethoxyoxalyl derivative (183) and reaction with an aldehyde to give a diketolactone (184) followed by base cleavage to yield the unsaturated product (55) has been reported[251].



Recently, a method[252] has been introduced in which methallyl alcohol dianion (186) addition to aldehydes and ketones yields allylic diols (187) that can subsequently be oxidised by activated manganese dioxide [243] to α-methylene-γ- lactone (162) via the presumed hemiacetal intermediate (188 - 189).

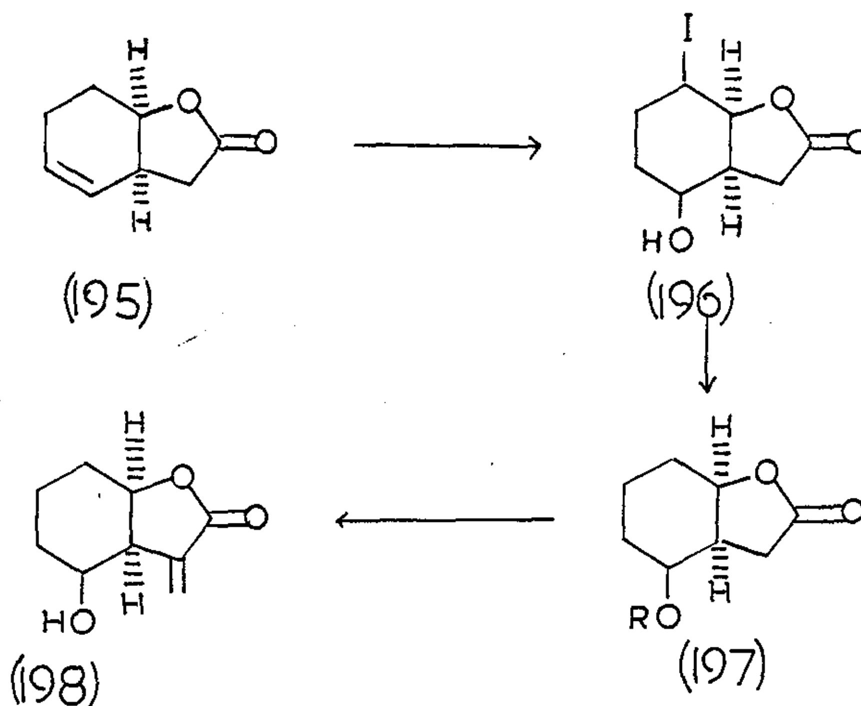


Analogous to the above scheme, Carlson [253,254] introduced a direct method which employs itaconic acid anion (194) instead of methallyl alcohol dianion (186) to obtain α-methylene lactones. Itaconic acid anion (194), a derivative of methylacrylic acid dianion (192), is a structural equivalent of methallyl alcohol dianion (186) (Scheme 5)



SCHEME 5

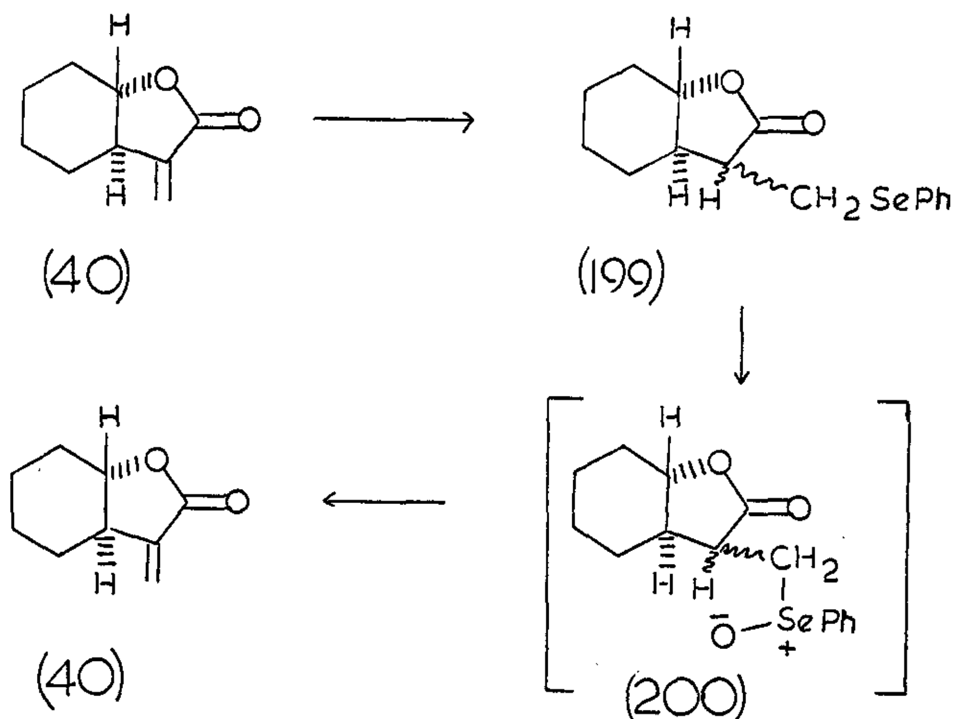
Since the presence of a lipophilic, conjugated ester side-chain located homoallylically to α-methylene-γ- butyrolactone contributes to the enhancement of the cytotoxic activity, [110,15C, 59] oxygenated α-methylene-γ-lactones have been synthesised[215].



The need to effect protection of an α-methylene function involving the synthesis of complex molecules possessing α-methylene moiety has led to a search for mild and potentially useful new blocking sequence. Kupchan[20, 226] required a method for protecting α-methylene unit conjugated with both γ- and -δ-lactones in order to determine the requirements for biological activity among derivatives of vernolepin (5). The protecting reagents employed included diethylamine [255], thiols [136,142] (propan-1-thiol[252], cysteine [28A, 142, 181]), phenylselenium anion [256] and thiophenoxide anion[257].

The regeneration of the α-methylene function involves the conversion of the diethylamino adduct and thio-ether into their methiodides and subsequent treatment with either saturated sodium hydrogen carbonate [255D] or pyrolysis [142, 255B]. Grieco's [256] approach employs a Michael-type addition of phenylselenium anion to the α-methylenelactone (40).and a

"retro-Michael" reaction via the corresponding selenoxide (200) which re-established the α-methylene function.



The above method is based on the observation [258,259] that alkylphenylselenoxides readily undergo elimination to give olefins. The thiophenoxide anion [257,258] is similarly eliminated.

Since the observation that the anti tumour activity of sesquiterpene α-methylenelactones is due to the α, β-unsaturated lactone system with an exocyclic methylene unit[5,11,18,19,133], it was decided to synthesise steroidal α-methylene lactones and evaluate their anti tumour activity. Lipophilicity appeared to enhance the cytotoxic activity of sesquiterpene α-methylenelactones [110, 15C,59] and as steroids are more lipophilic than sesquiterpenes, it was thought that this, in conjunction with αmethylenelactones moiety, would lead to important cytotoxic compounds. The distribution of these compounds should present very little difficult since steroids are present in the body as steroidal hormones.

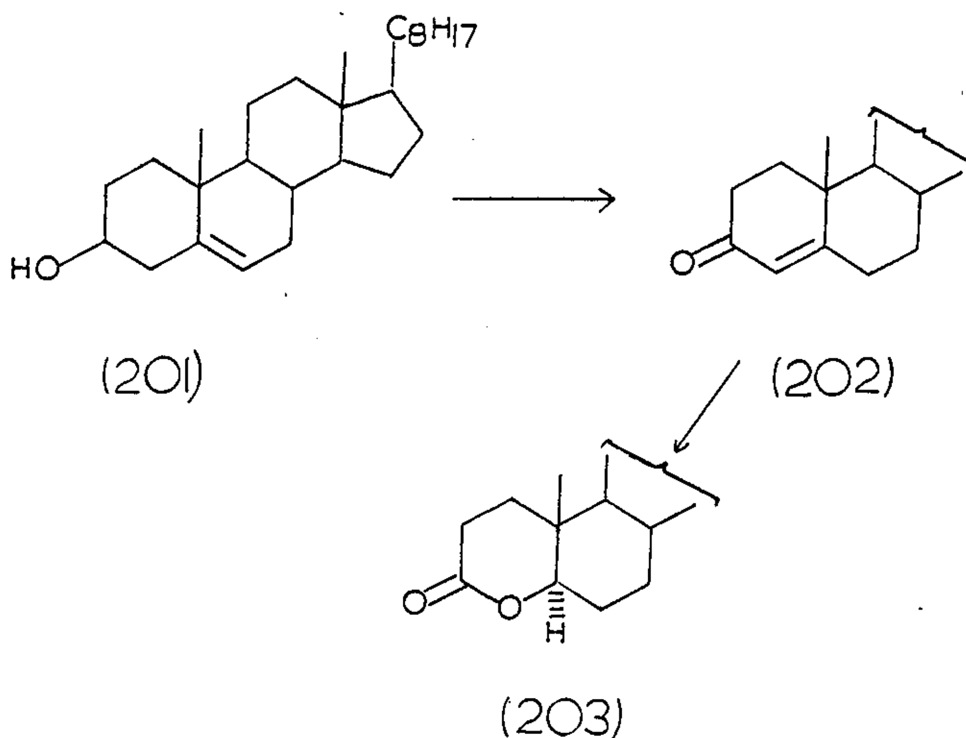
The fact that steroid hormones function through the recognisation of the receptor, at the active site, in the target tissue, this would appear to suggest that the synthetic steroidal α-methylenelactones may function in an analogous manner.

When the project was undertaken, there was no evidence of steroidal α-methylenelactones in the literature, but in the duration of the project, there appeared a publication describing the synthesis of steroidal α-methylenelactones[45] with anti tumour activity. The main aim of the project undertaken was to synthesise steroids containing an α-methylenelactone system as a part of the normal ring system of the steroid.

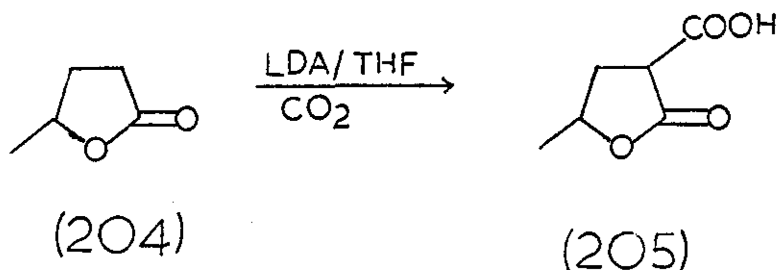
DISCUSSION

Peracid oxidation of α,β-unsaturated ketones, may, lead to enol esters, epoxy esters and epoxy ketones.[260] However, peracid oxidation of Δ^4-3-ketosteroids may result in an even larger variety of products, depending on the peracid being used. Thus, potassium persulphate and sulphuric acid furnished 3-keto-4-oxa-5α-steroids[261-264], whereas, perbenzoic acid containing perchloric acid in chloroform was reported to yield a mixture of enol-lactones and epoxylactones[265]. Recently, rearranged products, the 5α-aldehydo-3-keto-4-oxa-derivatives, were obtained when Δ^4-3-keto-steroids were treated with trifluoroperacetic acid[266].

Although there are many methods for the preparation of lactones,[267-282] the literature methods were employed in the synthesis of the steroidal lactones. Oppenauer oxidation [283] of cholesterol (201) gave cholest-4-en-3-one (202, 70%) which under the conditions of Baryer-Villiger oxidation [264] produced 3-oxo-4-oxa-5α-cholestane (203) in 40% yield.

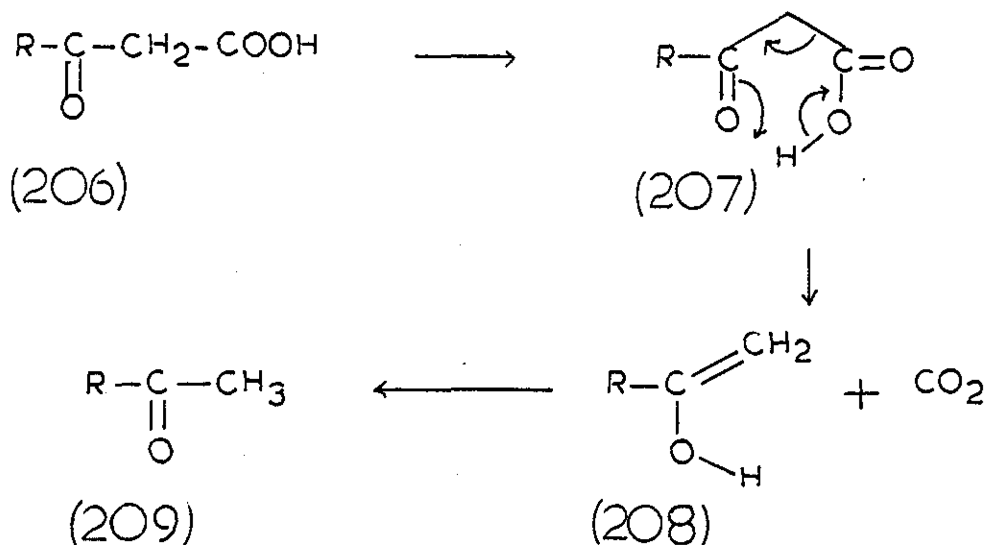


Once the formation of the lactone was successfully carried out, then the procedures for the synthesis of "α-methylene" structural unit on the preformed lactone were sought. The "α-carboxylation"[195] sequence was optimised with γ-methyl-γ- butrolactone (204) and the presence of an α-carboxylic acid lactone (205) was indicated by the presence of a polar spot on T.L.C. and by the infra-red spectrum (ν max, 3600-3200 cm^{-1} (-OH), 1780 cm^{-1} (γ-lactone C=O) and 1740 cm^{-1} (C=O)). The appearance of a D_2O-exchangeable peak at 1.77 (S, IH) in the ^1H n.m.r. spectrum was also indicative of the acidic proton.

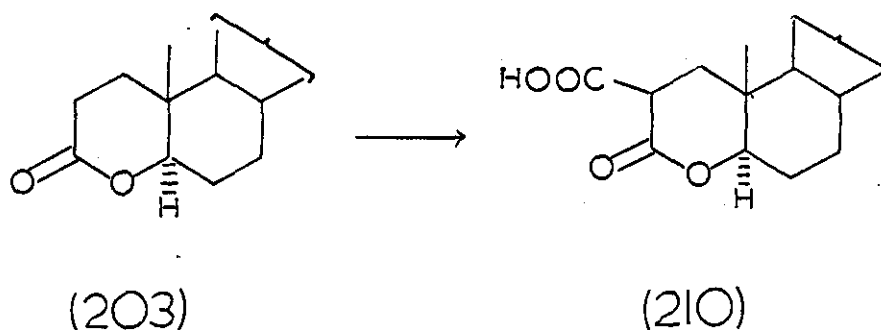


Application of this "α-carboxylation" procedure to 3-oxo-4-oxa-5α-cholestane (203) was unsuccessful, despite several attempts. It was thought that the α-carboxylated lactone formed was so unstable that it reverted to the starting lactone (203) during the workup, even though the reaction mixture was acidified with cold dilute hydrochloric acid and extracted with cold ether.

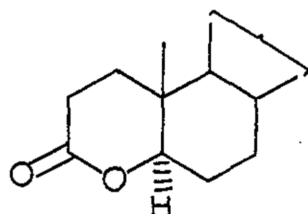
Many carboxylic acids can be successfully decarboxylated, either, as the free acid or in the salt form, but not simple fatty acids[284]. The decarboxylation of β-keto acids is well known[285] and is known to proceed by a cyclic, six-centre transition state (207)[286] leading to the enol form (208) which undergoes tautomerism to the stable keto-product (209).



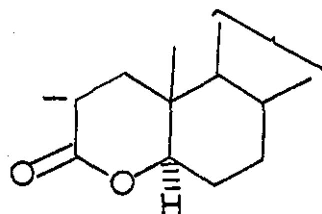
Assuming the reaction with lactone (203) proceeded as expected, then it must be concluded that the carboxylic acid lactone (210) is particularly unstable. However, it is possible that the carboxylation step did not take place. Although there was no direct evidence, the instability of the 2-formyl derivative (222) supports the former possibility.



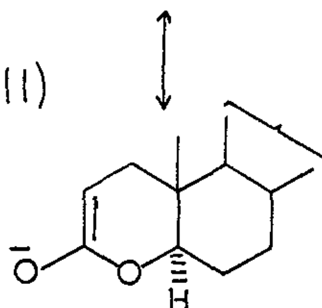
The rate of carboxylation is dependent upon the rate of formation of the enolate anion (211). The "stereoelectronic effect" results in the preferential loss of the axial proton [287,288]. This may be envisaged as arising from the possibility of overlap of the carbonyl π-orbital with the axial σ-bonding orbital (212), while no such overlap is possible with the equatorial σ-bonding orbital, which lies near the nodal plane of the carbonyl π-orbital (213).

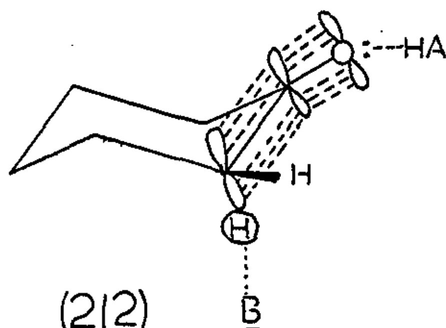


(203)



(211)





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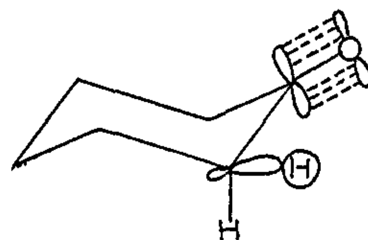
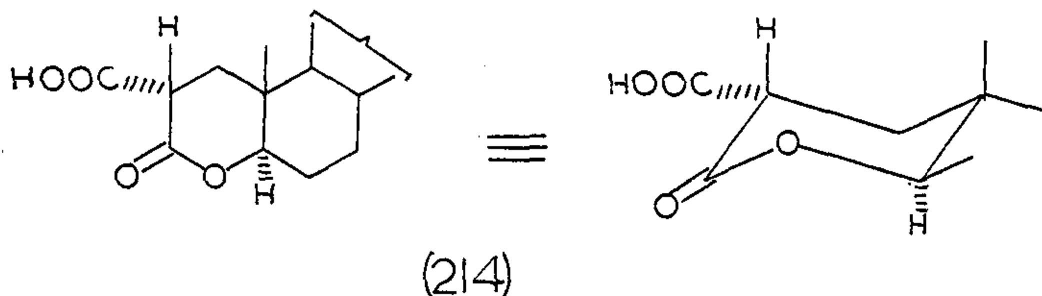


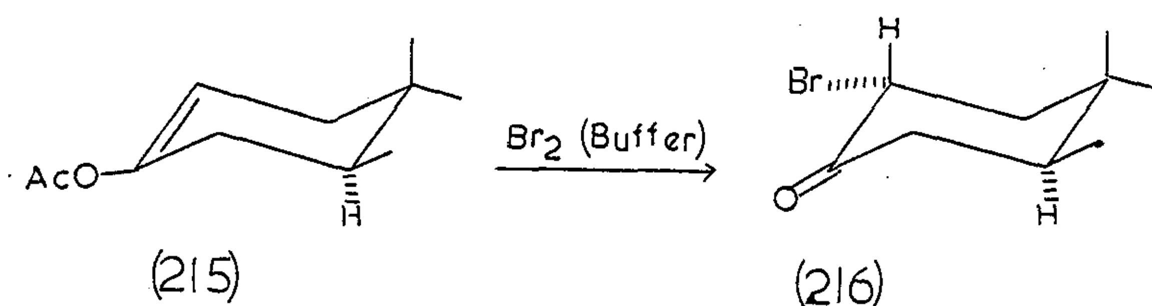
(213)

Orbital overlap in the transition state permits maximum electron delocalisation and a smooth transition from carbonyl π-bonding to the enolic structure with C=C π-bonding or in the base of the enolate anion, three centre π-bonding embracing the $\overline{C \cdots C \cdots O}^{-}$ system. Ringold[289] confirmed the operation of stereoelectronic control by revealing strong preferences for the loss of the 2β and 6β-protons during enolisation of 3-keto- and 7-keto-steroids respectively.

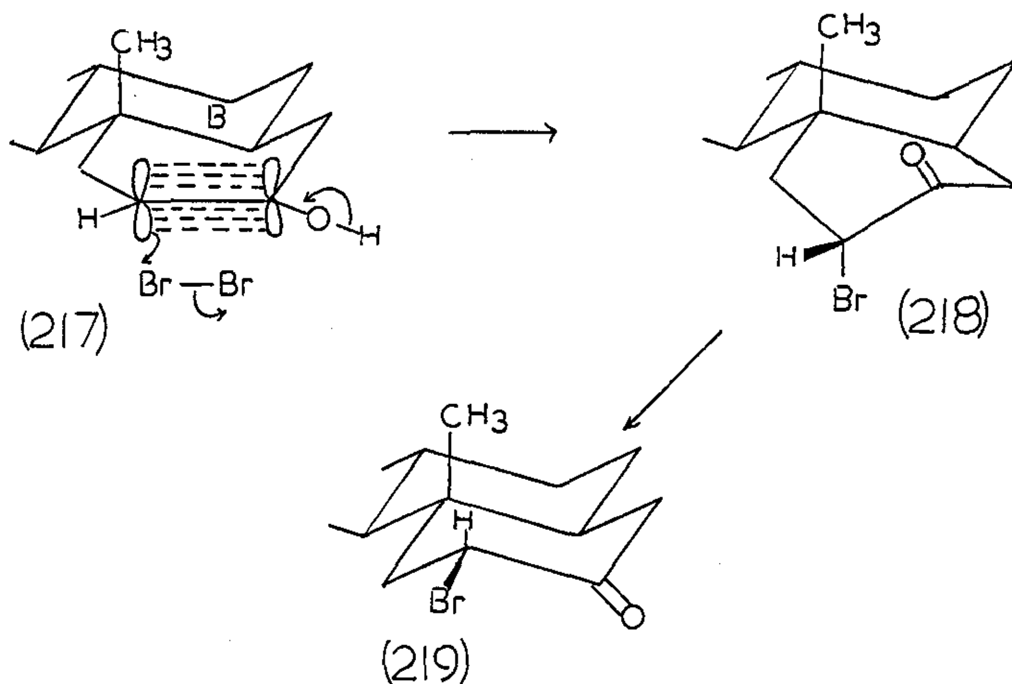
The electrophilic species, carbon dioxide, must approach in the plane of the π-obrital in order to secure maximum bonding in the reacting complex, probably resulting in the formation of 2 α-carboxylic acid lactone (214) by analogy with 2 α-bromination of 3-oxo-steroids.



Similar kinetically controlled bromination of the enol-acetate (215) was found to give the 2α-bromo-3-keto-product (216)[290].



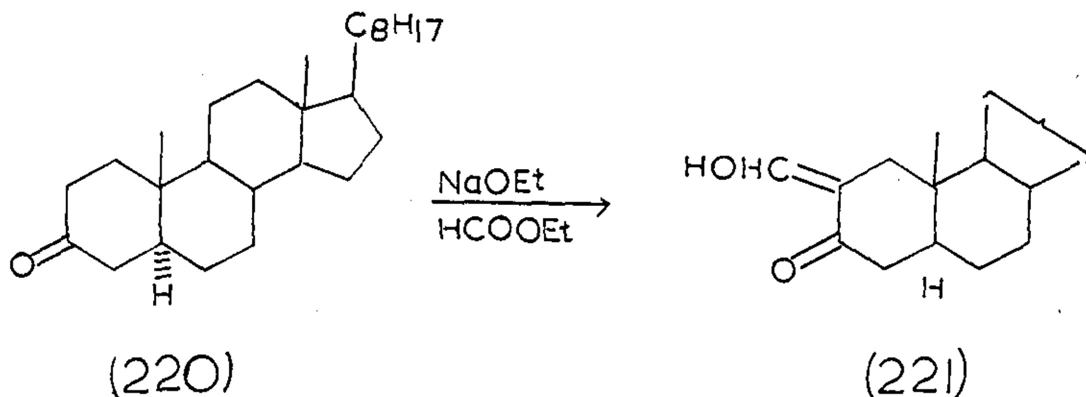
Also these results appear to contravene the principle that stereoelectronic control leads to the 2β-axial product, it was established that the stereoelectronic preference for axial attack is fulfilled by the molecular adopting a ring A boat or flexible conformation (218) during attack by bromine. Reversion of the chair conformation (219) leads to the bromine becoming equatorial.



Initial α-bromination is a consequence of steric hinderance to β-face attack up the enol due to the shielding effect of the C_{19}-methyl group, thus preventing perpendicular approach of bromine to the β-face of the double bond as required for maximum bonding. The α-face, however, is unhindered and permits approach of bromine to the α-lobe of the π-orbital of the double bond, although, as indicated, this requires ring A to adopt a boat-like conformation.

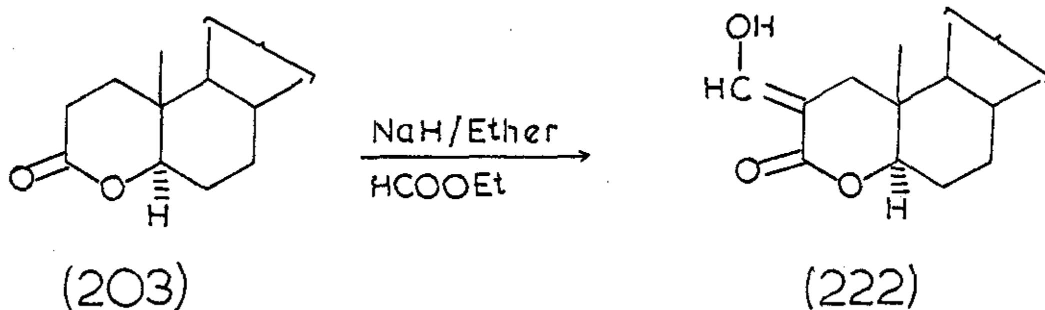
Carbon dioxide, being a larger reacting species, suffers considerably more 1,3-diaxial hinderance than bromine in the axial position. The above stated steric and conformational factors, operating in bromination of 3-keto-steroids, may also be operating in the carboxylation step and thus contribute towards this instability of the carboxy-lactone (210) or the difficulty in preparing this compound.

The "α-hydroxymethylenation" [291] of 5α-cholestan-3-one (220) with ethyl formate and sodium ethoxide gave 2-hydroxy methylene-5α-cholestan-3-one (221, 67%) as was indicated by a polar spot on TLC by the infra-red spectrum (ν max, 3600 - 3100 cm^{-1} (OH), 1562 cm^{-1} (α,β-unsaturated β-hydroxy ketone) and the U.V spectrum (λ max 283 nm (ε 16,837)).



The above α-formylation reaction was attempted with 3-oxo-4-oxa-5α-cholestane (203) under the same conditions but was unsuccessful.

The modification of the procedure of Yamada et al [160] was performed on 3-oxo-4-oxa-5α-cholestane (203), employing sodium hydride, a stronger base, instead of sodium ethoxide. The reaction was not terminated after twelve hours, as stated,[160] but continued until there was no starting lactone(203) observed on T.L.C., requiring approximately 90 hours for completion. The formation of 2-hydroxy methylene -3-oxo-4-oxa-5α-cholestane (222, 84%) was indicated by a polar spot on T.L.C, by the infra-red spectrum (ν max, 3600-2400 cm^{-1} (OH), 1715 cm^{-1} (C=O) and 1620 cm^{-1} (C=C) and the UV spectrum (λ max 251nm (ϵ 6.95 x 10^3)). The 'H n.m.r. spectrum displayed signals at τ 2.82 and τ 2.2(bs, 1H, Z - and E - HO-CH = C -). (See Experimental P 130 and 131).

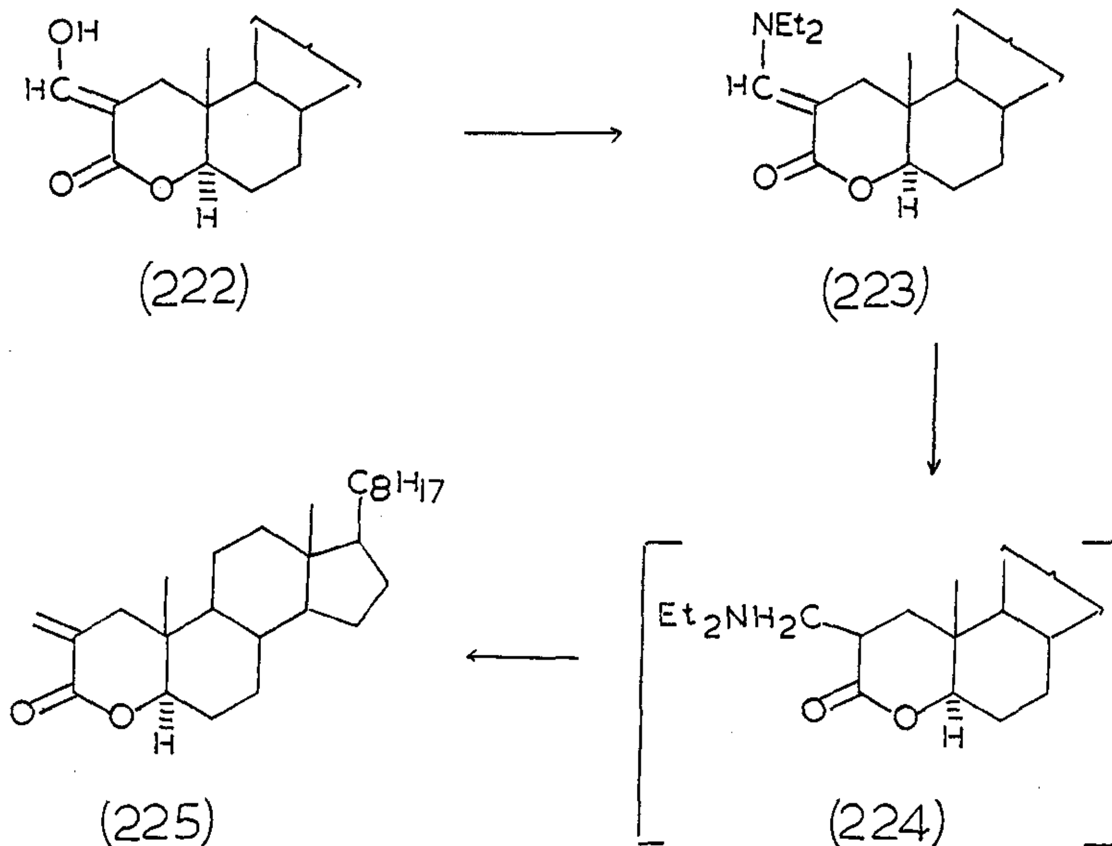


The 2-hydroxymethylene lactone (222) was not very stable as it reverted to the starting lactone (203) even on standing at -20°C. In the subsequent reactions, the 2-hydroxymethylene-lactone (222) was used directly with purification.

The 2-hydroxy methylene-3-oxo-4-oxa-5α-cholestane (222) was heated under reflex for 80 hours (the disappearance of the starting material (222) was followed by T.L.C) with diethylamine in benzene to obtain 2-(N,N-diethyl amino)-methylene-3-oxo-4-oxa-5α-cholestane (223, 90%).
The formation of the diethylamino adduct (223) was indicated by the infra-red spectrum (ν max. 1675 cm-1 (C=O) and 1560 cm-1 (C=C)) and

the U.V. spectrum (λ max 301nm (ϵ 2.84 x 10^4)). The 'H n.m.r. displayed signals at τ 2.82 and τ 2.30 (bs, 1H, Z - and E-Et-N CH = C -) [See P 131 and 132]

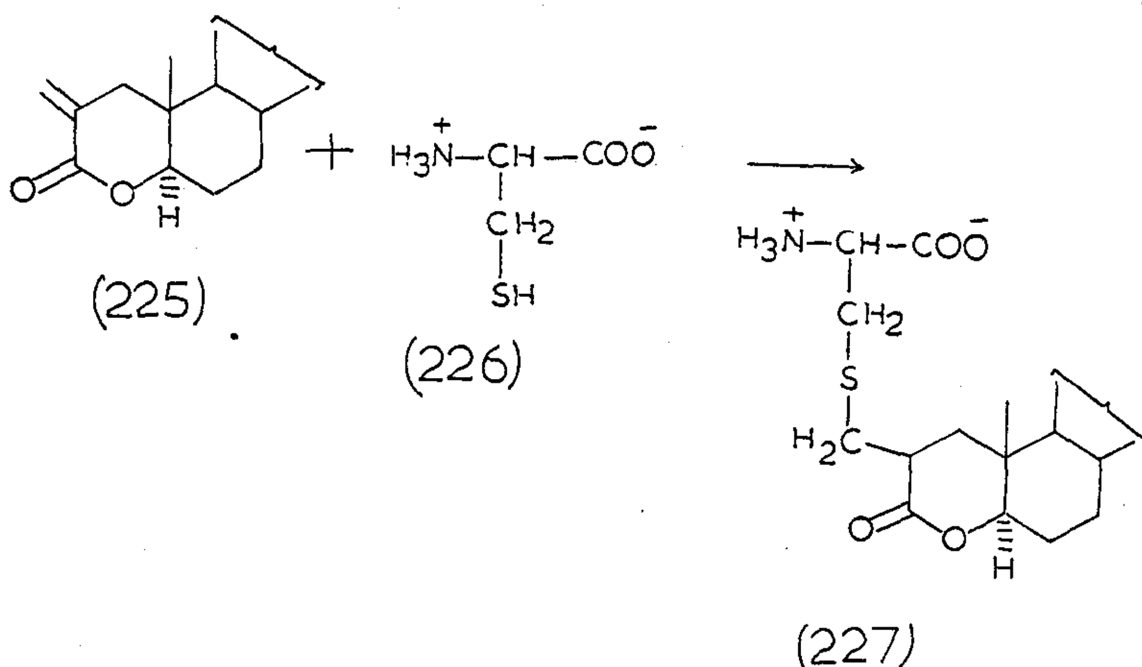
The diethylamino adduct (223) was hydrogenated in glacial acetic acid using platinum oxide catalyst to give the saturated product, 2-(N,N-diethylamino) methyl-3-oxo-4-oxa-5α-cholestane (224) which, without purification, was treated with sodium acetate in glacial acetic acid to yield the α-methylene-lactone, 2-methylene-3-oxo-4-oxa-5α-cholestane (225, 58% overall). It was shown by T.L.C. that during the hydrogenation step some of the saturated amino product (224) undergoes elimination to form the α-methylene lactone (225). However, it was necessary to heat, under reflux, the diethylamino adduct (224) with sodium acetate in glacial acetic acid for four hours to complete the elimination (Scheme 5).



SCHEME 5

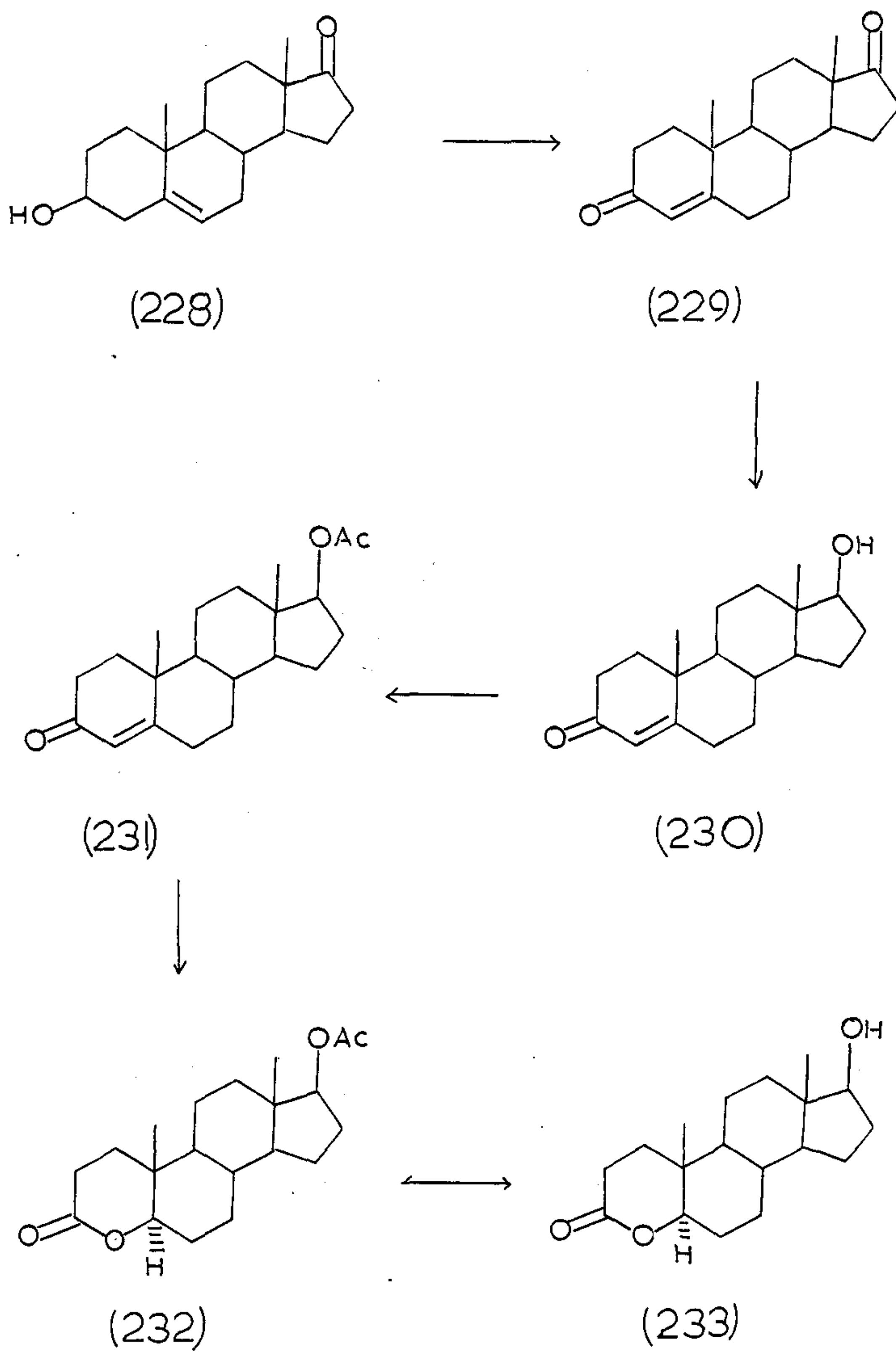
The formation of 2-methylene-3-oxo-4-oxa-5α-cholestane (225) was indicated by the infra-red spectrum (ν max. 1725 cm^{-1} (C=O), and 1620 cm^{-1} (C=C) and the U.V. spectrum (λmax 213nm (ε 7190)). The ^1H n.m.r. spectrum displayed signals at τ 4.43 and τ 3.50 (m,2 H, O -C(=O)-C=CH_2) corresponding to the C_2-methylene olefinic protons.

The 2-methylene-3-oxo-4-oxa-5α-cholestane (225), on reacting with L-cysteine (226) gave the cysteine-lactone adduct (227, 79%) under the conditions of Dalton and Elmes' procedure [186].



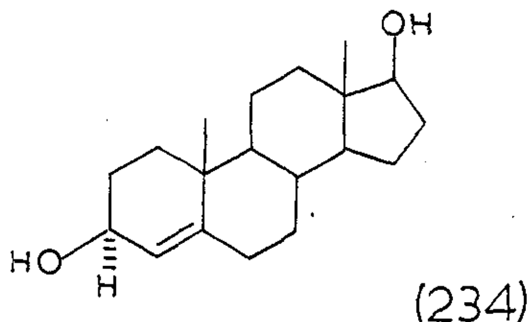
The formation of the cysteine-lactone adduct (227) was indicated by the absence of C_2-methylene olefinic protons in the ^1H' n.m.r. spectrum and the presence of (ν max 3700-2300 cm^{-1} (COOH and NH_2) 1725cm^{-1} (C=O of COOH and lactone) and 1630 cm^{-1} ($CO\bar{O}$), in the infra-red spectrum.

To observe if the side chain on C_{17} of the steroid nucleus has any effect on the cytotoxicity, it was decided to synthesise the α-methylene moiety in the A-ring of the androstane nucleus (Scheme 6).



Scheme 6

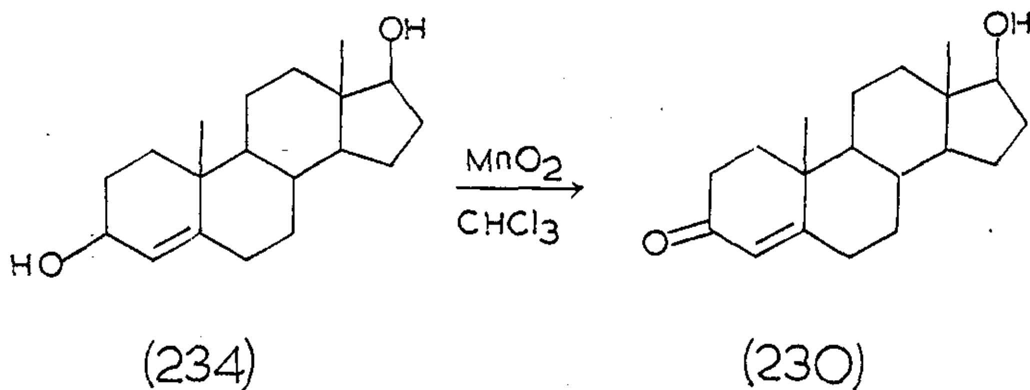
Oppernauer oxidation [283] of androst-5-en-3β-ol-17-one (228) gave androst-4-en-3,17-dione(229) in 85% yield. A partial reduction of androst-4-en-3,17-dione (229) was attempted to obtain androst-4-en-17β-ol-3-one (230). Sodium borohydride (1.5M equivalent)[292] in methanol at $0^{o}C$ was employed and the crude product contained not only the expected product, androst-4-en-17β-ol-3-one (230), but a more polar product, androst-4-en-3β, 17β-diol (234) was also present.



(234)

The disappearance of ν max $1735cm^{-1}$ and $1660cm^{-1}$ in the infra-red spectrum indicated the reduction at both the C-3 and C-17 carbonyl groups. A multiplet in the ^{1}H n.m.r. spectrum at 5.38 was also indicative of the 3 α-methine of the diol (234). The ratio of androst-4-en-17β-ol-3-one(230) to androst-4-en-3β,17β-diol (234) was approximately 2:1, as observed from the ^{1}H n.m.r. spectrum of the reduction product. It was then decided to reduce the concentration of sodium borohydride (1.0M equivalent). In this case, there was a small amount of androst-4-en-3β, 17β-diol (234, ≃ 10%). However, the starting material, androst-4-en-3,17-dione (229, ≃ 10%) was also present, as well as the required product (230). To circumvent this problem, the concentration of sodium borohydride was increased (1.2 M equivalent) and the reduction mixture consisted of androst-4-en-17β-ol-3-one (230) and androst-4-en-3β,17β-diol (234). The separation of the crude reduction products on alumina (activity III) column chromatography gave androst-4-en-17β-ol-3-one (230, 81%) and androst-4-en-3β, 17β-diol (234, 12%).

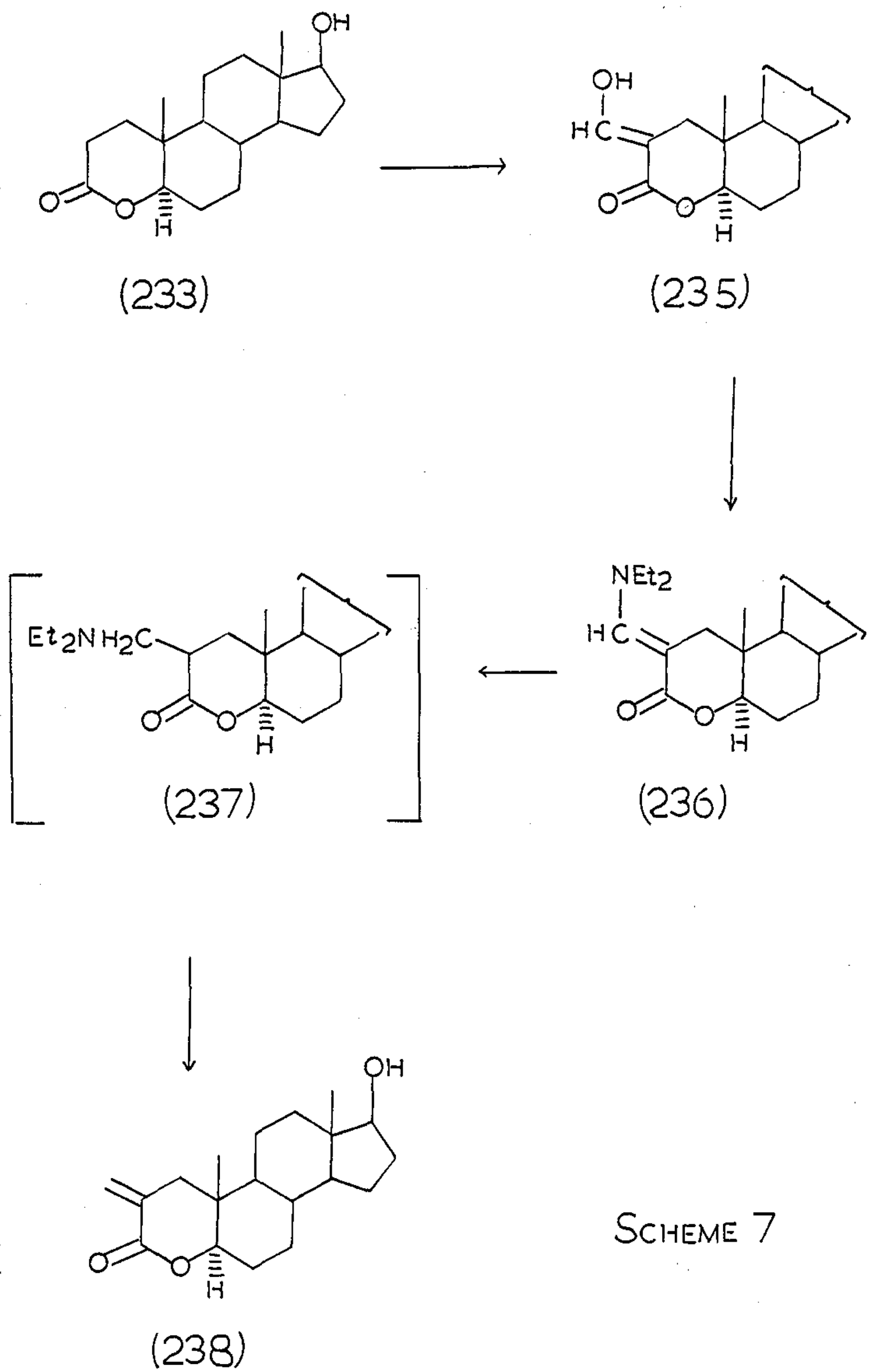
Androst-4-en-3β, 17β-diol (234) was oxidised[293] with activated manganese dioxide[294] to obtain androst-4-en-17β-ol-3-one (230, 87%).



Androst-4-en-17β-ol-3-one (230), after acetylation, gave testosterone acetate, 17β-acetoxy-androst-4-en-3-one (231, 88%) which under the conditions of Baeyer-Villiger oxidation[264] yielded the lactone, 17β-acetoxy-3-oxo-4oxa-5α-androstane (232·44%). The formation of the lactone (232) was indicated by the infra-red spectrum (ν max. 1720 cm^{-1} (C=O) and disappearance of 1660 cm^{-1} (C=O) and 1615 cm^{-1} (C=C) due to the conjugated α,β-unsaturated system of testosterone acetate (231).

17β-Acetoxy-3-oxo-4-oxa-5α-androstane (232) was hydrolysed[295] to obtain 17 β-hydroxy-3-oxo-4-oxa-5α-androstane (233, 77%). The formation of lactone (233) was shown by a polar spot on T.L.C., the infra-red spectrum (ν max. 3500 - 3200 cm^{-1} (OH)) and the disappearance of the C_{17}-β-methyl resonance at τ2.01 and a shift of C_{17} -α- methine chemical shift from τ 5.40 to τ6.34 in the ^{1}H n.m.r. spectrum.

The α-hydroxymethylenation procedure of Yamada <u>et al</u>[160] was employed to obtain 17 β-hydroxy-2-methylene-3-oxo-4-oxa-5α-androstane (238) (Scheme 7).



SCHEME 7

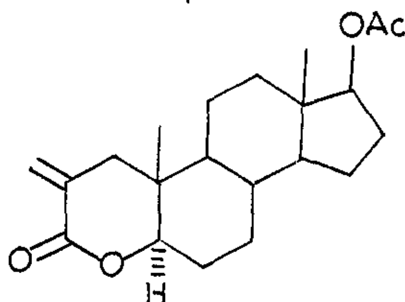
Reaction of ethyl formate and sodium hydride in ether with 17 β-hydroxy-3-oxo-4-oxa-5α-androstane (233) gave 17β-hydroxy-2-hydroxymethylene-3-oxo-4-oxa-5α-androstane (235, 86%). The reaction required approximately 90 hours for completion. The presence of 2-hydroxymethylene lactone (235) was indicated by a polar spot on T.L.C., the infra-red spectrum (ν max, 1706 cm^{-1} (C=O) and 1612 cm^{-1} (C=C) and the U.V. spectrum (λmax 252 nm (ε 7863)). The ^{1}H n.m.r. spectrum displayed signals at τ 2.78 and τ 2.16 (bs, 1H, Z- and E -HO-CH = CH). (see P131 and 144)

17β-Hydroxy-2-hydroxymethylene-3-oxo-4-oxa-5 α-androstane (235) was heated under reflux for 80 hours (the disappearance of the starting lactone (235) was followed by T.L.C) with diethylamine in benzene to obtain the diethylamino adduct, 17β-hydroxy-2- (N,N-diethylamino) methylene-3-oxo-4-oxa-5 α-androstane (236, 93%). The infra-red spectrum (ν max. 3440 cm^{-1} (OH) 1670 cm^{-1} (C=O), and 1570 cm^{-1} (C=C), the U.V spectrum (λ max 300-1nm (ε 1.3171 x 10^4)), the ^1H n.m.r. spectrum (τ 6.64 (q, 4H, N-CH_2 -CH_3), τ 8.78 (t, 6H, - N - CH_2-CH_3) τ 2.83 and τ 2.30 (bs, 1H, Z - and E - Et_2 N-CH = C)), and a polar spot on T.L.C. indicated the formation of the diethylamino-adduct (236). (See P 131 and 145)

17β-Hydroxy-2-(N,N-diethylamino)-methylene-3-oxo-4-oxa-5-α-androstane (236) was hydrogenated with platinum oxide in glacial acetic acid to obtain the saturated amino-adduct, 17β-hydroxy-2-(N,N-diethylamino) methyl-3-oxo-4-oxa-5 α-androstane (237) which, without isolation, was heated under reflux with sodium acetate in glacial acetic acid to eliminate diethylamine. The crude product appeared to be a mixture of two compounds on T.L.C. The compound corresponding to the lower spot had the same R_F value as the original lactone, 17β-hydroxy-3-oxo-4-oxa-5 α-androstane (233). However, on closer inspection, this compound appeared to be the α-methylene lactone, 17β-hydroxy-2-methylene-3-oxo-4-oxa-5 α-androstane (238, 28%). The presence of the α-methylene lactone (238) was confirmed by the infra-red spectrum (ν max. 3440 cm^{-1} (OH), 1712 cm^{-1} (C = O) and 1623 cm^{-1} (C=C), the U.V. spectrum (λ max 215 nm (ε 6680)

and the ^1H n.m.r. spectrum (τ 6.35 (t, 1H, $C_{17}\alpha$-H, J = 8.0 H_2)) and τ 4.42 (m, 1H, O-$\overset{O}{C}$-C=C^H-$\underline{H}$) and τ 3.51 (m, 1H, O-$\overset{O}{C}$-C=C^H-H) corresponding to the C_2-methylene olefinic protons.

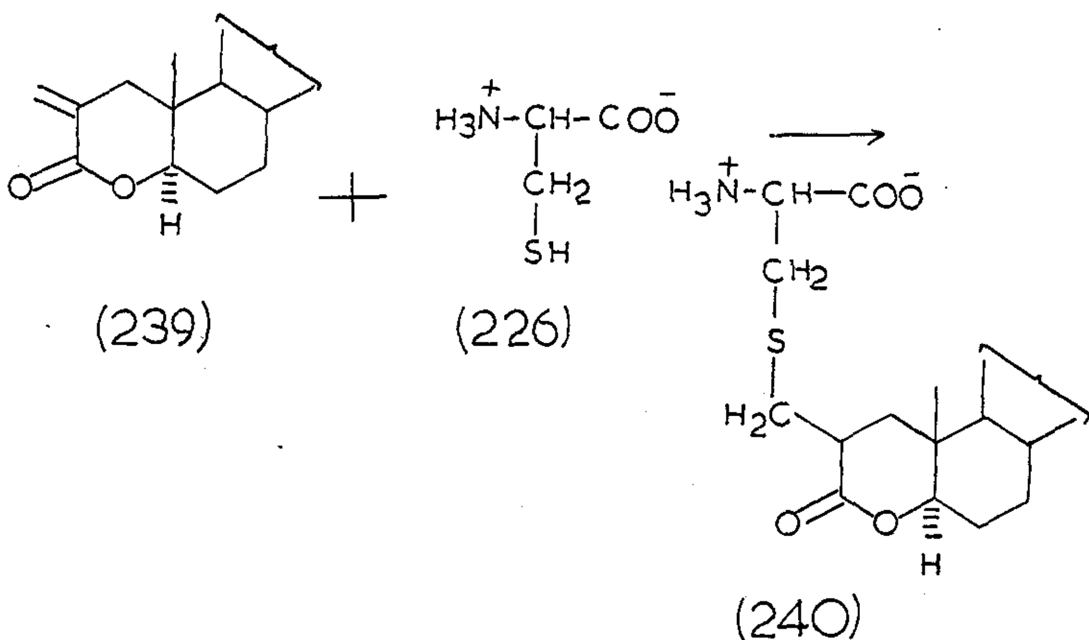
The product with a higher R_F value was shown to be the acetylated α-methylene lactone, 17β-acetoxy-2-methylene-3-oxo-4-oxa-5 α-androstane (239, 30%) by the infra-red spectrum. (ν max. 1735 cm^{-1} (C=O,ester), 1723 cm^{-1} (C=O, lactone), 1625 cm^{-1} (C=C) and the disappearance of the hydroxyl absorption), the U.V. spectrum (λ max 214-5 nm (ϵ 7936)) and the ^1H n.m.r. spectrum (τ 7.96, (S, 3H, $C_{17}\beta$-acetate methyl), τ 5.40 (t, 1H, $C_{17}\alpha$-H, J = 8.0H_2) and τ 4.39 (m, 1H, O-$\overset{O}{C}$-C=C^H-$\underline{H}$) and τ 3.48 (m, 1H, O-$\overset{O}{C}$-C =C^H-H).



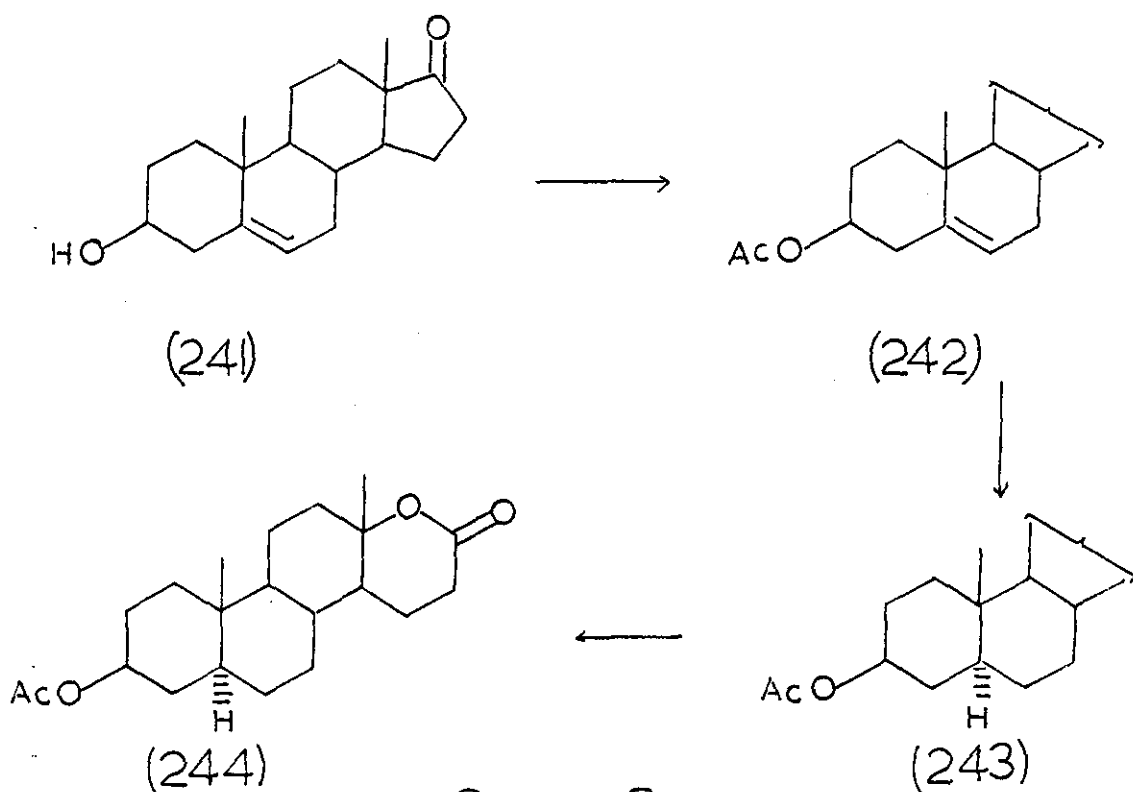
(239)

It appears that the acetylation of 17β-hydroxy-2-methylene-3-oxo-4-oxa-5 α-androstane (238) occurs during the elimination of diethylamine with sodium acetate in glacial acetic acid, as there is no evidence of this happening any earlier. The existence of 17β-hydroxy-2-methylene-3-oxo-4-oxa-5 α-androstane (238) was corroborated by conversion to 17β-acetoxy-2-methylene-3-oxo-4-oxa-5 α-androstane (239) with acetic anhydride in pyridine.

The reaction of L-cysteine (226) [185] with 17β-acetoxy-2-methylene-3-oxo-4-oxa-5 α-androstane (239) gave a cysteine-lactone adduct (240, 69%). The formation of the cysteine-lactone adduct (240) was indicated by the infra-red spectrum (ν max. 3700-2300 cm^{-1}(COOH and NH_2), 1735 cm^{-1}(C=O of carboxylic acid and lactone) and 1621 cm^{-1} (COO^-) and the absence of C_2-methylene olefinic protons in the ^1H n.m.r. spectrum.



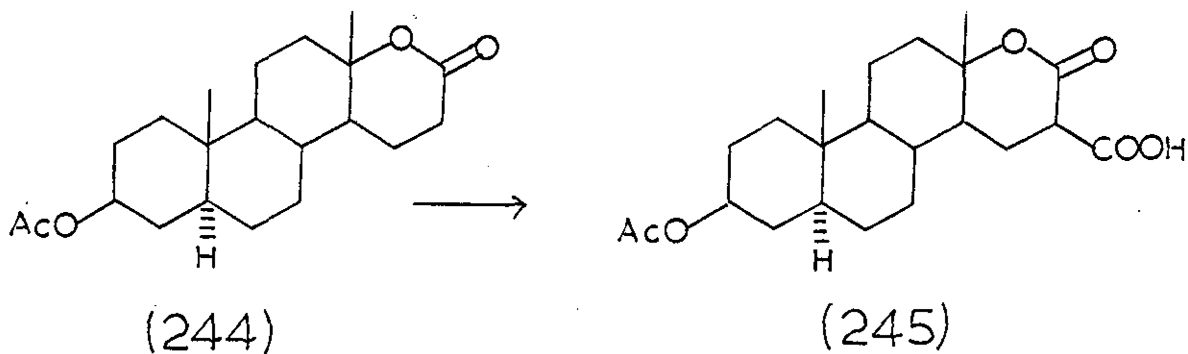
It was decided to synthesise α-methylene lactones in the D-ring of the steroid nucleus to compare the cytotoxic activity with the A-ring α-methylene lactones. Androst-5-en-3β-ol-17-one (241) was utilised for the formation of the D-ring lactone, 3β-acetoxy-17-oxo-17a-oxa-D-homo-5 α-androstane (244) (Scheme 8).



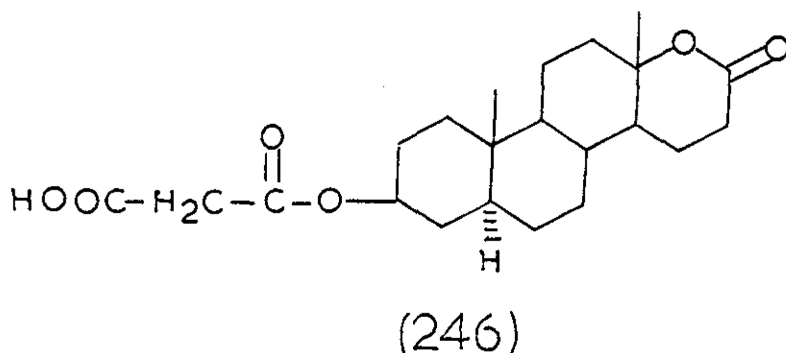
SCHEME 8

Acetylation of androst-5-en-3β-ol-17-one (241) with acetic anhydride in pyridine gave 3β-acetoxy-androst-5-en-17-one (242, 85) which was hydrogenated with palladium-charcoal (10%) catalyst in glacial acetic acid to obtain 3β-acetoxy-5 α-androstan-17-one (243, 88%). Baeyer-Villiger oxidation [295] of 3β-acetoxy-5 α-androstan-17-one (243) gave 3β-acetoxy-17-oxo-17a-oxa-D-homo-5 α-androstane (244), isoandrololactone acetate, in 65% yield. Recently an alternative, high yield conversion of cyclic ketones to a lactones employing hydrogen peroxide in glacial acetic acid was reported [296A, B] and also an application of selenylation and dehydroselenylation was utilised in the conversion of 17-oxo-steroids into D-homolactones [296 C].

α -Carboxylation of 3β-acetoxy-17-oxo-17a-oxa-D-homo-5 α-androstane (244), according to the procedure of Grieco and Hiroi [195], gave a mixture of the starting lactone and carboxylactones (245 and 246).

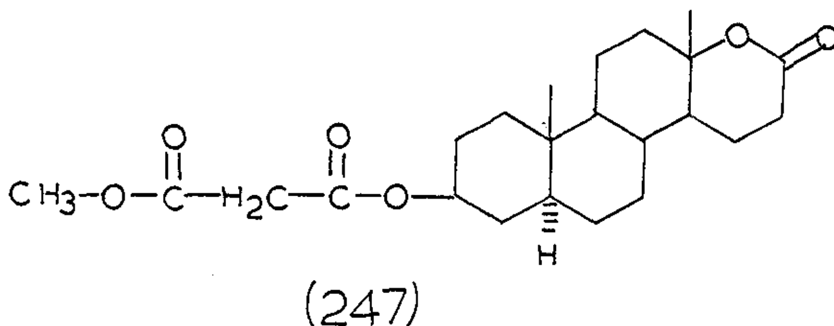


(244) (245)



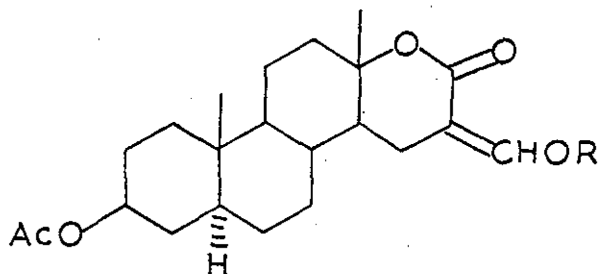
(246)

The presence of the carboxylactone (245) in the crude reaction product was indicated by ^{1}H n.m.r. spectrum (τ 7.9 (S, 3H, 3β-acetate CH_3), τ 5.2 (m, IH, C_3 α-H) and τ 0.23 (bs, 1H, C_{16} - COO H)). The presence of the carboxylactone (246), also in the crude product, was indicated by the ^{1}H n.m.r. spectrum (τ 7.38 (m, 2H, C_{16}-2H), τ 6.58 (S, 2H, C_3 - O - C(=O) - CH_2 - COOH), τ 5.2 (m, IH, C_3 α-H) and τ 0.23 (bs, 1H, - COOH)). The crude product was taken up in ether and washed with saturated sodium hydrogen carbonate solution followed by water. The neutral fraction obtained was the starting lactone (244) as indicated by the spectral data and T.L.C. The combined aqueous washing was acidified (pH $\simeq$ 3-4) and extracted with ether. The acidic fraction, consisting of carboxylactones (245 + 246), was unstable as the acidic proton signal in the ^{1}H n.m.r. spectrum at 0.23 had disappeared and the appearance of a multiplet at τ 7.36 (2H, C_{16}-H_2), which is characteristic of the starting material, indicated that the carboxylactone (245) had reverted to (244). The acidic fractions ^{1}H n.m.r. spectrum still exhibited the acidic proton at τ 1.05 which indicated that compound (246) is relatively stable. Further treatment of compounds (245+246) with saturated sodium hydrogen carbonate solution gave the neutral fraction, the starting lactone (244) (T.L.C. identification) and the acidic fraction, the carboxylactone (246), which was esterified with diazomethane to obtain the methylester (247). Although this was not fully characterised, its presence was indicated by the ^{1}H n.m.r. spectrum (τ 7.36 (m, 2H, C_{16}-H_2), τ 6.25 (S, 3H, Ester-CH_3) and τ 5.25 (m, IH, C_3 α-H).), the infra-red spectrum (no hydroxyl group absorption), a non-polar spot on T.L.C. and the mass spectrum (M$-$ CH_3 O - C(=O) - CH_2 - C(=O) - OH)$^+$ = 288).



(247)

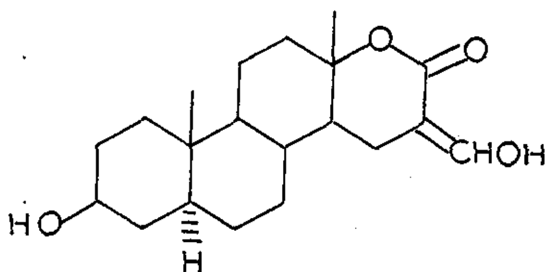
An " α-hydroxymethylenation" procedure of Volovelskii[291] was performed with 3β-acetoxy lactone (244) under the same conditions as for 5 α-cholestan-3-one (220). The crude product, a mixture of the starting lactone (244) and the α-hydroxymethylene lactone (248) was taken up in ether and triturated with acetone. The precipitate appeared to be the α-hydroxy-methylene lactone (248) as indicated by a polar-spot on T.L.C. and the infra-red spectrum (ν max. 3600-3300 cm^{-1} (OH), 1695 cm^{-1} (C=O) and 1610 cm^{-1} (C=C)).



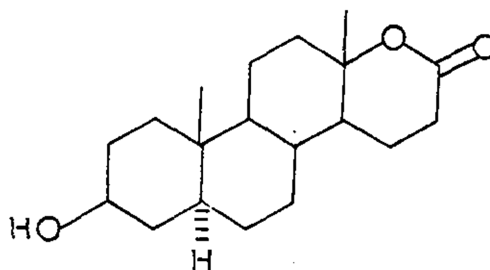
(248) R=H
(249) R=Ac

The acetone insoluble product was acetylated and, after chromatographic purification, the fraction that was thought to be the α-hydroxy-methylene lactone (249) showed no characteristic absorption, in the infra-red spectrum due to the α,β-unsaturated system of the lactone (249). It appeared that the product was either unstable on silica gel or it decomposes on standing for a time or both. The "α-hydroxymethylenation" procedure of Knox et al[297] was also unsuccessful on 3β-acetoxylactone (244).

The method of Yamada et al [160] was applied to 3β-acetoxy lactone (244) and the reaction was continued until there was no starting lactone (244) present, as observed on T.L.C. (90 hours). The crude product was crystallised from ethylacetate/pet ether (40-60°) to give the α-hydroxymethylene lactone, 3β-hydroxy-16-hydroxy methylene-17-oxo-17a-oxa-D-homo-5 α- androstane (250) as indicated by the infra-red spectrum (ν max. 3700-3100 cm^{-1} (OH), 1695 cm^{-1} (C=O) and 1610 cm^{-1} (C=C)). The mother liquors contained 3β-hydroxy-17-oxo-17a-oxa-D-homo-5 α-androstane (251) which presumably arises by hydrolysis during the reaction owing to traces of water and/or during workup.

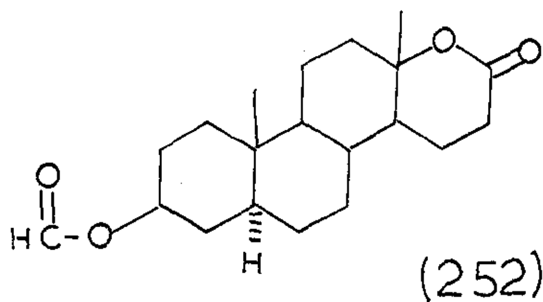


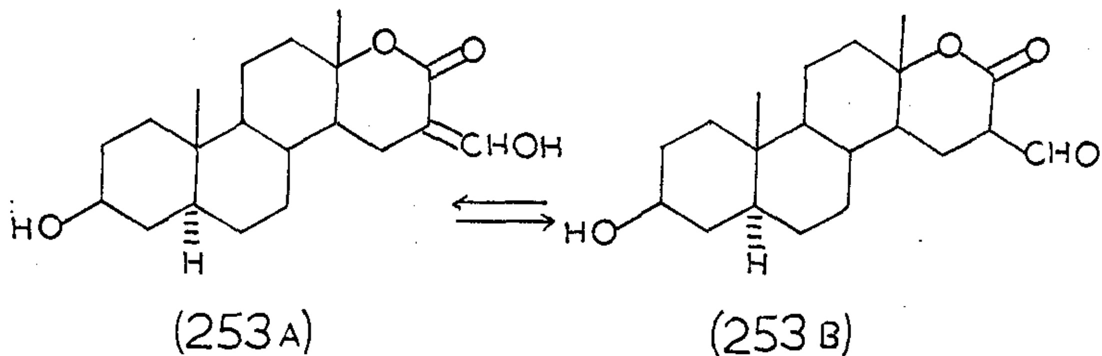
(250)



(251)

To avoid this complication, the " α-formylation" procedure [160] was applied to isoandrololactone (251) which was obtained from the hydrolysis of 3β-acetoxylactone (244). The crude product was shown to be a mixture of three compounds as indicated by T.L.C. and ^{1}H n.m.r. spectrum.

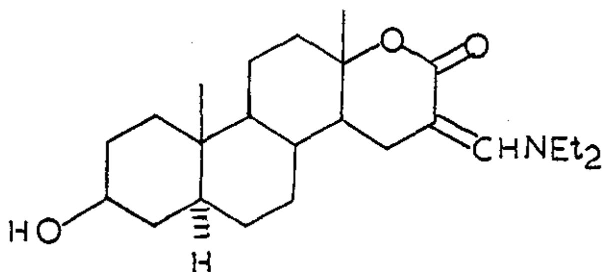




The presence of the starting lactone (251) was indicated by T.L.C. and the ^{1}H n.m.r. spectrum (τ 7.39 (m, 2H, C_{16}-2H)). 3β-Hydroxy-17-oxo-17a-oxa-D-homo-5 α-androstant-formate (252) was shown to be present by a non-polar spot on T.L.C. and ^{1}H n.m.r. spectrum (τ 2.0 (S, 1H, C_3 - O - $\overset{O}{\overset{\|}{C}}$ - $\underline{H}$)). The presence of (253) was indicated by a polar spot on T.L.C. and ^{1}H n.m.r. spectrum (τ 1.94 [S, 1H, C_{16} - C$\underline{H}$ =O). The product (253) may be in equilibrium with its hydroxymethylene isomer (253A-253B), but there was no evidence to support this.

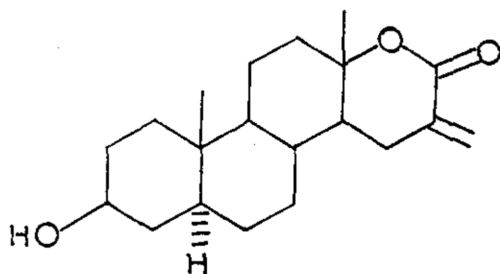
The above reaction was repeated using sodium ethoxide, instead of sodium hydride, as a base. Although the reaction was complete on T.L.C. the product, α-hydroxymethylene lactone (250) appeared to revert to the starting lactone (251) during workup. It was thought that the use of dilute hydrochloric acid for acidification of the aqueous fraction may cause decomposition of the α-hydroxymethylene lactone which may be unstable at pH 3-4.

When 5% aqueous acetic acid was employed to acidify the aqueous fraction (pH 6-7), the crude product was shown to be a mixture of the starting lactone (251) and the α-hydroxymethylene-lactone (250). The above crude product was heated under reflux with diethylamine in benzene, in an attempt to obtain the diethylamino adduct, 3β-hydroxy-16 (N, N-diethylamino)-methylene-17-oxo-17a-oxa-D-homo-5 α-androstane (254) but only 3β-hydroxylactone (251) was recovered.



(254)

It appears that the diethylamino adduct (254) is even more unstable than the α-hydroxymethylene lactone (250). When the diethylamino adduct (254) was formed in the reaction, as indicated by T.L.C., the infra-red spectrum and the U.V. spectrum (λmax 298-300 nm), attempts to carry out hydrogenation and subsequent elimination of diethylamine gave only 3β-hydroxylactone (251) but not the expected α-methylene lactone, 3β-hydroxy-16-methylene-17-oxo-17a-oxa-D-homo-5 α-androstane (255).



(255)

To obtain the D-ring α-methylene lactone (255), the α-hydroxymethylation" procedure of Grieco and Hiroi[203A] was applied to isoandrololactone (251) (Scheme 9).

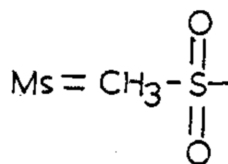
HO (251) —LDA/THF, HCHO→ HO (256) CH_2OH

↓

HO (257) CH_2OMs → HO (255)

MsO (258) CH_2OMs → (259)

(Scheme 9)



The yield of 3β-hydroxy-16-hydroxymethyl-17-oxo-17a-oxa-D-homo-5 α-androstane (256) was 18% with isoandrololactone (251) being recovered in a yield of 78%. The low yield of 16-hydroxymethyl lactone(256) was attributed to difficulties associated with the introduction of formaldehyde vapour into the reaction mixture. When the inlet carrying a nitrogenous stream of formaldehyde vapour, generated by heating paraformaldehyde to 180°C, was underneath the surface of the reaction mixture, the tip of the inlet became blocked owing to the polymerisation of the formaldehyde. When the tip of the inlet tube was placed just above the surface of the reaction mixture, after a few seconds, there appeared a film of polymerised formaldehyde at the surface, even though the rate of flow and stirring was quite fast.

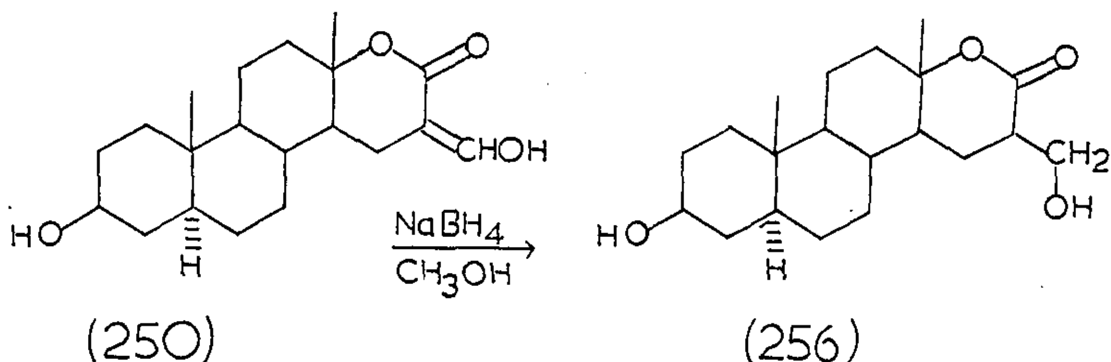
The procedure of Lucast and Wemple[223], in which the reaction mixture was kept at - 78°C (acetone/dry CO_2) instead of raising the temperature to - 20°C (carbon tetrachloride/dry CO_2),before introducing the formaldehyde vapour, did not improve the yield of the α-hydroxymethyl lactone (256). This procedure[223] was applied so as to reduce the time between the generation of the anion and the flow of formaldehyde vapour.

The formation of 3β-hydroxy-16-hydroxy methyl-17-oxo-17a-oxa-D-homo-5 α-androstane (256) was indicated by a polar spot on T.L.C. (solvent system, chloroform/methanol: 95/5) and the ^{1}H n.m.r. spectrum displayed a signal at τ 6.36 (d, 2H, J = 8.0HZ) corresponding to the methylene protons of the C16 - hydroxymethyl substituent.

An attempt[298] to obtain the mono-mesylate (257) was unsuccessful and the dimesylate (258, 82%) was obtained as indicated by a non-polar spot on T.L.C. (chloroform/methanol; 95/5), the infra-red spectrum (the absence of hydroxyl absorption, ν max 1724 cm^{-1} (C=O) and 1355 cm^{-1} and 1176 cm^{-1} ($-SO_2-O-$) and the ^1H n.m.r. spectrum, τ 6.97 and τ 7.02 (S, 6H, 2x - O - SO_2-$\underline{CH_3}$). It was expected that selective mesylation would have been possible owing to the fact that primary alcohols react faster than secondary alcohols and that the reaction was performed at a low temperature (-20°C) and further that selective sulphonation has previously been reported [298,299].

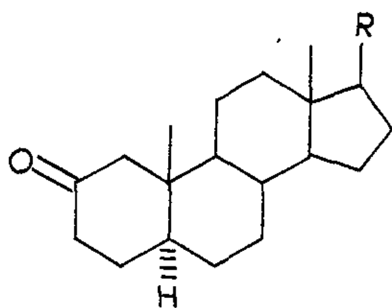
The di-methylsulphonate lactone (258), on heating under reflux with pyridine, resulted in the eliminated product, 16-methylene-17-oxo-17a-oxa-D-homo-5 α-androst-2-ene (259) in a low yield (28%). This has been confirmed by the infra-red spectrum (ν max. 3030 cm^{-1} (C=C), 1709 cm^{-1} (C=O) and 1620 cm^{-1} (C = C)), the U.V. spectrum (λ max 213-4nm, (ϵ 5939)) and the ^1H n.m.r. spectrum, τ 4.51 (m, 3H, C 2,3 H and O-C(=O)-C=C(H)-$\underline{H}$), τ 3.60 (m, 1H, O-C(=O)- C=C(H)-H).

As the α-hydroxymethylene D-ring lactone (250) could be obtained, even though unstable and impure, it was decided to synthesise the α-hydroxymethyl-D-ring lactone (256) by the reductive procedure of Minato and Horibe[176].

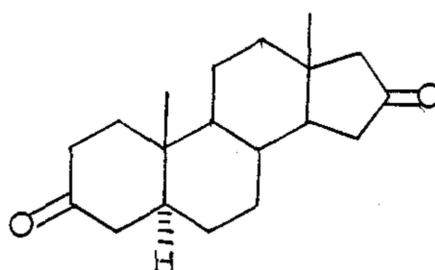


The above reductive method was unsuccessful even though sodium borohydride has been employed for reduction of α,β-unsaturated systems (250→256) in the synthesis of various natural cytotoxic sesquiterpene lactones[171, 177-180]. The use of palladium-charcoal (10%) in ethanol, ethylacetate and acetic acid and platinum oxide in ethyl acetate and acetic acid was also unsuccessful. The low reactivity of the olefinic bond towards reduction may be due to steric and conformational factors.

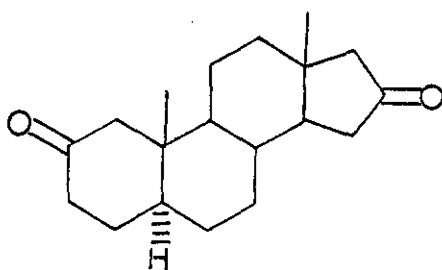
In studying the microbiological hydroxylation of steroids, Bridgemann et al [300] required oxygenated steroids of the less common type (e.g. 260 -262) prepared by the removal of 17-oxo-groups from the benzylidene ketones (263) by lithium aluminium hydride-aluminium chloride. The preparation of benzylidene-ketones(263) by a type of base catalysed aldol condensation has been reported.[301]



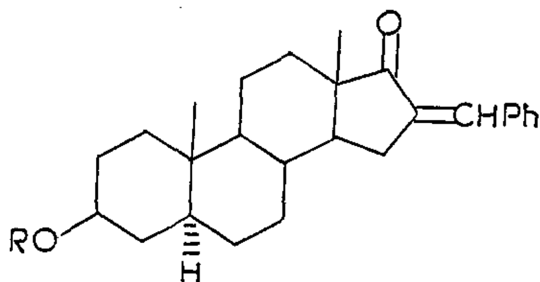
(260)



(261)

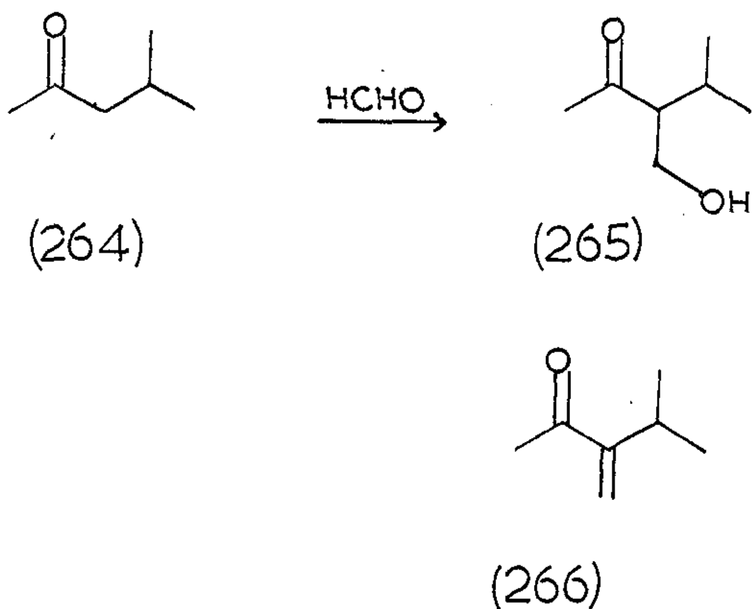


(262)

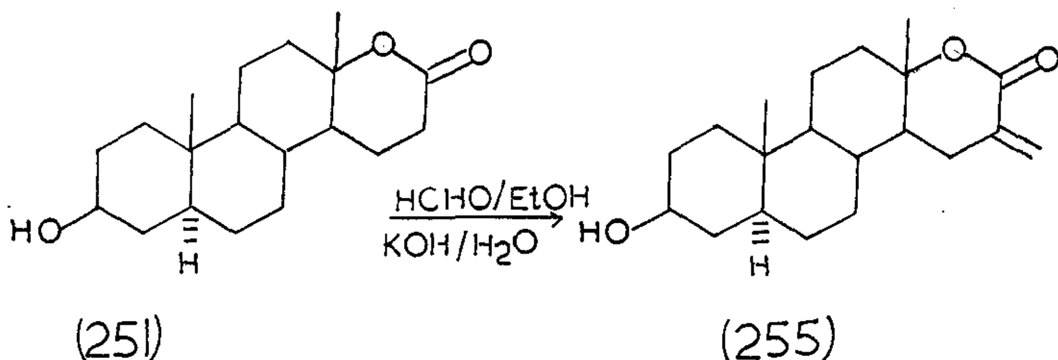


(263)

The ketone (264) on condensation with formaldehyde results in an α,β-unsaturated compound (266) after iodine-catalysed dehydration of the initially formed ketol (265).



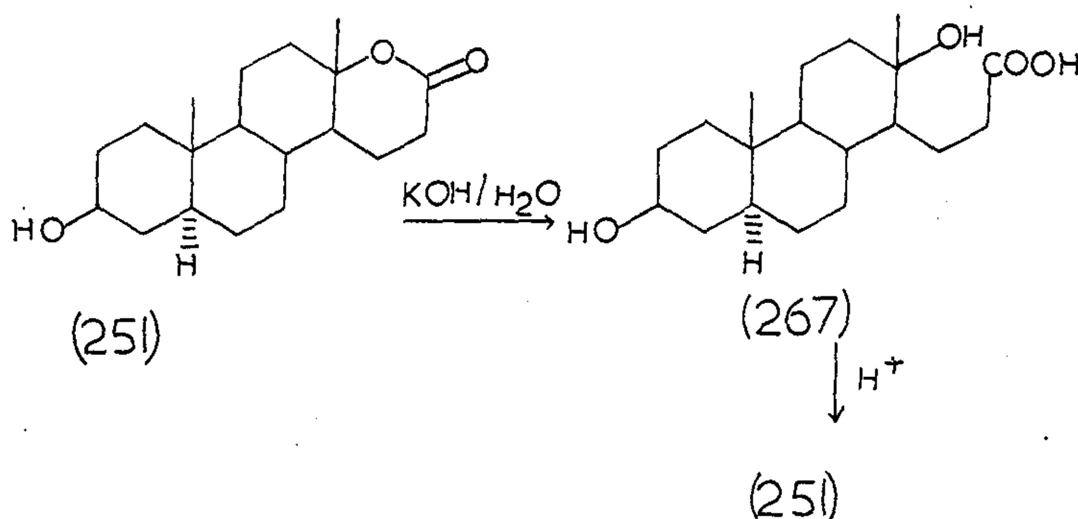
Bearing this in mind, the aldol condensation was attempted on the D-ring lactone (251) to obtain an α-methylene-D-ring lactone (255), although an aldol condensation with ester seems unfavourable owing to a lack of acidity of the α-methylene protons.



The yield of α-methylene lactone (255) was 27% with the starting lactone (251) being recovered in 34% yield. The formation of 3β-hydroxy-

16-methylene-17-oxo-17a-oxa-D-homo-5 α-androstane (254) was indicated by the infra-red spectrum (ν max. 3420 cm^{-1} (OH), 1715 cm^{-1} (C=O) and 1613 cm^{-1} (C=C)), the U.V. spectrum (λ max. 214-5 nm, (ϵ 3760)) and the ^{1}H n.m.r. spectrum, τ 4.30 (m, 1H, O-C(=O)-C=C(H)-H) and τ 3.52 (m, O-C(=O)-C=C-H)

The low yield of the α-methylene lactone (255) could be explained by the fact that the base, potassium hydroxide, does not only abstract a proton to form the lactone enolate but also causes the hydrolysis to the hydroxy acid (267) which on acidification relactonises to the starting lactone. Also, the equilibrium between the formation of the lactone enolate anion and hydrolysis may be in favour of hydrolysed product(267).

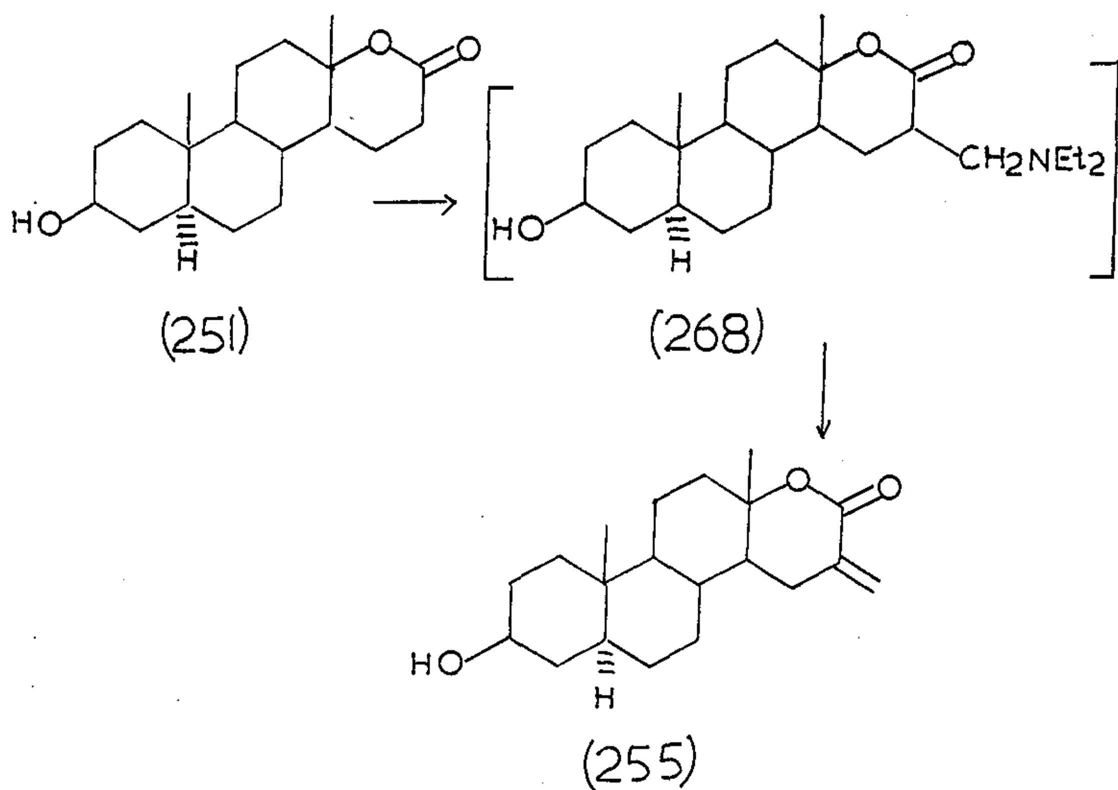


Condensation at the methylene group of acyclic ketones often fails because of the intermediate aldol prod uct undergoes a retrograde aldol reaction more rapidly than it dehydrates[302]. Apparently, there was no indication for the formation of the α-hydroxymethyl-lactone in the crude product.

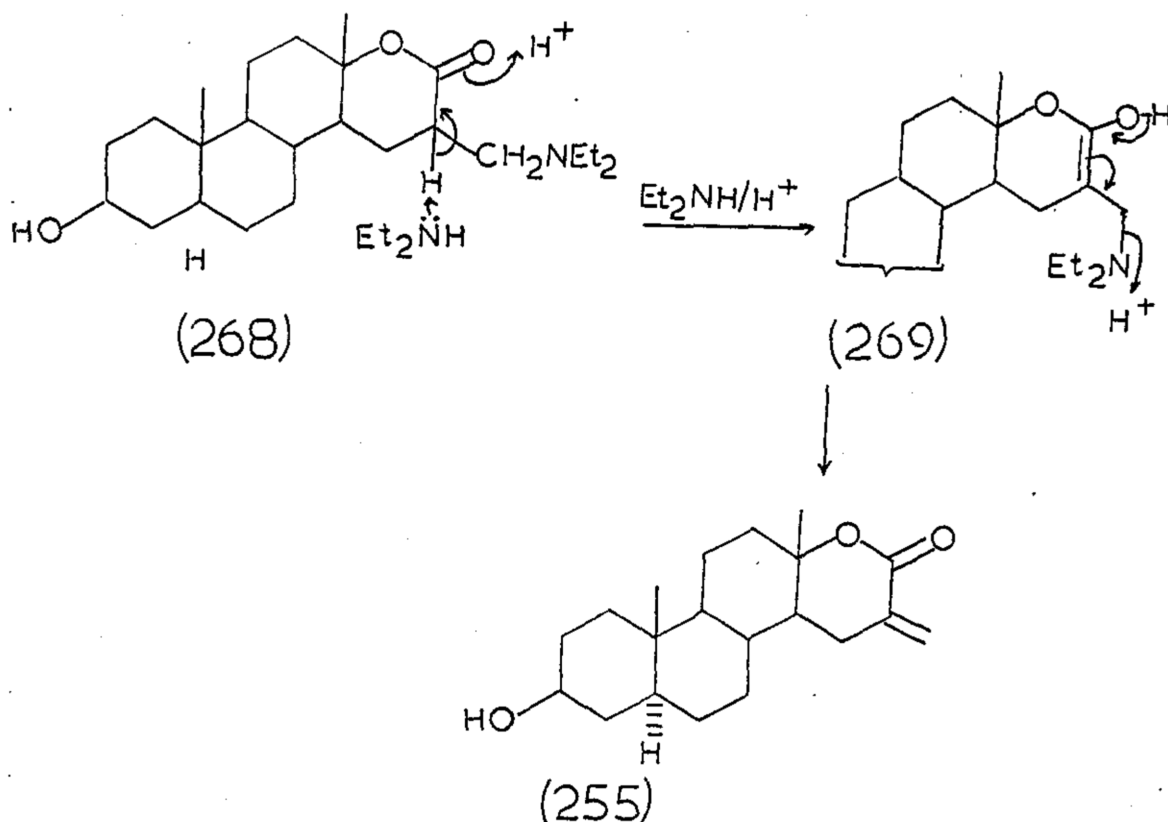
To overcome the problem of hydrolysis, sodium ethoxide, instead of potassium hydroxide, was employed as a base but there was no evidence of the α-methylene lactone (255) in the reaction mixture. There was some evidence for the formation of the α-hydroxymethyl lactone (256) but

repetition of the reaction was unsuccessful. The utilisation of dimethylsulphoxide (DMSO) and benzene as solvents and piperidine as a base also appeared to be unsuccessful.

A more satisfactory method for the introduction of a single carbon atom is the reaction of an active methylene compound with formaldehyde and an amine to form a β-amino-carbonyl compound - the Mannich reaction[303]. A literature survey revealed that although the Mannich reaction has been applied to carbonyl compounds [303,304] and activated lactones, i.e. α-carboxy-[181] and α-carboxyester-lactones [182-192], this has never been applied to lactones in the synthesis of α-methylenelactones. An application of the procedure of Miller and Smith [305] (37% aqueous formaldehyde, diethylamine and diethylamine hydrochloride in dioxan heated at 80^{0}C for 80 hours under nitrogen) to the D-ring lactone, isoandrololactone (251) gave the α-methylene lactone, 3β-hydroxy-16-methylene-17-oxo-17a-oxa -D-homo-5 α-androstane (255,27%) but there was no evidence of the diethylamino adduct, 3β-hydroxy-16-(N,N-diethylamino) methyl-17-oxo-17a-oxa-D-homo-5 α-androstane (268).



The formation of the α-methylenelactone (255) was confirmed by the spectral data which were identical to that of α-methylene-lactone (254) obtained with the base-catalysed aldol condensation with formaldehyde. The β-aminolactone (268) undergoes elimination of diethylamine, under the reaction condition, to form the α-methylene lactone (255).

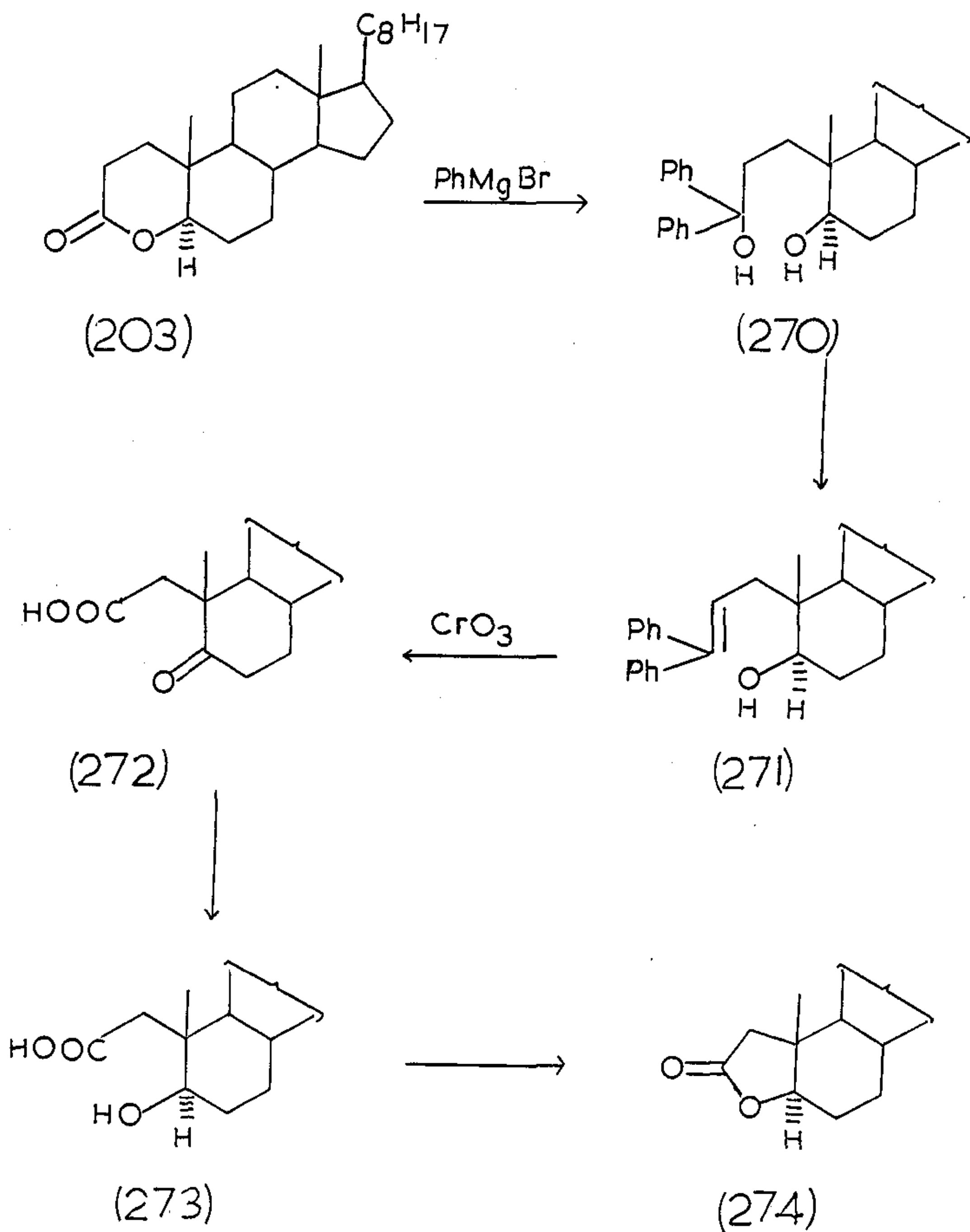


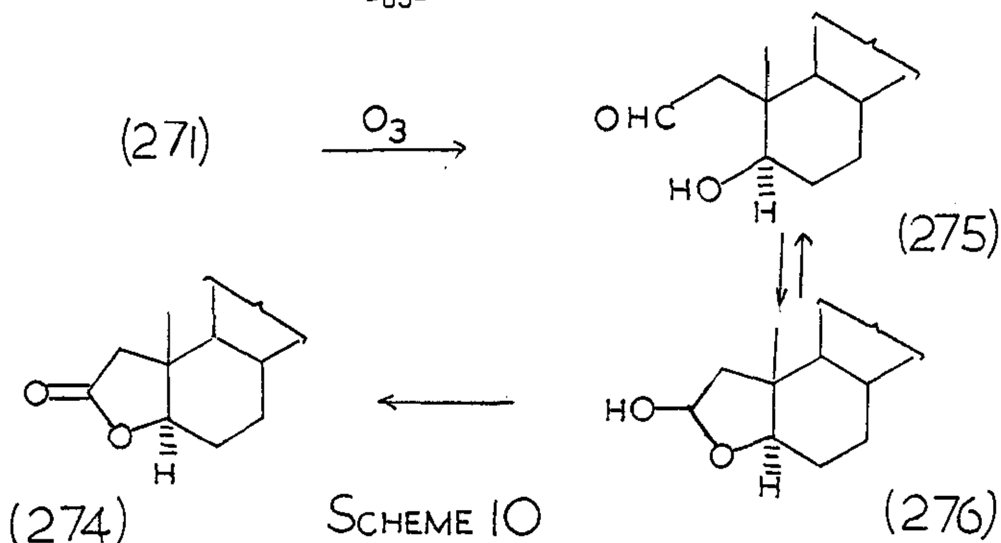
As there was a lot of starting lactone (251) recovered, it was decided to add more of the reagents after eight hours and keep the reaction mixture under reflux for further twelve hours. However, there was no improvement in the yield of the α-methylenelactone (255). Even keeping the reaction mixture under reflux for 72 hours showed no improvement.

The most widely used solvents in the Mannich reaction are ethanol, other alcohols such as methanol and isopropanol, water and acetic acid[306]. In some reactions involving weakly reactive substrates, 1-2,dimethoxy-ethane (glyme) has been used as a solvent[307,308]. The employment of glyme as a solvent[308] instead of dioxan did result in an α-methylene lactone (255) but in a very low yield whereas the use of glyme with pyrrolidine as a base instead of diethylamine was unsuccessful. Recently ketones have been allowed to react with preformed iminium ions [198A, 308, 309] to obtain β-amino adducts which, under the elimination conditions, could be converted to -methylene compounds.

Preliminary attempts using S-trioxan and N-methylanilinium trifluoroacetate (TAMA)[310] in dioxan were however unsuccessful but further work in this area including the use of Eschenimoser's salts, dimethyl (methylene) ammonium iodide [197,199] and dimethyl (methylene) ammonium chloride [198B] may be useful.

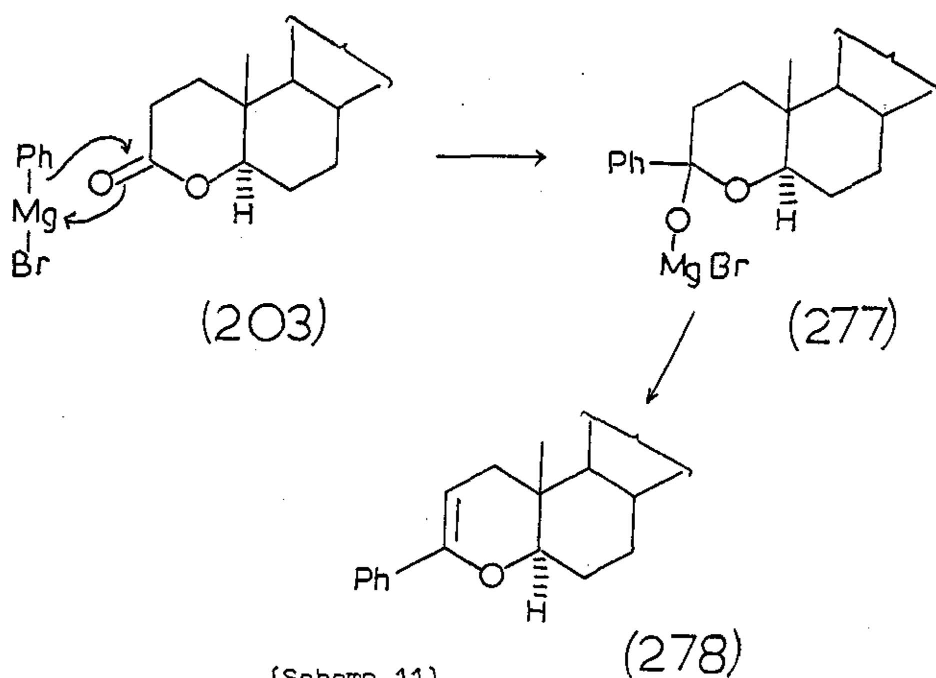
As an α-methylene-γ- lactone structural unit is present in most natural cytotoxic sequiterpene lactones, it was decided to synthesise γ- lactones, The application of the Barbier-Weland degradation [311] with the δ-lactone (203) was expected to lead to the γ-lactone (274) (Scheme 10).





SCHEME 10

The reactions of Grignard reagents with lactones were first investigated by Hauben[312] and in view of their relationship to the esters, the relatively simple γ-lactones gave tertiary alcohols. The reaction of a δ-lactone, 4-oxa-5α-cholestan-3-one (203) with Grignard reagent, phenylmagnesiumbromide,[313] was performed to obtain a tertiary alcohol (270). Unfortunately, the product obtained was not the expected product (270) but a dihydropyran, 4-oxa-3-phenyl-5α-cholest-2-ene (278), although the reaction of Grignard reagents with lactones forming tertiary alcohol products have been reported[314].

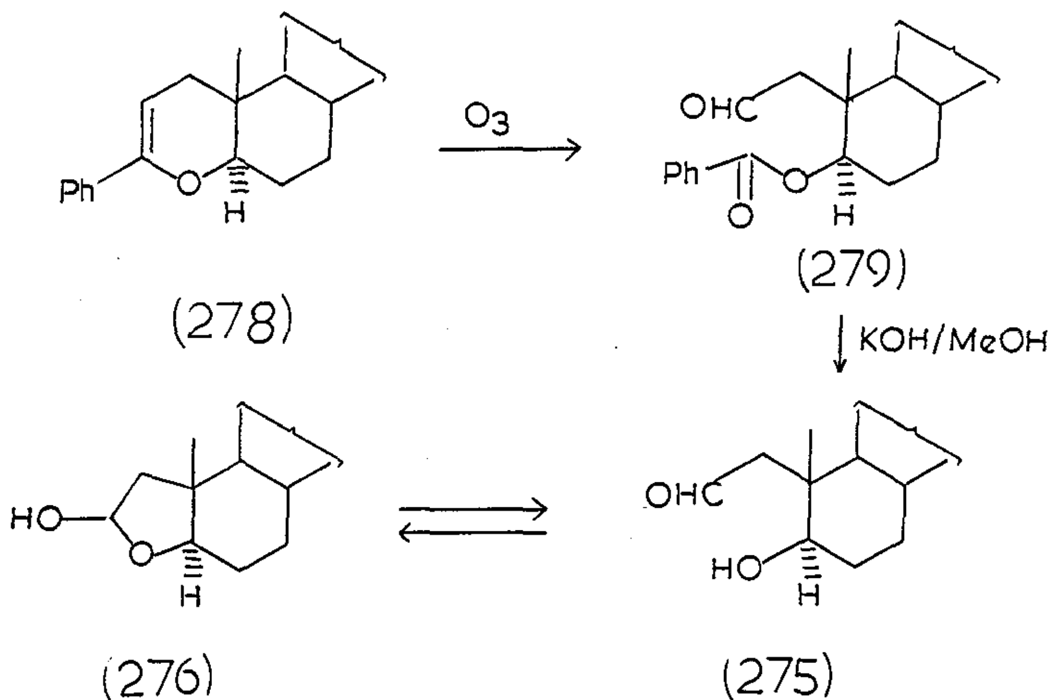


(Scheme 11)

At first glance it might appear that the reaction involves a rather unusual type of rearrangement. However, if it is assumed that the Grignard reaction terminates at the first stage with the formation of an intermediate (277), then the final product (278) is readily explicable as a result of dehydration of the resultant alcohol from (277) during work-up (Scheme 11). Similar dehydrated products, dihydropyran, have been obtained with androstane derivatives when treated with Grignard reagents[315]. The stability of the product, dihydropyran (278), may have been enhanced due to the conjugation of the olefinic bond with the aromatic ring.

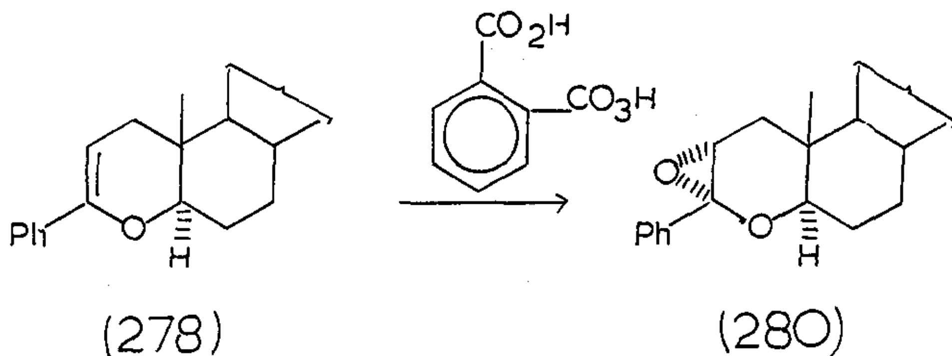
The dihydropyran (278, 50%) obtained was less polar than the starting lactone (203). The formation of dihydropyran (278) was indicated by the ^{1}H n.m.r. spectrum which showed a triplet at τ 4.72 (C_2-H, olefinic proton), a quartet centred at τ6.42 (C_5-α H) and a multiplet centred at τ2.58, the aromatic protons. The U.V. spectrum (λ max 264 nm, (ϵ 9250)) also supported this structure. There was no evidence of the formation of the tertiary alcohol, the expected product (270),in the crude reaction mixture.

Attempted ozonolysis of the dihydropyran (278), to cleave the C_2-C_3 bond, gave an aldehyde (279)although an oxidative work-up was followed. The presence of an aldehyde (279) was indicated by the ^{1}H n.m.r. spectrum (τ 4.91 (q, 1H, C_5 α-H), τ 1.9 - 2.73 (m, 5H, Aromatic protons) and τ 0.21 (1H, - CH)).

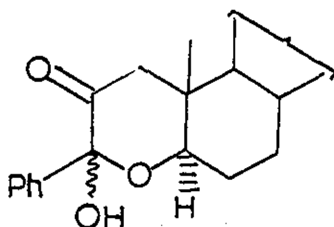


Hydrolysis of (279) was performed in an attempt to obtain the hydroxy-aldehyde (275). But, a number of products were obtained which were not characterised due to low yield. However, the hydroxy-aldehyde (275) may be in equilibrium with the lactol (276), although there was no evidence to support it.

The dihydropyran (278) was allowed to react with excess monoperphthalic acid[316]. It was envisaged that this would lead to the α-epoxide (280) or other products which could be utilised to cleave the C_2-C_3 bond.



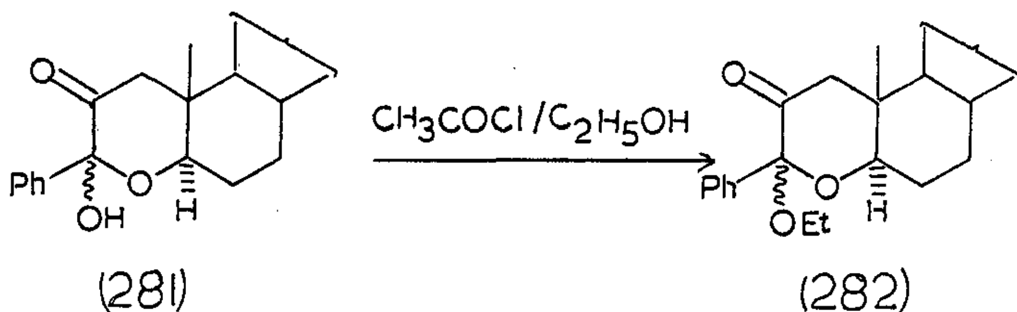
The product obtained was not the expected α-epoxide, 4-oxa-2α,3α-oxido-3-phenyl-5α-cholestane (280) as indicated by the absence of the C_2-H signal in the ^{1}H n.m.r. spectrum. The infra-red spectrum of the product showed the presence of the carbonyl (1730 cm^{-1}) and the hydroxyl (3300 cm^{-1}) groups. The presence of C_1-methylene protons as AB quartet (thus C_1-methylene protons adjacent to a carbonyl group) and the hydroxyl proton broad multiplet, exchangeable with deuterium oxide, in the ^{1}H n.m.r. spectrum indicated the structure of an hemiacetal ketone (281).



(281)

Although the composition of the epimeric mixture of hemiacetal ketones, 3-hydroxy-4-oxa-3-phenyl-5α-cholestan-2-one (281) was variable, its presence was confirmed by the presence of the carbonyl (1730 cm^{-1}) and the hydroxyl (3300 cm^{-1}) groups in the infra-red spectrum. The ^{1}H n.m.r. spectrum showed quartets at τ 5.7 and τ 6.45 which were assigned to the C_5 -α- methione in the 3α-hydroxy - and 3β-hydroxy-hemiacetal ketones (281) respectively. The methylene protons at C_1 displayed two overlapping AB quartets centred at τ 7.43 and 7.53, and the lower-field quartet was assigned to the 3α-hydroxy-hemiacetal ketone. The hydroxyl proton appeared as a broad multiplet at τ 6.8 which was exchangeable with deuterium oxide. The mixture of epimers could not be separated by crystallisation. The reaction of the hemiacetal-ketone (281)

with acetyl chloride in ethanol resulted in an acetal-ketone, 3-ethoxy-4-oxa-3-phenyl-5α-cholestan-2-one (282, 65%), thus giving support to the hemiacetal -ketone structure (281).



Crystallisation of the acetal-ketone (282) from ethanol resulted in a single epimer and was assigned the 3α-ethoxy configuration due to a single AB quartet of C_1-methylene protons centred at τ7.43 in the ^1H n.m.r. spectrum. The absence of absorption at 3300 cm^{-1} in the infra-red spectrum indicated the absence of the hydroxyl group. The methylene protons of the ethoxy substituent gave two overlapping quartets owing to their diastereospecificity. It appears that the 3α-ethoxy-epimer is the thermodynamically stable epimer and hence isolable on crystallisation. It was thought that this reaction was air oxidised to produce the 3-ethoxy compound, however, the same product was obtained when the reaction was performed in a nitrogen atmosphere.

It appears that the α-epoxide (280), which may have been formed in the reaction, undergoes rearrangement to give rise to the compound (281). The formation of the compound (281) can be formulated in a number of ways (Scheme 12)[317].

(A)

H^+ Ph O H

(280)

HO H Ph O + H

(283)

CO_2H CO_3H

H–O H+ O Ph O H

(285)

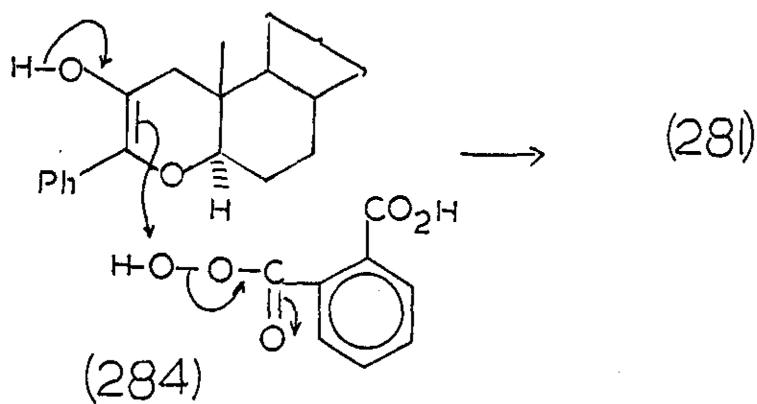
H–O Ph O H

(284)

O P OH O H

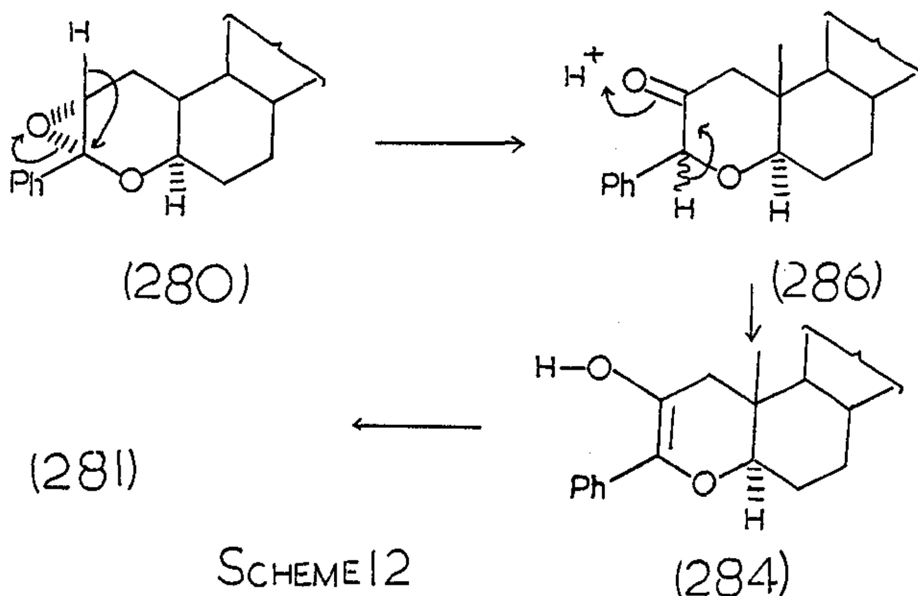
(281)

(B)



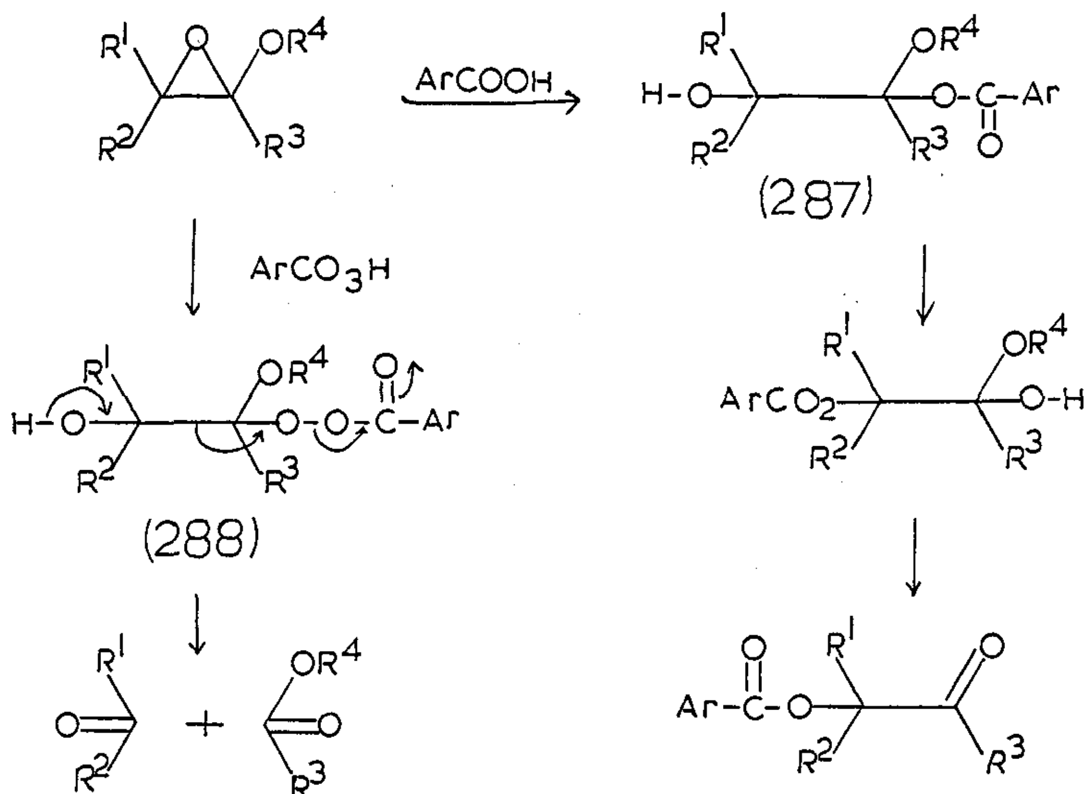
(281)

(c)



SCHEME 12

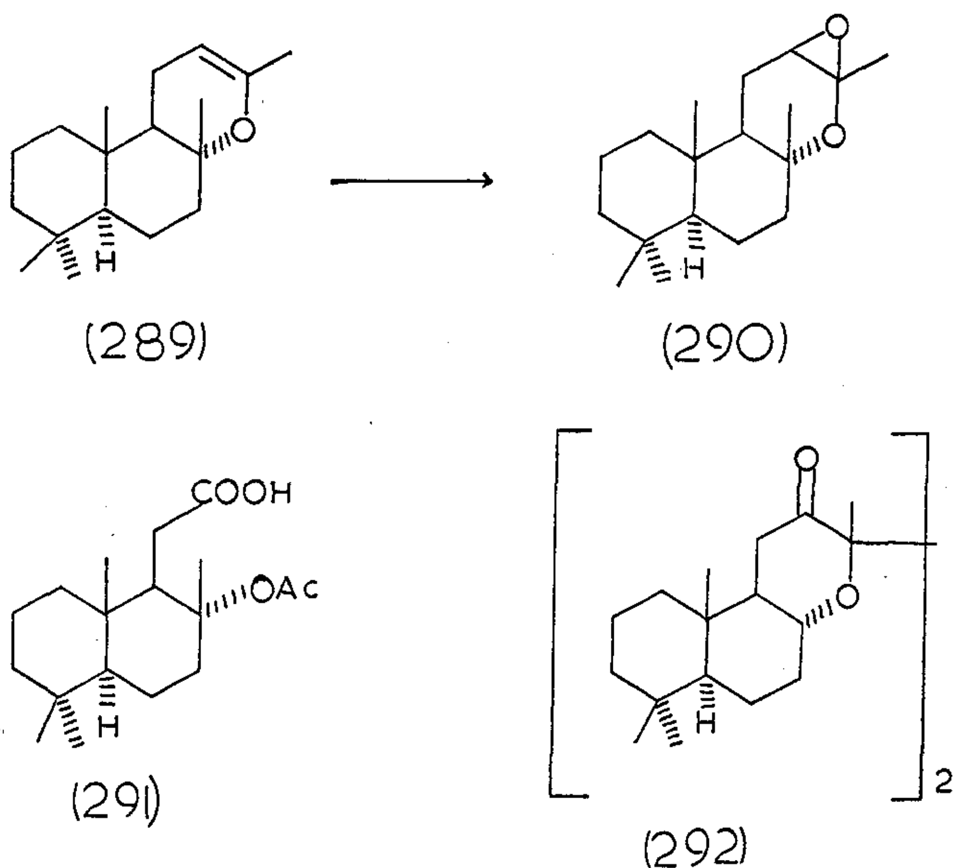
There have been essentially two processes reported for the reaction of enol-ethers with peracids[318-321]. The initially formed epoxides are attacked by the carboxylate or peroxycarboxylate anion to give the intermediates (287) or (288), respectively, which could either rearrange or fragment as shown (Scheme 13).



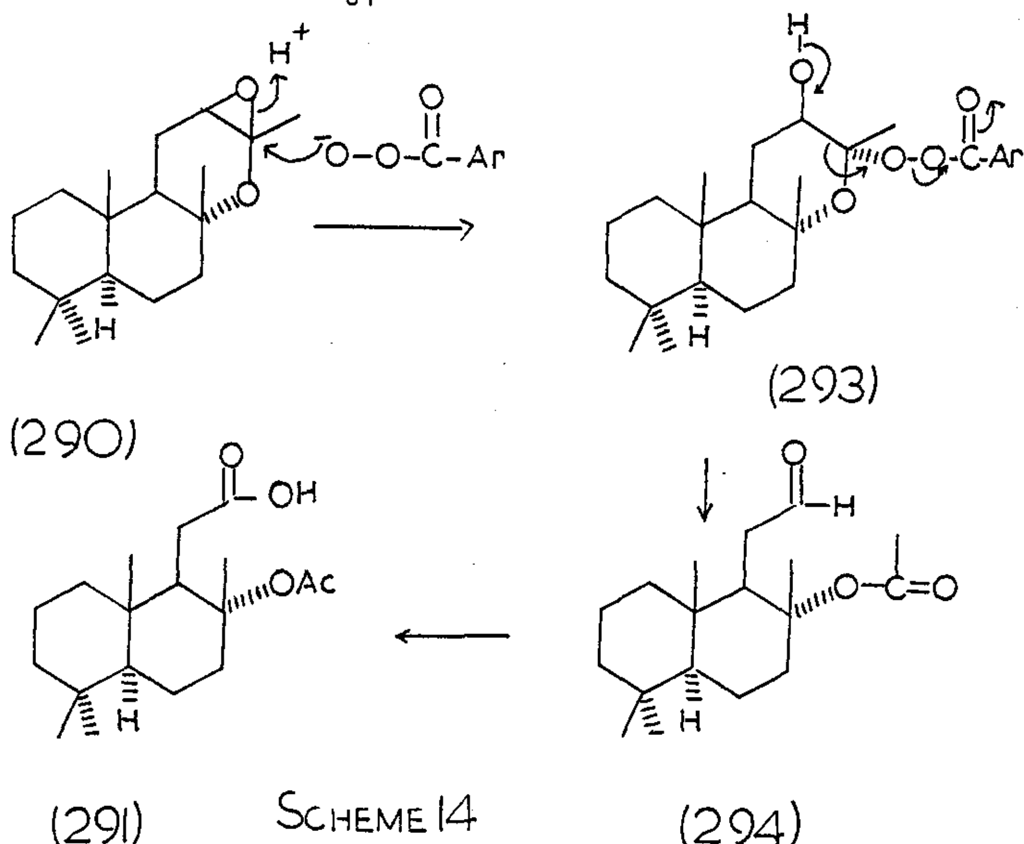
SCHEME 13

The product obtained from the epoxidation procedure with dihydropyran (278) was not the expected α-epoxide and also it did not appear to be the products corresponding to those in Scheme 13.

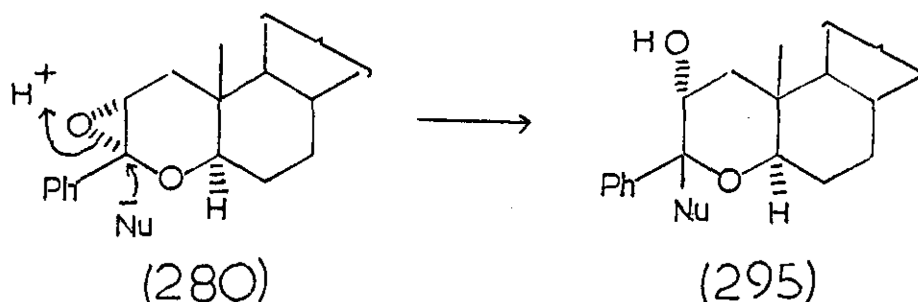
In order to obtain compounds which possess ambergris-type odours, Cambie et al[320] required the epoxide (290) whose formation was attempted by epoxidation of the enol-ether (289)[322] with monoperphthalic acid. However, although the successful isolation of the epoxides of some enol-ethers has been reported,[318] the only products isolated were the known acetoxy-acid (291)[322,323] and one which was identified as the dimer keto ether (292).



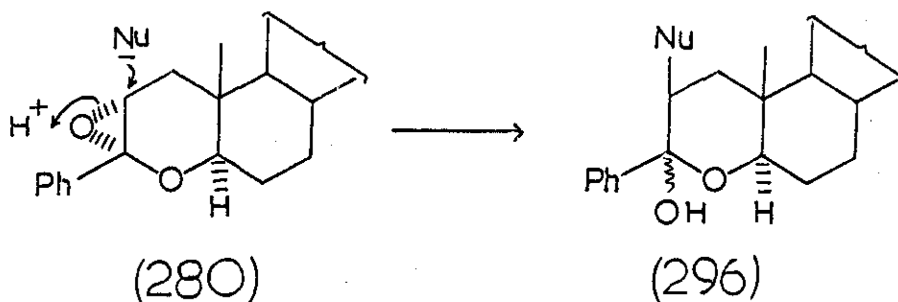
The acetoxy-acid (291) probably arises by a fragmentation reaction (Scheme 14) similar to that observed during the epoxidation of tetrahydrochromans[324] and certain steroidal enol-ethers[319].



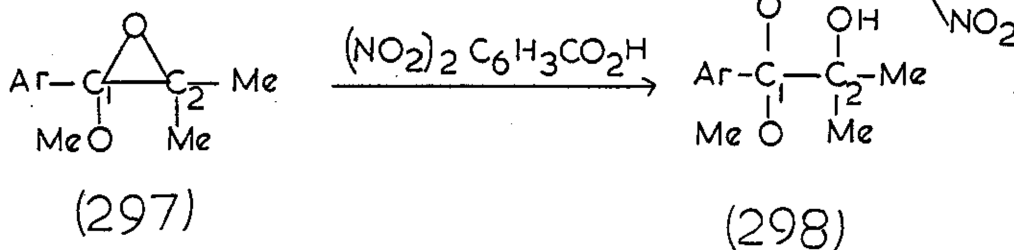
The stabilisation of the intermediate (283) by the 3-phenyl group may be of importance particularly since nucleophilic attack at C_3 in (280) by phthalate or perphthalate anion (Scheme 13) would not be favoured (stereoelectronically) assuming that α-epoxidation would mainly occur. The opening of epoxide ring to give "1,2-disubstituted" compounds occurs very readily under acidic catalysis, for protonation of the epoxide oxygen atom greatly enhances the normal polarisation of the C-O bond, permitting nucleophilic attack by an anion or solvent molecule in concert with breaking of a C-O bond. Epoxide-opening reactions have the stereoelectronic feature that the attacking nucleophile approaches the epoxide ring to give a <u>trans</u> diaxially substituted product.



Accordingly, the formation of (295) would be unfavourable and the opening to (296) would be stereoelectronically favoured.



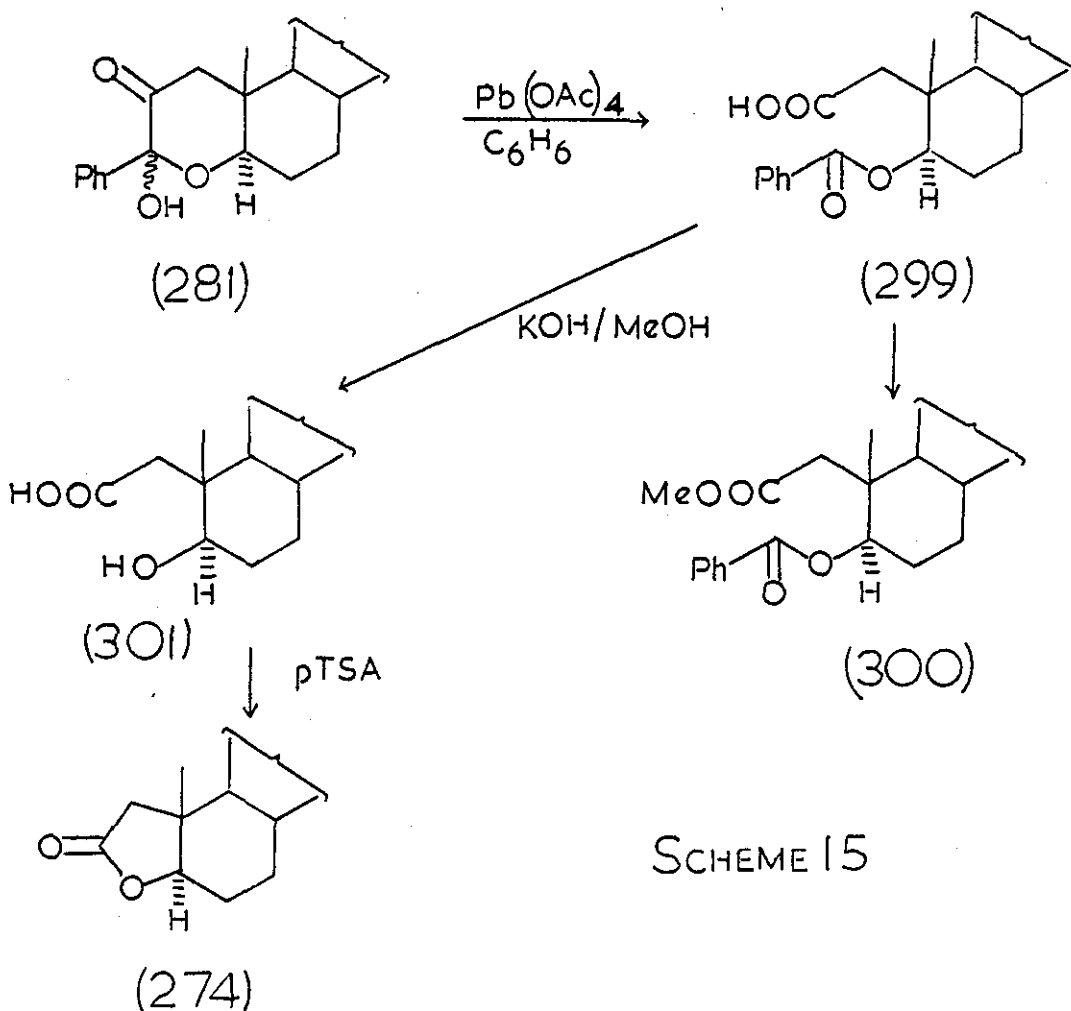
However, the epoxide ring of epoxyethers (297) has been opened with acid at the C_1 atom holding two oxygen functions to give the ester of a hemiketal (298).[319B] It is of interest to note that (297) which contains a stabilising aryl group similar to (280) follows the normal reaction pathway (Scheme 13).



The low solubility of phthalic acid in ether may also be of some importance in inhibiting the efficiency of the epoxide opening reactions.

The structure of the hemiacetal-ketone (281) was further supported by the evidence that the treatment of the hemiacetal-ketone with lead tetra-acetate in benzene gave 2,3-seco-5β-benzoate-5α-cholestan-2-carboxylic acid (299). The presence of the carboxylic acid (299) was indicated by the T.L.C., the infra-red spectrum (ν max 3600-2800 cm^{-1} (OH), 1720 cm^{-1} (C=O)) and a downfield shift of C_5 α -methine proton to τ 4.91 in the ^1H n.m.r. spectrum.

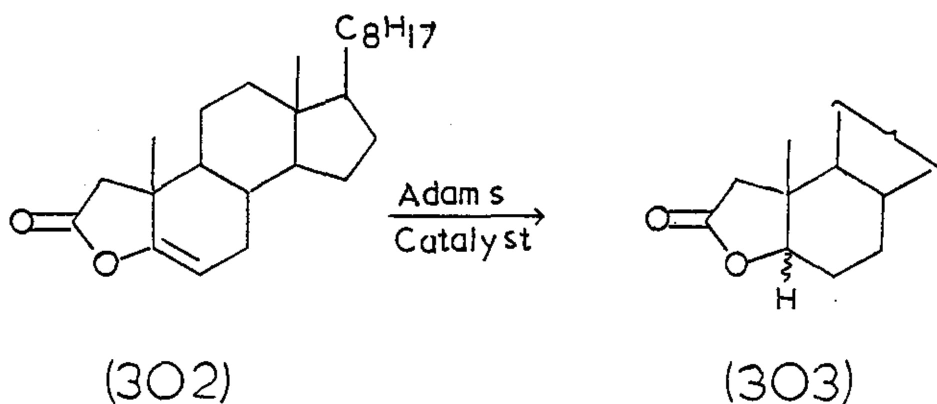
Further confirmation of the structure of the carboxylic acid (299) was afforded by its reaction with diazomethane to obtain the methyl ester (300). The presence of a singlet at τ 6.43 in the ^{1}H n.m.r. spectrum and an absence of the hydroxyl absorption in the infra-red spectrum indicated the formation of the methyl ester (Scheme 15).



SCHEME 15

The carboxylic acid (299) was hydrolysed with potassium hydroxide/ methanol to obtain the hydroxy-acid (301). The absence of aromatic protons in the ^{1}H n.m.r. spectrum and the presence of a polar compound on T.L.C. was indicative of the formation of the hydroxy-acid (301). Treatment of the hydroxy-acid with toluene-p-sulphonic acid in benzene afforded a γ-lactone, 3-nor-4-oxa-5α-cholestan-2-one (274) indicated by the infra-red spectrum (ν max 1785 cm^{-1} (C=O)) (Scheme 15).

In their studies on the effect, on physiological properties, of modification of the size of the A-ring in various steroids Jacobs and Takahashi[325] obtained a γ-lactone, 3-nor-4-oxa-cholestan-2-one (303) from the hydrogenation of the enol-lactone, 3-nor-4-oxa-cholest-5-en-2-one (302).

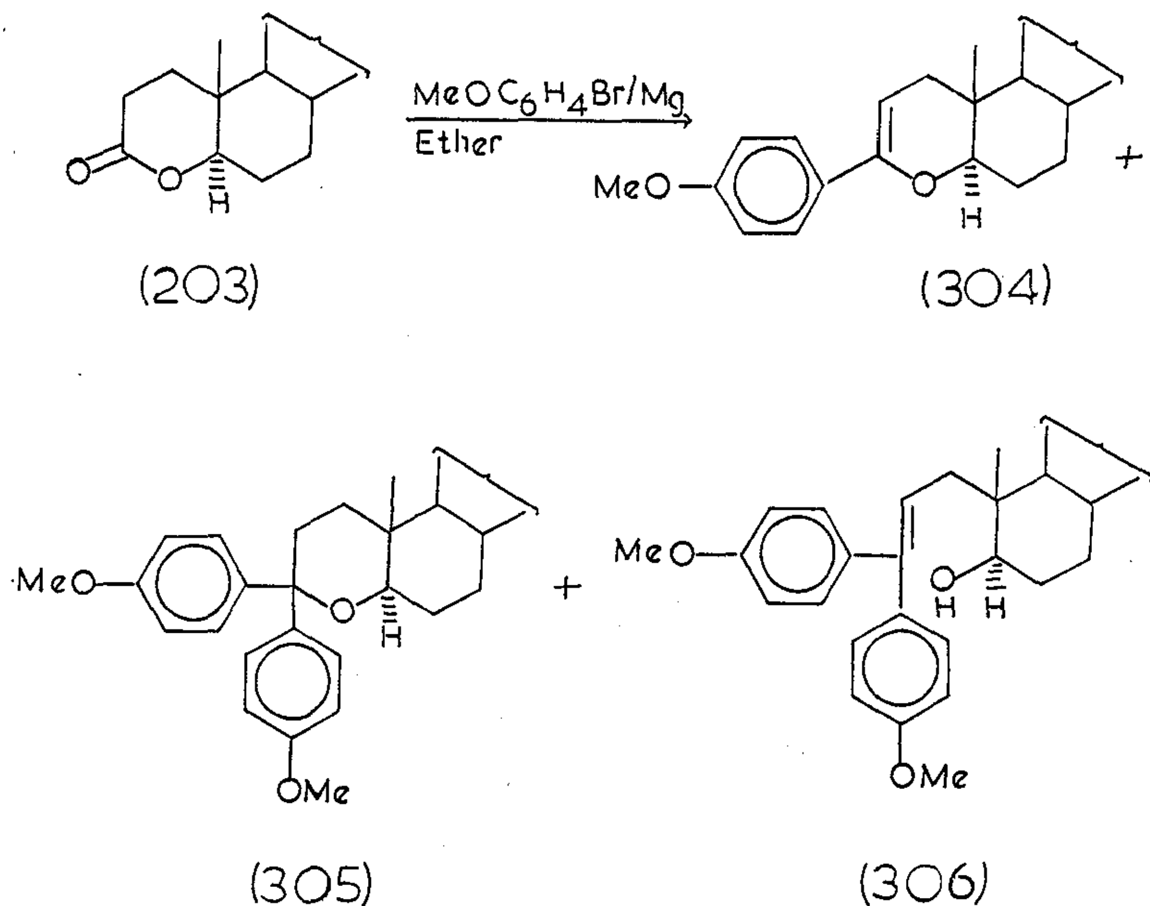


This γ-lactone (303) (M.P. 126-127°C (methanol), $(\alpha)_{D}^{25}$ + 8.8 ($CHCl_3$)) differed from the γ-lactone (274) (M.P. 105-106°C (methanol), $(\alpha)_{D}^{25}$ + 25.07 ($CHCl_3$)). The stereochemistry of γ-lactone (303) at C_5 has not been specified whereas γ-lactone (274) has its stereochemistry determined (5α) by its starting δ-lactone[203] and it is presumed that the earlier prepared lactone (303) in the 5β-epimer.

The overall yield of the γ-lactone (274) is very low and thus α-methylenation procedure was not performed.

To determine the importance of electronic effects in the Grignard reaction with δ-lactone (203) and subsequent epoxidation step, the reaction of 4-methoxy phenyl bromide with δ-lactone was performed. The crude product, on separation by preparative T.L.C., gave the

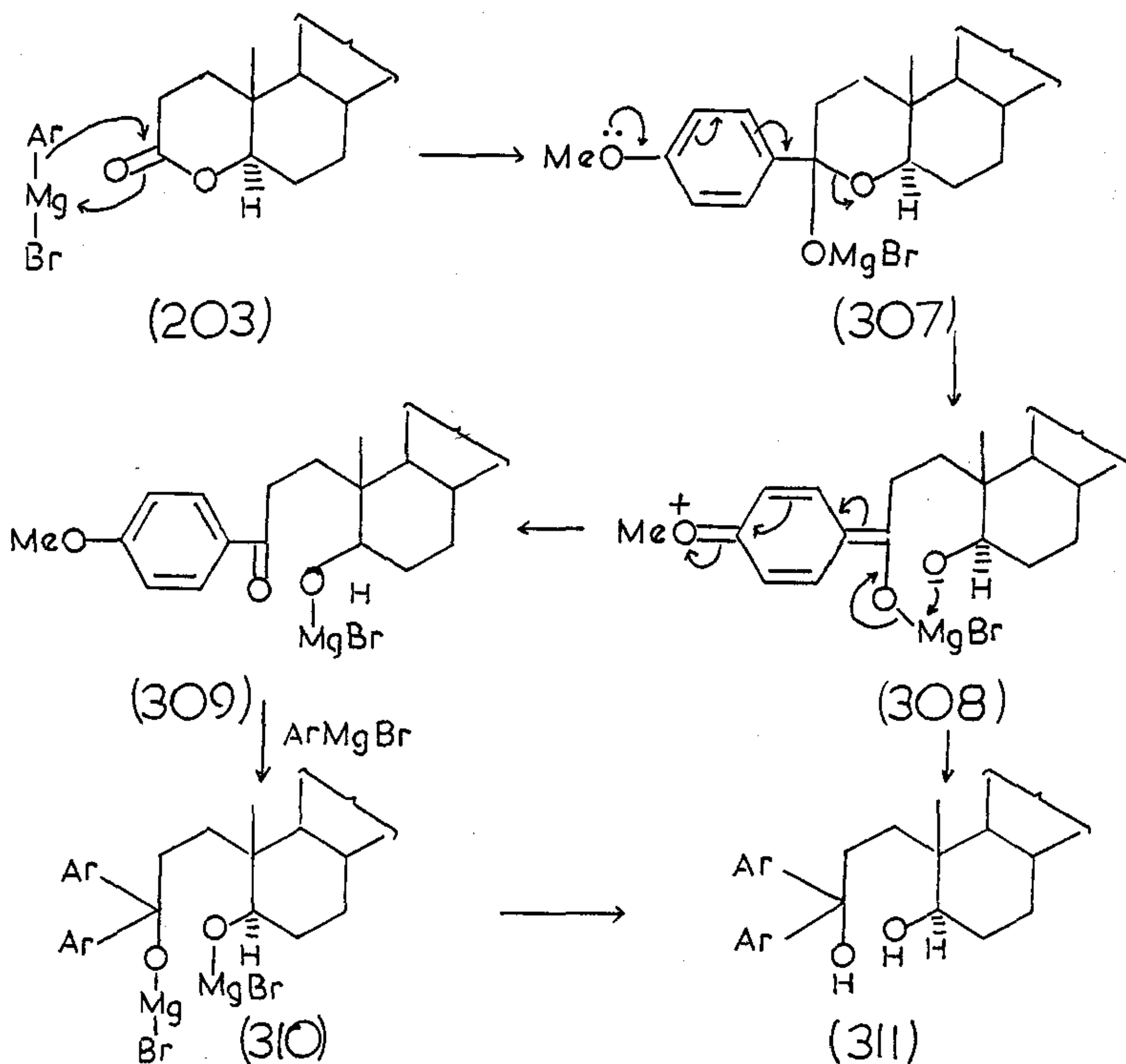
dihydropyran, 3-(4-methoxy phenyl)-4-oxa-5α-cholest-2-ene (304), the tetrahydropyran, 3,3-di (4-methoxyphenyl)-4-oxa-5α-cholestane (305) and the hydroxyolefin, 3,4-seco-5β-hydroxy-3,3-di (4-methoxyphenyl) - 5α-cholest-2-ene (306). There were also three other minor products which were not identified.

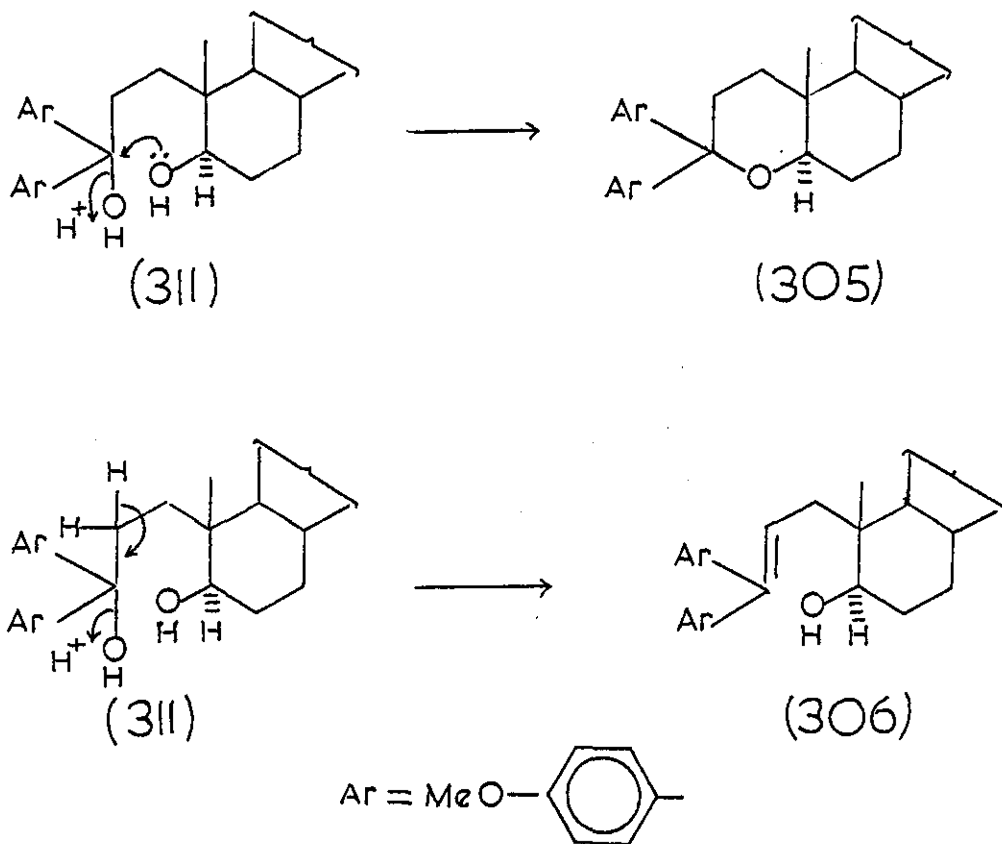


The spectroscopic data of the dihydropyran (304) were similar to those for the dihydropyran (278). The ^{1}H n.m.r. spectrum of the dihydropyran (304) displayed a singlet at τ 6.2 (3H, -O - CH_3) and a triplet at τ 4.92 (1H, C_2-H). The occurence, in the ^{1}H n.m.r. spectrum of the major product (37%), of two singlets at τ 6.17 and τ 6.25 (2x -O - CH_3), a multiplet at τ 2.5 - 3.4 (8H), confirming the presence of two phenyl groups and a multiplet at τ 6.96 assigned to the C_5 α-

methine, confirmed the tetrahydropyran structure (305). Kuhn[326] reported that when an ether solution of a γ-lactone is permitted to react vigorously with 3 equivalents of phenyl-magnesium bromide, the product is a tetrahydropyran derivative.

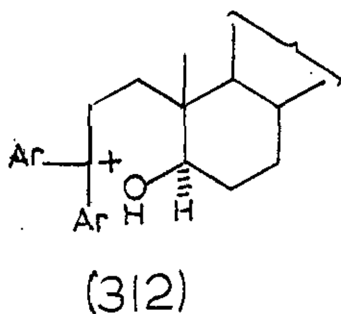
The identity of the hydroxyolefin, 3,4-seco-5β-hydroxy-3,3-di-(4-methoxyphenyl)-5α-cholest-2-ene (306, 17%) was confirmed by the ^{1}H n.m.r. spectrum which displayed two singlets at τ6.2 and τ6.27 (6H, C_3 - MeO-Aromatics), a multiplet at τ 2.8 - 3.4 (8H, Aromatic H) and a triplet at τ4.1 (1H, C_2-H) and by the infra-red spectrum (ν max. 3640 - 3200 cm^{-1} (OH)). The formation of the tetrahydropyran (305) and the hydroxyolefin (306) could be postulated as shown (Scheme 16).



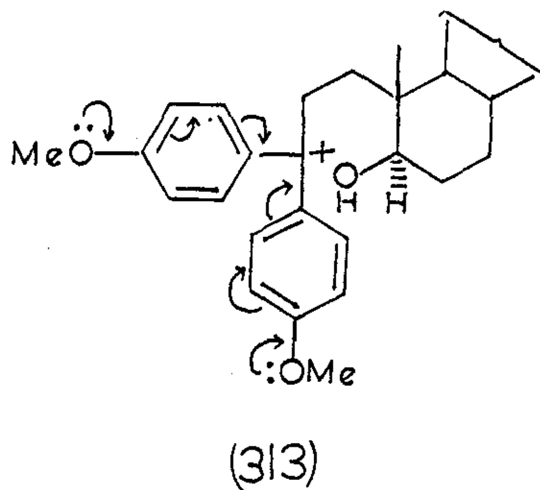


SCHEME 16

The compound (310), on workup, would give rise to compound (311) which would be an intermediate forming the tetrahydropyran (305) and the hydroxyolefin (306) by cyclisation and dehydration, respectively. It could be stated that the removal of the hydroxyl group from C_3, as a water molecule, would form a benzylic carbocationic intermediate (312) which could cyclise to form the tetrahydropyran (305) and abstract a proton from C_2-methylene to form the hydroxyolefin (306). The benzylic cationic intermediate (312) would be favoured by the strong electron donating properties of the 4-methoxyphenyl substituent as indicated in (313).

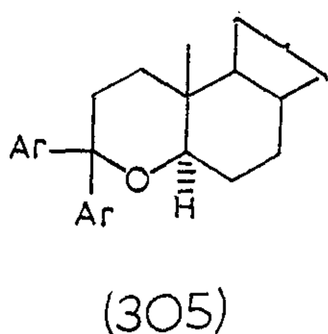


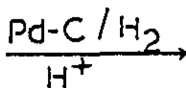
(312)

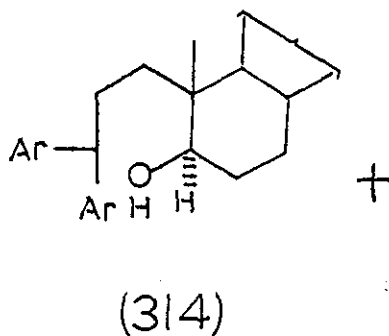


(313)

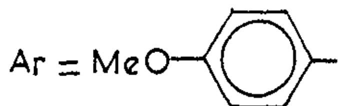
The structure of the tetrahydropyran (305) was further supported by its palladium/charcoal acid catalysed hydrogenolysis to the saturated alcohol, 3,4-seco-3,3-di-(4-methoxy-phenyl)-5α-cholestan-5β-ol (314,) which was accompanied by the ketone, 3,4-seco-3,3-di-(4-methoxyphenyl)-cholestan-5-one (315).

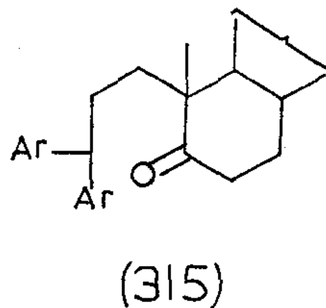






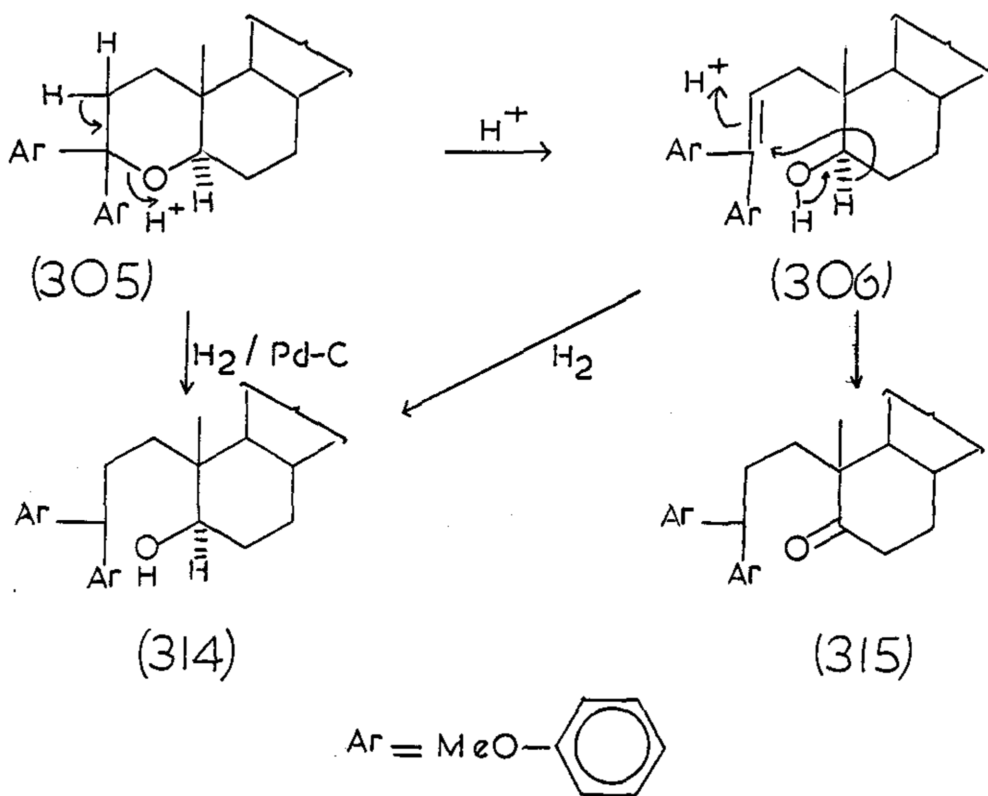
(305) (314)





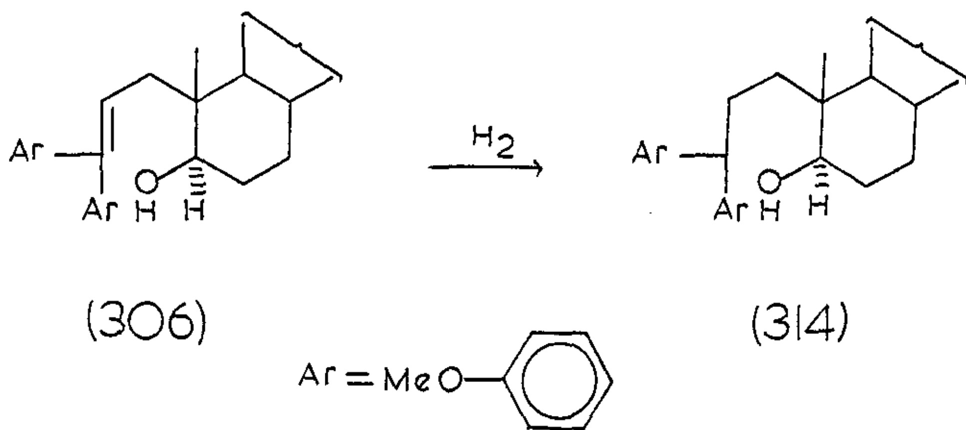
(315)

The formation of the saturated alcohol (314) was indicated by the infra-red spectrum (ν max 3300 cm^{-1} (OH)) and the ^{1}H n.m.r. spectrum in which a downfield shift of C_5 α-methine from τ 6.96 to τ 6.51 occurred. The ketone (315) was indicated by a non-polar spot on T.L.C., the infra-red spectrum (ν max 1705 cm^{-1} (C=O)) and the absence of the C_5 α-methine in the ^{1}H n.m.r. spectrum. The formation of the saturated alcohol (314) and the carbonyl compound (315) has been postulated as shown (Scheme 17).

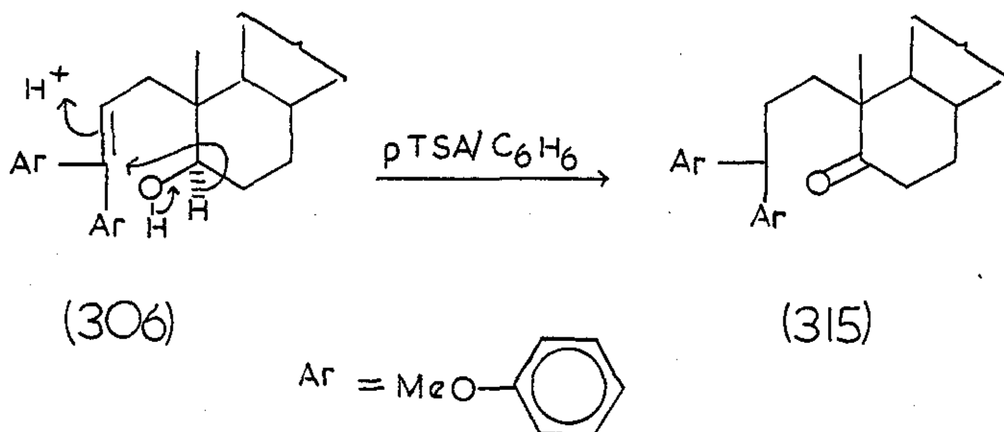


SCHEME 17

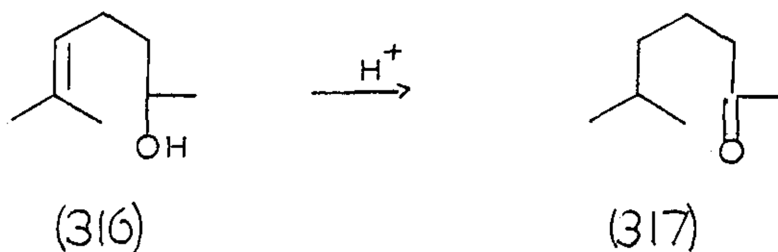
The structure of the saturated alcohol (314) was further supported by its formation from the hydroxyolefin (306) by hydrogenation.



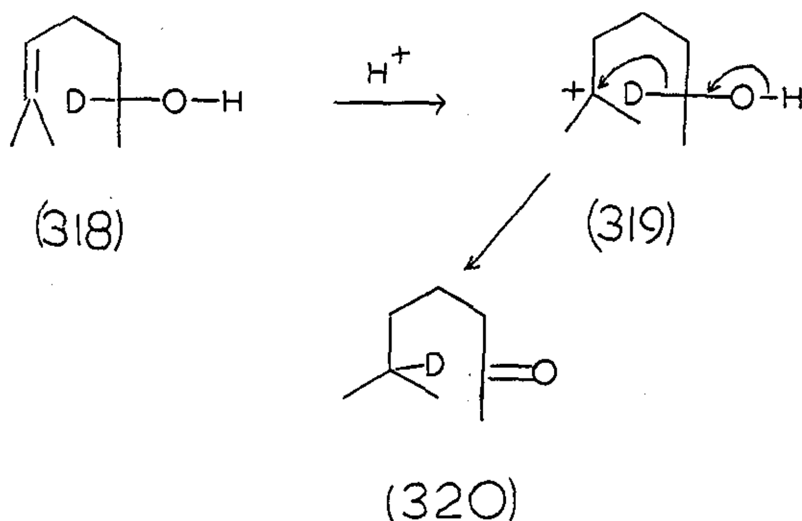
Confirmation of the transformation of (306) to the carbonyl compound (315) was obtained when it was treated with toluene-p-sulphonic acid in benzene.



This rearranegement, which involves a hydride shift from C_5 to C_3, appears to be novel in the steroid field, although a similar rearrangement of (316) to (317) has been reported[327].



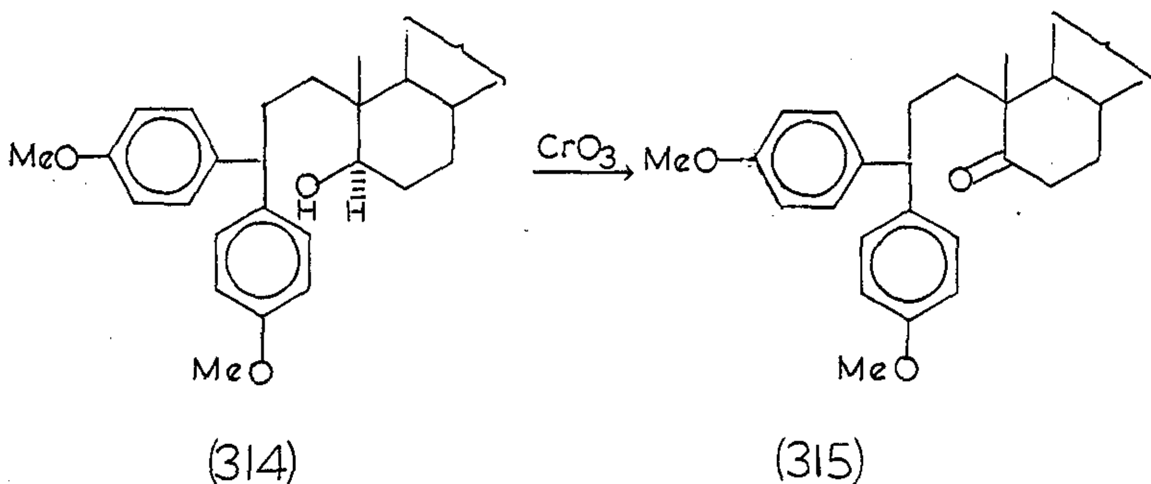
Thus, 1,5 hydride transfer, rearrangement was supported with deuterium labelling experiment, as shown below.



(318) (319)

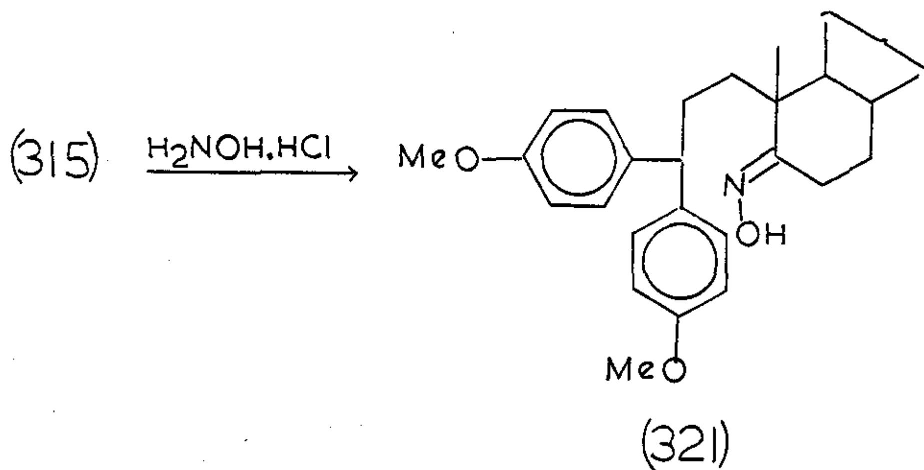
(320)

The saturated alcohol (314), on oxidation with Jones' reagent, resulted in a carbonyl product which was identical to the carbonyl compound (315) obtained from the acid-catalysed hydrogenolysis and from the hydroxyolefin (306) on treatment with toluene-p-sulphonic acid in benzene.

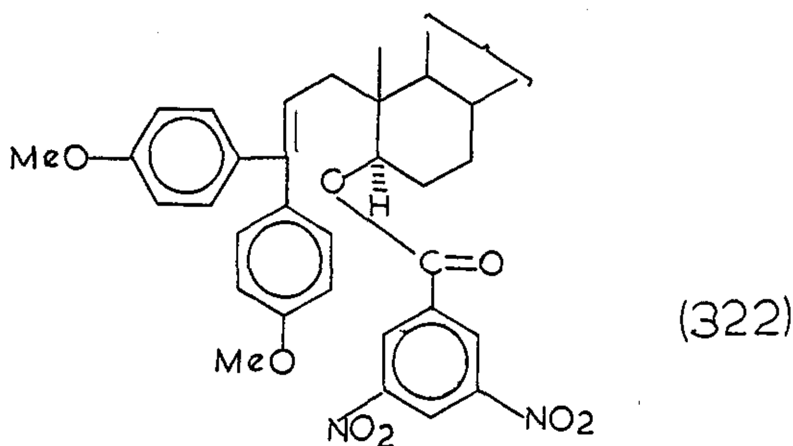


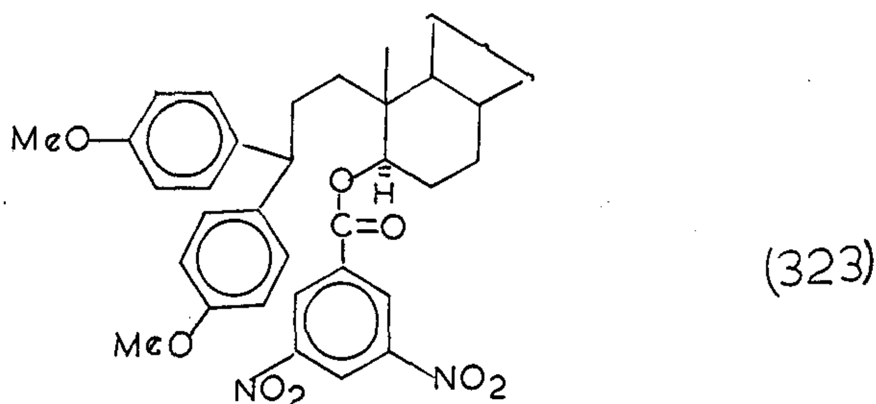
(314) (315)

This also corroborated the structure of the saturated alcohol (314) as well as that of the ketone (315).Crystallisation of the carbonyl product (315) was not successful and thus it was converted to the oxime (321).



The structure of the hydroxyolefin (306) and the saturated alcohol (314) was further supported by the formation of 3,5-dinitrobenzoate esters (322 and 323) respectively.



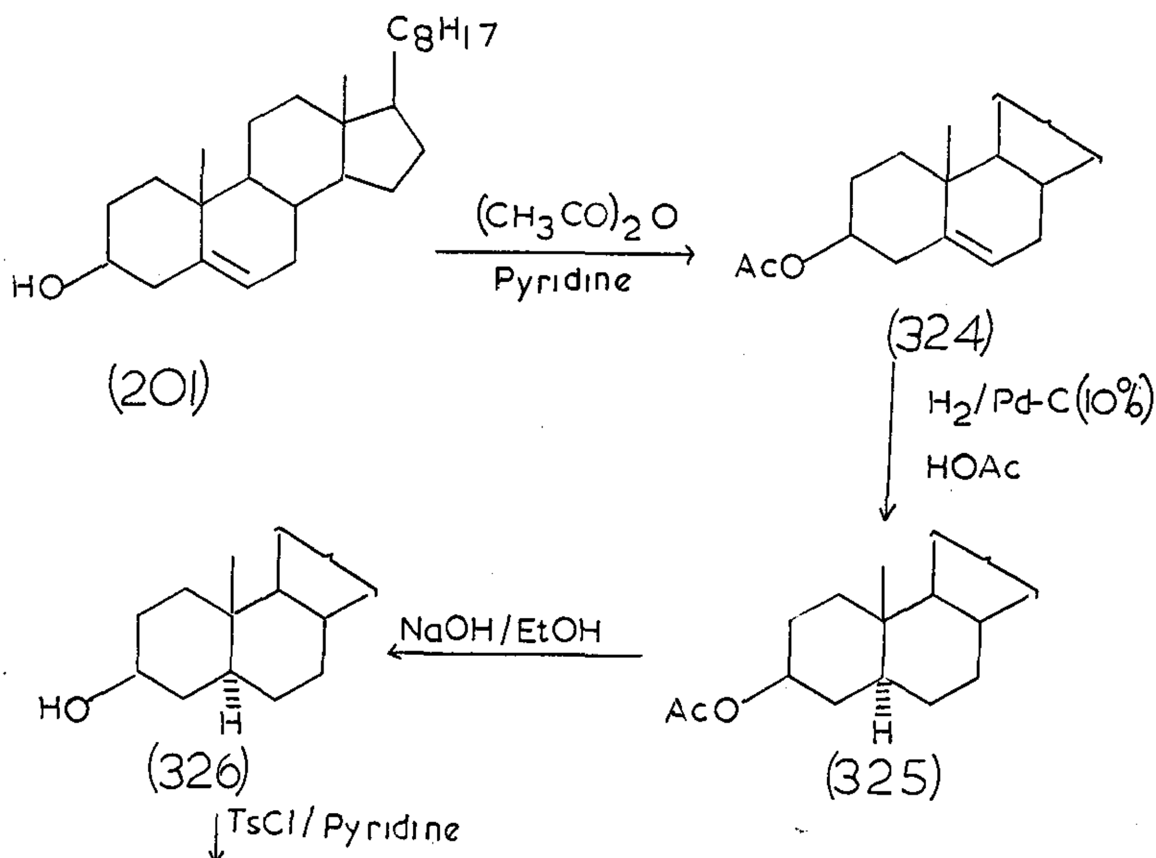


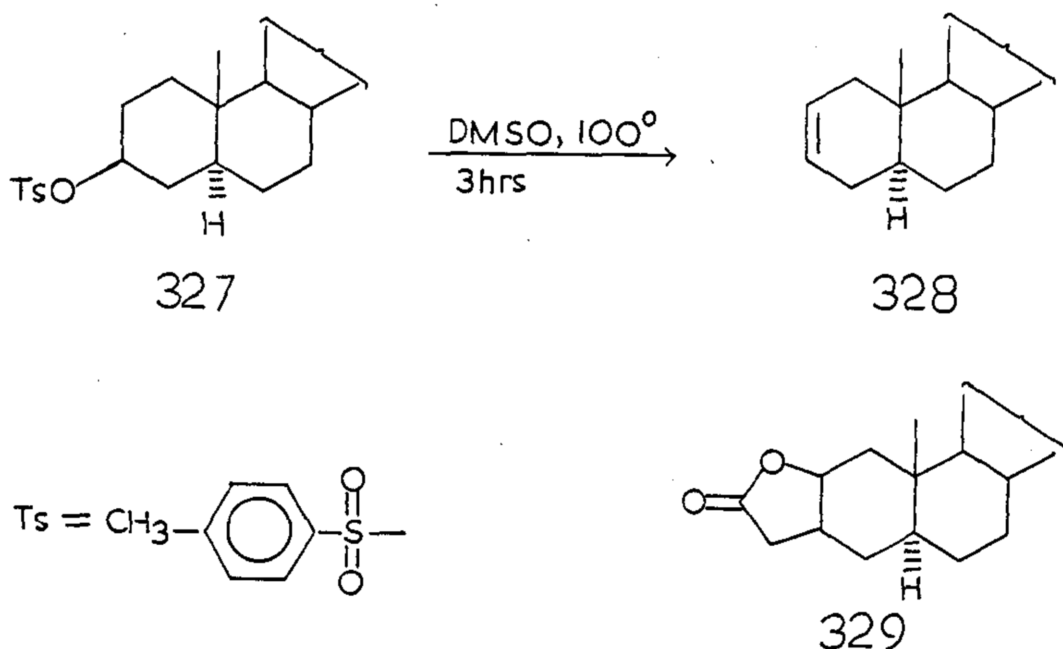
There is a need for further work to be carried out on the dihydropyran (305) to ascertain whether an epoxidation would result in a normal epoxide or some rearranged product(s). The low yield of this product thus far has prevented this investigation.

As α-methylene-γ-lactones display various biological properties, it was decided to synthesise a γ-lactone onto rather than in the steroid nucleus. In their studies on the oxidation mechanism of metal carboxylates, Heiba _et al_[269Ai, 269Bii, 328] observed a novel reaction of manganic and ceric carboxylates with olefins leading to γ-butyrolactones in excellent yields.

The procedure of Heiba _et al_[269F] employing manganic acetate was applied with cholest-4-en-3-one and there was no reaction. The lack of reactivity of cholest-4en-3-one may be due in part to the electron deficiency in the conjugated double bond. Reaction with cholest-5-en-3β-acetate was also unsuccessful and suggest steric hinderance may also be important[269F].

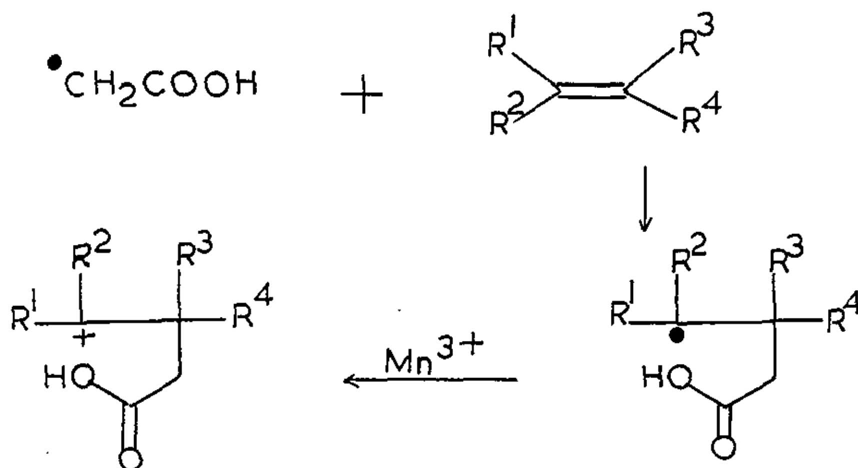
Reaction with 5 α-cholest-2-ene, which was synthesised from cholesterol (Scheme 18) was, more successful.

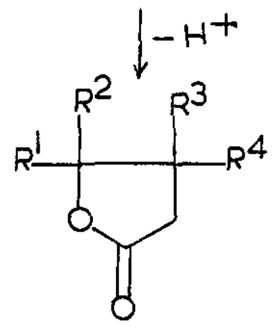




SCHEME 18

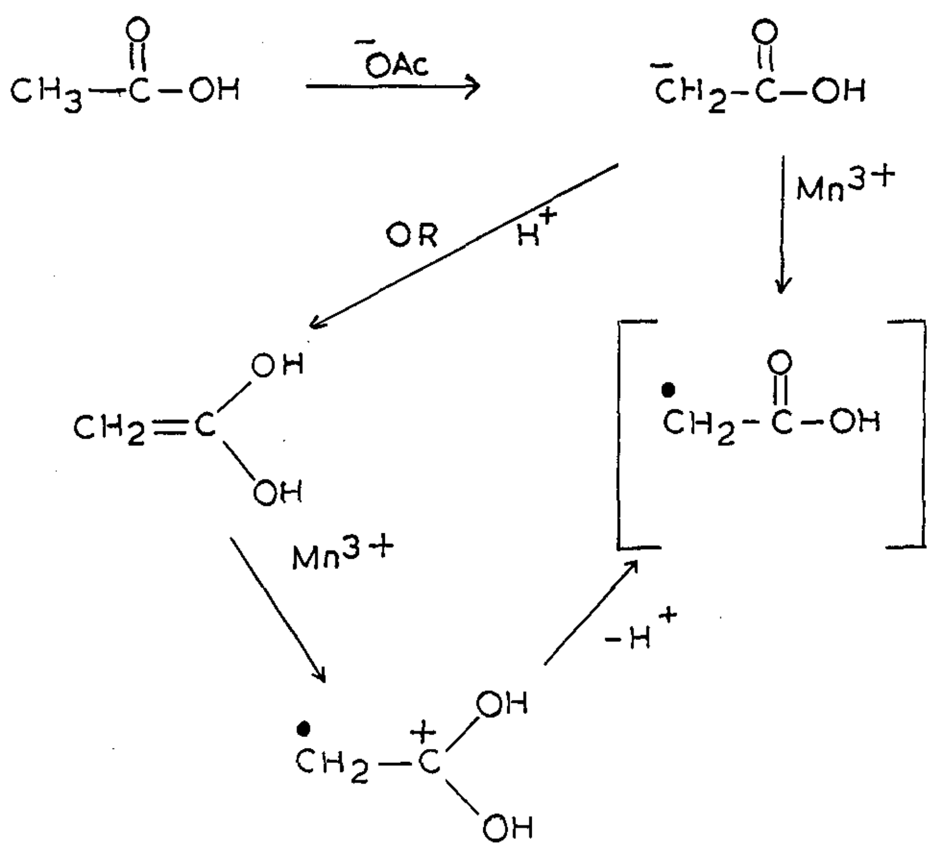
The formation of a γ-butyrolactone, 3-(2'-acetic acid)-2-hydroxy-5α-cholestan-γ- lactone (330) (See later) was indicated by the infra-red spectrum (ν max. 1768 cm^{-1} (C = O)). The thermal decomposition of manganic and ceric acetate [269Ai, 269Bii, 328, 329, 330] has demonstrated the intermediacy of the free-carboxy-methyl radical. The first step in the lactone synthesis (Scheme 19) is the addition of this radical to the olefin.





(Scheme 19)

The direct formation of carboxyalkyl radicals from carboxylic acids by manganese (III) most probably involves initial enolisation of the carboxylic acid followed by electron transfer from either the enol or the enolate anion (Scheme 20).



(Scheme 20)

This has been indicated by the fact that highly enolisable carboxylic acids such as cyanoacetic acid react much more rapidly with manganese (III) than do simple alkyl carboxylic acids. The importance of base-catalysed enolisation in these reactions was demonstrated by the relative rates of disappearance of *p*-substituted benzyl phenyl ketones, where electron-withdrawing groups increased the reaction rate with manganic acetate[269Bi]. The preference for formation of the least substituted α-keto radical from unsymmetrical ketones [269Bi] is further support for the intermediacy of the enolate anion.

The lactone synthesis depends upon five basic mechanistic requirements:

(1) the selective direct generation of carboxyalkyl radicals;

(2) the difficult oxidation of the initially formed carboxyl radical;

(3) the rapid and selective addition of this radical to the olefin;

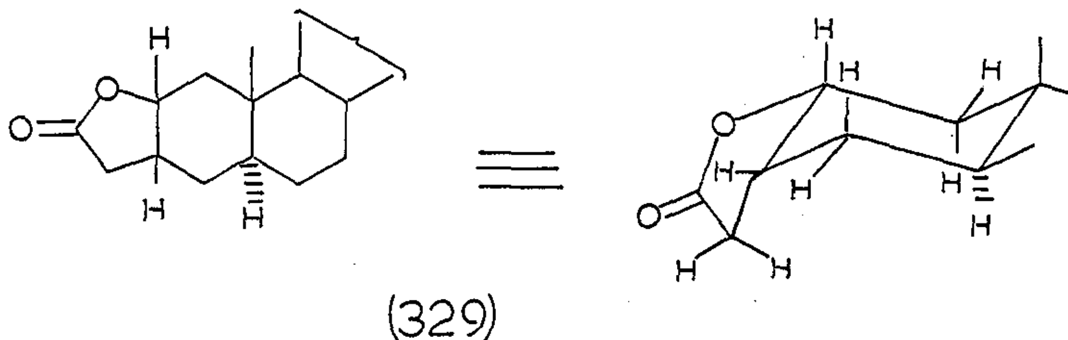
(4) the fast oxidation of the resulting adduct radical to the carbonium ion, and

(5) the rapid cyclisation of the carbonium ion to the lactone.

The low yield of the γ-butyrolactone (329) obtained may be directly attributed to problems related to one or more of the mechanistic requirements.

The reaction of the carboxymethyl radical, $^{\bullet}CH_2COOH$, with 5α-cholest-2-ene (328) may result in eight regio-and stereo-isomeric γ-butyrolactones. The structure of the major product isolated (329)

was assigned from the ^{1}H n.m.r. spectrum in which the signal (5 lines) for the low field methine at C-2 was an overlapping doublet (J = 12.0 H_z) of triplets (J = 6.0 Hz) (Fig 1A).



(329)

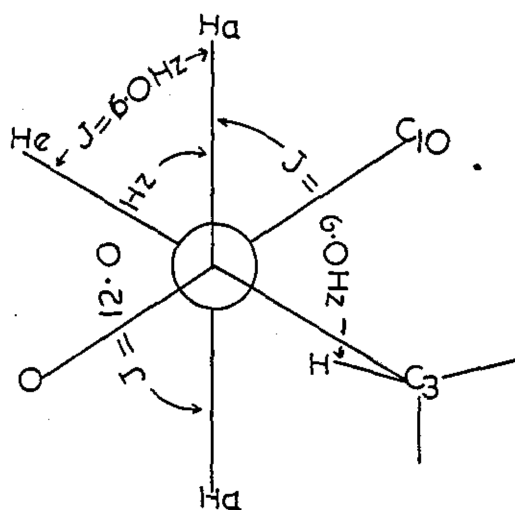


FIG 1B

FIG 1A

The smaller spin-spin coupling is to the equatorial protons at C_1 and C_3 and the larger spin-spin coupling is to the axial proton at C_1 (Fig 1 B).

The carboxyalkyl radical would be expected to attack preferentially at the C-3-position, from the unhindered α-face, giving rise to a radical at C-2, which on oxidation would form a carbonium ion which in turn

would give the thermodynamically stable _cis_ - γ-lactone (333).

The hydrolysis of the lactone resulting in a 2-hydroxy-3-(2-acetic acid)-5α-cholestane and the oxidation of the hydroxy-acid to 2-oxo-acid may give further support for the structure of the γ-lactone. However, the low yield of the γ-lactone prevented further investigation, of the structural assignment. This low yield also prevented the investigation of reactions leading to the preparation of an α-methylene -γ- lactone.

MATERIALS:

Human carcinoma of the nasopharynx (KB) cells were obtained from Flow Laboratories Limited, Irvine, Scotland.

Minimal Essential Medium (MEM) containing Earle's salts, L-Glutamine (200 mM; 1%) and Non-Essential Amino Acids (1%), Foetal Bovine Serum (10%), Phosphate Buffered Saline (PBS). Dulbecco's formula (modified) without calcium and magnesium. Trypsin (2.5%) in PBS were obtained from Gibco Bio-Cult Limited, Scotland. Sodium hydrogen carbonate was of analytical grade. Penicillin G (Potassium benzylpenicillin) and Streptomycin Sulphate were obtained from Sigma Chemical Company Limited, Poole, Dorset. Dimethylsulphoxide (DMSO) was distilled from Calcium hydride under vacuum and stored over molecular sieve.

PROCEDURE:

Minimum Essential Medium (MEM) pre-mixed powder was dissolved in sterilised distilled water (approximately 500 ml) with stirring. Sodium hydrogen carbonate (2.0 g/l) was dissolved in sterilised water (100 ml) and added to the MEM solution, with the flask being well rinsed with water. The pH of the medium was adjusted with IN hydrochloric acid. The medium was stirred slowly all the time during the addition of the acid. As the pH of the medium rises during filtration, the pH of the medium was adjusted such that the pH was 0.2 - 0.3 units lower than the final desired pH value (7.4). The medium was made up to a final volume of one litre with sterilised water. Penicillin G (potassium benzyl-penicillin; 40.0 mg/l) and streptomycin sulphate (50.0 mg/l) were added to the medium under sterile conditions.

The medium was filtered, under a positive pressure to prevent the loss of carbon dioxide, through a sterile membrane filter. Foetal Bovine Serum was added to the medium to give a final concentration of 10%. The medium was aseptically dispensed into sterile 500 ml containers and stored at $4^{o}C$. Trypsin (0.25%) was prepared by the addition of trypsin solution (2.5 % w/v; 10 ml) to Dulbecco's PBS solution without calcium and magnesium and stored at $-20^{o}C$.

The (KB) cells were incubated at $37^{o}C$ for 24 hours,upon receipt, in an atmosphere of 5% CO_2 - 95% air. The medium was removed, from the culture vessel, by suction, and sufficient trypsin solution (0.25%, 1.0 ml) was added so that the monolayer of cells was adequately covered. The culture vessel was then incubated at $37^{o}C$ for about 5-10 minutes. The resultant cell suspension was centrifuged for 5 minutes at a speed of 250g/minute. The medium was removed and the pellet of cells was resuspended in MEM solution. The suspension was aspirated thoroughly and the above sequence was repeated to obtain trypsin free cells. The cells were suspended in fresh MEM solution and incubated at $37^{o}C$ for 4-6 days to obtain a monolayer of cells.

By employing the above procedure actively dividing cells, ready for assay; were obtained. The drugs were assayed following a rapid visual assay method [331] and the active drugs were assayed following the Cancer Chemotherapy National Service Centre (CCNSC) procedure[332]. The resuspended cells, obtained after the trypsination sequence, were counted using a haemocytometer. The cells were dispersed in MEM

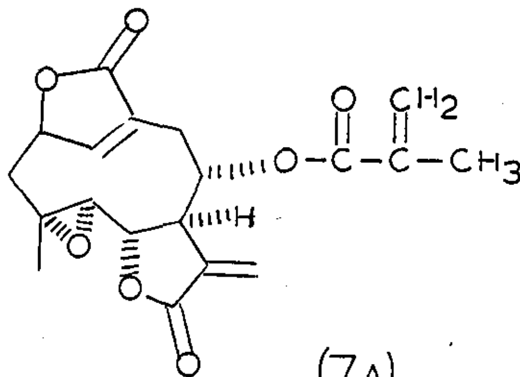
solution to ensure even distribution for the cytotoxic assay and approximately 5 x 10^5 cells were transfered to Gibco's Screw-cap tissue culture tubes containing 4.0 ml of the medium. Cells were incubated for 24 hours. Two tubes were used to obtain base-line cell number.

Dimethylsulphoxide (DMSO; 10 μl) was added to two further tissue culture tubes and incubated to act as a control, and to the rest of the tubes, α-methylene-S-lactones (0.1 - 5.0 μg/ml) in DMSO (10μl) were added and incubated for 72 hours. All the assays were run in duplicate. After incubation, the cells were isolated employing the above trypsinisation sequence. The pellet of cells was taken up in 0.5 ml of the medium and cells were counted using a Coulter Counter (Model Z, Coulter Electronics Limited, Herts, England). Dilution of samples was necessary to enable realistic number of cells to be counted. After conversion of Coulter Counter readings to total cell number, a graph of percentage death against concentration was plotted and an LD_{50} value, the concentration at which 50% of the total cells were added, was determined.(See Appendix).

RESULTS AND DISCUSSION

The potent cytotoxic action of many sesquiterpene plant products and their ability to inactivate selected enzymes *in vitro* have been attributed to the presence of an α-methylene butyrolactone moiety[4A,11C]. These compounds can be viewed as a special class of naturally occurring alkylating agents, though the exact biological role these agents might have in plants remains undefined. Interest in α-methylene butyrolactone derivatives as medicinal agents has been stimulated by the possibility that some of them might show enough selective cytotoxicity against neoplastic cells to be of therapeutic value as anti cancer agents. A number of sesquiterpenes containing the α-methylene butyrolactone function have shown high levels of cytotoxicity against tumour cells *in vitro*[11D,19,25,29,32,333].

Preliminary cytotoxic testing, employing a Rapid-Microtitre method, indicated that all the synthetic steroidal α-methylenelactones were active at a concentration under 10 μg/ml. The procedure of the Cancer Chemotherapy National Service Centre[332] was employed to obtain an accurate LD_{50} value and all the steroidal α-methylene-δ-lactones exhibited approximately equal significant cytotoxic activity at a level of 1.0 μg/ml or less against KB cells. (Table 1).

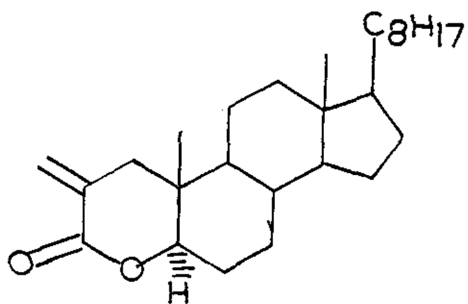


(7A)

TABLE 1

COMPOUND	LD 50 (μg/ml)	
	MICROTITRE METHOD[331]	CCNSC METHOD [332]
7A	< 10	0.31 (0.32) [331]
225	< 10	0.80
230	< 10	1.27; (0.58,0.44)[331]
233	NO ACTIVITY	-
238	< 10	0.80
239	< 10	0.72
255	< 10	1.0
259	< 10	0.92
329	NO ACTIVITY	-

Elephantopin, vernolèpin, eriolangin and erioflorin were used as standards in the microtitre procedure. Elephantopin, the most active of the natural compounds and the one of comparable cytotoxic activity to the synthetic α- methylene lactones, was employed solely in the CCNSC procedure.



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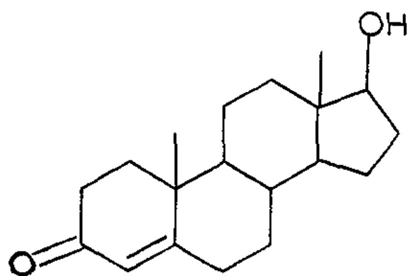

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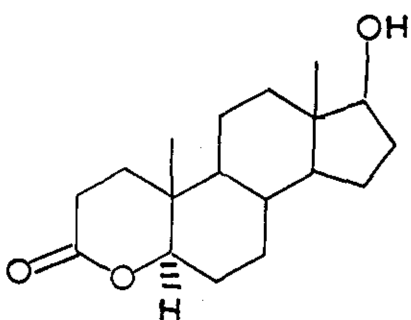

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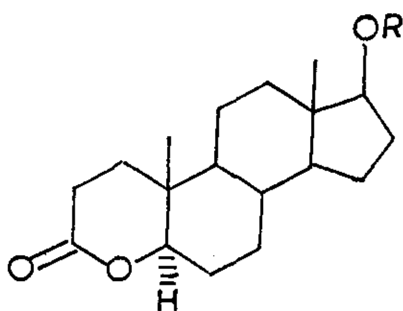
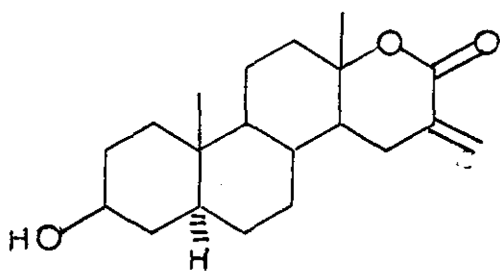


(238) R = H

(239) R = OAc



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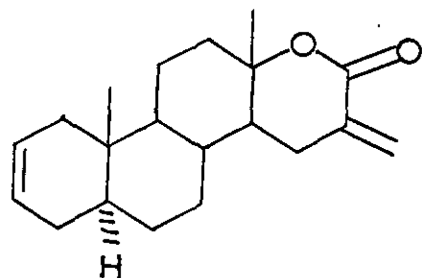

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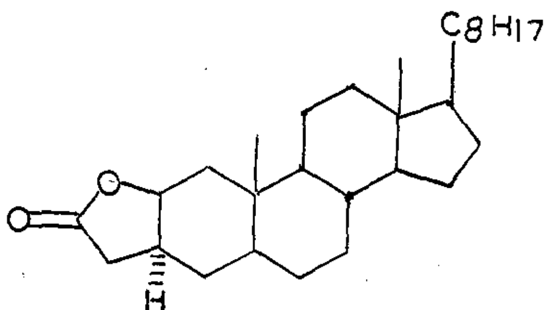
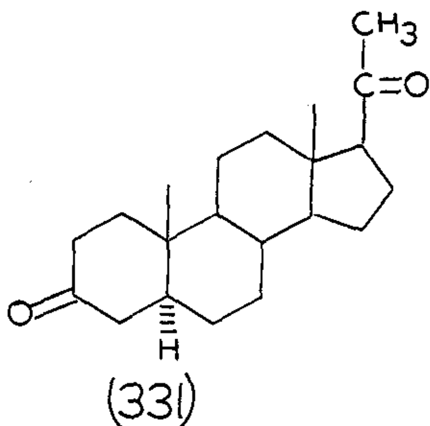


(329)

Although only a few steroidal α-methylenelactones structures were evaluated, the degree of activity appears to be independent, irrespective of whether the α-methylene lactone moiety was in the A or D ring of the steroid nucleus. However, the A-ring α-methylene lactones are relatively more active than the D-ring counterparts which could be due to a change in the configuration of the D-ring in the steroid nucleus.

The simple synthetic α-methylene lactones are approximately as cytotoxic as more complex natural products[29]. This supports the tentative conclusion that in cell cultures, as opposed to the whole animal assays, the α-methylene lactone, the alkylating function, is sufficient to confer cytotoxic properties upon the molecule, even in the absence of other reactive functionalities. Selective modification of the α-methylene lactones moiety by hydrogenation[110] or alkyl substitution[32] always resulted in complete loss of cytotoxicity, thus identifying the activity conferring functionality.

The cytotoxic activity of testosterone (230), confirms previous finding on HEp-2-cell line[331], and of 5 α-pregnan-3,20-dione (331)[45] suggests that cytotoxicity may be associated with steroid nucleus. In addition, it has been learned in clinical practice[334,335] that testosterone can cause regression of neoplasm in certain women with advanced mammary cancer and several related steroids, such as 17 α-methyltestosterone(332), and 5 α-dihydrotestosterone (333) have been found to have similar beneficial activity. The mechanisms involved in the restraint of human mammary cancer by androstane compounds are unclear. Δ^1-Testololactone (334), which lacks hormone properties in man and animals[336] was studied for the effectiveness in patients with advanced breast cancer and 30% remission was observed[337].



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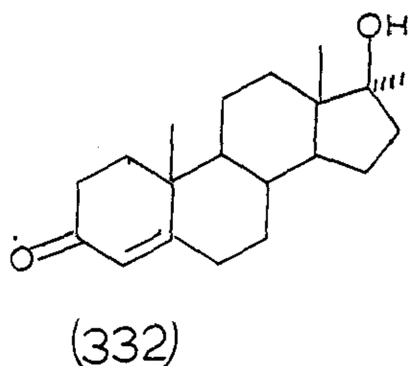

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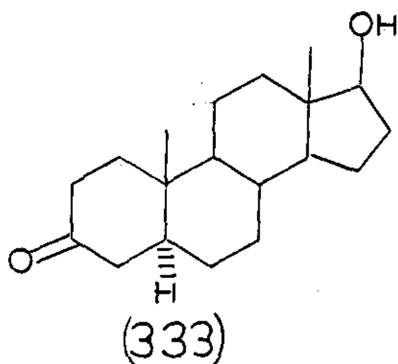

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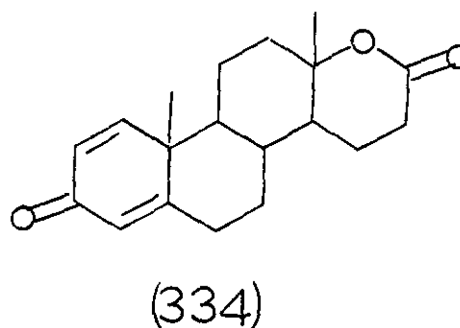

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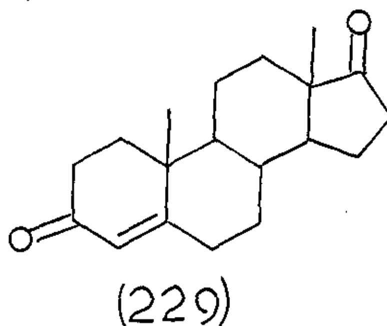


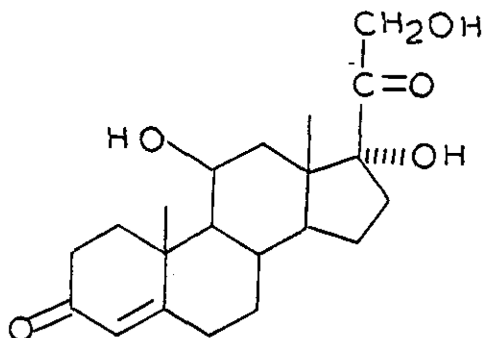
(229)

In certain androgen-sensitive tissues, 5 α-dihydrogen-testosterone (333) is believed to be an active form of androgen[338,339] and the presence of 5 α-reductase, membrane bound, in mammalian tissues[340-355] and in bacteria[356,357] may help to explain the activity of testosterone. However, all attempts to reverse the reaction utilising mammalian enzymes have been unsuccessful, because although the first step in reversal (enolisation) occurs the energy barrier for hydride abstraction is extremely high[358,359]. Recently, the findings that human breast cancer can metabolise testosterone in vitro to androstendione (229) and the potent androgen 5 α -dihydrotestosterone (333)[360] has been confirmed[361], but contradict that 5 α-reductase activity is absent in normal breast[361].

The androgen-dependent Shionogi 115(S 115) and the androgen-independent Shionogi 42 (S 42) tumours[362] contain the NADPH-dependent 5 α-reductase enzyme necessary for forming 5 α-dihydrotestosterone (333) [363, 364], although the activity is somewhat higher in S115 cells[364]. It was also indicated that numerous active metabolites are necessary for the full expression of the androgenic response as this cannot be elicited by 5 α-dihydrotestosterone (333) alone[365].

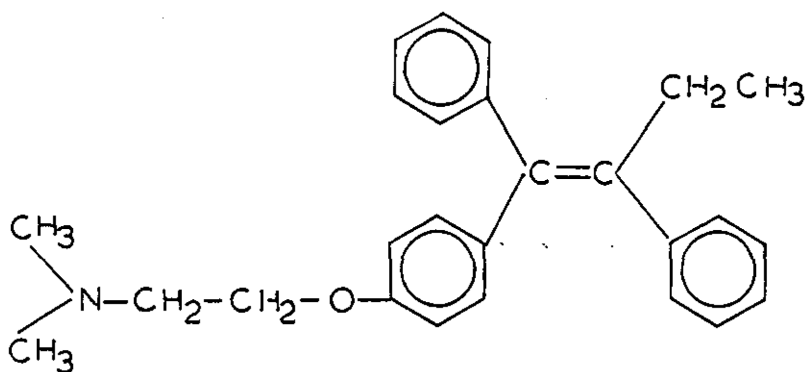
Since the early observations of Beatson,[366,367] hormonal manipulations have become accepted modalities of therapy in leukaemia and lymphomatous disease[368], breast cancer,[369] endometrial[370] and prostatic cancer[371]. A principle deterrent to wider use of these compounds has been the inherent hormonal side-effects. From the knowledge that most, if not all classes of steroid hormones result from relatively similar interactions of hormone with target tissue,[372] it seems reasonable to assume that steroid receptor is at least a necessary, if not sufficient, condition for steroid hormone responsiveness in a target tissue.

The responsiveness of some human leukaemias to glucocorticoids is demonstrated in previously untreated childhood acute lymohoblastic leukaemia (ALL)[373,374] though many patients fail to respond. Unfortunately, glucocorticoid administration is associated with numerous serious and potentially life threatening complications. The failure of glucocorticoids to respond to ALL has been demonstrated[375] to be due to lack of steroid receptors in cancer cells. Corticoids cause lysis of several cultured cell lines, one of the fast effects being the inhibition of precursor uptake of nucleic acids[376] and testosterone has been shown to be active in such systems. Glucocorticoids, such as cortisol (335) are employed in the treatment of ALL, if the tumour cells have steroid receptors and only if the glucocorticoids are active hormones as the receptors shown critical specificity in differentiating biologically active steroids from their inactive congenus. The 11β-hydroxyl configuration strongly favours binding to the receptor and the more potency of the glucocorticoid the greater the activity against ALL[374].



(335)

Experiments with rat mammary tumours[377-379] and preliminary data in human patients[380] indicate that tumours which contain appreciable cytoplasmic oestrogen receptor regress after endocrine ablation therapy whereas tumours without receptors fail to respond.. The cytoplasmic oestrogen receptor in human mammary tumours has properties similar to that from rat tumours[381-383]. Lack of oestrogen receptors satisfactorily predicts a lack of hormone responsiveness in human breast cancer although the presence of a receptor is clearly no guarantee that a tumour is responsive to the hormone. It has been shown that a breast tumour cell line (MCF 7) which does contain receptors is stimulated by low doses of oestrogens but inhibited by high doses[384]. In addition, these cells are killed by the anti-oestrogen Tamoxifen (336), used in the treatment of breast cancer. Tamoxifen has no action on cell lines that do not respond to oestrogen (e.g. Hela and other breast cell lines and it binds to the oestrogen with 1000 x less the affinity of oestrogen; its action is reversible by oestrogens. The suppression of growth of population of leukaemia L5178Y cells in vivo and in vitro by glucocorticoids was not surprising since[385] the growth inhibitory activity of this class of steroids against other cellular populations of mesenchymal origin is well established.[386-393]



(336)

It has been proposed[28,141] that α-methylenelactones can act as the alkylating centre for cytotoxic anti tumour lactone via Michael-type addition between biological nucleophiles. However, the target of attack for α-methylenelactone is unlikely to be cysteine-scavenger, since KB cells have no absolute requirement for cysteine, as has been demonstrated from the studies on CCRF-CEM human lymphoblastic leukaemia cells in culture.[29] In addition, the concentration of L-cysteine in medium is far greater than the concentration of the α-methylenelactone. Thus, the cytotoxicity of these compounds cannot be due only to cysteine scavenging.

Several studies have indicated that cancer cells are dependent on the glycolytic and aerobic pathway for energy[394-398] which is critical for the cells survival in view of the energy required for rapid nucleic acid and protein synthesis in proliferating cells. Recently,[399] helenalin has been demonstrated to inhibit both aerobic and anaerobic energy process of tumour cells.

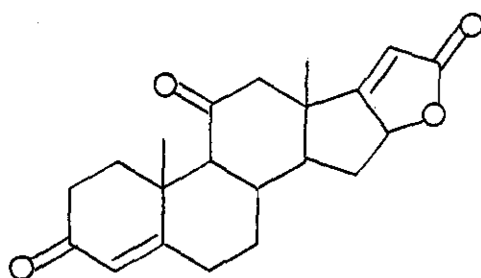
The mechanism of growth inhibition (i.e. cytotoxic activity) involves a mechanism other than (or in addition to) scavenging of free cysteine. One possibility is rapid, irreversible S-alkylation of various metabolically essential intracellular proteins (e.g. respiratory and other enzymes)[288]. Whereas the inhibition of glycolytic metabolism is probably not the major mechanism of action of helenalin[146], as an anti tumour (ie the mechanism most likely is inhibition of nucleic acid and protein synthesis although it had little effect on ribonucleic acid synthesis of Ehrlich tumour cells[400]) the fact that the energy available to cells for synthetic purposes is seriously hampered and rate limiting in the presence of helenalin has an additive effect on the drug's action cellular metabolism and tumour inhibition. The inhibition of the respiratory enzymes is

probably of secondary importance; the important mechanism is of inhibition of DNA synthesising enzymes. It has been shown for sesquiterpene lactones, helenalin and tenulin that they do not alkylate nucleophiles of purine bases and this would agree with negative finding for steroid α-methylene lactones in an Ames test,[401] rather appear to be reactive with thiol groups of enzymes necessary for DNA replication. Furthermore, these agents can cause an increase in cAMP levels of tumour cells which may be correlated with the suppression of chromatin protein phosphorylation necessary for cell replication and differentiation[146].

A second possibility is S-alkylation of sulphydryl groups in cell surface protèins, as has been suggested for the action of 6,6'-dithionicotinic acid and related cytotoxic pyridine disulphides[402].

The cytotoxic activity of the synthesised steroidal α-methylene-lactones is approximately in the same range as the plant sesquiterpene lactone, elephantopin. This higher cytotoxic value of elephantopin is contributed by two active alkylating sites, whereas the epoxide and the endocyclic α,β-unsaturated lactone moieties do not contribute to the cytotoxicity. The cytotoxic activity was absent for lactones lacking the α-methylene function, thus indicating the requirement of the α-methylene lactone structure for cytotixicity in this system. The presence of an 17β-acetate, conferring increased lipophilicity, appears to increase the activity slightly which may suggest the increase in transport of the α-methylene lactone structure.

Preliminary work shows the steroidal α-methylene-lactones to be inactive against Gram positive and Gram negative organisms at a concentration of up to 100 μg/ml[403]. Testosterone and its metabolites, however, show antibacterial activity against both G +ve and G-ve organisms at a concentration of 20 μg/ml[404]. The α,β-unsaturated lactones (337-339) were shown to be inactive against various G +ve and G-ve micro-organisms at concentrations of 100 μg/ml[405].



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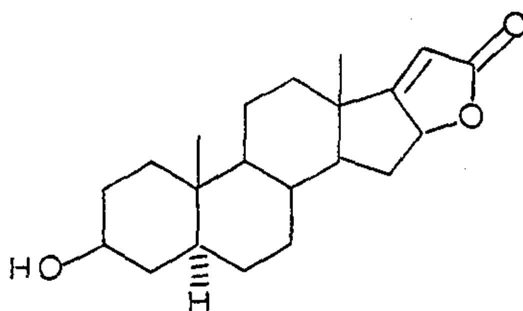
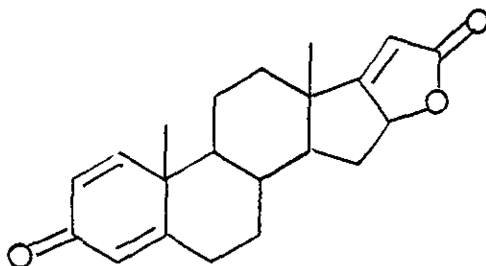


(338)



(339)

Although the preliminary results are encouraging, the compounds must be tested for activity in vivo, since varied success has been achieved previously with steroid derivatives substituted with alkylating functions. The steroid receptor is a protein which contains an essential -SH function[406] and synthesis of hormonally active steroids containing α-methylene-lactone functions could thus provide steroids which irreversibly bind to hormone receptors in tumour cells.

EXPERIMENTAL

EXPERIMENTAL

All solvents were distilled and dried, as appropriate, by the conventional methods before use. Solvents were removed *in vacuo* on a rotary evaporator.

Analytical T.L.C. was performed using 0.75 mm thick layers of silica-gel (P F_{254} according to Stahl) and preparative T.L.C. was performed using 1 metre plates of 0.75 mm thickness. Column chromatography was carried out using 'CAMAG'alumina (Bröckmann activity 1).

Infra-red spectra were determined for K Br discs, thin films or solutions in chloroform, on a Perkin-Elmer 177 and 257 spectrophotometer. Ultra-violet spectra was determined in ethanol, unless stated otherwise, on a Pye-Unicam SP 8000 spectrophotometer. ^{1}H.N.M.R. spectra were determined for solutions in deutero-chloroform with tetramethylsilane as as internal standard, at 60 M Hz on Varian EM 360A or 90 M Hz on Perkin-Elmer R32 spectrophotometer, respectively.

Mass spectra were recorded on an A.E.I. M.S.12 mass spectrophotometer. High resolution mass spectrometry was carried out on an A.E.I. M.S. 9 at P.C. M.U by courtesy of the S.R.C.

Melting points were determined on a Koffler block and are uncorrected. Optical rotations were measured for solutions in chloroform at 25°C with a Bendix polarimeter 143C.

1. PREPARATION OF 2-HYDROXYMETHYLENE-5α- CHOLESTAN-3-ONE

Sodium metal (1.38g; 60.0 mM) was added to dry ethanol (200 ml) with stirring. After all the sodium had dissolved, it was refluxed for 45 minutes and then excess ethanol was evaporated to dryness.

Benzene (150 ml) was added to sodium ethoxide (formed as above) and nitrogen was passed through the solution. After fifteen minutes, 5α-cholestan-3-one (772.0 mg; 2mM) was added. After ten minutes of stirring, ethyl formate (4.44g; 60.0 mM) was added and the solution was stirred at room temperature for five hours. Water (60 ml) was added and the stirring was continued for another fifteen minutes.

The contents were transferred into a separating funnel and the aqueous layer was separated. The benzene layer was washed with water (2 x 50 ml). The combined aqueous extract was washed with benzene (3 x 100 ml) and the combined benzene extract was washed with water (3 x 50 ml). The benzene extract was dried over magnesium sulphate and evaporated to dryness. The crude product (97.5 mg;) was shown to be the starting material, 5α-cholestan-3-one,as observed from T.L.C. and spectroscopic data.

The aqueous washing was acidified to Congo Red with dilute hydrochloric acid and extracted with ether (3 x 150 ml). The combined ether extract was dried over anhydrous magnesium sulphate, and evaporated to dryness. The crude product was crystallised from chloroform-methanol to obtain crystals of 2-hydroxy-methylene-5α-cholestan-3-one.

M.P. 179-181°C (Lit MP[407] 180-182°C)

Yield: 592.0 mg; 71.5%

I.R. ν max 3640-2300 (OH, H-bonded), 2975, 2950, 2885, 2870, 1562 (α, β-unsaturated-β-hydroxyketones), 1455, 1419, 1390, 1353, 1332, 1279, 1255, 1235, 1219, 1146, 950, 943, 920, 806, 740, 709.

NMR. τ 9.31 (S, 3H, C_{18}-CH_3), 9.22 (S, 3H, C_{19}-CH_3), 7.81 (ABq, 2H, C_4-H, J = 15.0 Hz, 18.0 Hz). 2.60 (S, 1H, C=CH-OH), 1.34 (S, 1H, C = CH - OH)

U.V. λ max 283 nm (ϵ 16,837).

2.PREPARATION OF CHOLEST-4-EN-3-ONE (202)[283]

Distilled toluene (400 ml) was added to a 1 litre three-necked flask, equipped with a stirrer, a dropping funnel and a Dean-Stark trap. The openings of the dropping funnel and the condenser were protected with drying tubes. A portion of toluene (40 ml) was distilled off, in order to dry the system by azeotropic distillation, then cholesterol (201, 20.0g, 0.052M) and cyclohexanone (100 ml) was added to the flask. After an additional toluene (10 ml) had been distilled off, a solution of aluminium isopropoxide* (5.6g, 0.028M) in toluene (80 ml) was added dropwise over a period of approximately 30 minutes. During this time, toluene was distilled at a rate slightly greater than the rate of addition of the catalyst solution, so that when the addition was complete, about 120 ml of toluene had been distilled. After an additional toluene (60 ml) had been distilled, the murky-orange coloured reaction mixture was allowed to cool to room temperature.

A saturated solution of Rochelle salt (potassium sodium tartarate, (80 ml) was added to the reaction mixture and the organic layer became of clear orange colour. The stirrer assembly was removed and the mixture was steam-distilled until 1200 ml of distillate had been collected. The residual mixture was cooled and extracted with chloroform (3 x 100 ml). The combined extract was washed with water (2 x 50 ml), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The residual viscous oil (amber) was dissolved by heating in methanol (15 ml). When the solution had cooled to about 40°C, seeds of cholest-4-en-3-one were added and the flask was wrapped with a small towel to ensure slow cooling. After the bulk of the material had crystallised, which required several hours, the flask was stored overnight at 0°C.

The product was collected by suction filteration washed with methanol (4-5 ml), previously cooled in an ice-salt bath, and then dried at reduced pressure at room temperature obtaining light cream coloured crystals of cholest-4-en-3-one (202).

M.P. 77-79°C (Lit. M.P.[283] 79-81°C)

Yield: 13.95g, 70%

IR ν max 3020, 2950, 2935, 2870, 1673 (C=O), 1612 (C=C), 1470, 1443, 1430, 1415, 1380, 1375, 1330, 1265, 1232, 1227, 1190, 1172, 1125, 955, 945, 929, 899, 868, 772, 729, 681.

NMR ($CDCl_3$) τ 9.27 (S, 3H, C_{18}-CH_3), 8.81 (S, 3H, C_{19}-CH_3), 7.62 (m, 2H, C_2-H), 4.25 (bs , 1H, C_4-H)

* Aluminium isopropoxide was taken up in dry, distilled chloroform and filtered rapidly to remove aluminium hydroxide (white gelatinous precipitate). The filterate was evaporated to dryness and solid then employed in the reaction.

3. PREPARATION OF 4-OXA-5α-CHOLESTAN-3-ONE (203)[264]

Potassium persulphate (27.0g) and concentrated sulphuric acid (30.0g) were mixed in a mortar and diluted with glacial acetic acid (300 ml). The resulting mixture was added to a solution of Cholest-4-en-3-one (202, 9.0g) in glacial acetic acid (600 ml). Following a 7-day period of intermittent shaking at room temperature, in the absence of light, the mixture was cooled and treated with aqueous 50% potassium hydroxide (180 ml). The precipitated salt was removed by filtration and the filterate was evaporated to dryness (in vacuo at 60°C). The residue was taken up in ether (250 ml), washed successively with water (3 x 100 ml), 5% sodium hydrogen carbonate (3 x 100 ml) and water (3 x 100 ml). The ether solution was dried over anhydrous magnesium sulphate and evaporated to dryness. The crude solid was crystallised from ethanol to obtain light cream-yellow crystals of 4-oxa-5α-cholestan-3-one (203).

M.P. 113-115°C (Lit M.P.[264] 116-118°C)

Yield: 3.64g (40%)

IR ν max: 2950, 2935, 2870, 2855, 1730 (C=O), 1467, 1459, 1390, 1380, 1374, 1364, 1259, 1190, 1130, 1112, 1076, 1047, 991, 955, 940, 664.

NMR ($CDCl_3$) 9.32 (S, 3H, C_{18}-CH_3), 9.06 (S, 3H, C_{19}-CH_3) 7.38 (dd, 2H, C_2-H, J = 6.0H_Z, J 10.0 H_Z) 6.02 (q, 1H, C_5-αH, J = 4.6 and 11.4 H_Z).

4. PREPARATION OF 2-HYDROXYMETHYLENE-4-OXA-5α-CHOLESTAN-3-ONE (222)[160]

Sodium hydride (960 mg; 20.0 mM, 50% dispersion) was weighed into a dry three-necked 500 ml round-bottom flask and washed twice with dry benzene followed by dry ether with nitrogen passing through the reaction flask all the time. To a suspension of sodium hydride in dry ether (200 ml), 4-oxa-5α-cholestan-3-one (203, 388.0 mg; 1.0 mM) was added with stirring. After about 5 hours of stirring, distilled, dry ethyl formate (1.58g; 20.0 mM) was added and the reaction mixture was left stirring at room temperature under a nitrogen atmosphere. (The course of the reaction was checked, at intervals, by thin-layer-chromatography (T.L.C)).

When the reaction was complete (on T.L.C.), after about 70 hours, the reaction mixture was acidified carefully with dilute hydrochloric acid and washed with water (3 x 100 ml). The ether solution was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The light-yellowish residue was crystallised from ethanol to obtain crystals of 2-hydroxymethylene-4-oxa-5α-cholest-3-one (222).

M.P. 238 - 240°C

Yield: 374.0 mg (84%).

IR ν max 3600-2400 (H bonded OH), 3020, 2940, 2880, 2860, 1715, (C=O) 1620 (C=C), 1450, 1388, 1345, 1282, 1171, 1140, 1105, 1040, 1025, 998, 950, 937, 920, 760, 742, 668.

U.V. λ_{max} 251 mm (ϵ 6.95 x 10^{3})

Acc. Mass. Cal: 416.3291

Found: 416.3293 (M, $C_{27}H_{44}O_3$)

$[\alpha]_D$ (+) 104.17 (C, 0.12)

NMR ($CDCl_3$/DMSO)τ 9.33 (S, 3H, C_{18}-CH_3), 9.15 (S, 3H, C_{19}-CH_3) 6.13 (m, 1H, $C_5\alpha$-H), 2.82 and 2.2 (bs, 1H, Z-and E- HO - CH = C -).

The 'H n.m.r. spectrum of the hydroxymethylene lactone (222) displayed signals for both Z-(minor) and E- (major) isomers whereas the absence of H-bonding evidence in the infra-red (K Br disc) was characteristic of the E- isomer only. It appears that the E-isomer preferentially crystallises and equilibrates to give a mixture of E - and Z isomers in $CDCl_3$ solution. Similar observations were made for compounds (235), (223) and (236).

5. PREPARATION OF 2- (N,N-DIETHYLAMINO METHYLENE) -4-OXA-5 α- CHOLESTAN-3-ONE (223)[160].

To a solution of 2-hydroxymethane-4-oxa-5 α-cholestan-3-one (222, 374.0 mg; 0.9 mM) in dry benzene (150 ml), distilled diethylamine (1.46g; 20.0 mM) was added and the reaction mixture was refluxed using a Dean-Stark trap. When the reaction was complete; after about 85 hours (T.L.C.) the solution was evaporated to dryness. The crude residue was crystallised from acetone/pet-ether (60-80°) to obtain light-yellowish crystals of 2-(N, N-diethylamino) methylene-4-oxa-5 α-cholest-3-one (223).

M.P. 156-158°C

Yield: 382.0 mg (90.0%)

IR ν max 2920, 2850, 1675 (C=O), 1560 (C=C), 1455, 1375, 1350, 1312, 1237, 1170, 1120, 1070, 1051, 939, 805, 751, 739

U.V. λ max 301 nm (ϵ 2.84 x 10^4)

Acc. Mass. Cal: 471.4076

Found: 471.4087 (M, $C_{31} H_{53} NO_2$)

$[\alpha]_D$ (-) 16.20 (C, 0.46)

NMR τ 9.32 (S, 3H, C_{18}-CH_3), 9.11 (S, 3H, C_{19}-CH_3), 8.79 (t, 6H, 2X - N-CH_2-$\underline{CH}_3$, J = 7.0 H_Z), 6.64 and 6.73 (q, 4H, E- and Z- CH_3-$\underline{CH_2}$-N-CH=C-, J = 7.0 H_Z), 6.11 (q, 1H, C_5- H), 2.82 and 2.30 (1H, bs, Z- and E-$(CH_3\ CH_2)_2$ N $\underline{CH}$ = C -).

6. PREPARATION OF 2-METHYLENE-4-OXA-5 α-CHOLESTAN-3-ONE (225)[160]

2-N,N-Diethylamino methylene-4-oxa-5 α-cholestan-3-one (223), 375.0 mg; 0.8 mM) was taken up in glacial acetic acid (40 ml) and hydrogenated using the Adams' catalyst-Platinum oxide (50.0 mg). The hydrogenated product was filtered and evaporated to dryness. The residue (224) was taken up in glacial acetic acid (40.0 ml) and sodium acetate (400 mg) was added. The reaction mixture was heated under reflux for four hours and the course of the reaction followed on T.L.C.

On completion of the reaction, the reaction mixture was concentrated and treated with saturated sodium hydrogen carbonate solution until there was no more evolution of carbon dioxide. The aqueous solution was extracted with ether (3 x 100 ml). The combined ether extract was washed with water (2 x 50 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was crystallised from methanol giving white crystals of 2-methylene-4-oxa-5 α-cholestan-3-one (225).

M.P. 105-106°C

Yield: 243.0 mg (76% from 224); 58% overall

IR ν max 2940, 2870, 1725 (C=O), 1620 (C=C), 1473, 1460, 1383, 1310, 1283, 1260, 1190, 1151, 1078, 1042, 1029, 995, 945, 912, 805.

NMR ($CDCl_3$) τ 9.3 (S, 3H, C_{18}-CH_3), 9.07 (S, 3H, C_{19}-CH_3). 5.95 (q, 1H, C_5-αH, J = 11.0 and 5.0 Hz) 4.43 (m, 1H, O - C(=O) - C = C(H) - H), 3.50 (m, 1H, O -C(=O) - C = C(H) - H)

U.V λ_{max} (EtOH) 213 nm (ϵ. 7.19 x 10^3)

(Hexane) 217-8 nm (ϵ 5.0 x 10^3)

Acc. Mass Cal: 400.3341

Found: 400.3329 (M, $C_{27}H_{44}O_2$)

$[\alpha]_D$ (+) 90.51 (C, 0.16)

Analysis: Expected:C, 80.94; H, 11.07

Found: C, 80.7, H, 11.2

7. REACTION OF L-CYSTEINE WITH 2-METHYLENE-4-OXA-5 α-CHOLESTAN-3-ONE (225)[186].

2-Methylene-4-oxa-5 α-Cholestan-3-one (225, 40 mg; 0.4 mM) and L-cysteine (12.1 mg; 0.4 mM) were mixed in 60% aqueous ethanol (5.0 ml) and heated under reflux for one hour over a water-bath.

The reaction mixture was allowed to cool to room temperature. The white precipitate was collected, washed well with the solvent (60% aqueous ethanol) and dried. Recrystallisation from aqueous ethanol gave white crystals of the Cysteine adduct (227).

M.P. 186-189°C

Yield: 41.2 mg, (7.9%)

IR ν max 3700 - 2300 (COOH and NH_2), 2930, 2865, 1725 (C=O) of carboxylic acid and lactone) (COO^-) 1466, 1381, 1345, 1238, 1219, 1185, 1131, 1075, 1040, 990, 950.

Acc. Mass. Found (M - $C_3H_5NO_2S$)+ = 402.3492, $C_{27}H_{46}O_2$ (Cal: 402.3947)

($M - C_3H_6NO_2S$)+ ; 401.3388, $C_{27}H_{45}O_2$ (401.3419)

($M - C_3H_7NO_2S$)+ = 400.3333, $C_{27}H_{44}O_2$ (400.3341)

8. PREPARATION OF ANDROST-4-EN-3, 17-DIONE (229)[283]

To a litre three-necked round bottom flask, equipped with a stirrer, a dropping funnel and a Dean-Stark trap, toluene (200 ml) was added. The openings of the dropping funnel and the condenser were protected with drying tubes. A portion of toluene (20 ml) was distilled in order to dry the system by azeot ropic distillation. Androst-4-en-3-β-ol-17-one (228, 10.0g; 30.0 mM) and cyclohexanone (50 ml) were added to the flask. After an additional toluene (5 ml) had been distilled, a solution of aluminium isopropoxide* (2.8g; 0.028M) in toluene (40 ml) was added dropwise over a period of approximately 30 minutes. During this period, toluene was distilled at a rate slightly greater than the rate of addition of the catalyst solution, so that when the addition was complete, about 60 ml of toluene had been distilled. After an additional toluene (30 ml) had been distilled, the orange-yellowish solution was allowed to cool to room temperature.

A saturated solution of Rochelle salt (potassium-sodium tarterate, 40ml) was added to the reaction flask and the organic layer became clear and light yellow. The reaction mixture was steam -distilled until about 600 ml of the distillate had been collected. The residual mixture was cooled and extracted with chloroform (3 x 100 ml). The combined extract was washed with water (2 x 50 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. Crystallisation of the crude solid from acetone-hexane (Pet-ether 60-80°) gave white crystals of androst-4-en-3,17-dione (229).

M.P. 169-171°C (Lit. M.P.[331] 173-174°C)

Yield: 8.44g; (85%)

IR ν max 3030, 2960, 2925, 2855, 2825, 1730 (C_{17}C=O), 1660 (C_3-C=O), 1615 (C=C), 1452, 1431, 1421, 1407, 1380, 1350, 1331, 1292, 1272, 1250, 1235, 1227, 1192, 1175, 1155, 1123, 1111, 1093, 1056, 1031, 1016, 950, 936, 930, 919, 900, 870, 831, 825, 780, 713, 670, 655.

NMR ($CDCl_3$) τ 9.07 (S, 3H, C_{18}-CH_3), 8.77 (S, 3H, C_{19}-CH_3),

7.60 (m, 2H, C_2-H)

4.24 (bs, 1H, C_4-H)

* See footnote for preparation of cholest-4-en-3-one

9. SODIUM BOROHYDRIDE REDUCTION OF ANDROST-4-EN-3,17-DIONE (229)[292]

To a solution of androst-4-en-3,17-dione (229, 3.50g; 12.24 mM) in methanol (400 ml) at $0^{o}C$, sodium borohydride (555.0 mg; 14.7 mM; 1.2 equiv) was added. After stirring for one hour at $0^{o}C$, glacial acetic acid (15-20 drops) was added and the solution was evaporated to dryness in vacuo. The residue was taken up in hot ethyl-acetate and the precipitate was filtered. The precipitate was washed thoroughly with hot ethyl acetate and the filtrate was evaporated to dryness. The crude product (3.38g) was separated on silica (solvent; chloroform/ methanol; 99/1) and obtained two products.

The non-polar crude product was crystallised from acetone and obtained white crystals of androst-4-en-17β-ol-3-one (230).

M.P. 151-153°C (Lit MP[292] 154-154.5^{o}C)

Yield: 2.85g: (81%)

IR ν max 3700 -3100 (OH) 3030, 2955, 2930, 2875, 2855, 1660 (C=O) 1612 (C=C), 1469, 1448, 1432, 1418, 1382, 1348, 1331, 1320, 1301, 1278, 1271, 1231, 1192, 1173, 1131, 1111, 1079, 1058, 1020, 955, 939, 915, 861, 828, 780, 685.

NMR ($CDCl_3$)τ 9.21 (S, 3H, C_{18}-CH_3), 8.80 (S, 3H, C_{19}-CH_3), 7.62 (m, 2H, C_2-H) 6.57 (bs, 1H , C_{17}-OH), 6.36 (t, 1H, C_{17}-α-H, J = 8.0 Hz), 4.28 (bs, 1H, C_4-H).

The polar compound was crystallised from acetone to obtain white crystals of androst-4-en-3β,17β-diol (234).

M.P. 150-2°C (Lit. M.P.[292] 153-4°C)

Yield: 428.0 mg; (12.0%)

NMR ($CDCl_3$) τ 9.26 (S, 3H, C_{18}-CH_3), 8.95 (S, 3H, C_{19}-CH_3), 7.41 (bs, 2H, C_3 ,C_{17}-OH), 6.39 (t, 1H, C_{17} α-H, J = 8.0 Hz), 5.89 (m, 1H, C_3 α-H), 4.70 (bS, 1H, C_4-H)

10. ALLYLIC OXIDATION[293] WITH MANGANESE DIOXIDE

Androst-4-en-3β,17β-diol (234, 3.0g, 10.0mM) was suspended in chloroform (67.0 ml) and stirred with manganese dioxide[294] (11.9g) for ten hours at room temperature. Manganese dioxide was removed by filteration. The precipitate was washed thoroughly with hot chloroform and the combined washing was dried over anhydrous magnesium sulphate and evaporated to dryness. The crude solid was crystallised from acetone to obtain white needles of androst-4-en-17β-ol -3-one (230).

M.P. 152-154°C (Lit M.P[293] 154-154.5^{o}C)

Yield: 2.59g (87%)

IR ν max 3620-3100 (OH), 2955, 2930, 2875, 2855, 1675 (C=O), 1612, (C=O), 1469, 1448, 1432, 1418, 1381, 1347, 1331, 1320, 1305, 1280, 1231, 1180, 1172, 1131, 1111, 1079, 1065, 1057, 1020, 995, 956, 939, 915, 861, 828, 780, 685.

NMR ($CDCl_3$) τ 9.21 (S, 3H, C_{18}-CH_3), 8.80 (S, 3H, C_{19}-CH_3), 7.62 (m, 2H, C2-H,) 6.57 (bs 1H, C_{17}-OH), 6.36 (t, 1H, C_{17} -α-H, J = 8.0 H_Z), 4.28 (bs, 1H, C_4-H).

11. PREPARATION OF 17β-ACETOXY-ANDROST-4-EN-3-ONE (TESTOSTERONE ACETATE) (231)

To a solution of androst-4-en-17β-ol-3-one (230 3.6g; 12.5mM) in pyridine (60 ml), acetic anhydride (30 ml) was added with stirring. The solution was stirred for two hours and then left to stand overnight at room temperature.

The reaction mixture was poured over ice-water and extracted with ether (3 x 150 ml). The combined ether extract was washed with dilute hydrochloric acid (3 x 50 ml) to remove pyridine, followed by water (3 x 50 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. Crystallisation of the crude product from aqueous acetone gave white crystals of 17β-acetoxy-androst-4-en-3-one (231).

M.P. 137-139°C (Lit M.P.[331] 140-1°C)

Yield: 3.63g (88%)

IR. ν max 3030, 2940, 2910, 2880, 2855, 1735 (Acetate C=O), 1670 (C_3 - C=O), 1618 (C=C), 1450, 1435, 1421, 1390, 1375, 1356, 1332, 1310, 1290, 1270, 1245, 1187, 1130, 1118, 1070, 1031, 1021, 998, 975, 958, 939, 919, 860, 831, 775, 750, 685, 661.

NMR ($CDCl_3$) τ 9.16 (S, 3H, C_{18}-CH_3), 8.82 (S, 3H, C_{19}-CH_3) 7.97, (S, 3H, $C_{17}\beta$ - O - $\overset{O}{\overset{\|}{C}}$ - CH_3), 7.63 (m, 4H, C_2, C_{16}-H), 5.40 (t, 1H, $C_{17}\alpha$-H J = 8.0H_Z), 4.27 (bs 1H, C_4 H).

12. PREPARATION OF 17β-ACETOXY-4-OXA-5α- ANDROSTAN-3-ONE (232)[264]

Potassium persulphate (12.1g) was grounded with concentrated sulphuric acid (13.4g) and diluted glacial acetic acid (160 ml). The resulting mixture was added to a solution of 17β-acetoxy-androst-4-en-3-one (231, 3.0g, 9.1mM) in glacial acetic acid (320 ml). The reaction mixture was shaken intermittently and left in the dark at room temperature for seven days.

The reaction mixture was cooled and treated with 50% potassium hydroxide solution (60 ml). The precipitated salts were removed by filteration and the filterate was evaporated to dryness. The residue was taken up in ether (250 ml), washed with water (3 x 100 ml), 5% sodium carbonate solution (3 x 100 ml) and finally with water (3 x 100 ml). The ether solution was dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was crystallised from acetone-pet ether (40-60°) to obtain white crystals of 17β-acetoxy-4-oxa-5α-androstan-3-one (232).

M.P. 152-4°C

Yield: 1.35g (44%)

IR ν max 2940, 2865, 1735 (C=O), 1450, 1403, 1388, 1376, 1363, 1255, 1235, 1212, 1201, 1143, 1121, 1083, 1060, 1049, 1042, 1025, 1017, 963, 950, 924, 910, 874, 845, 815, 803, 788, 752,

NMR ($CDCl_3$) τ 9.27 (S, 3H, C_{18}-CH_3), 9.09, (S, 3H, C_{19}-CH_3)
8.01 (S, 3H, C_{17}β - O-C(=O)-CH_3), 7.44 (m, 4H, C_2 and C_{16}-H),
6.10 (q, 1H, C_5-αH, J = 4.4 and 10.4 Hz), 5.50
(t, 1H, C_{17}αH, J = 8.0 Hz)

13. PREPARATION OF 4-OXA 5-α- ANDROSTAN-17β-OL-3-ONE (233)[295]

17β-Acetoxy-4-oxa-5α-androstan-3-one (232, 1.47g; 4.37 mM) in methanol (58.4 ml) and water (7.3 ml) was saponified by refluxing with 10% methanolic potassium hydroxide (8.03 ml) for one hour. Then the solution was acidified with 3N hydrochloric acid (8.03 ml), diluted with water (73 ml) and heated on a steam-bath for one hour in a current of nitrogen.

The reaction mixture was allowed to cool to room temperature and extracted with ether (3 x 100 ml). The combined ether extract was washed once with water (50 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was crystallised from acetone/pet-ether (60-80°C) to obtain white crystals of 4-oxa-5 - androstan-17β-ol-3-one (233).

M.P. 173-175°C (Lit. M.P.[264] 178-180°C)

Yield: 986.0mg (77%)

IR ν max 3700 - 3100 (OH), 2945, 2875, 1720 (C=O), 1451, 1411, 1397, 1386, 1350, 1318, 1274, 1260, 1232, 1210, 1173, 1140, 1120, 1080, 1051, 1010, 958, 910, 901, 868, 835, 800, 743,

NMR ($CDCl_3$) τ 9.27 (S, 3H, C_{18}-CH_3), 9.08 (S, 3H, C_{19}-CH_3), 7.97 (bs, 1H, C_{17} -OH), 7.39 (dd, 2H, C_2-2H, J = 6.2 Hz), 6.34 (t, 1H, C_{17}α-H, J = 8.4 Hz), 6.03 (q, 1H, C_5α-H, J = 4.4 and 12.0 Hz).

14. PREPARATION OF 2-HYDROXYMETHYLENE-4-OXA-5 α-ANDROSTAN-17β-OL -3-ONE (235)[160].

Sodium hydride (1.574g; 32.8 mM; 50% dispersion) was weighed into a 250 ml three-necked round bottom flask and washed with dry benzene followed by dry ether with nitrogen passing through the reaction flask. To a suspension of sodium hydride in ether (150 ml), 4-oxa-5 α-androstan-17β-ol-3-one (233, 480.0 mg; 1.64 mM) was added with stirring. After five hours of stirring, distilled ethyl formate (2.34g; 32.8 mM) was added and the reaction mixture was allowed to stir at room temperature under a nitrogen atmosphere.

When the reaction was complete (as observed on T.L.C.), after about 90 hours, the reaction mixture was acidified carefully with dilute hydrochloric acid. The ether solution was washed with water (3 x 50 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was crystallised from acetone-pet ether (40-60^{0}) to obtain white crystals of 2-hydroxymethylene-4-oxa-5 α-androstan-17β-ol-3-one (235).

M.P. 221-3^{0}C

Yield: 458.0 mg, 87%

IR ν max 3420 (OH), 3700-2400 (H bonded OH), 2950, 2940, 2860, 1706 (C=O) 1612 (C=C), 1446, 1385, 1334, 1276, 1208, 1168, 1134, 1101, 1016, 945, 931, 760, 665,

UV λ max (EtOH) 252.3 nm (ϵ 7863)

$(\alpha)_D$ (+) 94.87 (C, 0.18)

Acc. Mass. Expected: 320.1987

Found: 320.1986 (M, $C_{19}H_{28}O_4$)

NMR τ 9.25 (S, 3H, C_{18}-CH_3), 9.11 (S, 3H, C_{19}-CH_3)

6.40 (t, 1H, $C_{17}\alpha$-H, J = 8.0 Hz), 6.11 (q, 1H, C_5 H, J = 5.0 and 11.0 Hz), 2.78 and 2.16 (bs, 1H, Z- and E- HO-CH=CH-)

15. PREPARATION OF 2 N,N-DIETHYLAMINO METHYLENE-4-OXA-5 α-ANDROSTAN-17β-OL-3-ONE (236).

To a solution of 2-hydroxymethylene-4-oxa-5 α-androstan-17β-ol-3-one (235; 450.0 mg; 1.41 mM) in dry benzene (150 ml), distilled diethylamine (2.06g; 28.2mM) was added and the reaction mixture was heated under reflux employing a Dean-Stark trap. When the reaction was complete (as observed on T.L.C.), after about 85 hours, the solution was evaporated to dryness (Yield: 490.0 mg (93.0%). The crude product was crystallised from acetone/pet-ether (60-80^{0}) to obtain light yellowish crystals of 2-N,N-diethylamino methylene-4-oxa-5α -androstan-17b-ol-3-one (236).

M.P. 121-3^{0}C

Yield: 490.0 mg (93.0%)

IR ν max 3420 (OH), 2980, 2950, 2880, 2860, 1670 (C=O), 1570 (C=C), 1471, 1449, 1385, 1355, 1330, 1252, 1192, 1168, 1134, 1097, 1088, 1045, 1030, 949, 927, 885, 857, 825, 819, 767, 753,

NMR ($CDCl_3$) τ 9.23 (S, 3H, C_{18}-CH_3), 9.05 (S, 3H, C_{19}-CH_3), 8.78 (t, 6H, 2(-N-CH_2-$C\underline{H}_3$, J = 7.4 Hz), 7.68 (q, 2H, C_1-2H, J = 14.2 Hz), 6.64 (q, 4H, 2(N-$C\underline{H}_2$-CH_3, J= 8.8Hz), 6.32 (t, 1H, $C_{17}\alpha$-H) 6.04 (q, 1H, $C_5\alpha$-H, J = 5.0 and 11.0H) 2.83 and 2.30 (bs, 1H, Z- and E-$(CH_3CH_2)_2NC\underline{H}$= C)

UV. λ max (EtOH) 300-1nm (ε 1.3171 x 10^4)

$(\alpha)_D$ (+) 41.74 (C, o.11)

Acc. Mass. Calculated: 375.2774

Found: 375.2771 (M, $C_{23}H_{37}N_1O_3$)

16. PREPARATION OF 2-METHYLENE-4-OXA-5α-ANDROSTAN-17β-OL-3-ONE[238]

2-N,N-Diethylamino methylene-4-oxa-5α-androstan-17β-ol-3-one (236, 530 mg; 1.41 mM) was taken up in glacial acetic acid (40.0 ml) and hydrogenated by using the Adam's catalyst-Platinum oxide (75.0 mg). The hydrogenated product was filtered to remove the catalyst and evaporated to dryness.

The residue, saturated product (237) was dissolved in glacial acetic acid (40.0 ml) and sodium acetate (726.0 mg) was added. The reaction mixture was refluxed for four hours. The solution was concentrated and treated with a saturated solution of sodium hydrogen carbonate until there was no evolution of carbon dioxide. The aqueous solution was extracted with ether (3 x 100 ml). The combined ether extract was washed with water (3 x 50 ml), dried over magnesium sulphate and evaporated to dryness.

The crude product (407.0 mg) appeared to be a mixture of two compounds. The crude product was separated on preparative thin-layer-chromatography (solvent system; chloroform/methanol; 85/15). The crude compound, with higher Rf value, was crystallised from aqueous methanol to obtain white crystals of -17β-acetoxy-2-methylene-4-oxa-5α-androstan-3-one (239).

M.P. 141-3°C

Yield 147.0 mg, 30%

$[\alpha]_D$ (+) 72.35 (C, 0.11)

Acc. Mass Found: 346.2147 (M, $C_{21}H_{30}O_4$)

Calculated: 346.2144.

IR ν max 2965, 2940, 2880, 2845, 1735 (C=O), Acetate), 1723 (C=O) 1625 (C=C), 1460, 1437, 1393, 1369, 1355, 1315, 1303, 1285, 1246, 1220, 1181, 1141, 1117, 1079, 1027, 999, 948, 917, 879, 853, 832, 755, 660.

NMR ($CDCl_3$) τ 9.18 (S, 3H, C_{18}-CH_3), 9.07 (S, 3H, C_{19}-CH_3), 7.92 (S, 3H, C_{17} -OC(=O)-CH_3), 7.52 (q, 2H, C_1-2H, J = 16.0 H_Z), 5.94 (q, 1H, $C_5\alpha$-H, J = 11.0 and 4.0 H_Z), 5.36 (t, 1H, $C_{17}\alpha$ -H, J = 8.0 H_Z), 4.39 (m, 1H, O - C(=O) - C = C(H)-H), 3.48 (m, 1H, O - C(=O) - C = C(H) -H)

UV λ max 214-5mm (ϵ 7936)

ANALYSIS;EXPECTED: C, 71.80; H, 8.73; FOUND: C, 72.45; H, 8.9.

The more polar of the two compounds was crystallised from aqueous methanol to obtain white crystals of 2-methylene-4-oxa-5 -androstan-17β-ol-3-one.

M.P. 144-6°C

Yield: 120.0 mg 28%

Acc. Mass. Found: 304.2071 (M, C_{19} H_{28} O_3)

Calculated: 304.2079

IR ν max 3440 (OH), 3015, 2955, 2930, 2855, 1712 (C=O), 1623 (C=C), 1460, 1445, 1375, 1317, 1280, 1215, 1189, 1145, 1040, 1026, 950, 808, 664.

NMR ($CDCl_3$) τ 9.23 (S, 3H, C_{18}-CH_3), 9.07 (S, 3H, C_{19}-CH_3), 7.53 (q, 2H, C_1-2H, J = 15.0 H_Z), 6.34 (t, 1H, $C_{17}\alpha$-H, J = 8.0 Hz), 5.88 (q, 1H, $C_5\alpha$-H, J = 11.0 and 4.0 H_Z), 4.42 (m, 1H, O-C(=O)-C=C(H)-H), 3.51 (m, 1H, O-C(=O)-C=C(H)-H).

UV λ max 214-5 nm (ϵ 6680).

17. REACTION OF L-CYSTINE WITH 17β-ACETOXY-4-METHYLENE-4-OXA-5α-ANDROSTAN-3-ONE (239)[85]

17β-Acetoxy-2-methylene-4-oxa-5α-androstan-3-one (239, 57.0 mg; 0.16 mM) and L-cystine (29.0 mg; 0.16 mM) were dissolved in 60% aqueous ethanol (7.5 ml) and heated over water-bath for one hour.

The reaction mixture was allowed to cool to room temperature. The light-yellowish crystals were filtered, washed thoroughly with 60% aqueous ethanol and dried. Recrystallisation from aqueous ethanol gave white crystals of the cysteine-adduct (240).

M.P. 168-71°C

Yield: 53.0 mg, (69%)

IRνmax 3700-2300 (COOH and NH_2), 2940, 2870, 1735 (C=O of carboxylic acid and lactone), 1621 (COO^-), 1586, 1489, 1412, 1385, 1341, 1300, 1249, 1196, 1129, 1091, 1046, 967, 878, 852, 783, 676.

MS $[M-C_3H_7O_2NS]^+ = 346$

18. PREPARATION OF 3β-ACETOXY-ANDROST-5-EN- 17-ONE (242)

To a solution of androst-5-en-3β-ol-17-one (241), 10.0g; 0.03M) in pyridine (100 ml), acetic anhydride (100 ml) was added with stirring. The solution was stirred for two hours and left to stand overnight at room temperature. The reaction mixture was poured onto ice-water and extracted with ether (3 x 100 ml). The combined ether extract was washed with ice-cold water (3 x 100 ml), dilute hydrochloric acid (3 x 50 ml), followed by water (3 x 50 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was crystallised from methanol to obtain white crystals of 3β-acetoxy-androst-5-en-17-one (242).

M.P. 169-171°C (Lit[331] M.P. 171-173°C)

Yield: 9.74g (85%)

I.R. ν max 3040, 2965, 2875, 1740 (C=O), 1475, 1448, 1415, 1384, 1373, 1259, 1228, 1148, 1069, 1039, 1020, 965, 915, 855, 841, 822, 810, 690, 676,

NMR ($CDCl_3$) τ 9.08 (S, 3H, C_{18}-CH_3), 8.92 (S, 3H, C_{19}-CH_3), 7.94 (S, 3H, C_3-O-$\overset{O}{\overset{\|}{C}}$-$CH_3$) 7.65 (m, 4H, C_2, C_{16}-H) 5.35 (m, 1H, C_3-αH), 4.55 (d, 1H, C_6-H, 5.0 H_Z)

19. PREPARATION OF 3β-ACETOXY-5α-ANDROSTAN-17-ONE (243)

To a solution of 3β-acetoxy-androst-5-en-17-one (242, 5.0g) in glacial acetic acid (200 ml), 10% palladium charcoal (200 mg) was added. The solution was hydrogenated until there was no more uptake of hydrogen as observed from the manometer. The reaction mixture was filtered, concentrated to half of its original volume and poured onto ice-water. The product was extracted with ether (3 x 100 ml). The combined ether extract was washed with saturated sodium hydrogen carbonate solution (3 x 100 ml) followed by water (2 x 100 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was crystallised from aqueous methanol to yield white crystals of 3β-acetoxy-5α-androstan-17-one (243).

M.P. 94-95°C (Lit. M.P[331] 96-97°C)

Yield: 4.45g (88%)

I.R ν max 2940, 2860, 1730 (C=O), 1480, 1460, 1410, 1380, 1260, 1160, 1140, 1110, 1065, 1030, 990, 975, 960, 910, 900.

NMR ($CDCl_3$) τ 9.13 (S, 6H, $C_{18,19}$,-CH_3), 7.96 (S, 3H, C_3 -O-C(=O)-CH_3) 5.30 (m, 1H, C_3 α-H).

20. BAEYER-VILLIGER OXIDATION[295] OF 3β-ACETOXY-5α-ANDROSTAN-17-ONE (243).

A solution of 3β-acetoxy-5α-androstan-17-one (243, 500 mg; 1.55mM) in glacial acetic acid (4.0 ml) and 28% aqueous hydrogen peroxide (3.0 ml) was allowed to stand in the dark at 35°C for 53 hours. A large volume of water was added dropwise until crystallisation began to precipitate. The precipitate was extracted with ether (3 x 100 ml) and the combined ether extract was washed with saturated sodium hydrogen carbonate solution (2 x 100 ml), followed by water (2 x 100 ml). The ethereal solution was dried over magnesium sulphate and evaporated to dryness. The crude product was crystallised from benzene-pet ether (60-80°C) to obtain white crystals of 3β-acetoxy-17-oxo-17a-oxa-D-homo-5α-androstane (244).

M.P. 155-157°C (Lit. M.P.[295] 158-159.5°C)

Yield: 340.0 mg (65%)

IR ν max 2960, 2870, 1730 (C=O), 1460, 1386, 1364, 1332, 1303, 1283, 1244, 1225, 1171, 1153, 1137, 1122, 1105, 1090, 1066, 1032, 994, 974, 960, 948, 915, 897, 867, 855, 824, 808, 732, 700, 670.

NMR ($CDCl_3$) τ 9.21 (S, 3H, C_{19}-CH_3), 8.70 (S, 3H, C_{18}-CH_3), 7.97 (S, 3H, C_3-O-C(=O)-CH_3) 7.36 (m, 2H, C_{16}-CH_2), 5.30 (m, 1H, C_3-α H).

21. PREPARATION OF 3β-HYDROXY-17-OXO-17a-OXA-D-HOMO-5α-ANDROSTANE (251)[295]

A solution of 3β-acetoxy-17-oxo-17a-oxa-D-homo-5α-androstane (244, 3.07g; 10.0 mM)in methanol (124 ml) and water (15.5 ml) was saponified by refluxing for one hour with 10% methanolic-potassium hydroxide (17.5 ml). The reaction mixture was acidified with 3N hydrochloric acid (17.5 ml), diluted with water (155 ml) and heated on a water-bath for one hour in a current of nitrogen.

The reaction mixture, on cooling, was extracted with ether (3 x 100 ml) and the combined ether extract was washed with water (2 x 50 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was crystallised from ethyl acetate-pet ether (60-80°) to yield white crystals of 3β-hydroxy-17-oxo-17a-oxa-D-homo-5α-androstane (251).

M.P. 168-169°C (Lit M.P.[295] 169.7 - 169.9°C).

Yield: 2.21g (82%)

IR. ν max: 3420 (OH), 2940, 2865, 1723 (C=O), 1455, 1389, 1367, 1305, 1218, 1170, 1153, 1110, 1087, 1064, 1045, 990, 963, 956, 944, 852, 665,

NMR ($CDCl_3$) τ 9.20 (S, 3H, C_{19}-CH_3) , 8.68 (S, 3H, C_{18}-CH_3), 7.36 (m, 2H, C_{16}-CH_2), 6.35 (m, 1H, C_3 -α H).

22. PREPARATION OF 3β-HYDROXY-16-HYDROXYMETHYL-17-OXO-17A-OXA D-HOMO-5α-ANDROSTANE (256)[203A]

Nitrogen gas was passed through tetrahydrofuran (THF, - 100 ml) for about 30 minutes at -78°C (acetone/dry CO_2). Diisopropylamine (1.93g, 19.3 mM) was added with stirring and after fifteen minutes, butyllithium (3.0 ml, 2.0M,) was added via a dry syringe. The solution was left at -78°C for one hour to equilibrate.

To the solution of lithium diisopropylamide at -78°C, was added very slowly a solution of 3β hydroxy-17-oxo-17a-oxa-D-homo-5α-androstane (251, 175.0 mg; 0.57 mM) in THF (5.0 ml) under nitrogen. After thirty minutes, the temperature of the solution was raised to -20°C (carbon tetrachloride/dry CO_2) and treated with formaldehyde vapours (prepared by heating paraformaldehyde (3.0g, Xs) to 180°C and vapours were carried by a nitrogen gas flow into the reaction mixture). After all the paraformaldehyde had been consumed, stirring was continued for further thirty minutes. The reaction was quenched by the addition of 10% hydrochloric acid and extracted with ether (3 x 100 ml). The combined ether extract was washed with saturated sodium chloride (3 x 50 ml) followed by water (3 x 50 ml), dried over magnesium sulphate and evaporated to dryness. The crude product (265.0 mg) was separated on silica preparative plate (solvent system, chloroform/methanol, 97/3) and obtained two fractions. The non-polar fraction (136.0 mg; 78%) was the starting lactone as concluded from T.L.C. and spectroscopic data.

The polar compound was shown to be the α-hydroxymethyl lactone, 3β-hydroxy-16-hydroxymethyl-17-oxo-17a-oxa-D-homo-5α-androstane (256).

Yield = 34.0 mg; (18.0%)

I.R ν_{max} = 3430 (OH), 2945, 2875, 1725 (C=O), 1460, 1391, 1370, 1355, 1338, 1309, 1288, 1225, 1178, 1158, 1120, 1092, 1070, 1050, 994, 962, 950. 911, 899, 805, 673.

$(\alpha)_D$ (-) 26.37 (c, 0.06)

NMR τ 9.21 (S, 3H, C_{14}-CH_3), 8.64 (S, 3H, C_{18}-CH_3), 6.36 (d, 2H, C_{16}-CH_2 -OH, J = 8.0 Hz), 6.04 - 6.52 (m, 1H, C_3 α-H).

Mass Spectrum: $(M - 30)^+$ = 306.

Acc. Mass. Found ($M-H_2O)^+$ = 318.2222 (M, $C_{20}H_{30}O_3$)

Calculated: = 318.2195

Found: $(M - CH_2O)^{\dot{}+}$ = 306.2198 (M, $C_{19}H_{30}O_3$)

Calculated: = 306.2195

23. REACTION OF 3 β-HYDROXY-16-HYDROXYMETHYL-17-OXO-17a-OXA-D-HOMO-5 α-ANDROSTANE (256) WITH METHANE SULPHONYL CHLORIDE[203A].

3β-Hydroxy-16-hydroxymethyl-17-oxo-17a-oxa-D-homo-5 α-androstane (256, 81.0 mg; 0.24 mM) was taken up in pyridine (2.0 ml) and methanesulphonyl chloride (10 drops; 0.5 ml) added. The temperature of the reaction mixture was kept at -20°C (carbon tetrachloride/ dry carbon dioxide) for ten hours and then at 0°C overnight.

The reaction mixture was poured over ice-water and extracted with ether (4 x 50 ml). The combined ether extract was washed with ice-cold water (3 x 50 ml), dried over anhydrous magnesium sulphate and evaporated to dryness to obtain 3β-hydroxy-16-hydroxy-methyl-17-oxo-17a-oxa-D-homo-5 α-androstan-3, 16-dimethane sulphonate (258).

Yield: 97.4 mg, 82%

IR ν max. 2042, 2865, 1724, (C=O), 1456, 1355 (- SO_2-O), 1235, 1176 (- SO_2 O),

NMR τ 9.18 (S, 3H, C_{19}-CH_3), 8.64 (S, 3H, C_{18}-CH_3), 6.98, 6.92 (S, 6H, C_3 and C_{16} - O - SO_2 CH_3), 5.08 - 5.78 (m, 3H, C_3 α-H, C_{16}-CH_2 - O - Ms)

$(\alpha)_D$ (-) 13.07 (C, 0.1).

24. PREPARATION OF 16-METHYLENE-17-OXO-17a-OXA-D-HOMO-5α-ANDROST-2-ENE (259)[203A].

A solution of 3β-hydroxy-16-hydroxymethyl-17-oxo-17a-oxa-D-homo-5α-androstan-3b-16-dimethanesulphonate (258, 101.7 mg; 0.21mM) in pyridine (2.0 ml) was heated under reflux for 6 hours. (The reaction was followed by T.L.C. for the disappearance of the starting material, 258). The reaction mixture, on cooling, was poured over ice-water and extracted with ether (3 x 15 ml). The combined ethereal extract was washed with water (3 x 15 ml), 5% hydrochloric acid (3 x 15 ml) and water (3 x 15 ml), dried over magnesium sulphate and evaporated to dryness. The crude product was separated on preparative T.L.C. (Solvent system, ether/Pet ether; 25/75, 2 runs) to obtain 16-methylene-17-oxo-17a-oxa-D-homo-5α-androst-2-ene (259).

Yield: 17.5 mg, 28.3 %

I.R νmax 3030, 2925, 2855, 1709, (C=O), 1620 (C=C), 1597, 1543, 1521, 1460, 1455, 1381, 1344, 1202, 1106, 980, 951, 925, 872, 851, 780, 723, 661.

NMR τ 9.28 (S, 3H, C_{19}-CH_3), 8.71 (S, 3H, C_{18}-CH_3), 4.51 (m, 3H, $C_{2,3}$-H and -O-C(=O)-C = C(H)-H), 3.60 (m , 1H, -O-C(=O)-C = C(H) - H)

U.V λ max 213-4 nm (ϵ 5939)

Mass Spec. M^+ = 300

25. REACTION OF 3β-HYDROXY-17-OXO-17a-OXA-D-HOMO-5α-ANDROSTANE (251) WITH FORMALDEHYDE[300].

A solution of 3β-hydroxy-17-oxo-17a-oxa-D-homo-5α-androstane (251, 53.0 mg; 0.16 mM) and paraformaldehyde (30.0 mg; 0.8 mM) and potassium hydroxide (10.0 mg) in ethanol (1.0 ml) - water (0.5 ml) were mixed, refluxed for ten minutes and diluted with water (3.0 ml). After standing it overnight at room temperature, the reaction mixture was cooled to 10°C, acidified with dilute hydrochloric acid and diluted with water (5.0 ml). The reaction mixture was extracted with ether (3 x 20 ml) and the combined ether extract was washed with water (10 ml), dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was separated on silica preparative plate (solvent system, ether/chloroform; 80/20) and obtained two products. The polar material (18.0 mg, 34%) was the starting lactone as observed on T.L.C. and spectroscopic data. The non-polar fraction was crystallised from methanol and obtained white crystals of 3β-hydroxy-16-methylene-17-oxo-17a-oxa-D-homo-5α-androstane (252).

M.P. 259-60°C

Yield: 15.0 mg (27%)

IR ν max 3420 (OH), 3030, 2965, 2875, 1715, (C=O), 1631 (C=C), 1455, 1389, 1310, 1220, 1169, 1155, 1115, 1089, 1065, 1045, 989, 958, 945, 907, 853, 668.

NMR $(CDCl_3)$ τ 9.22 (S, 3H, C_{19}-CH_3), 8.71 (S, 3H, C_{18}-CH_3)
4.37, (m, 1H, C_3-α H), 4.30 (m, 1H, O-C(=O)-C = C(H)-H)
3.52 (m, 1H, O-C(=O)-C = C(H)-H)

$(\alpha)_D$ + 89.0 (C, 0.04)

UV λ max 214-5 nm (ε 3760)

Acc. Mass. Found 318.2241, (M, $C_{20} H_{30} O_3$)

Calculated: 318.2238

26. MANNICH REACTION WITH 3 β-HYDROXY-17-OXO-17a-OXA-HOMO-5 α-ANDROSTANE (251) [305].

To a mixture of 3β-hydroxy-17-oxo-17a-oxa-D-homo-5 α-androstane (251, 136.0 mg; 0.44 mM) and 37% aqueous formaldehyde (0.5 ml, 4.84 mM) was added a solution containing diethylamine (150 mg; 2.05 mM), diethylamine hydrochloride (170.0 mg, 1.55 mM) and dioxane (10 ml). The resulting mixture was refluxed for 8 hours under nitrogen atmosphere. Then sodium hydroxide (2N) was added until basic and the reaction mixture was extracted with benzene (3 x 25 ml). The combined benzene extract was washed with saturated sodium chloride solution, dried over anhydrous magnesium sulphate and evaporated to dryness. The crúde product was separated on silica preparative plate in ether/chloroform (80/20; 2 runs). The polar fraction (45.0 mg) was the starting lactone as observed on T.L.C. and by spectroscopic data. The non-polar fraction was crystallised from methanol to obtain white crystals of 3β-hydroxy-16-methylene-17-oxo-17a-oxa-D-homo-5 α-androstane (255).

M.P. 259-60°C

Yield: 38.0 mg (27%)

IR ν max 3420 (OH), 3030, 2965, 2875, 1715 (C=O), 1631 (C=C), 1455, 1389, 1310, 1220, 1169, 1155, 1115, 1089, 1065, 1045, 989, 958, 945, 907, 853, 668,

NMR ($CDCl_3$) τ 9.22/S, 3H, $C_{19}-CH_3$), 8.71 (S, 3H, $C_{18}-CH_3$), 4.37 (m, 1H, C_3α-H), 4.3 (m, 1H, -O-C(=O)-C = C(H), -H), 3.92 (m, 1H, - O-C(=O)-C = C(H)-H)

UV λ_{max} 214-5 nm (ϵ 3760)

Acc. Mass. Found: 318.2241 (M, $C_{20}H_{30}O_3$)

Calculated: = 318.2238

$[\alpha]_D$ + 89.0, C, (0.04).

27. PREPARATION OF 3-PHENYL-4-OXA-5 α-CHOLEST-2-ENE (278)[313]

Dry magnesium (2.25g, 93.8 mM), (Magnesium turnings washed with dry ether and dried in an oven) was added to a three-necked flask fitted with a condenser and nitrogen passing through the flask. Dry ether (250 ml) was added and the solution stirred.

In order for the reaction to proceed a crystal of iodine was added to the reaction mixture followed by a few drops of bromobenzene (180.3g, 115.0 mM), and the solution was warmed to reflux over a water-bath. Once the reaction mixture had started refluxing, water-bath was removed and bromobenzene was added at such a rate that the reaction mixture was under reflux all the time.

After 15 minutes of the addition of bromobenzene, the ether-benzene solution of 4-oxa-5 α-chlostan-3-one (203, 3.20g, 8.25mM) was added dropwise with stirring. The reaction mixture was allowed to stir overnight at room temperature. The reaction was worked up by the dropwise addition of saturated ammonium chloride solution (13.8 ml). The clear ether layer was decanted and the precipitated magnesium salts were washed with ether (3 x 100 ml). The combined ether washings were dried over anhydrous magnesium sulphate and evaporated to dryness. Reddish-orange oily residue was crystallised from methanol to yield light-yellow crystals of 3-phenyl-4-oxa-5 α-cholest-2-ene (278).

M.P. 123 - 125 $^{\circ}$C

Yield: 1.84 g (50%)

I.R. 2960, 2860, 1655 (C =C), 1602, 1580, 1497, 1468, 1445, 1380, 1365, 1335, 1320, 1306, 1280, 1260, 1209, 1161, 1128, 1080, 1071, 1017, 995, 973, 940, 930, 910, 900, 880, 861, 750, 715, 690, 640.

NMR: τ 9.33 (S, 3H, C_{18}-CH_3), 9.12 (S, 3H, C_{19}-CH_3),

8.03 (d, 2H, C_1-2H, J = 4.4Hz), 6.43 (q, 1H, C_5 α-H, J = 4.2, 11.0 H_Z), 4.71 (t, 1H, C_2-H, J = 4.0H_Z), 2.3 -2.82 (m, 5H, C_3-Aromatic H).

$(\alpha)_D$ (+) 91.70 (C, 0.10)

Acc. Mass: Calculated: 448.3705 (M, $C_{32}H_{48}O$)

Measured: 448. 3702

U.V. λ max 264 nm (ε 9250).

28. REACTION OF 3-PHENYL-4-OXA-5α-CHOLEST-2-ENE (278) WITH MONOPERPHTHALIC ACID.

3-Phenyl-4-oxa-5α-cholest-2-ene (278, 1.16g, 2.59mM) was taken up in dry ether (150 ml) and monoperphthalic acid[316] (25 ml, 0.51M, 13.0mM, 5XS) was added. The reaction mixture was stirred at room temperature for 48 hours. The solution was filtered and the precipitate washed with dry ether (2 x 50 ml). The combined ether solution was washed with saturated sodium hydrogen carbonate solution (3 x 50 ml), dried over magnesium sulphate and evaporated to dryness. The crude product (1.24g), a mixture as appeared on T.L.C. (ether/ pet ether 40-60) (50/50), was plated. The major fraction was crystallised from acetone/pet-ether (40-60) to obtain white crystals of 3-hydroxy-3-phenyl-4-oxa-5α-cholestan-2-one (281).

M.P. 170-172°C

Yield: 659 mg (53%)

I.R.: 3300 (OH), 2940, 2875, 1730 (C =O) 1585, 1468, 1448, 1382, 1379, 1368, 1315, 1272, 1160, 1118, 1080, 1030, 1001, 947, 914, 860, 697.

NMR: τ 3β-HYDROXY EPIMER 9.37 (S, 3H, C_{18}-CH_3), 9.17 (S, 3H C_{19}-CH_3) 7.53 (AB q, 2H, C-2H, J = 13.6 Hz).
6.45 (q, 1H, C_5-α-H, J = 4.6, 10.4Hz), 2.51 (m, 5H, C_3-Aromatic H).
3α -HYDROXY EPIMER: 9.33 (S, 3H, C_{18}-CH_3), 9.11 (S, 3H, C_{19}-CH_3) 7.43 (ABq, 2H, C_1-2H, J = 14.0 Hz), 5.7 (q, 1H, C_5α- H, J=4.8,10.4Hz) 2.51 (m, 5H, C_3-Aromatic H).

$(\alpha)_D$ (+) 85.2 (c, 0.08).

29. REACTION OF 3-HYDROXY-3-PHENYL-4-OXA-5 α-CHOLESTAN -2-ONE (3) WITH ACETYLCHLORIDE/ETHANOL.

3-Hydroxy-3-phenyl-4-oxa-5 α-Cholestan-2-one (281, 66.5 mg; 0.14 mM) was taken up in ethanol (10 ml) and acetyl chloride/ ethanol solution (10 drops) was added. The reaction mixture was left standing at room temperature for 48 hours and then the solvent was evaporated to dryness. The crude product was crystallised from ethanol to obtain white cyrstals of 3-ethoxy-3-phenyl-4-oxa-5 α-cholestan-2-one (282).

M.P. 126 - 128°C

Yield: 46.0 mg (65%)

I.R: 2945, 2875, 1730 (C=O), 1605, 1468, 1450, 1385, 1369, 1317, 1279, 1177, 1160, 1119, 1087, 1071, 1029, 1005, 984, 950, 932, 920, 700

NMR: τ 9.32 (S, 3H, C_{18}-CH_3), 9.04 (S, 3H, C_{19}-CH_3) 8.80 (t, 3H, -O-CH_2-CH_3, J = 7.0Hz), 7.42 (q, 2H, C_1-2H J = 14.0Hz), 6.68 (overlapping quartets-diastereotopic -O-CH_2-CH_3) 5.96 (q, 1H, $C_5\alpha$-H, J = 5.0, 10.0 Hz)2.56 (m, 5H, C_3-Aromatic-H)

$(\alpha)_D$ (+) 183.69 (c, 0.22)

Acc. Mass: Calculated = 508.3917 (M, $C_{45}H_{52}O_3$)

Found = 508.3890

30. REACTION OF 3-HYDROXY-3-PHENYL-4-OXA-5 α-CHOLESTAN-2-ONE (281) WITH LEAD TETRA ACETATE[409].

Lead tetra-acetate paste was washed with acetic acid followed by dry benzene. The crystals were dried by employing suction pump.

Lead tetra-acetate crystals (130.0 mg; 0.29 mM) were added to a rapidly stirring solution of 3-hydroxy-3-phenyl-4-oxa-5 α-cholestan-2-one (281, 130 mg, 0.27 mM) in the dry benzene (30 ml). The reaction mixture was stirred for five hours at room temperature. The solution was filtered to remove inorganic salts and washed with dry benzene. The filtrate was evaporated to dryness. The residue was plated (silica, solvent: ether/pet-ether 40-60; 50/50) to obtain the major product which was crystallised from ether/pet-ether 40-60, giving white crystals of 3,4-seco 5β-benzoate, 5 α-cholestan-2-carboxylic acid (299).

M.P. 220-222°C

Yield 86.5 mg (64.4%)

I.R: 3640 - 2600 (OH) 2950, 2870, 1720, (C=O) 1700, 1600, 1580, 1467, 1449, 1381, 1315, 1270, 1173, 1149, 1109, 1068, 1024, 972, 930, 905, 805, 710, 683, 677.

NMR τ 9.32 (S, 3H, C_{18}-CH_3), 9 08 (S, 3H, C_{19}-CH_3), 7.68 (ABq, 2H, C_1-2H, J=14.0 H_Z), 4.91 (q, 1H, C_5-α-H, J=4.0, 11.0H_Z) 1.82 - 2.60 (m, 5H, Aromatic H).

$(\alpha)_D$ (-) 25.1 (c, 0.09)

$(M - C_6H_5 COOH)^+ = 374$

31. REACTION OF 3,4-SECO-5β- BENZOATE-5x-CHOLESTAN-2-CARBOXYLIC ACID (299) WITH DIAZOMETHANE.

3,4-Seco-5β-benzoate-5 α-cholestan-2 -carboxylic acid (299), 29.0 mg; 0.06 mM) was taken up in dry ether and diazomethane was added dropwise until there was a slight excess of diazomethane[410] as observed by the presence of a yellow colour of the reaction mixture. The solution was left overnight in a fume-cupboard and then the colourless solution was evaporated to dryness. The crude product (29.6 mg) was crystallised from methanol giving white crystals of 3,4-seco-5β-benzoate-5 α-cholestan-2-methoxy carboxylic acid (300).

M.P. 109-111°C

Yield: 23.5 mg (79%)

IR 2955, 2935, 2865, 2855, 1735, 1721 (C=O), 1600, 1580, 1466, 1451, 1440, 1392, 1381, 1363, 1329, 1313, 1271, 1228, 1217, 1191, 1173, 1140, 1115, 1096, 1068, 1024, 973, 932, 917, 902, 886, 807, 711, 679, 640.

NMR τ 9.32 (S, 3H, C_{18}-CH_3), 9.08 (S, 3H, C_{19}-CH_3), 7.6 (ABq, 2H, C_1-2H, J = 14.0 H_Z), 6.53 (S, 3H, Ester-CH_3), 4.97 (q, 1H, C_5α- H, J = 5.0, 11.0 H_Z), 1.84-2.61 (m, 5H, Aromatic-H)

$(\alpha)_D$ (-) 68.4 (C, 0.07)

Acc. Mass: Calculated: 510 • 3709 (M, $C_{33}H_{50}O_4$)

Found: 510.3724

32. PREPARATION OF - 3,4-SECO-2-CARBOXY-5 α-CHOLESTAN-5β-OL (301)

3,4-Seco-5β-benzoate-5 α-cholestan-2-carboxylic acid (299, 35.6 mg; 0.091mM) was taken up in methanol (10.0 ml) and 5% potassium-hydroxide-methanol (3.0 ml) was added. The reaction mixture was heated under reflux for thirty minutes. After cooling to room temperature, the reaction mixture was extracted with ether (3 x 15 ml), washed with water (2 x10 ml), dried over anhydrous magnesium sulphate and evaporated to dryness, to obtain 3,4-seco-2-carboxy-5 α-cholestan-5β-ol (301).

Yield: 25.5 mg (91%)

IR ν max 3600-2500 (OH), 2960, 2930, 2855, 1709 (C=O), 1461, 1442, 1380, 1313, 1300, 1195, 1155, 1120, 1079, 1021, 987, 949, 926, 907, 895, 669.

NMR τ 9.33 (S, 3H, C_{18}-CH_3), 9.05 (S, 3H, C_{19}-CH_3) 7.49 (ABq, 2H, G-2H, J = 14.0 H_Z), 6.29 (q, 1H, C_5-α H, J = 4.4, 11.0 H_Z) 5.20 (b m 1H, C_5-β-OH).

33. PREPARATION OF 3,NOR-4-OXA-5 α-CHOLESTAN-2-ONE (274)

A solution of 3,4-seco-2-carboxy-5 α-cholestan-5β-ol (301, 24.0 mg; 0.061 mM) and toluene p-sulphonic acid monohydrate[411] (1-2 mg) in benzene (25 ml) was heated under reflux with stirring under nitrogen for 15 minutes using a Dean-Stark azeotrope set-up. The solution was cooled in an ice-bath, washed with saturated sodium hydrogen carbonate solution (3 x 20 ml), followed by water (2 x 10 ml), dried over magnesium sulphate and evaporated to dryness. The crude product was crystallised from methanol to obtain white crystals of 3-nor-4-oxa-5 α-cholestan-2-one (274)

M.P. 104-106°C

Yield 17.5 mg (73%)

$(\alpha)_D$ (+) 25.1 (c, 0.04)

IR 2950, 2930, 2870, 2855, 1785, (C=O, γ-lactone), 1467, 1445, 1422, 1381, 1365, 1273, 1250, 1180, 1148, 1126, 1103, 1082, 1075, 1031, 1016, 1000, 982, 935, 925, 836, 695, 662.

NMR τ 9.33 (s, 3H, C_{18}-CH_3), 9.02 (S, 3H, C_{19}-CH_3)
7.73 (bs,,2H, C_1-2H), 6.15 (q, 1H, C_5-α H, J = 4.0, 11.0 Hz)

Mass spec. M^+ 374

Acc Mass. Found 374.3192 (M, $C_{25}H_{42}O_2$)
Calculated = 374. 3185

34. REACTION OF 4-OXA-5 α-CHOLESTAN-3-ONE(1) WITH p-METHOXY PHENYL-MAGNESIUM BROMIDE.

Dry magnesium (330.0 mg, 13.6 mM) was placed in a dry three-necked flask fitted with a condenser and nitrogen passing through the flask. Dry ether (150 ml) was added and the stirrer was set in motion.

In order for the reaction to proceed a crystal of iodine was added to the reaction mixture followed by a few drops of distilled p-bromo anisole (2.65g), 14.2 mM) and the solution was warmed to reflux on a water-bath. Once the reaction mixture had started refluxing, the water-bath was removed and 4-bromo anisole was added at such a rate that the reaction mixture was under reflux all the time.

After 15 minutes of the addition of p-bromo-anisole, the ether benzene solution of 4-oxa-5 α-cholestan-3-one (203, 385.0 mg; 1.0 mM) was added dropwise. The reaction mixture was stirred overnight at room temperature. The reaction was worked up by the dropwise addition of saturated ammonium chloride solution (1.7 ml). The clear ether layer was decanted and the precipitated magnesium salts were washed with ether (3 x 100 ml). The combined ether fractions was dried over magnesium sulphate and evaporated to dryness. The only residue was evaporated under vacuum to remove anisole. The crude product was plated (solvent, ether-pet-ether (40-60°); 10/90) to obtain three major products.

The first, non-polar fraction was crystallised from ethanol to obtain white crystals of 3-(4-methoxy phenyl)-4-oxa-5 α-cholest-2-ene (304).

M.P. 113- 114°C

Yield: 51.2 mg (10.8%)

IR ν max 2960, 2940, 2880, 2860, 1661 (C =C), 1616, 1580, 1515, 1470, 1446, 1383, 1368, 1339, 1321, 1306, 1267, 1250, 1166, 1113, 1087, 1045, 1000,977, 944, 932, 917, 885, 866, 839, 809, 795, 780, 770, 701, 669.

NMR τ 9.31 (S, 3H, C_{18}-CH_3), 9.11 (S, 3H, C_{19}-CH_3) 8.03 (d, 2H, C_1-H, J = 4.0 H_Z), 6.42 (q, 1H, C_5 -α H, J = 4.0, 11.0 H_Z) 6.18 (S, 3H, - O-CH_3), 4.92 (t, 1H, C_2-H, J = 4.0 H_Z), 2.48 - 3.15 (,4H, Aromatic H).

U.V. λ max 265 nm (ϵ 8118)

Acc. Mass: Found 478.3826 (M_1 C_{33} H_{50} O_2)

Calculated: 478.3810

$(\alpha)_D$ (+) 111.0 (C,0.34)

The second fraction (401.0 mg) was crystallised from ethanol to obtain white crystals of 3,3-(di - (4-methoxy phenyl)) - 4-oxa-5 α-cholestane (305).

M.P. 151-3°C

Yield: 215.0 mg (37%)

IR ν max 2960, 2935, 2875, 1611, 1585, 1512, 1466, 1445, 1386, 1368, 1309, 1276, 1250, 1178, 1079, 1039, 994, 959, 935, 889, 835, 812, 789, 669.

NMR τ 9.36 (S, 3H, C_{18}-CH_3), 8.93 (S, 3H, C_{19}-CH_3) 6.96 (q, 1H, C_5 -α H, J = 5.0, 10.0 H_Z), 6.17, 6.25 (S, 6H, 2X O-CH_3), 2.5 - 3.4 (m, 8H, Aromatic H).

Acc Mass: Expected: 586.4386 (M. C_{40} H_{58} O_3

Found: 586.4388

$(\alpha)_D$ = (+) 165.7 (c, 0.29)

The third more polar-fraction -3,4-seco-3,3-di-(4-methoxyphenyl,)-5 α cholest-2-en-5β-ol (306) was obtained as an oil.

Yield: 98.0 mg (17%)

IR ν max ($CHCl_3$) 3640 - 3200 (OH), 2960, 2945, 2875, 1609, 1575, 1515, 1467, 1445, 1418, 1386, 1369, 1290, 1250, 1182, 1175, 1112, 1085, 1040, 990, 913, 838, 810, 679,

NMR τ 9.39 (S, 3H, C_{18}-CH_3), 9.26 (S, 3H, C_{19}-CH_3), 6.64 (1, 1H, C_5 -α H, J = 3.6, 10.0Hz) 6.20, 6.27 (S, 6H, 2 x - O - CH_3), 4.09 (t, 1H, C_2-H, J = 8.0 Hz), 2.91 - 3.38 (m, 8H, Aromatic H)

$(\alpha)_D$ (+) 40.8 (C, 0.22).

35. HYDROGENOLYSIS OF 3,3 -DI- (4 -METHOXYPHENYL)) -4-OXA-5α-CHOLESTANE (305)

3,3-(Di (4 - methoxyphenyl)-4-oxa-5 α-cholestane (305, 43.3 mg, 0.074 mM) was taken up in ethyl acetate (10.0 ml) and concentrated hydrochloric acid (3.0 drops) and hydrogenated for 36 hours using 10% Palladium/Charcoal (13.0 mg) as a catalyst. The reaction mixture was filtered through high flow-super cel filter aid and washed with ethyl acetate. The solvent was evaporated to dryness. The crude product (46.4 mg) was separated on silica gel, preparative-plate, solvent system; ether/pet ether (50/50), to obtain three products.

The non-polar fraction was the starting material, 3,3-(di-(4-methoxyphenyl)-4-oxa-5 α-cholestane (305, 15.6 mg; 36.0%) as indicated by T.L.C. and spectral data.

The middle fraction was 3,4-seco-3,3-di-(4 -methoxyphenyl)) -cholestan-5-one (315).

Yield: 5.2 mg; 12.0%

IR νmax 2965, 2876, 1706 (C=O), 1609, 1582, 1510, 1469, 1441, 1383, 1379, 1365, 1334, 1303, 1250, 1175, 1169, 1161, 1117, 1040, 977, 953, 876, 830, 810, 670.

NMR τ 9.31 (S, 3H, C_{18}-CH_3), 9.01 (S, 3H, C_{19}-CH_3) 6.25 (S, 6H, 2 x - O - CH_3), 2.75 - 3.30 (m, 8H, Aromatic H).

The third more polar fraction was 3,4-seco-3,3-di (4-methoxyphenyl) - 5α-cholestan-5β-ol (314).

Yield: 18.3 mg; 42.0%

IR ν max 3640 - 3200 (OH), 2965, 2875, 1610, 1585, 1511, 1469, 1444, 1385, 1303, 1250, 1176, 1122, 1103, 1082, 1040, 986, 956, 933, 890, 875, 830, 810, 700, 668.

NMR τ 9.38 (S, 3H, C_{18}-CH_3), 9.28 (S, 3H, C_{19}-CH_3) 6.50 (q, 1H, C5-αH, J = 4.8, 11.2 H_Z), 6.23 (S, 6H, 2x - O CH_3), 2.76 - 3.31 (m, 8H, Aromatic H).

36. PREPARATION OF 3,4-SECO-3,3- Di (4 -METHOXYPHENYL)- 5α CHOLESTAN - 5β-OL (314)

3,4- Seco-3,3-di (4 -methoxyphenyl)-5 -cholest-2-en-5b-ol (306, 24.6 mg; 0.042 mM) was taken up in ethyl acetate (10 ml) and hydrogenated using palladium-charcoal (10%, 12.0 mg) until there was no more uptake of hydrogen. The solution was filtered and the flask was washed with ethyl acetate (20 ml). The combined ethyl acetate was evaporated to dryness to obtain 3,4-seco-3,3-di-(4-methoxyphenyl) - 5α-cholestan-5β-ol (314).

Yield: 20.7 mg (84%)

IR ν max: 3640 - 3200 (OH), 2965, 2875, 1610, 1585, 1511, 1469, 1444, 1385, 1303, 1250, 1176, 1122, 1103, 1082, 1040, 986, 956, 933, 890, 875, 830, 870, 700, 668.

NMR ($CDCl_3$) τ 9.37 (s, 3H, C_{18}-CH_3), 9.27 (S, 3H, C_{19}-CH_3), 6.50 (q, 1H, C 5α-H, J = 4.8, 11.2 Hz), 6.23 (S, 6H, 2X - O - CH_3), 2.76 - 3.31 (m, 8H, Aromatic H).

37. REACTION OF 3,4-SECO-3,3-Di (4 -METHOXYPHENYL) -5α-CHOLESTAN-5β-OL (306) WITH JONES' REAGENT.

3,4-Seco-3,3-di (4-methoxyphenyl)-5 -cholestan-5b-ol (306, 24.5 mg; 0.042 mM) was taken up in acetone (3.0 ml) and cooled to $0^{o}C$ (ice-water) followed by a dropwise addition of Jones' reagent (4 drops) with stirring. The reaction mixture was stirred for 5 minutes and washed up by adding water(5.0 ml) and extracted with ether (4 x 10 ml). The combined ether extract was washed with water (2 x 10 ml), dried over magnesium sulphate and evaporated to dryness to yield 3,4-seco-3,3-di-(4-methoxyphenyl)-cholestan-5-one (315).

Yield: 17.0 mg (70%)

IR 2965, 2876, 1706 (C=O), 1609, 1582, 1510, 1469, 1441, 1383, 1379, 1365, 1334, 1303, 1250, 1175, 1169, 1161, 1117, 1040, 977, 953, 876, 830, 810, 670.

NMR τ 9.31 (S, 3H, C_{18}-CH_3), 9.01 (S, 3H, C_{19}-CH_3),
6.25 (S, 6H, 2 (- O CH_3))
2.75 - 3.30 (m, 8H, Aromatic-H).

38. REACTION OF 3,4-SECO-3,3-Di (4-METHOXYPHENYL)-5 α-CHOLEST-2-EN-5β-OL (306) WITH TOLUENE-p-SULPHONIC ACID.

A solution of 3,4-seco-3,3-di (4-methoxyphenyl)-5 α-cholest-2-en-5β-ol (306, 4.7 mg; 0.008 mM) in benzene (5.0 ml) was heated under flux for two hours in the presence of toluene-p-sulphonic acid (1.0 mg). The reaction mixture was washed with water (2 x 5 ml) and the benzene layer evaporated to dryness to obtain 3,4-seco-3,3-di- (4-methoxy-phenyl)-cholestan-5-one (315).

Yield: 3.2 mg; 68%

NMR τ 9.31 (S, 3H, C_{18}-CH_3), 9.01 (S, 3H, C_{19}-CH_3),
6.25 (S, 6H, 2 (-O-CH_3),
2.75 - 3.30 (m, 8H, Aromatic-H).

IR 2965, 2876, 1706 (C=O), 1609, 1582, 1510, 1469, 1441, 1383, 1379, 1365, 1334, 1303, 1250, 1175, 1169, 1161, 1117, 1040, 977, 953, 876, 830, 810, 670.

39. REACTION OF 3,4-SECO-3,3-D$_1$ (4-METHOXYPHENYL)-CHOLESTAN-5-ONE (315) WITH HYDROXYLAMINE-HYDROCHLORIDE.

To a solution of 3,4-seco-3,3-di (4-methoxyphenyl)-cholestan-5-one (315, 17.0 mg; 0.029mM) in ethanol (3.0 ml) was added a solution of hydroxylamine-hydrochloride (10.4 mg; 0.149 mM; 5M xs) and sodium acetate (18.6 mg; 0.23 mM; 8 M xs) in water (2 drops) and diluted with ethanol (5 drops). The reaction was heated under reflux for 30 minutes, water (3 drops) added and allowed to cool.

A white precipitate, not crystalline, was extracted with ether (3 x 15 ml). The combined ether extract was washed with water (2 x 10 ml), dried over magnesium sulphate and evaporated to dryness. The crude product was crystallised from aqueous ethanol to obtain white crystals of 3,4-seco-3,3-di (4-methoxyphenyl)-cholestan-5-one-oxime (321).

M.P: 52-54°C

Yield: 6.8 mg; 39%

IR ν max ($CHCl_3$); 3620-2700 (OH, 4- bonded), 2940, 2875, 1609, 1585, 1510, 1465, 1443, 1379, 1303, 1250, 1218, 1177, 1118, 1040, 995, 951, 928, 852, 830, 810, 668.

NMR: τ 9.33 (S, 3H, C_{18}-CH_3) 9.03 (S, 3H, C_{19}-CH_3), 6.22 (S, 6H, 2X-O CH_3) , 2.63 - 3.30 (m, 8H, Aromatic H)

$(\alpha)_D$ + 10.6 (C, 0.09)

U.V. λ max 276-7 nm (ϵ = 4036), ((Me O-C_6H_4)-$_2$ CH_2; max 281.4 nm; ϵ = 4150)) [412].

(M- (MeO $C_6H_4)_2$ CH_2)+ = 373.

40. REACTION OF 3,4-SECO-3,3 (Di - (4-METHOXYPHENYL)- 5α-CHOLEST-2-EN-5β-OL (306) WITH 3,5-DINITROBENZOYL CHLORIDE.

3,4-Seco-3,3-di-(4-methoxyphenyl)5α-cholest-2-en-5β-ol (306, 22.6 mg; 0.05 mM) was taken up in pyridine (5.0 ml) and 3,5-dinitrobenzoyl chloride (34.0 mg; 0.15 mM, 3.75 M Xs) was added and the solution was stirred for 48 hours at room temperature. The reaction mixture was poured into ice-water and extracted with ether (3 x 50 ml). The combined ether extract was washed with ice-cold water (2 x 20 ml), saturated sodium hydrogen carbonate solution (2 x 20 ml) followed by water (2 x 20 ml). Ether extract was dried over magnesium sulphate and evaporated to dryness. The crude product was crystallised from acetone-methanol to obtain yellow crystals of 3,4-seco-3,3-di (4-methoxyphenyl) - 5α-cholest-2-en-5β - ($3^1 5^1$-dinitrobenzoate) (322).

M.P. 181-3°C

Yield: 22.6 mg, (78.5%)

I.R: 2940, 2875, 1730 (C=O) , 1630, 1605, 1550 (NO_2), 1512, 1465, 1345 (NO_2), 1282, 1250, 1175, 1121, 1075, 1035, 971, 922, 875, 835, 830, 808, 732, 723, 667.

NMR: τ 9.31 (S, 3H, C_{18}-CH_3), 8.90 (S, 3H, C_{19}-CH_3). 6.21, 6.46 (S, 6H, C_3-2X-OCH_3), 5.10 (q, 1H, C_5αH, J = 4.0, 10.4H_Z) 6.12 (t, 1H, C_2-H, J = 6.0H_Z), 2.91 - 3.70 (m, 8H, C_3-Aromatic H $_5$), 0.8 - 1.33 (m, 3H, C_5-EsterAromatics).

Analysis: Calculated: C, 72.32; H, 7.69; N, 3.59

Found: C, 72.20; H, 7.9; N. 3.5

(M, C_{47} H_{60} O_8 N_2).

41.REACTION OF 3,4-SECO-3,3 Di-(4-METHOXYPHENYL)-5 α-CHOLESTAN-5β-OL (314) WITH 3,5-DINITROBENZOYL CHLORIDE.

3,4-Seco-3,3-di- (4-methoxyphenyl)-5 -cholestan-5b-ol (314, 23.4 mg; 0.04mM) was taken up in pyridine (5.0 ml) and 3,5-dinitrobenzoyl chloride (33.0 mg, 0.14mM, 3.5M Xs) was added and the solution was stirred at room temperature for 48 hours. The reaction mixture was poured into ice-water and extracted with ether (3 x 50 ml). The combined ether extract was washed with ice-cold water (2 x 25 ml) sodium hydrogen carbonate solution (2 x 25 ml) and water (2 x 25 ml). Ether extract was dried over magnesium sulphate and evaporated to dryness. The crude product was crystallised from acetone-methanol yielding yellow crystals of 3,4-seco-3,3-di-(4-methoxyphenyl)- 5 α-cholestan-5b-($3^1 5^1$ -dinitrobenzoate) (323).

M.P. 157-9°C

Yield: 25.4 mg; (82.0%)

I.R 2960, 2945, 2875, 2855, 1728 (C=O), 1630, 1610, 1550 (NO_2), 1511, 1463, 1346 (NO_2), 1280, 1255, 1175, 1121, 1076, 1035, 970, 921, 875, 829, 806, 731, 722, 688.

NMR τ ($CDCl_3$) 9.32 (S, 3H, C_{18}-CH_3), 8.97 (S, 3H, C_{19}-CH_3), 6.58, 6.29 (S, 6H, 2X O-CH_3), 5.04 (q, 1H, $C_5\alpha$H, J = 4.8, 11.0H_Z), 2.83 - 3.90 (m, 8H, Aromatic H_S), 0.76 - 1.34 (m, 3H, Aromatic ester H_S).

Analysis: Calculated, C, 72.10; H, 7.98; N, 3.58

Found; C, 72.10; H, 8.2; N, 3.40

(M, $C_{47}H_{62}O_8N_2$).

42. PREPARATION OF CHOLESTERYL-3β-ACETATE (324)

To a solution of cholesterol (201, 20.0g; 0.052M) in pyridine (200 ml), acetic anhydride (30 ml) was added and the solution was stirred for one hour and left to stand overnight at room temperature. The reaction mixture was poured over ice-water and extracted with ether (5 x 100 ml). The combined ether extract was washed with dilute hydrochloric acid (3 x 50 ml) followed by water (3 x 50 ml), dried over magnesium sulphate and evaporated to dryness. The crude product was crystallised from acetone to obtain white crystals of cholesteryl-3β-acetate (324).

M.P. 111 - 113°C (Lit MP[408] 114-115°C).

Yield: 19.85g (89.5%)

I.R.ν max 2960, 2930, 2890, 2850, 1733 (C=O), 1665 (C=C), 1468, 1445, 1386, 1375, 1366, 1335, 1316, 1289, 1250, 1235, 1175, 1162, 1145, 1135, 1114, 1088, 1037, 1025, 997, 982, 963, 957, 943, 929, 921, 906, 895, 886, 859, 846, 833, 825, 819, 805, 795, 771, 743, 705, 690, 665.

NMR ($CDCl_3$)τ 9.32 (S, 3H, $C_{18}-CH_3$), 8.98 (S, 3H, $C_{19}-CH_3$) 7.98 (S, 3H, $3\beta-O-\overset{O}{\overset{\|}{C}}CH_3$), 5.41 (m, 1H, $C_3\alpha H$), 4.62 (d, 1H, C_6-H, J = 4.6 H_Z).

43. PREPARATION OF 5α-CHOLESTAN-3β-ACETATE (325)

Cholesteryl acetate (324, 5.0g; 11.68 mM) was taken up in ether (25.0 ml) and glacial acetic acid (50.0 ml) and hydrogenated using platinum oxide (106 mg), as a catalyst, at room temperature, until there was no more uptake of hydrogen. The reaction mixture was filtered through high-flow supercel filter aid, using ether to dissolve any cyrstallised product, and the filtrate was evaporated to dryness. The crude product was crystallised from ethylacetate-methanol to obtain white crystals of 5α-cholestan-3β-acetate (325).

M.P. 108°-110°C (Lit MP[413] 110-111°C).

Yield: 4.83g (96.2%)

I.R. ν max: 2956, 2940, 2880, 2865, 1740 (C=O), 1472, 1450, 1388, 1379, 1368, 1362, 1242, 1176, 1160, 1152, 1135, 1125, 1078, 1030, 963, 933, 913, 890, 867, 842, 801, 757, 745, 727, 670.

NMR: τ 9.34 (S, 3H, C_{18}-CH_3), 9.19 (S, 3H, C_{19}-CH_3), 8.0 (S, 3H, C_3 - O - C(=O) - CH_3), 5.35 (m, 1H, C_3 -αH)

44. PREPARATION OF 5α-CHOLESTAN-3β-OL (326)[413]

5α-Cholestan-3β-acetate (325, 4.80 g; 11.16 mM) was taken up in ethanol (22 ml) and a solution of sodium hydroxide (1.4g) in water (5.6 ml) was added. The reaction mixture was heated on steam bath for three hours and then allowed to cool. The precipitate was collected washed and crystallised from ethanol to yield white crystals of 5α-cholestan-3β-ol (326).

M.P. 139 -140°C (Lit. MP 413 140-141·5°C)

Yield 4.08g (94%)

IR ν max 3700-3100 (OH), 2945, 2925, 2875, 2855, 1475, 1453, 1392, 1373, 1345, 1309, 1176, 1160, 1144, 1085, 1045, 967, 938, 890, 786, 679,

NMR ($CDCl_3$) τ 9.35 (S, 3H, C_{18}-CH_3), 9.20 (S, 3H, C_{19}-CH_3), 7.02 (Cs, 1H, C_3-OH) 6.43 (m, 1H, C_3 α H)

45. PREPARATION OF 5α-CHOLESTAN-3β-TOLUENE-p-SULPHONATE (327)

To a solution of 5α-cholestan-3β-ol (326, 4.0g; 10.31 mM) in pyridine (100 ml), toluene-p-sulphonyl chloride (18.6g, 103.1 mM) was added. The reaction mixture was stirred for two hours and then allowed to stand overnight at room temperature. The reaction mixture was poured onto ice-water and extracted with ether (3 x 150 ml). The combined ether extract was washed with dilute hydrochloric acid (2 x 50 ml) followed by ice-cold water (3 x 50 ml). Ether extract was dried over magnesium sulphate and evaporated to dryness. The crude product (5.35g) was crystallised from acetone to obtain white crystals of 5α-cholestan-3β-toluene-p-sulphonate (327).

M.P: 131-133°C (Lit. M.P.[414] 134-5°C)

Yield: 4.77g (85.4%)

IR ν max 2955, 2935, 2870, 1600 (C=C Aromatic), 1495, 1469, 1455, 1447, 1404, 1385, 1360 (SO_2-O-), 1335, 1312, 1298, 1210, 1192, 1180 (-SO_2-O-), 1156, 1132, 1121, 1100, 1073, 1040, 1021, 1015, 1001, 985, 953, 940, 931, 907, 890, 870, 855, 817, 810, 755, 731, 708, 689, 672, 665.

NMR ($CDCl_3$) τ 9.36 (S, 3H, C_{18}-CH_3), 9.22 (S, 3H, C_{19}-CH_3) 7.54 (S, 3H, - C_6H_4 - CH_3), 5.57 (m, 1H, 3α-H), 2.20-2.66 (4H, Aromatic J = 8.0 H_Z).

46. PREPARATION OF 5 α- CHOLEST-2-ENE (6)[415]

A solution of 5 α-cholestan-3β-toluene-p-sulphonate (327, 4.0g) in dry dimethylsulphoxide (60 ml) was heated at 100°C for three hours. (The temperature of the oil-bath was 115-120°C). The reaction mixture was poured into water (100 ml) and extracted with ether (3 x 100 ml). The combined ether extract was washed with water (4 x 100 ml), 2N hydrochloric acid (2 x 100 ml), water (100 ml), saturated sodium hydrogen carbonate (2 x 100 ml) and finally with water (3 x 100 ml). Ether extract was dried over magnesium sulphate and evaporated to dryness. The crude product was columned on alumina. Elution with pet-ether (40-60) gave 5α-cholest-2-ene (328), pet-ether-benzene gave 5α-cholestan-3-one (220), ether-benzene gave 5α-cholestan -3α-ol) and washing the column with chloroform afforded a gum which was mainly 5α-cholestan-3β-ol (326).

5α-Cholest-2-ene-(328) was crystallised from acetone to obtain white crystals.

M.P.: 69-71°C (Lit. M.P.[415] 72-73°C)

Yield: 1.84g; 67.4%

IR: ν max 3030 (C=C) 2970, 2950, 2875, 2855, 1471, 1445, 1431, 1385, 1380, 1375, 1348, 1299, 1256, 1211, 1186, 1180, 1170, 1155, 1138, 1117, 1050, 1032, 996, 988, 968, 960, 943, 932, 920, 909, 890, 875, 848, 829, 810, 773, 731, 667, 650.

NMR ($CDCl_3$) τ 9.33 (S, 3H, C_{18}-CH_3), 9.25, (S, 3H, C_{19}-CH_3)
4.46 (bs, 2H, $C_{2,3}$-H)

5α-Cholestan-3-one (220, 0.82g) was crystallised from ethanol to obtain white crystals.

M.P. 125-127°C (Lit. M.P.[408] 128-129°C)

Yield 0.62g; 21.8%

I.R. ν max 2980, 2940, 2880, 2865, 1715 (C=O) 1471-1446, 1421, 1387, 1377, 1368, 1355, 1336, 1315, 1287, 1277, 1258, 1232, 1214, 1205, 1187, 1176, 1157, 1133, 1076, 1033, 1001, 959, 945, 932, 905, 875, 850, 811, 762, 738, 710, 687.

NMR τ 9.31 (S, 3H, $C_{18}-CH_3$), 8.97 (S, 3H, $C_{19}-CH_3$) 7.51 - 7.96 (m, 4H, C_2 and C_4-H).

Crystallisation of the crude third fraction (0.21g) from methanol produced white crystals of 5α-Cholestan-3α-ol.

M.P. 183-185°C (Lit M.P. [408] 187-188°C)

Yield: 0.18g; 6.3%

IR ν max 3700-3100 (OH), 2940, 2925, 2875, 2855, 1470, 1447, 1386, 1377, 1368, 1335, 1310, 1277, 1252, 1231, 1211, 1185, 1168, 1127, 1119, 1107, 1075, 1035, 1005, 977, 960, 938, 919, 906, 884, 846, 832, 729.

NMR τ 9.35 (S, 3H, $C_{18}-CH_3$), 9.22 (S, 3H, $C_{19}-CH_3$), 7.63 (S, 1H, C_3 α-OH) 5.93 (d, 1H, C_3-βH, J = 2.0H_Z)

Crystallisation of the fourth, the very polar fraction (0.13g) from ethanol yielded white crystals of 5α-cholestan-3β-ol (326).

M.P. 138-140°C (Lit. M.P.[408] 140-141.5°C)

Yield: 95 mg; 3.3%

I.R. ν max 3600 - 3160 (OH), 2945, 2880, 1475, 1453, 1392, 1373, 1345, 1309, 1176, 1160, 1144, 1085, 1045, 967, 938, 890, 786, 679.

NMR τ 9.35 (S, 3H, $C_{18}-CH_3$), 9.20 (S, 3H, $C_{19}-CH_3$), 7.02 (bs, 1H, C_3-OH) 6.43 (m, 1H, C_3 -αH).

47. PREPARATION OF 3-(2^{1}-ACETIC ACID)-2-HYDROXY-5 α-CHOLESTAN-γ-LACTONE 269F

Manganous acetate tetrahydrate (530 mg, 2.2 mM) was[329] dissolved in glacial acetic acid (10 ml) by raising the temperature to 90°C. At this temperature potassium permanganate (100 mg, was added with stirring. When the exothermic reaction had subsided and temperature dropped to 90°C, acetic anhydride (0.8 ml) was added followed by sodium acetate (1.20g).

5α-Cholest-2-ene (328, 350 mg; 0.96 mM) was added and the reaction mixture was refluxed until the brown colour had disappeared. (approximately one hour). The reaction mixture was diluted with water (50 ml) and extracted with ether (3 x 150 ml). The combined ether extract was washed with saturated sodium hydrogen carbonate solution (2 x 150 ml) and water (2 x 100 ml). The ether extract was dried over anhydrous magnesium sulphate and evaporated to dryness. The crude product was separated by preparative T.L.C. (solvent system, Pet-ether/Ether; 60/40). The fraction corresponding to the γ-lactone was crystallised from methanol to obtain white crystals of 4 (2^{1}-acetic acid)-2-hydroxy-5α-cholestan- γ-lactone (329).

M.P. 121-3°C

Yield: 41.0 mg; 10.0%

IR ν max 2960, 2945, 2880, 1768 (C=O), 1470, 1460, 1451, 1420, 1385, 1378, 1346, 1332, 1298, 1247, 1174, 1111, 1000, 974, 941, 869, 840.

NMR τ 9.35 (S, 3H, C_{18}-CH_3), 9.22 (S, 3H, C_{19}-CH_3), 5.34 (quintet, 2-overlapping triplets, 1H, C_2-H).

$(\alpha)_D$ (+) 20.5 (C, 0.15)

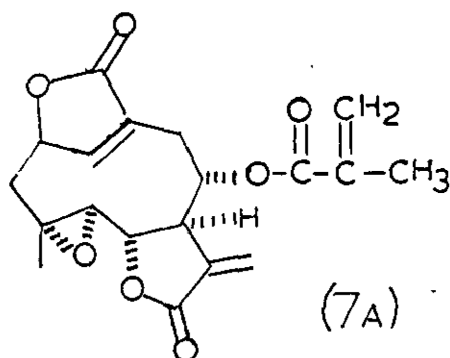
Acc. Mass: Exp. 428. 3654

Found 428.3648 (M, $C_{29}H_{48}O_2$).

APPENDIX

TABLE 2

TOTAL VIABLE CELLS X 10^6	% GROWTH	% DEATH	CONCENTRATION (μg/ml)
17.96	100.0	0.0	0.0
13.58	75.6	24.4	0.1
8.42	46.85	53.1	0.25
5.50	30.6	69.4	0.50
3.85	21.4	78.6	0.75
1.19	6.7	93.3	1.0
1.02	5.7	94.3	5.0



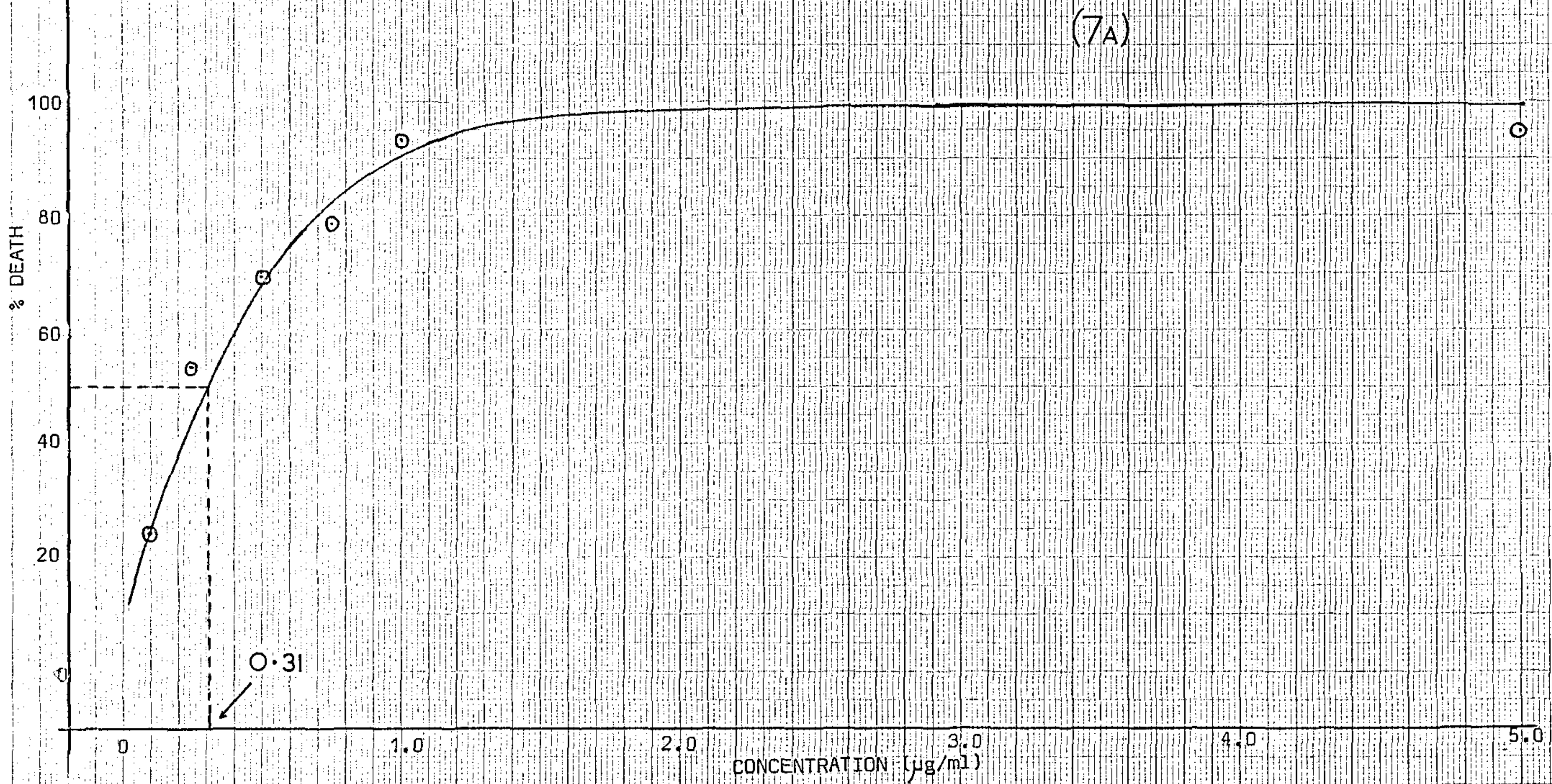
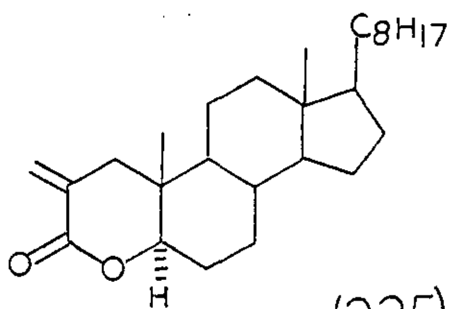
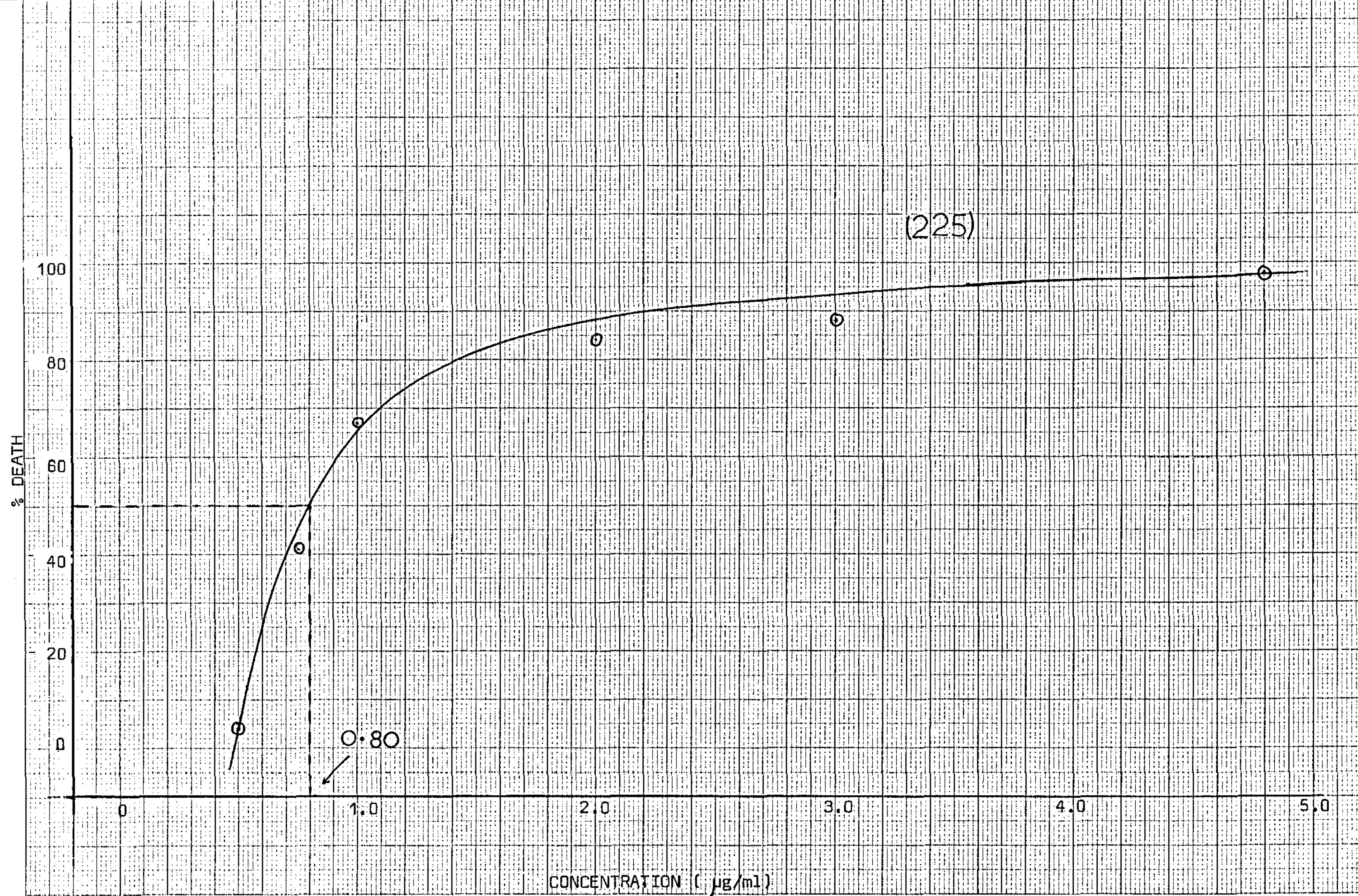

(7A)
% DEATH
100
80
60
40
20
0
0·31
0
1.0
2.0
3.0
4.0
5.0
CONCENTRATION (µg/ml)

TABLE 3

TOTAL VIABLE CELLS X 10^6	% GROWTH	% DEATH	CONCENTRATION (μg/ml)
7.85	100.0	0.0	0.0
7.55	96.0	4.0	0.5
4.65	59.0	41.0	0.75
2.56	33.0	67.0	1.0
1.25	16.0	84.0	2.0
0.97	12.0	88.0	3.0
0.18	2.0	98.0	5.0

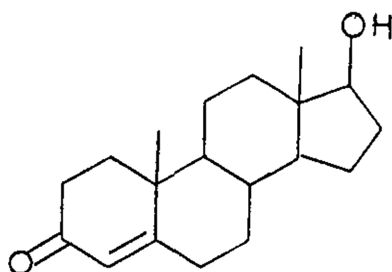




(225)
100
80
60
40
20
0
% DEATH
0·80
0
1.0
2.0
3.0
4.0
5.0
CONCENTRATION (µg/ml)

TABLE 4

TOTAL VIABLE CELLS X 10^6	% GROWTH	% DEATH	CONCENTRATION (µg/ml)
43.14	100.0	0.0	0.0
25.76	59.7	40.3	0.5
23.71	55.0	45.0	1.0
19.03	44.1	55.9	2.5
4.40	10.2	89.8	5.0
2.57	6.0	94.0	7.5
1.25	2.9	97.1	10.0



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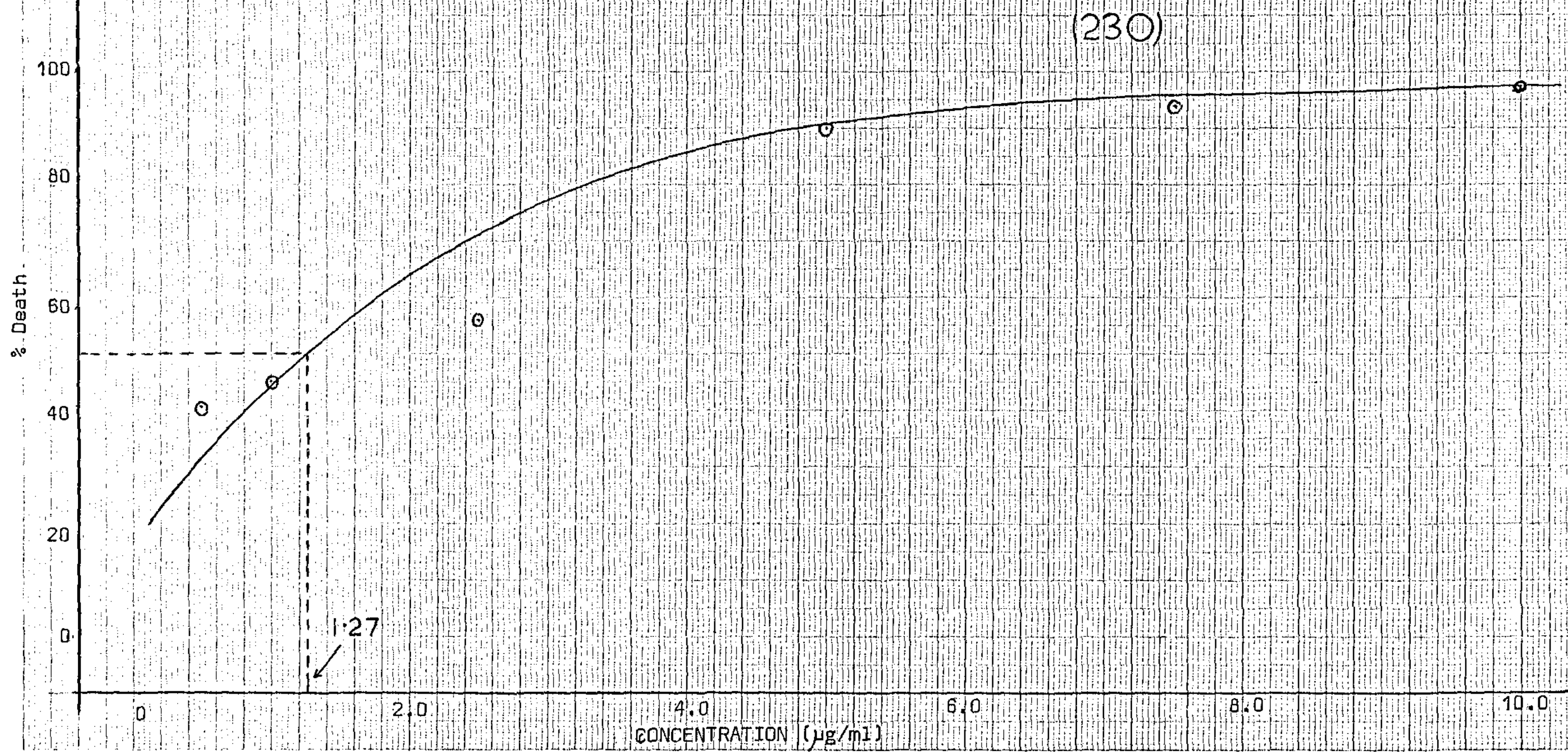
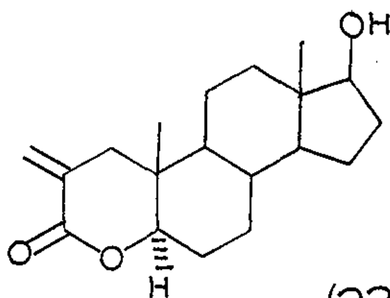

(230)
% Death
100
80
60
40
20
0
1·27
0
2.0
4.0
6.0
8.0
10.0
CONCENTRATION (μg/ml)

TABLE 5

TOTAL VIABLE CELLS X 10^6	% GROWTH	% DEATH	CONCENTRATION (μg/ml)
42.11	100.0	0.0	0.0
34.10	81.0	19.0	0.5
23.15	55.0	45.0	0.75
21.05	50.0	50.0	0.85
9.26	22.0	78.0	1.0
5.05	12.0	88.0	2.5
0.00	0.00	100.0	5.0



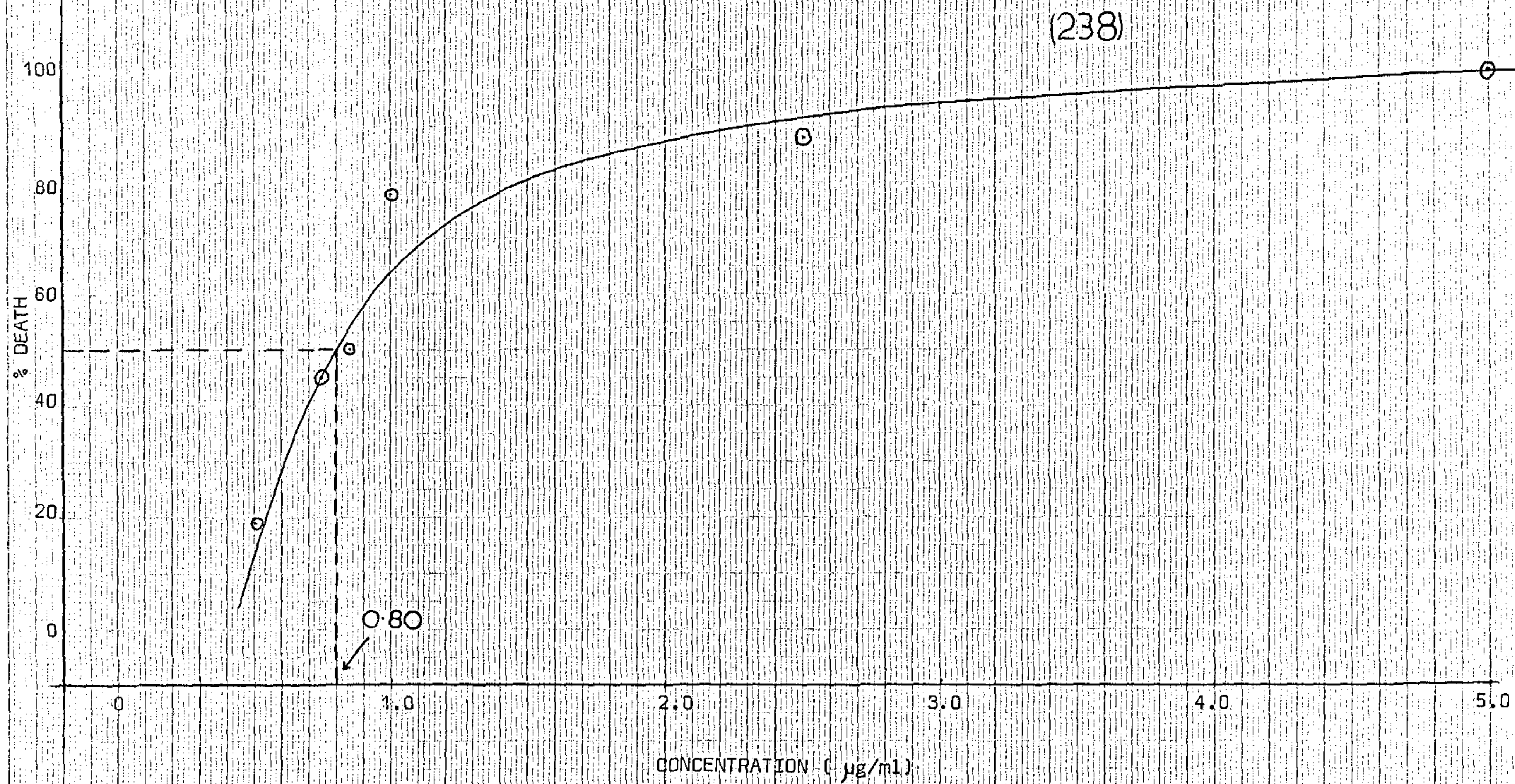
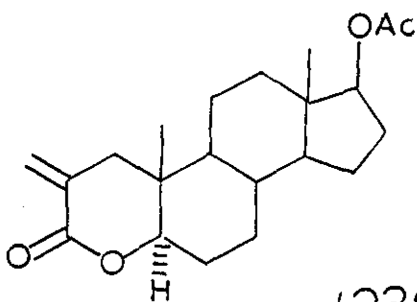

(238)
100
80
60
40
20
0
% DEATH
0·80
0
1.0
2.0
3.0
4.0
5.0
CONCENTRATION (µg/ml)

TABLE 6

TOTAL VIABLE CELLS X 10^6	% GROWTH	% DEATH	CONCENTRATION (μg/ml)
42.11	100.0	0.0	0.0
32.00	76.0	24.0	0.5
18.99	45.0	55.0	0.75
17.76	42.0	58.0	0.85
6.32	15.0	85.0	1.0
2.08	5.0	95.0	2.5
0.00	0.0	100.0	5.0



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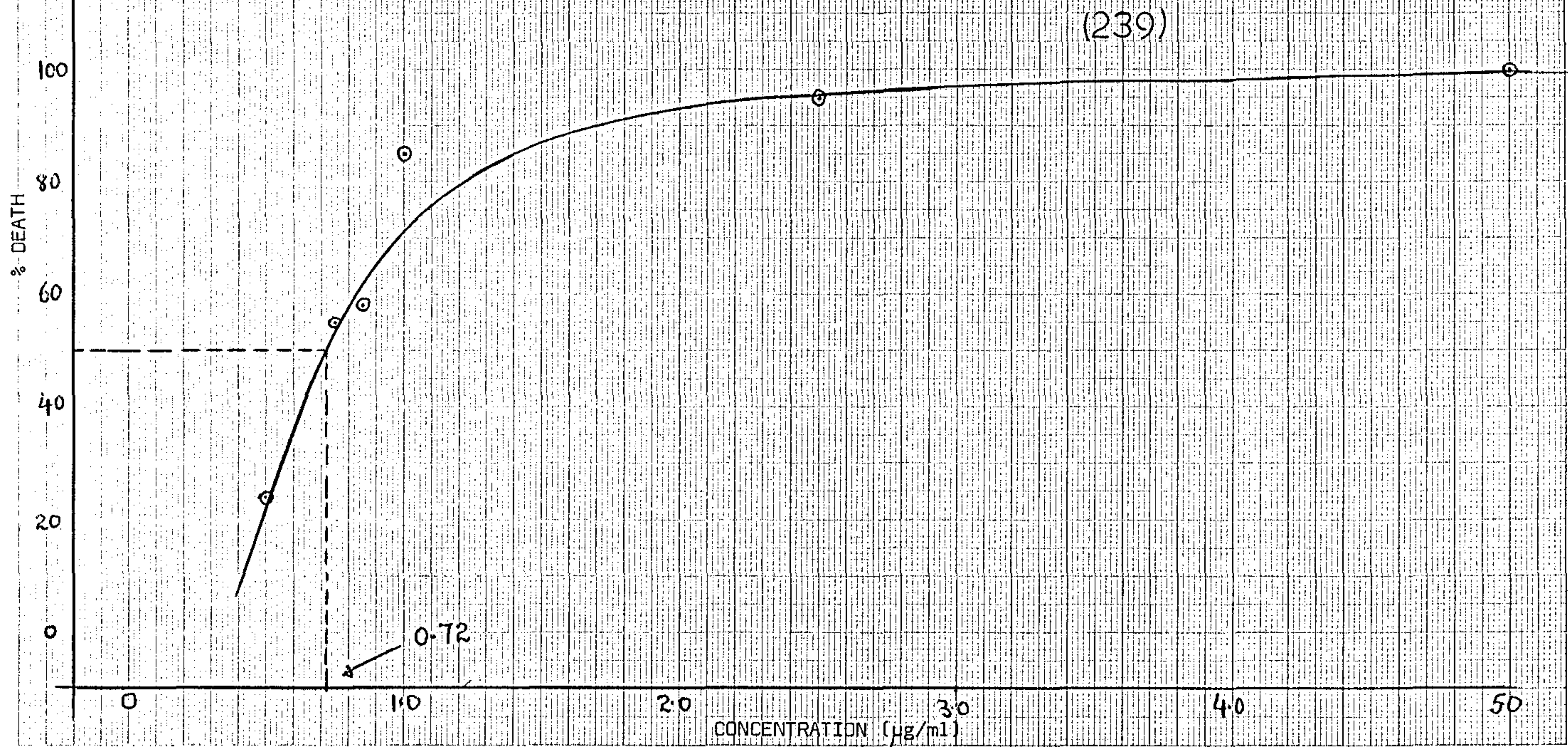
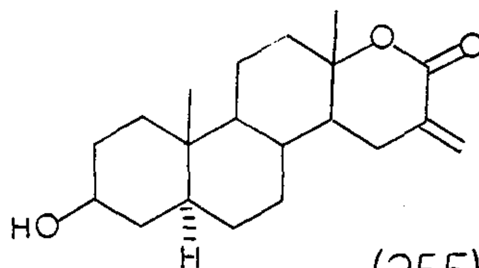

(239)
100
80
60
40
20
0
% DEATH
0·72
0
1·0
2·0
3·0
4·0
5·0
CONCENTRATION (µg/ml)

TABLE 7

TOTAL VARIABLE CELLS x 10^6	% GROWTH	% DEATH	CONCENTRATION (μg/ml)
2.89	100.0	0.0	0.0
2.31	80.0	20.0	0.5
1.87	65.0	35.0	0.75
1.42	49.0	51.0	1.0
1.18	41.0	59.0	2.0
0.51	18.0	82.0	3.0
0.096	3.0	97.0	5.0



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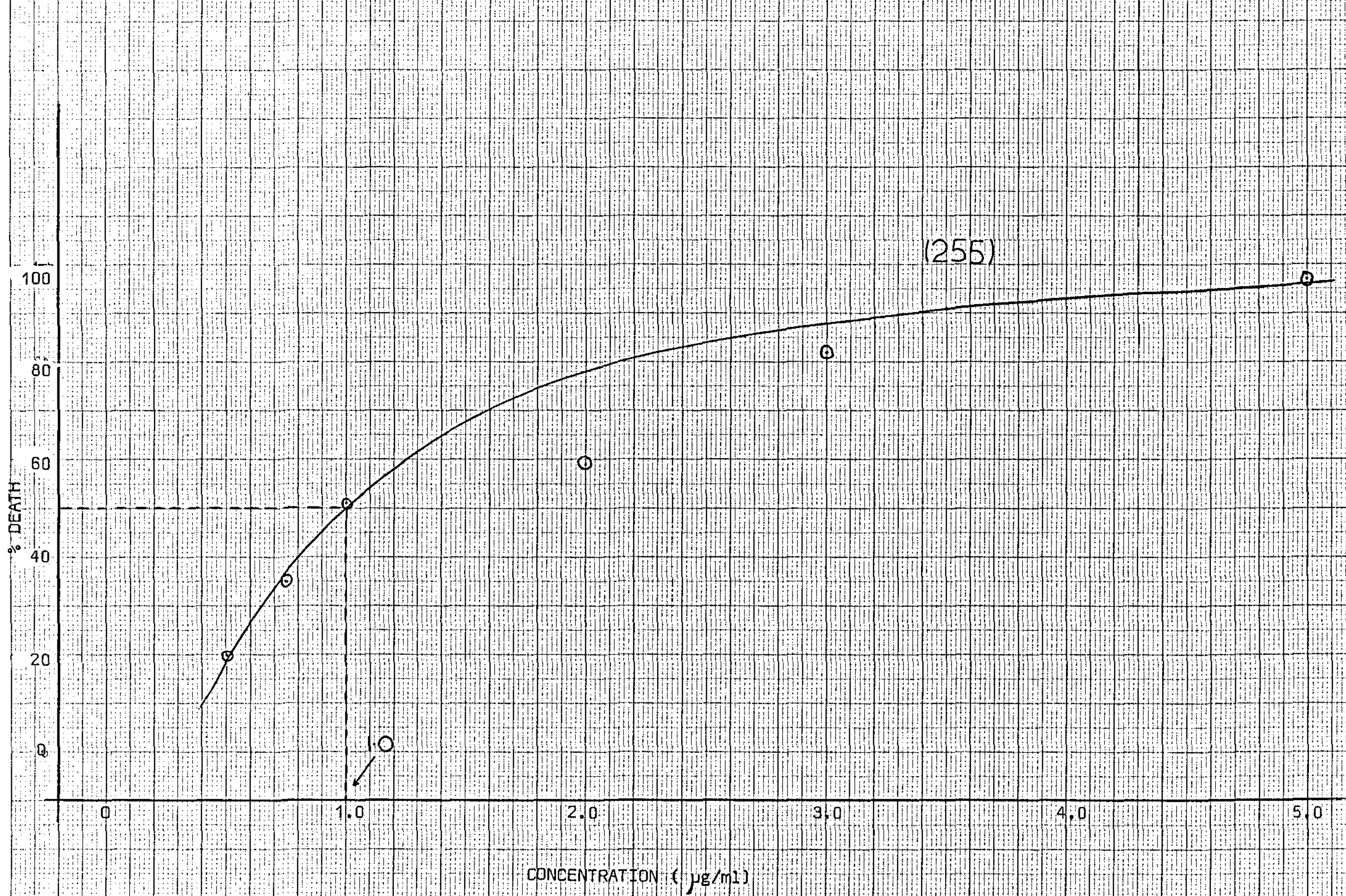
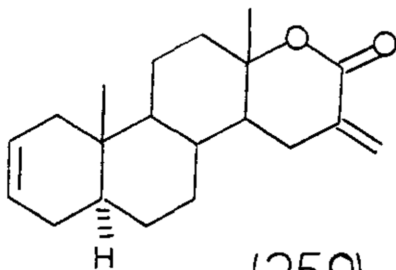
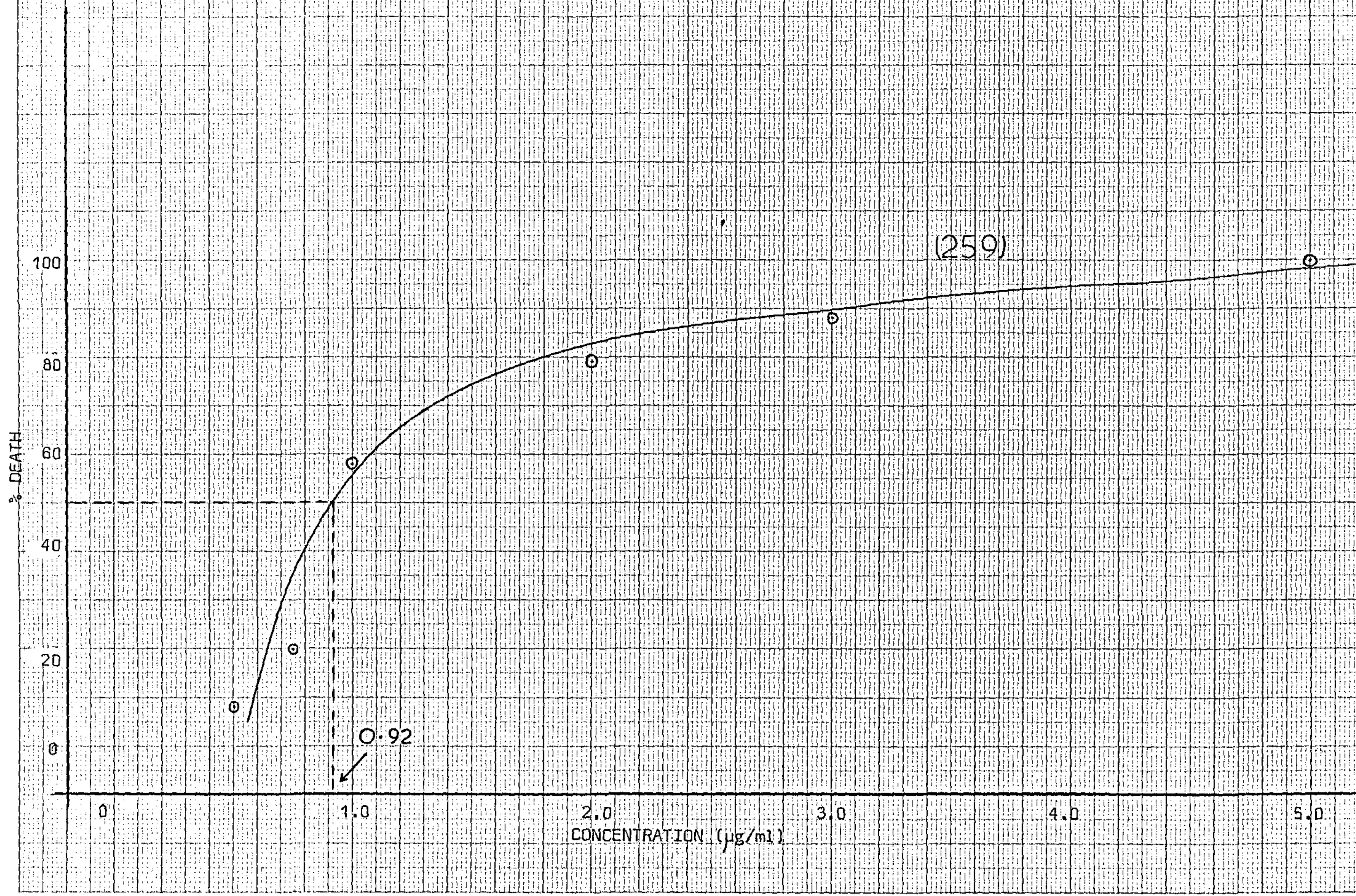

(255)
100
80
60
40
20
0
% DEATH
0
1.0
2.0
3.0
4.0
5.0
CONCENTRATION (µg/ml)
1.0

TABLE 8

TOTAL VIABLE CELLS $\times 10^5$	% GROWTH	% DEATH	CONCENTRATION (μg/ml)
6.48	100.0	0.0	0.0
5.96	92.0	8.0	0.5
5.17	80.0	20.0	0.75
2.75	42.0	58.0	1.0
1.37	21.0	79.0	2.0
0.77	12.0	88.0	3.0
0.0	0.0	100.0	5.0





(259)
100
80
60
40
20
0
% DEATH
0.92
0
1.0
2.0
3.0
4.0
5.0
CONCENTRATION (μg/ml)

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