A Thesis Entitled

"STEREOCHEMICAL AND MECHANISTIC STUDIES IN A BIOSYNTHETIC MODEL SYSTEM"

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by

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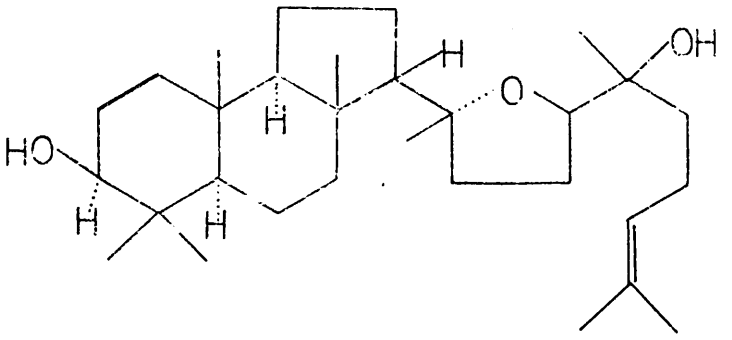
PART I

Stereochemical and Mechanistic Studies in a Biosynthetic Model System

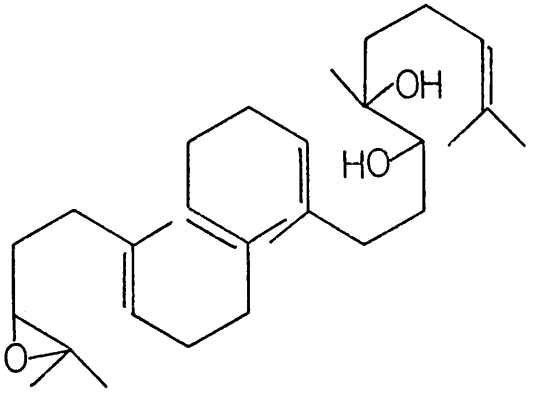
INTRODUCTION

In recent years it has become possible to elucidate the stereochemistry of some enzymically mediated reactions, at least with regard to substrates and co-enzymes. This has been due in part to improvements in techniques for the isolation of enzyme systems and the growing use of cell-free preparations which are more easily handled under reproducible conditions than whole plants or organisms. Spectacular advances too have been made in the determination of the chirality of isotopically labelled substrates on a micro-scale[1] and in the preparation of stereospecifically labelled precursors for use in enzymic reactions[2].

The most striking feature of enzymic processes is the high degree of stereoselectivity which they exhibit and a very reasonable question which arises is why such reactions should be stereoselective. In an attempt to answer this question it is usual to state that since proteins are chiral then enzymes are also chiral and will possess active sites and specific binding regions which will orientate a suitable substrate in one preferred conformation in which reaction will occur. The concept that use of a chiral reagent can induce chirality in the product it forms with an achiral substrate is not a new one and has been put to effective use in the laboratory[3]. However, the stereoselectivity exhibited in such processes does not match that of enzymic reactions which are commonly totally stereoselective. That enzymes are chiral does not necessarily explain their stereoselectivity since this may be attributable to selective binding of the substrate which is an essential prerequisite for catalytic activity. In general the catalytic groups are so disposed at an active site that they cannot allow the reaction



(1)



(2)

on the substrate to exhibit the alternative stereospecificity. Many non-enzymic processes also occur with high selectivity and the success of many "biogenetic type" syntheses of natural products has been quite remarkable. The formation of d,l-malabaricanediol (1)[4], for example, by cyclisation of the acyclic substrate (2) requires the stereospecific formation of six chiral centres and yet the reaction proceeds 'in vitro' to the extent of 7%. Many examples of similar cyclisations have now been investigated[5] particularly by the schools of Johnson[6] and van Tamelen[7], and in many cases acceptable yields of complex cyclic compounds have been produced stereospecifically by a simple cation-initiated process. It seems possible that many enzymic transformations of a similar nature may be obliged to be stereospecific on purely 'chemical' grounds, such as orbital symmetry or the steric requirements of chain folding in acyclic precursors. The very high yields of polycyclic compounds produced by cyclisation of long chain acyclic substrates, such as squalene-2,3-oxide, in enzymic systems probably reflect the ability of the enzyme to fold the chain in the correct orientation for cyclisation, whereas non-enzymic processes depend very markedly on the conformation of the substrate in solution, and often the choice of solvent or initiator[4] can affect critically the yield and the number of products formed.

That most of the reactions utilised by Nature in the production of secondary metabolites are stereospecific is consistent with the view that efficiency in synthesis requires stereospecificity. The geometries of transition states will therefore be important and the relative disposition of interacting groups in a substrate will affect rate constants. It has been argued[8] that the most favoured pathways will be those in which maximum bonding is maintained through the transition state, and motion of all atoms involved in the reaction is kept at a minimum. In suggesting mechanisms for enzymically mediated reactions,

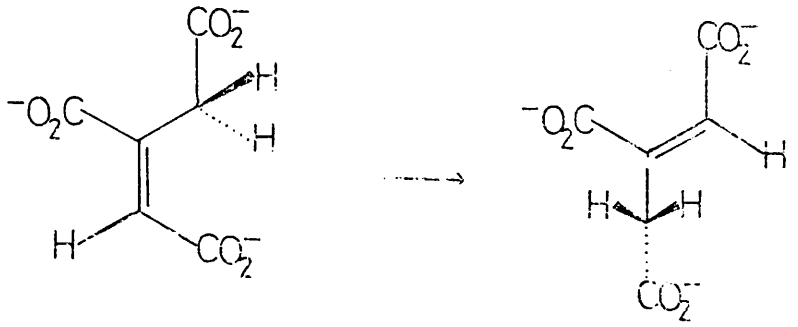
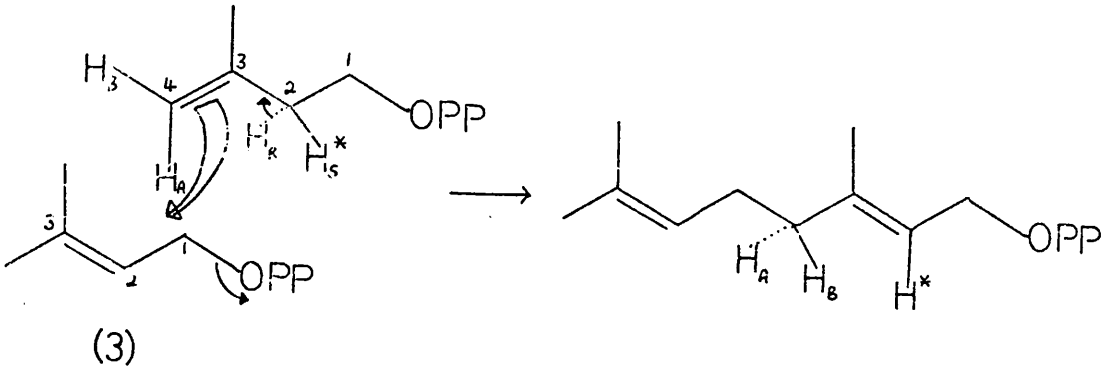
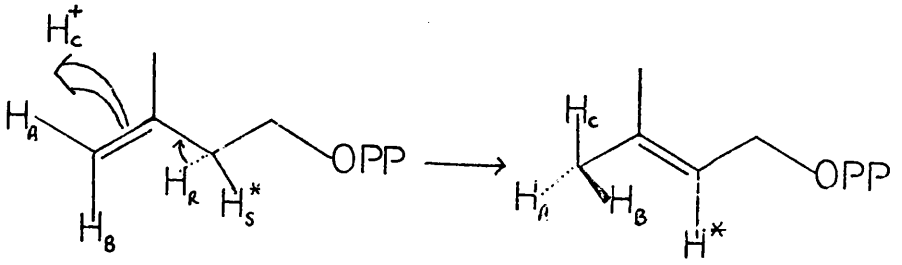


Fig.1



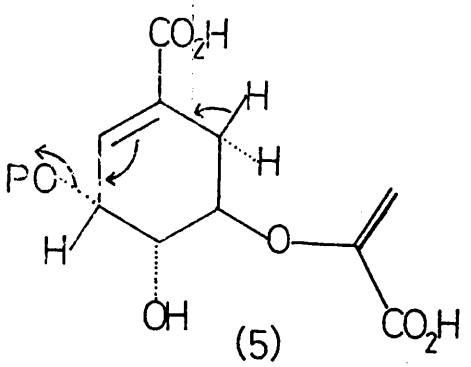
(3)

Fig. 2



(4)

Fig,3



(5)

Fig.4

comparisons with similar non-enzymic processes are often made. If there exists a well-documented stereochemical course for a non-enzymic reaction and a formally similar reaction occurs enzymically, then it is tempting to assume a similar mechanism. Likewise, selection rules derived from molecular orbital theories have been used as arguments for and against proposed enzymic mechanisms. For example, in the trans-cis isomerisation of aconitate[9], Figure (1), stereospecific hydrogen transfer occurs in a suprafacial manner. This is contrary to The Woodward-Hoffmann Rules[10] for a concerted 1,3-sigmatropic shift and the conclusion is therefore that the reaction must be stepwise.

Interest in the comparison of 'in vivo' and 'in vitro' mechanisms stemmed from the findings of the Cornforth group that, (a), the condensation of 3-methyl-2-butenyl pyrophosphate (3) with 3-methyl-3-butenyl pyrophosphate (4) proceeds with net <u>syn</u> stereochemistry[11], Figure (2), and (b), the stereochemistry of the formally related process, the isomerisation of 3-methyl-3-butenyl pyrophosphate (4) to 3-methyl-2-butenyl pyrophosphate (3) occurs with net <u>anti</u> stereochemistry[12], Figure (3). Another related reaction (c), also of biochemical importance, that of 1,4-eliminations, has been studied and the stereochemistry shown[13] to be <u>anti</u> in the formation of chorismate from 5-enolpyruvoyl-3-phosphoshikimate (5), Figure (4). The stereochemistries of the two reactions investigated by Cornforth, both formally S_E2' processes, have therefore been shown to be different, one being <u>syn</u> and the other <u>anti</u>.

It is pertinent to examine the theoretical predictions for the stereochemistry of such reactions and to compare them with the observed results. Orbital symmetry rules have been very successful in predicting the course of <u>concerted</u> reactions[10], particularly those which are electrocyclic in nature. An approach, similar to that of

Woodward and Hoffmann but claimed to be of greater generality, has been extensively developed by Fukui[14], in which orbital interactions of the highest occupied molecular orbital of one reactant and the lowest unoccupied molecular orbital of the other are used to rationalise the stereochemistry observed in many chemical reactions. This method of orbital interactions has been extended to apply to S_N2' and S_E2' reactions, and 1,4-additions to dienes, in all of which syn stereochemistry is predicted. It is of interest to note that Fukui, in a review of "The Selection of Stereochemical Paths by Orbital Interactions" [14] in 1971, states that there is a preference for syn-stereochemistry in a concerted S_N2' reaction, while Anh[15] in 1968 proposed a more qualitative view in which he concludes that the reaction should be anti-if concerted and synchronous, and syn -if concerted but non-synchronous. Drenth[16] has also carried out some simple Huckel molecular orbital calculations for the S_N2' reaction and concludes that the stereochemistry should be syn making no comment on concertedness but by implication assuming it. While Drenth makes reference to Fukui, neither Anh nor Fukui comment on each other's findings which is strange since they appear to predict exactly the opposite course for the same reaction, and it is therefore appropriate to question whether the basis of their theoretical analysis is valid. Of the many putative examples of the S_N2' reaction recorded in the literature that of Stork and White[17] is one of the few that may actually qualify for the label, though the possibility exists that the reaction proceeds through an intimate ion pair[18]. The three theoreticians quoted above all claim that the syn stereochemistry demonstrated in this reaction supports their predictions. Fukui and Drenth have no problem in accommodating this example by their theories but Anh is forced to propose a concerted reaction which is non-

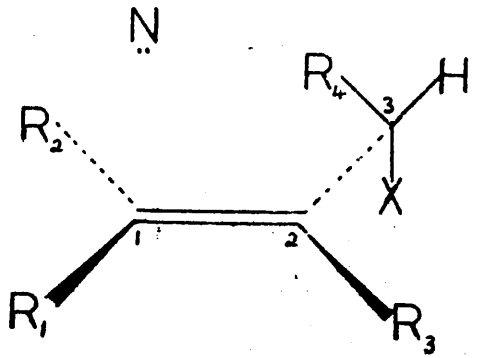


Fig.5

synchronous. If, as he suggests, approach of a nucleophile (N) to C-1 of the allyl group, Figure (5), will be more favourable if there is some X - C-3 bond breaking to create positive charge at C-1, then it is difficult to envisage the case in which a wholly concerted and synchronous reaction will occur.

A recurring problem in reaction mechanisms and one posed by the theories of Anh is the question of concertedness and the problem of experimentally establishing whether a given reaction is concerted or not[19]. This problem is further compounded in enzymic reaction mechanisms by the lack of knowledge about which groups in the enzyme participate in a given reaction. If theoretical predictions are to be of any use in shedding light on such a problem then they must produce a strict rule, for example, "if the observed stereochemistry is anti then the mechanism cannot be concerted". Unfortunately although such rules are highly developed for electrocyclic processes the more common enzymatic reactions, such as those described above, do not fall into this category and such theories as are available for these reactions seem to be divergent in their predictions. A reaction is usually considered to be 'concerted' if there is equal bond making and bond breaking in the transition state and such a process should require a lower activation energy than the corresponding dissociation-recombination process. Such a "definition" of 'concertedness' makes the distinction by Anh of 'concerted reactions' into synchronous and non-synchronous illogical and the reactions must either be concerted or non-concerted. However it has been pointed out[19] that although a two-step reaction may not be energetically concerted (i.e. there may exist a small minimum in the potential energy surface) it is not inconsistent for such a process simultaneously to be bondingly and orbitally concerted. It has also been postulated[19] that as the

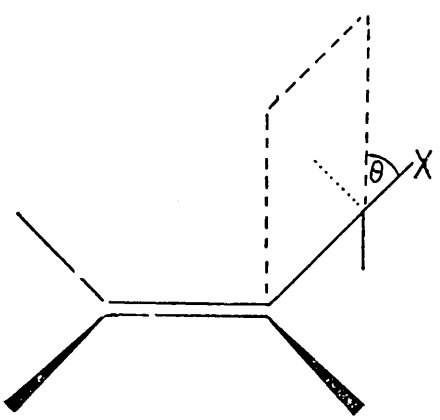


Fig.6

number of atoms involved in a reacting system increases then the activation energy required to contain an intermediate for 10^{-12} sec falls rapidly for any given internal energy, for example while 27 Kcal mol^{-1} may be required for a 4-atom intermediate, only 5 Kcal mol^{-1} is required to contain a 14-atom intermediate for 10^{-12} sec.

In an extensive review on base induced 1,2-eliminations Bordwell[20] concludes that possibly one of the few cases of a concerted E_2 elimination occurs in non-activated primary halides, such as $R-CH_2-CH_2-Br$, although S_N2 substitution is the predominant reaction for such systems. All other base-induced eliminations can be considered to merge imperceptibly into reaction types proceeding through either cationic or anionic intermediates. Although conceptually pleasing, a concerted mechanism demands a transition state of ordered geometry and charge distribution. If there is such doubt about the concertedness or otherwise of 1,2-eliminations, then there must be even greater doubt concerning 1,3-allylic substitutions or 1,4-eliminations[18] since they would require even more extensive bond ordering in the transition state. In an allyl system such as in Figure (6) there will be interaction of the π-bond with the allylic C-X σ-bond, the magnitude of which will depend on the torsional angle θ, being greatest when $\theta = 0^\circ$ or 180°. This interaction, sometimes termed hyperconjugation, causes allylic bonds such as C-X to be weaker than they would be in a fully saturated system, and results in the molecule acquiring a small dipole moment. Intuitively it does not seem reasonable to expect that on approach of a nucleophile or electrophile to this system, there will not be considerable polarisation of the allyl system which will in turn affect the strength of the allylic bond C-X. Indeed many of the reactions of α- alkyl allyl

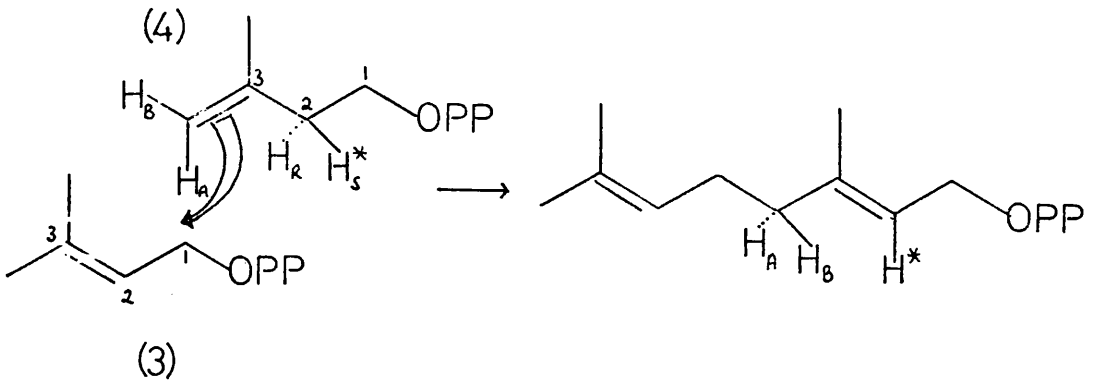


Fig.2

Note: H_B precedes H_A in the "Sequence Rule"

chlorides represented as occurring by an S_N2' mechanism can be explained by a carbonium ion type process and in support of this view[18] it is observed that substitution of electron withdrawing groups on the allylic halide prevents an S_N2' type displacement from occurring or forces it to proceed by a carbanion mechanism.

Consider now in detail the mechanism proposed for the three enzymic reactions and the arguments employed to substantiate them. The stereochemical course of the C-5 condensation process (a) was defined by the Cornforth Group in a series of elegant labelling studies prior to 1966. They established that (i) the pyrophosphate leaving group is expelled with inversion of configuration at the primary allylic carbon atom, C-1 of (3); (ii) the new carbon-carbon bond is formed on the 4 re face of (4); and (iii) the 2-pro-R-hydrogen of (4) is lost to form the new trans-double bond as illustrated in Figure (2). Cornforth favours a two step process for the following reasons.

(i) Since complete inversion of configuration at C 1 of (3) is observed the condensation is not initiated by formation of the primary carbonium ion, unless rotation about the C-2/C-1 bond in (3) is prevented, or formation of the new bond happens before rotation can occur. The simplest interpretation based on comparison with non-enzymic substitution reactions would be to suppose that the two steps are concerted and the reaction is of the normal S_N2 type.

(ii) It is possible to extend the idea of concertedness to the whole reaction, formally an S_E2' type, in which a continuous drift of electrons from the $C\text{-}H_R$ bond of (4) to the C-1 position of (3) is supposed, but this is deemed unlikely. If the electrons of the $C\text{-}H_R$ bond are being used to form the new double bond in a concerted fashion then C-3 of (4) is having electrons supplied to it (for formation of the new double bond) and withdrawn from it (to form a new

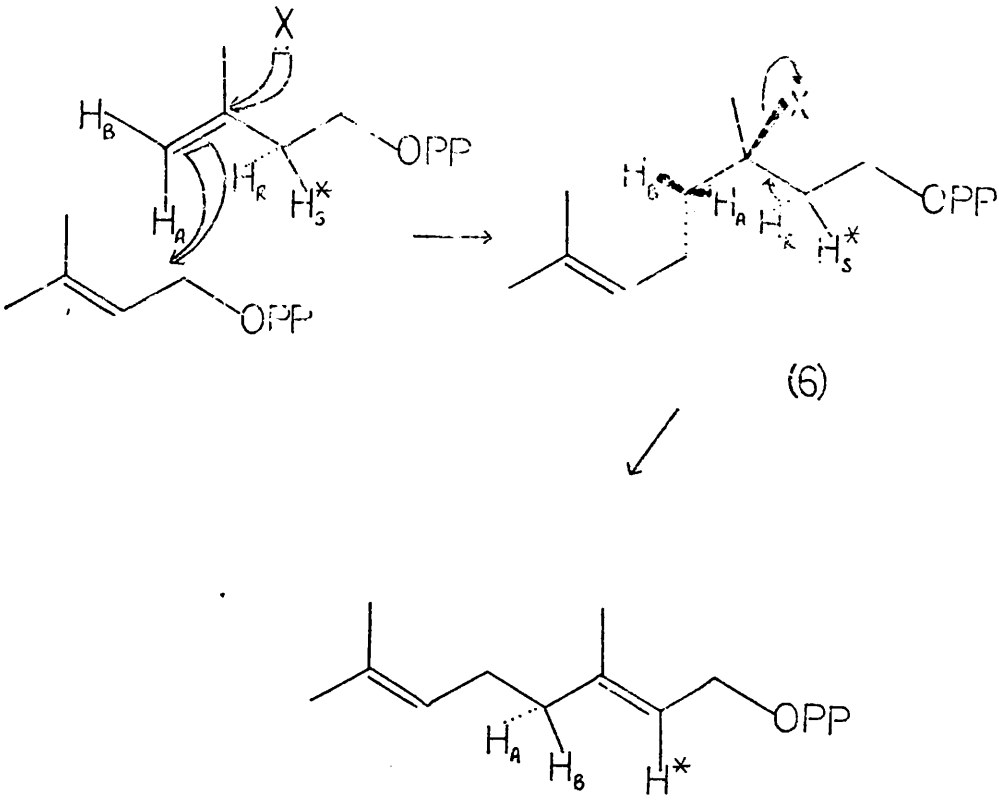

X
H_B
H_A
H_R
H_S^*
OPP
OPP
H_B
H_A
H_R
H_S^*
OPP
(6)
H_A
H_B
H^*
OPP

Fig.7

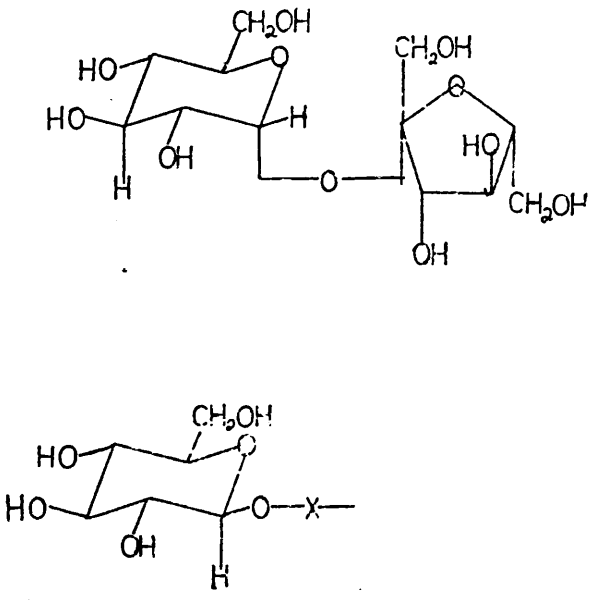
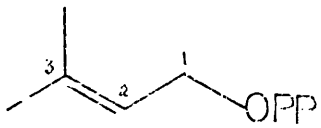

CH_2OH
HO
HO
H
OH
H
O
CH_2OH
O
HO
CH_2OH
OH
CH_2OH
HO
HO
O
X
OH
H

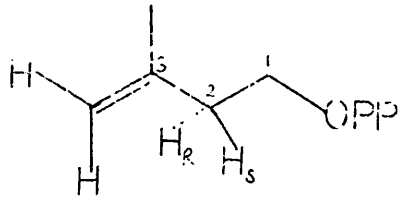
Fig.8

carbon-carbon bond) on the same side, and it would be reasonable to suppose this process would be less favourable than a mechanism where electrons are supplied to, and withdrawn from, opposite sides of C-3. Clearly this does not happen in practice since this would lead either to formation of a cis-double bond or loss of H_s, both contrary to observation.

A two-stage mechanism was therefore proposed by Cornforth in which an electron donating group X participates (The X-group mechanism). The nature of X is not specified but it could be, for example, an enzyme-bound water molecule, or the oxygen atom of a pyrophosphate group[21]. The overall reaction then reduces to a trans-addition of the allylic group (3) and of X to the double bond of (4), followed by a second stage in which H_R and X are eliminated in trans-fashion from the intermediate (6), Figure (7). While these postulates account for the observed stereochemistry they are open to criticism and other plausible schemes can be advanced[22]. One objection to the X-group mechanism is that elimination of H-X leads to an isolated double bond, whereas most enzymic eliminations produce double bonds only if they are conjugated (for example, with CO_2^- or phenyl) and thus X must be a very good leaving group. However there is some evidence from another system to support an X-group mechanism. Voet and Abeles[23] isolated an enzyme-X-β-Glucoside from the reaction of sucrose phosphorylase with sucrose, in which a covalent glucosyl-enzyme bond is formed as shown by the fact that glucose is not released from the complex on treatment with acid or passage through sephadex. Since the glucose released in the normal enzymic process has the β-configuration it is concluded that the formation of the complex occurs with inversion at C-1 of the glucosyl moiety, and a carboxyl group is advanced as being the most likely nucleophile, X, Figure (8).


3
2
1
OPP

(3)


H
3
2
1
OPP
H
H_R
H_S

(4)

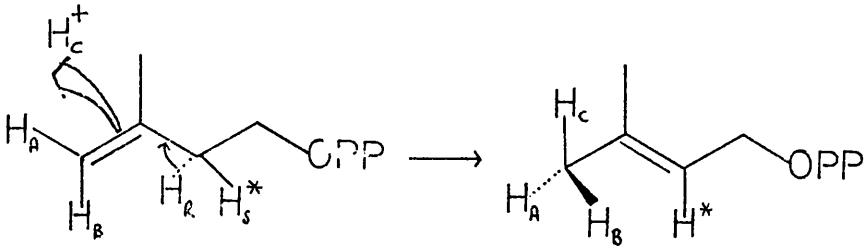

H_C^+
H_A
H_B
H_R
H_S^*
OPP
H_C
H_A
H_B
H^*
OPP

Fig.4

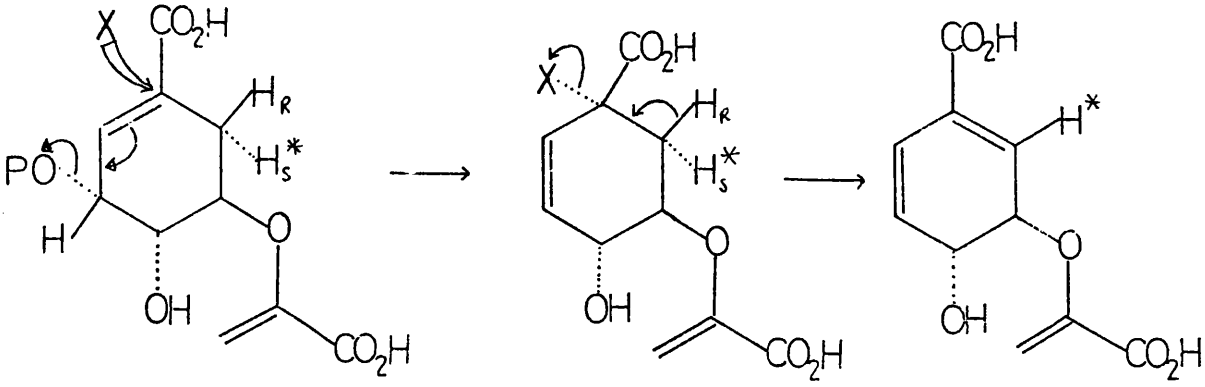

X
CO_2H
H_R
H_S^*
PO
H
OH
O
CO_2H
X
CO_2H
H_R
H_S^*
OH
O
CO_2H
CO_2H
H^*
OH
O
CO_2H

Fig.9

In general, however, formal bonding is not required between X and substrate, since if X has a full negative charge an intimate ion-pair would be sufficient. If the displacement of pyrophosphate from (3) is an S_N2 process then the isolated double bond is acting as a nucleophile, which in the presence of other better nucleophiles, such as water, would not be expected to compete successfully for the potential electrophilic site. The implication therefore is that the binding of the two reactants (3) and (4) on the enzyme must be in a well defined manner such that competition by other nucleophiles is excluded. If such restrictions on the relative dispositions of these reactants are imposed, then an S_E2' process will require less motion than the X-group mechanism and is only objectionable <u>if it is stereoelectronically prohibited</u>.

In the related isomerisation reaction (b), the stereochemical course was established as <u>anti</u> by showing that the 2-pro-R-hydrogen of (4) is lost and that hydrogen is added to the <u>re</u>-face of the double bond in (3), Figure (4). A consecutive 1,2-addition and 1,2-elimination does not explain the observed stereochemistry as it does above, and a wholly concerted addition-abstraction of hydrogen atoms has been proposed[12]. In the 1,4-elimination (c), Floss has suggested[24] that since the net stereochemical result is one of <u>anti</u>-elimination and previous theoretical predictions indicate that concerted 1,4-eliminations should occur in a <u>syn</u>-fashion[14] then the process is non-concerted and may proceed by a <u>syn</u>-S_N2' displacement of phosphate by attack of X at C-1, followed by a normal <u>anti</u>-elimination of HX, Figure (9). Thus Cornforth on the one hand rejects a <u>syn</u> S_E2' reaction on the basis of a wholly empirical view of the movement of electrons in a transition state, while Floss invokes a <u>syn</u> S_N2' reaction to explain the observed stereochemistry in chorismate formation. Such a dichotomy of

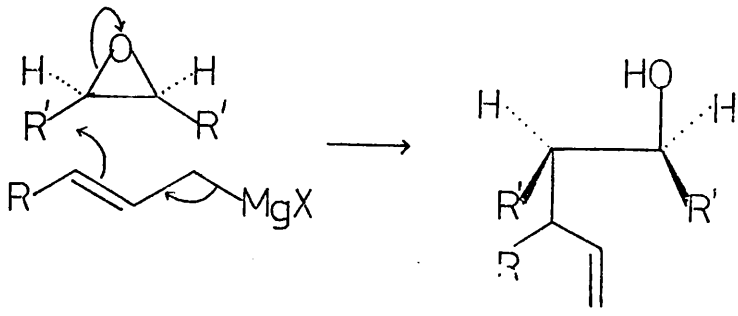
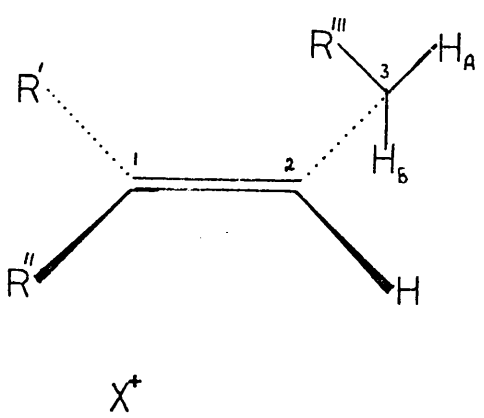


Fig.10

mechanism is inconsistent with a theoretical analysis of the S_E2' and S_N2' reactions since they are based on the same orbitals with the same symmetry, the only difference being in their electronic populations. It is interesting to note also that Cornforth, while suggesting a concerted mechanism for the isomerisation of (4) to (3) on the same empirical grounds used to reject a concerted process for (a), makes no reference whatsoever to the theoretical predictions extant at that time. Clearly then any predictions made about the concertedness or non-concertedness of these reactions have no justification from either a theoretical analysis or from comparison with non-enzymic examples.

There appeared then to be a clear need to devise a non-enzymic model system in which the stereochemistry of an S_E2' reaction could be determined and to compare the results with (i) the stereochemistry observed in the corresponding enzymic reactions and (ii) the observed stereochemistry in S_N2' reactions, and this we set out to fulfil. A survey of the literature revealed only one previous report of an S_E2' reaction in which Felkin proposed[25] that the opening of epoxides by allylic Grignard reagents proceeded by such a mechanism, Figure (10). While the case examined by Stork is taken to show that the S_N2' reaction is syn in nature, no stereochemical study of the S_E2' reaction has been reported. By determining the stereochemistry of an S_E2' reaction evidence for or against the necessity to invoke an X-group mechanism should be obtained, and in conjunction with the necessary experimental work on this problem molecular orbital calculations have been performed on several allylic systems in an attempt to obtain a more secure theoretical basis for these reactions than is available from the literature.

DISCUSSION



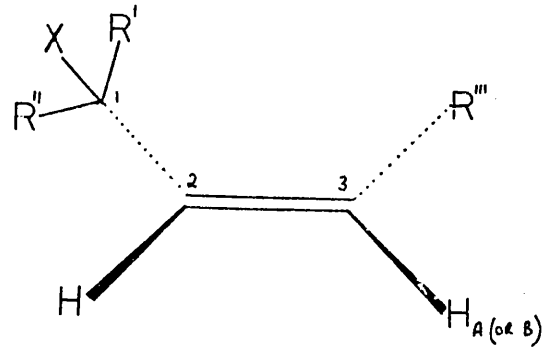
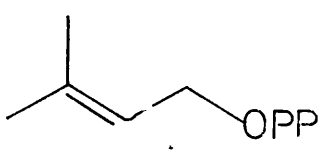


Fig.11



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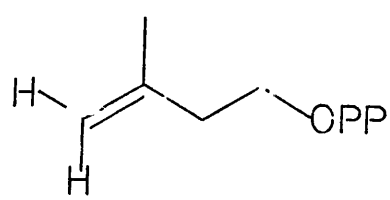


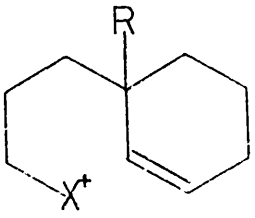
(4)

DISCUSSION

(i) Selection of a Model System

In order to define the stereochemistry of an S_E2' reaction it is necessary to determine (i) which hydrogen (H_A or H_B) is lost from the allylic position at C-3 and (ii) the configuration at C-1 in the product to which the electrophilic group (X) becomes attached, Figure (11). To distinguish between H_A and H_B requires that one of them, but preferably both, can be stereoselectively replaced by either deuterium, or tritium at tracer level. One of the simplest ways to achieve this is to incorporate the allyl system into a ring of defined geometry such that one hydrogen will become quasi-axial and the other quasi-equatorial, and to choose a ring in which it is possible to stereoselectively label axial and equatorial positions in the allylic methylene group. By inclusion of the allyl group into a ring the problem of determining the configuration at C-1 reduces to finding if X becomes attached in an axial or equatorial position. As is implied in the diagram it is also necessary to have only one allylic methylene group available for reaction, since the presence of two would lead to unnecessary complications. Having specified that a ring system is required, the model must be chosen so that there is a good chance that the desired reaction will occur. In general electrophilic attack on an isolated double bond leads to addition across the bond, therefore an electrophile must be chosen such that neither its counter-ion nor the solvent is sufficiently nucleophilic to add to the carbonium ion created by addition of an electrophile, or is prevented from so doing by steric restrictions.

By analogy with the enzymic condensation of (3) and (4)[11] it is desirable to generate the electrophile in close proximity to the weakly



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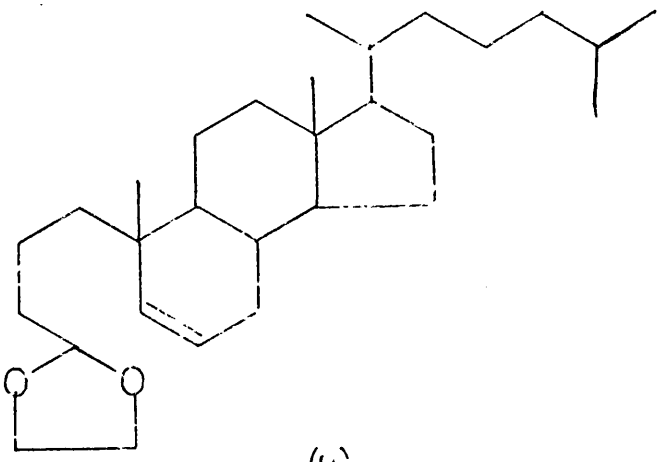

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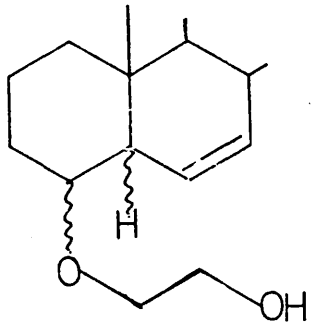
Note: In all the following diagrams which involve the basic cholestane skeleton, the structures are abbreviated so that only rings A and B are shown.



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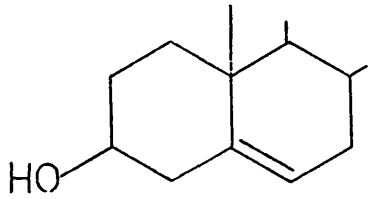

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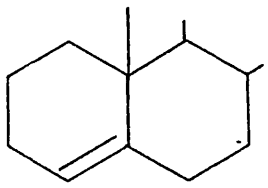

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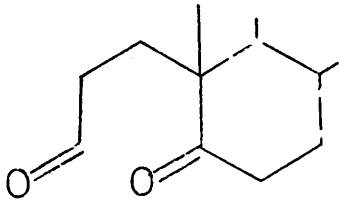

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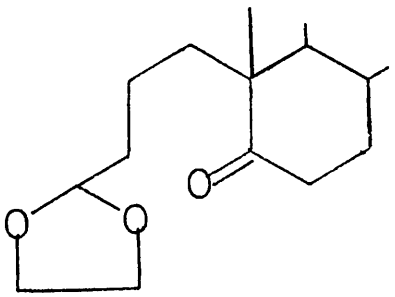

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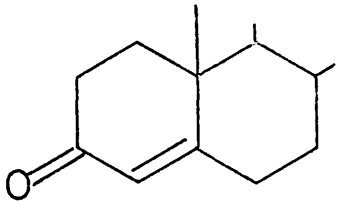


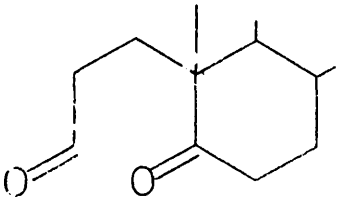
(14)

nucleophilic olefin, and preferably in such a manner that it will interact with only one of the two olefinic carbon atoms, C-1. This is best achieved by an intramolecular process in which the electrophilic centre is generated at the end of a carbon chain of appropriate length. The partial structure (7) satisfies the above requirements. An important practical consideration in the selection of a model system is that it should be synthetically accessible in good yield. The steroidal substrate (8) therefore seemed an attractive possibility since it appeared that an unremarkable synthesis from cholesterol could be readily accomplished and it was known that the allylic 7α - and 7β - hydrogens in cholesterol could be stereoselectively replaced by deuterium[26]. The choice of an acetal as a potential electrophilic centre was based on the extensive work by Johnson[6] on olefinic cyclisations. Our initial objective was to synthesise such a model system (8) from cholesterol and to subject it to conditions which might induce cyclisation to give a product or products of general form (9).

(ii) Synthesis of the Olefin-Acetal(8), and its Cyclisation.

The most direct route from cholesterol (10) to the olefin-acetal (8) appeared to be by way of cholest-4-ene (11) followed by ozonolysis to give the keto-aldehyde (12). If the aldehydic group in the keto-aldehyde could be selectively converted to the keto-acetal (13), then reduction of the ketone followed by elimination of water should give the desired precursor (8).

Cholesterol was converted to cholestenone (14) by standard methods[27] and the enone reduced to give cholest-4-ene (11) by the method of Brown[28] with modification of the stoichiometry of the chloroaluminium hydride used. Cholest-4-ene (11), readily purified by crystallisation from ether-methanol, was ozonised in hexane at -78° and the resulting ozonide reductively cleaved using zinc in acetic acid. Attempts to isolate and



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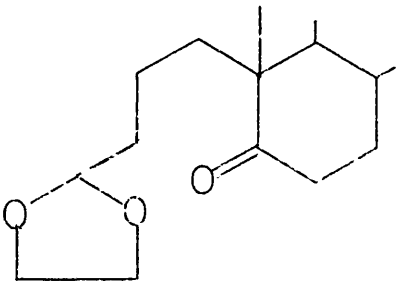

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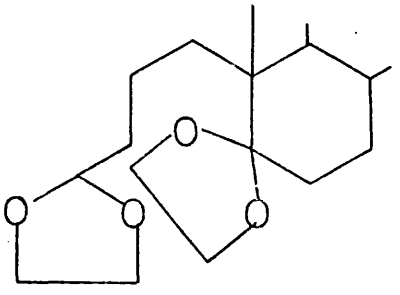

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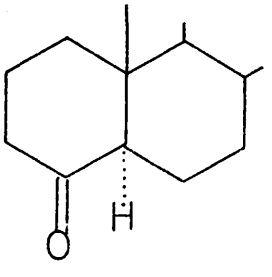

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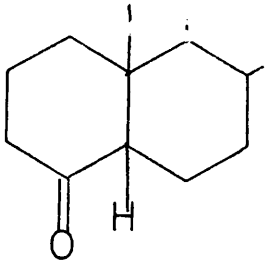

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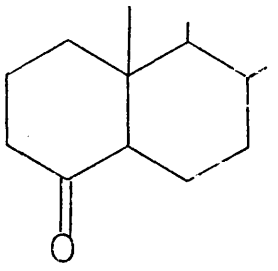
purify the resulting oil by preparative layer chromatography failed to give a pure sample of the desired keto-aldehyde (12) due to its lability on the absorbent, but the i.r. and n.m.r. spectra of the crude oil showed features characteristic of a keto-aldehyde (ν_{max} 2720, 1730, and 1710 cm^{-1}, τ 0.26). In a subsequent experiment the crude keto-aldehyde (12) was treated immediately with 1.1 equivalents of ethylene glycol in refluxing benzene with continuous water separation in an attempt to form the keto-acetal (13). However analytical t.l.c. showed that a major proportion of the oily product consisted of very polar components with only a small amount corresponding to the expected polarity of the keto-acetal (13), where two incompletely separated bands were evident. Attempted separation of these two components on preparative t.l.c. afforded only a small sample of each (< 5% yield) still contaminated with a little of the other. These components were identified as the keto-acetal (13) and probably the diacetal (15) on the basis of i.r. and n.m.r. data. Due to the high lability of the intermediate keto-aldehyde under basic or acidic conditions and the low yields of products obtainable, this route to the olefin-acetal (8) was not further pursued.

Hydroboration of cholest-4-ene followed by oxidative work up was known[29] to produce an equal mixture of cholestan-4-ones isomeric at C-5, which could be equilibrated under basic conditions to produce predominantly 5α - cholestan-4-one (16) and a trace amount of 5β -cholestan-4-one (17). Baeyer-Villiger oxidation of these ketones and opening of the lactones produced therefore seemed an attractive route towards 4,5-seco-derivatives. The above ketones were obtained in high yield by this procedure and isolated by preparative t.l.c. G.l.c. was used in an attempt to assay the purity of the samples obtained, however it was found that for samples homogeneous on t.l.c.



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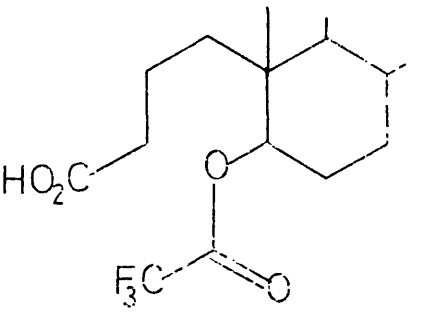

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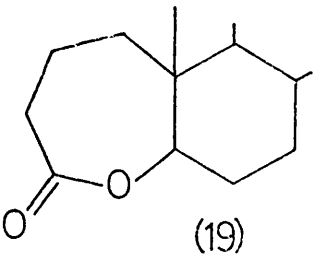

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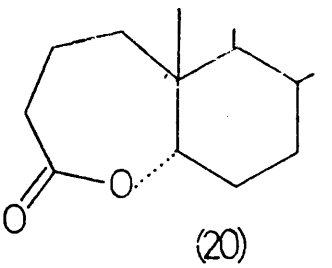

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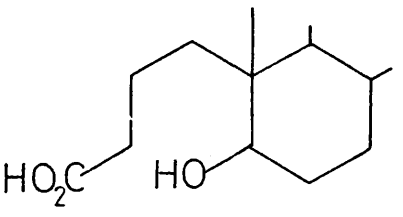

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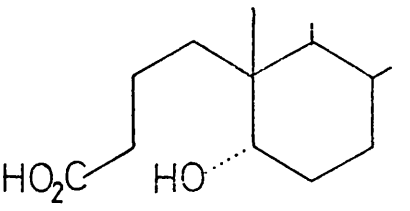

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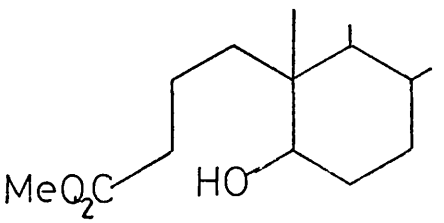

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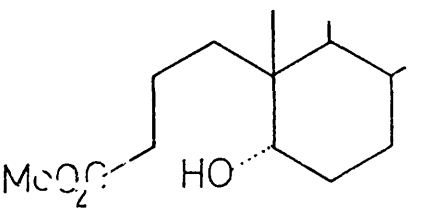

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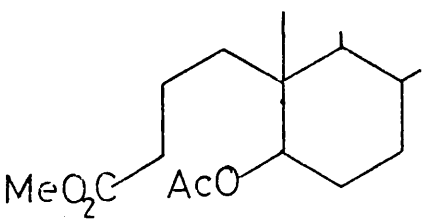

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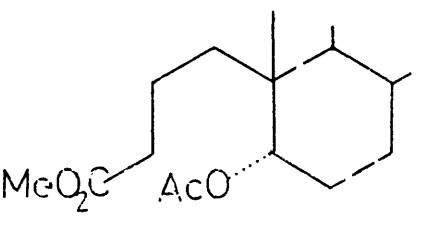

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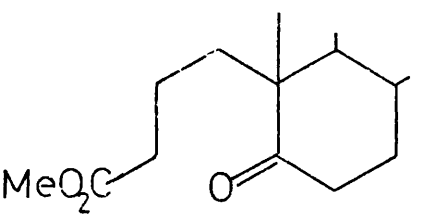
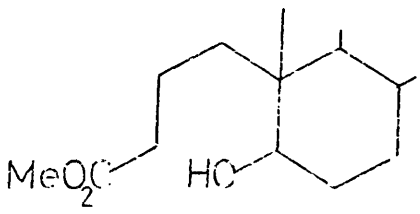


(27)

two peaks appeared in each case, due to equilibration on the column used. The 5β -isomer showed peaks at 2917 and 2975 (1:1) whereas the 5α -isomer (16) gave the same peaks but in a ratio of 1:8. Since the 5α -isomer is the more stable, the peak with highest retention value may be ascribed to it.

Baeyer-Villiger oxidation of the mixture (9:1) of 5α - and 5β-cholestan-4-ones using peroxytrifluoroacetic acid in methylene chloride[30] resulted in a mixture, the i.r. spectrum of which showed ν_{max} 3600-2500, 1785 and 1740 cm^{-1} and which appeared on t.l.c. to consist of an equal mixture of a very polar and a more mobile component. It seems likely that under the acidic conditions esterification had occurred after oxidation of the ketone to give the trifluoroacetate (18) of the hydroxy-acid (21). To prevent this occurring the reaction mixture was buffered using disodium hydrogen phosphate[31] and yields of 85-90% of the lactones (19) and (20) were routinely obtainable. Opening of the lactones to give the corresponding hydroxy-acids (21) and (22) was effected by stirring with methanolic potassium hydroxide, and the acids characterised by conversion (i) to the hydroxy-esters (23) and (24) and then to the acetates (25) and (26), and (ii) by oxidation and methylation to give the keto-ester (27).

Attempts to introduce the Δ^5 double bond met with difficulties. Since the Baeyer-Villiger oxidation proceeds with retention of configuration the major proportion of the mixture of lactones produced had the C-5/O bond β and equatorial (19). An attempt to effect elimination of the corresponding hydroxy-acid (21) with phosphyoryl chloride might not then seem too hopeful but there is precedent for elimination of neo-pentyl equatorial alcohols by this reagent without rearrangement (for example dihydrolanosterol $\longrightarrow$ lanosta -2,8-diene[32]); however the major product obtained on reaction was the lactone (19).



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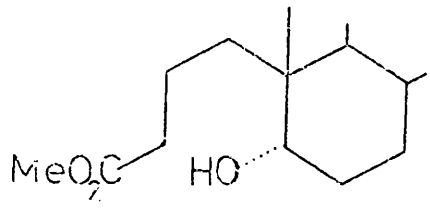

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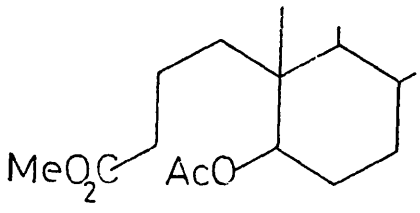

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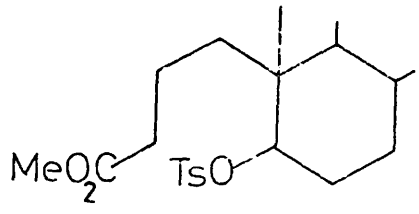
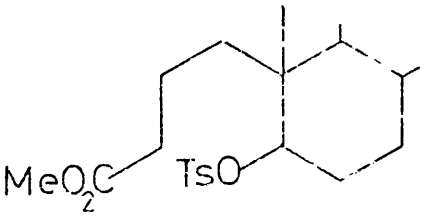


(28)

Similar treatment of the hydroxy-ester (23) with phosphoryl chloride afforded a complex mixture from which was obtained an unidentified methyl-ester in low yield.

Pyrolysis of the corresponding acetate (25) as a method of effecting elimination of the β-hydroxyl group was then investigated. Passage of a small sample of the acetate (25) through a silica tube at 570° packed loosely with glass wool resulted in recovery of the acetate and a trace of two components more mobile on t.l.c. Introduction of a larger sample into the tube by sublimation at reduced pressure allowed these two components to be isolated. The more mobile component appeared to be an olefinic hydrocarbon and was not further investigated, while the second component appeared to be an olefin-ester [ν_{max} 3010, 1740, and 1640 cm^{-1}, τ 4.53(1.8H,q), 5.30(small), and 6.36(3H,s)] which was still a mixture, and on g.l.c. showed the presence of one major component and a minor component as a shoulder on the peak. Encouraged by this result a preparative pyrolysis procedure was sought. By clamping the pyrolysis tube vertically in an oven a solution of the acetate (25) could be introduced dropwise in a stream of nitrogen and the product collected in a receiver below. The results obtained were variable, depending critically on the rate of introduction of the acetate solution, and to ensure maximum utilisation of the acetate several passes through the tube were required for each sample, which not only diminished the overall recovery of material but increased the proportion of hydrocarbon at the expense of the olefin-ester.

Elimination of the tosylates of equatorial steroidal alcohols by various methods have been reported[33, 34, 35] and our attention was next focussed on this procedure. The tosylate (28) of the β-hydroxy-ester (23) formed readily in good yield but the corresponding α-hydroxy-ester (24)



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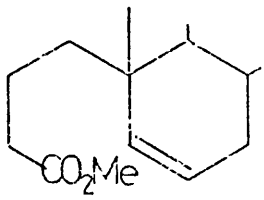

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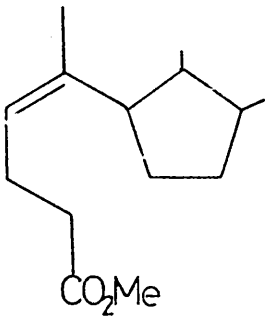

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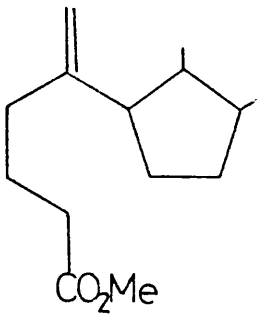
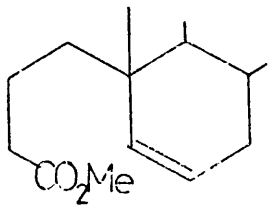


(31)

did not react so rapidly and in general yields were some 30-40% lower. Adsorption of equatorial tosylates on alumina, studied by Meakins[36] and Shoppee[37] has been shown to result in olefin formation, but adsorption of the tosylate (28) on alumina for 80h resulted in recovery of half of the tosylate and a small amount of a component with the same R_F on t.l.c. as the olefin-ester obtained by pyrolysis. Tosylate eliminations have also been carried out in dipolar aprotic solvents[38,39] and our next choice of conditions was to heat the tosylate (28) in dry dimethylsulphoxide[40] at 115° for 17h. This resulted in 75% elimination to give apparently one product on t.l.c. corresponding to that obtained from elimination of the tosylate on alumina; however t.l.c. on silver nitrate plates showed the presence of at least three components and correspondingly g.l.c. showed three peaks in a ratio of 6:4:1 in order of increasing retention times. A pure sample of the desired olefin-ester (29) was obtained from this mixture by preparative layer chromatography on silver nitrate plates with multiple elution. This compound (29), characterised by its spectral properties was shown to be identical on g.l.c. to the major component from acetate pyrolysis by cross injection. Impure samples of the two other components present in the mixture were obtained. The first, probably a mixture (3:1) of the rearranged olefin-esters (30) and (31) was homogeneous on g.l.c. and t.l.c. The structure (31) is suggested from the n.m.r. spectrum, τ 5.37 (d), which in fact is probably not a doublet but two singlets from the exomethylene protons. The isomeric olefin-ester (30) is proposed to account for the presence of a vinyl methyl group (τ 8.38) and an apparent doublet at 7.67τ, which collapses to a broad singlet on irradiation at the olefinic resonance (4.95τ). This doublet, which is not symmetrical, may well result from accidental equivalence of the methylene protons adjacent to the ester and those allylic to the double



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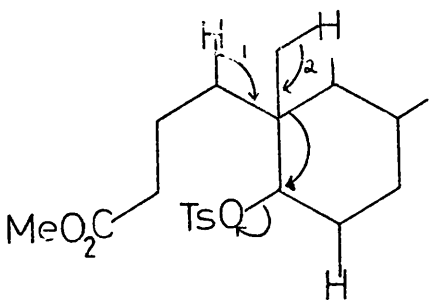

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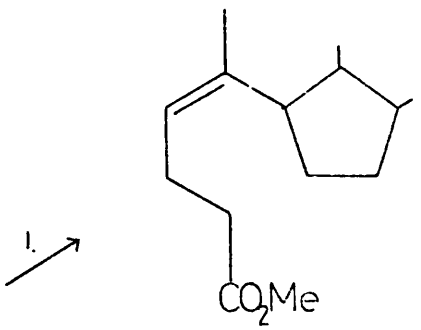

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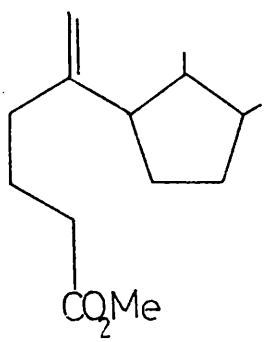
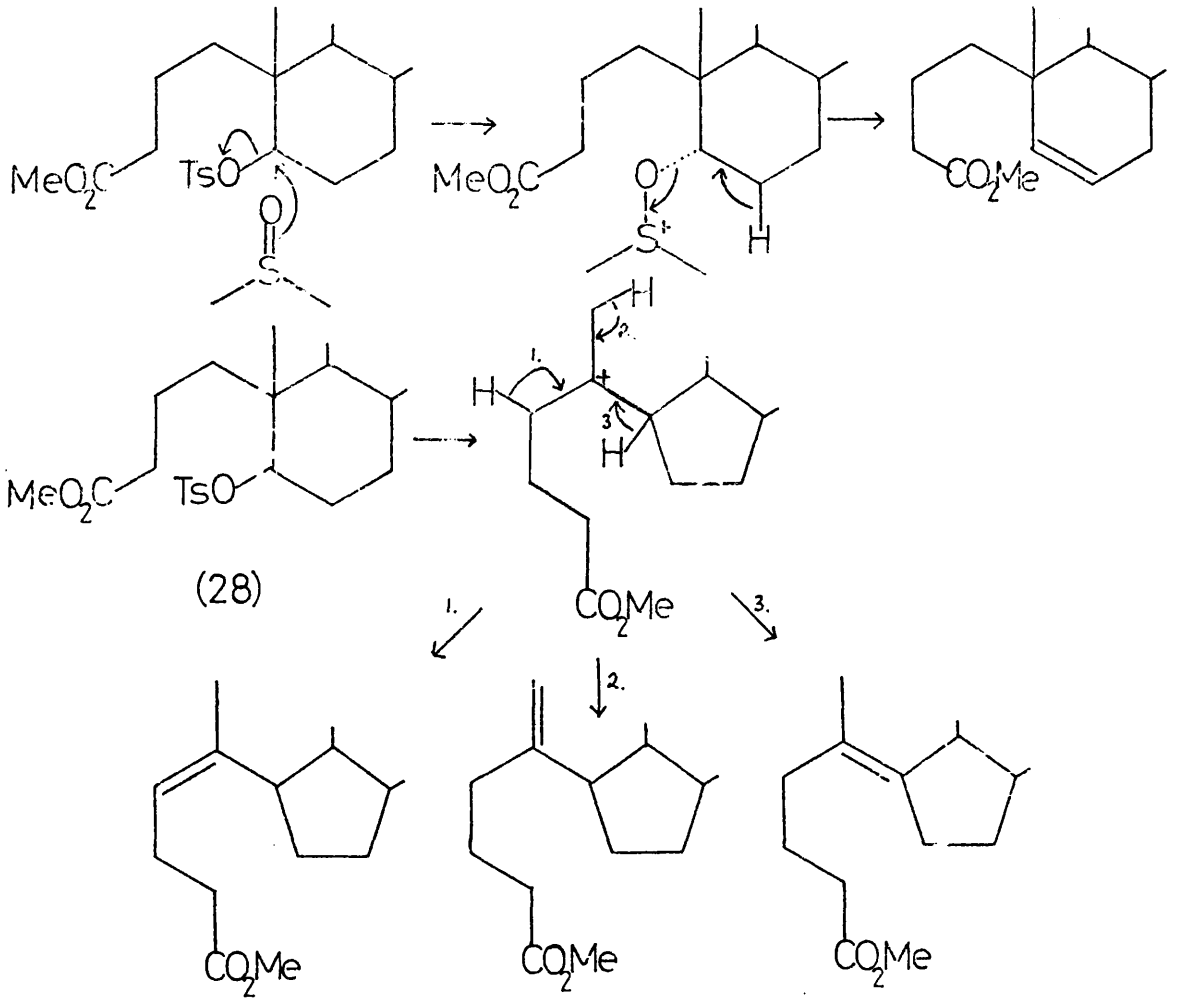


(31)

Fig.12

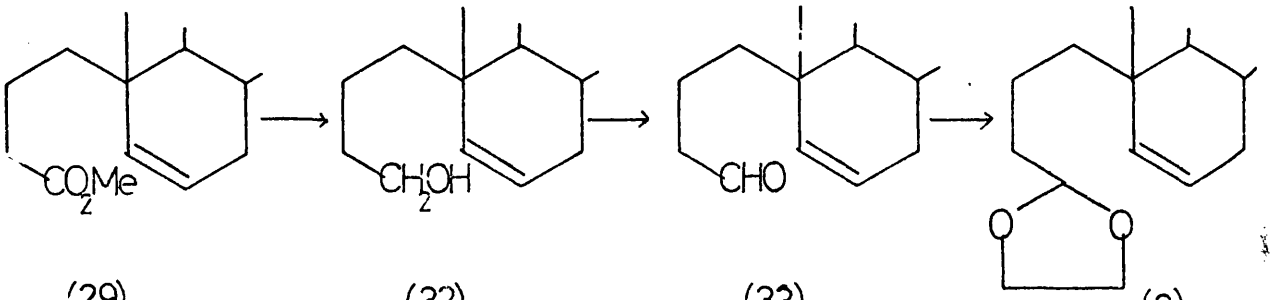
bond. The predicted structure for such a signal would be a singlet (from the methylene group adjacent to the ester) superimposed on a doublet (from the allylic methylene group which couples with the vinyl proton). Double irradiation experiments are consistent with these proposals. The second component, also a mixture (5:1) showed the presence of a vinyl methyl group, a vinyl proton and a methyl ester but remains unidentified. Both of these impure samples correspond to the peak with shortest retention time on g.l.c. although the second showed a trace of the component with longest retention time while the required olefin-ester (29) corresponds to the central peak. From this elimination one structure has been established, two others are suggested and a fourth remains unidentified. Such a range of products was not expected but is readily rationalised in terms of the geometry of the tosylate (28) in which the 9,10 - C-C bond is parallel to the departing β-tosylate group. Migration of this bond as shown in Figure (12) results in a contracted ring B, and proton loss from C-1 or C-19 leads to (30) and (31) respectively. Conditions were then sought to minimise this competitive rearrangement and experiments were assayed by g.l.c..

Addition of potassium t-butoxide improved the ratio of products from 6:4:1 to 2:3:1 while refluxing the tosylate (28) in pyridine gave a ratio of 1.5:3:1 and use of sodium acetate in acetic acid[41] resulted in a ratio of 2.5:2:1. Elimination in dry dimethylformamide however proved more encouraging giving a ratio of 2:3.5:1. Lithium halides have been used as nucleophiles in aprotic solvents to effect reactions such as demethylation of phenols or esters[42] and we were prompted by previous work[43] to use lithium bromide in dimethylformamide. Accordingly, when the tosylate (28) was treated with a two-fold excess of lithium bromide in dry dimethylformamide with lithium carbonate added to neutralise any acid formed, the product ratio improved



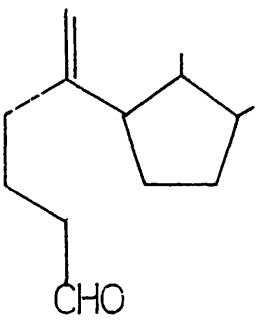
(28)

Fig.13

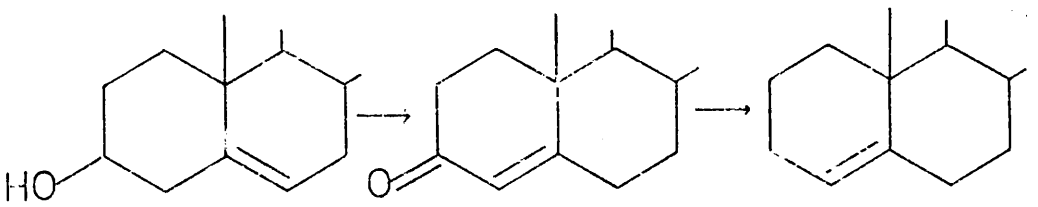


(29) (32) (33) (8)

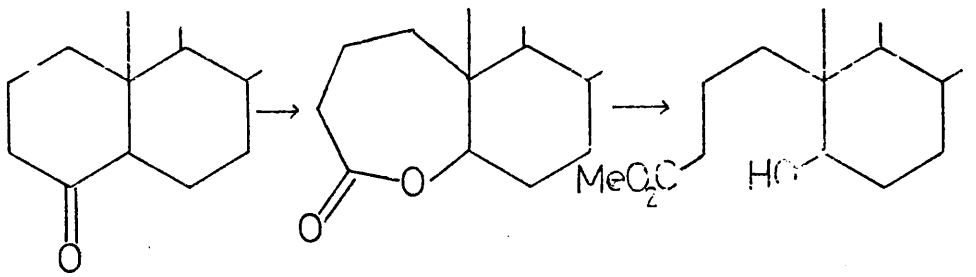
Fig.14



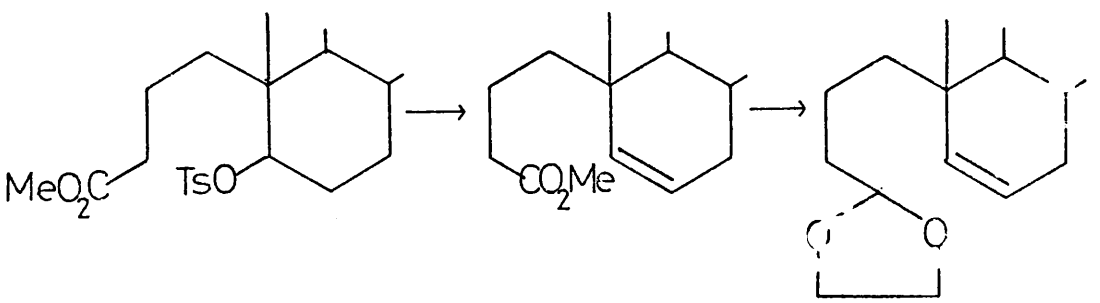
(34)



(10) (14) (11)



(16) (19) (23)



(28) (29) (8)

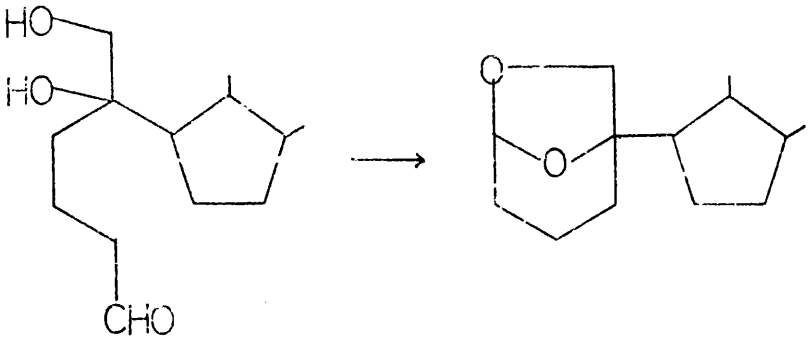
Fig.15

markedly to 1.5:6:1, and on increasing the proportion of lithium bromide to a ten-fold excess the desired olefin-ester (29) was obtained as the predominant product (>90%) in the olefinic fraction.

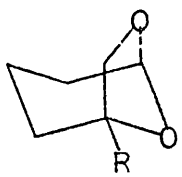
It seems likely that in dimethylsulphoxide two processes are operating; (i) S_N2 displacement of tosylate by dimethylsulphoxide[40] followed by trans-diaxial elimination to give the olefin-ester (29) and (ii) departure of the β-tosylate assisted by concomitant C-C bond migration to leave a carbonium ion which can lose a proton in three ways, Figure (13). Use of a better nucleophile, bromide ion, in dimethylformamide evidently competes strongly in the tosylate elimination with rearrangement, presumably by a similar mechanism of S_N2 displacement followed by elimination of HBr, and by saturating the solution with bromide ion the best yield of pure olefin-ester (29) was obtained.

Elaboration of the olefin-ester (29) to the olefin-acetal (8) was accomplished by unexceptional means, Figure (14).

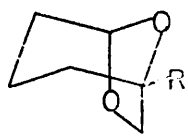
Having established this synthetic route from cholesterol, summarised in Figure (15), further production of the olefin-acetal (8) was carried out without purification of all intermediates and a yield of 10% of (8) was obtainable starting from cholestenone (14). In one such process an impurity was detected at the olefin-aldehyde stage. Analytical t.l.c. showed the presence of a minor component just separated from the olefin-aldehyde (33). Isolation of this isomeric rearranged aldehyde by preparative t.l.c. and spectral analysis [ν_{max} 3080, 2705, 1730, 1630 and 890 cm^{-1}, τ 0.22(1H), 5.32(1H), and 5.52(1H)] led to the proposed structure (34) in accord with the previous suggestion of the structure (31) for one of the rearranged olefin-esters arising from elimination of the tosylate (28) in dimethylsulphoxide. In an attempt to confirm this structure the rearranged aldehyde (34) was



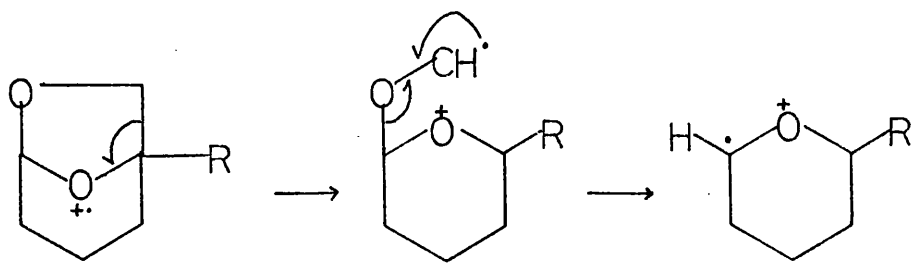
(35)



(36)

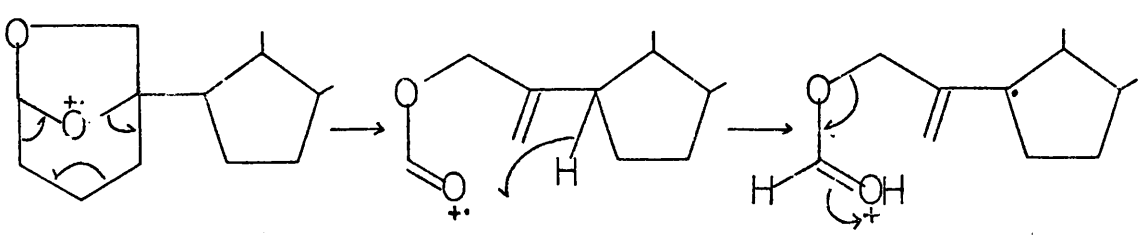


(37)



m/e 402

m/e 372



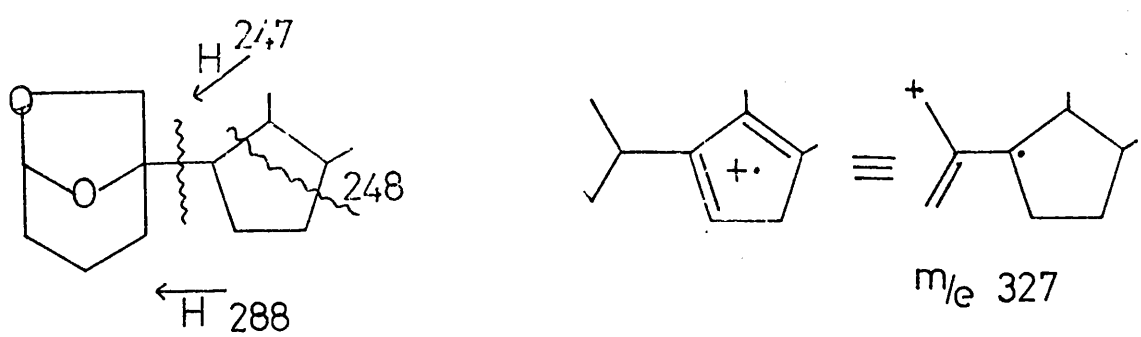
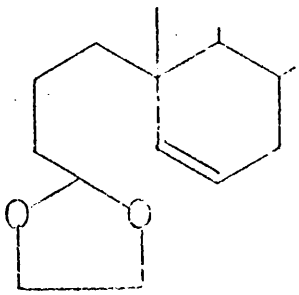


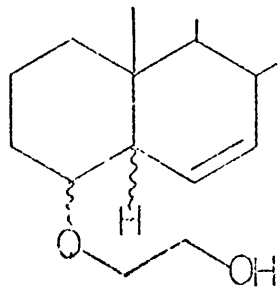
Fig.16

osmylated but instead of isolating the corresponding diol (35), work up and evaporation of the benzene solution produced a very non-polar compound, homogeneous on t.l.c. but a mixture (2:5) on g.l.c.. The most probable explanation is that a mixture of isomeric internal acetals (36) and (37) were formed, part structures of which are illustrated. G.c.-m.s. studies of the mixture showed the two components to be isomeric and in conjunction with i.r. and n.m.r. spectra are consistent with the proposed structures and the major fragment ions in the m.s. are readily accommodated[44] as shown in Figure (16).

Our next concern was to seek conditions which would induce cyclisation of the olefin-acetal (8) to produce hydroxy-ethers (9). Once more drawing on the work of Johnson[45] stannic chloride was chosen as the Lewis Acid initiator and the cyclisation was initially attempted in benzene; however this resulted in recovery of unchanged acetal as judged by t.l.c.. On changing the solvent to nitromethane and mixing the olefin-acetal (8) and stannic chloride in a molar ratio of 1:4 an intractable mixture was produced after 40 min. However mixing of the olefin-acetal and stannic chloride (1:2) and quenching the reaction after 15 sec gave a more promising mixture which on t.l.c. appeared to consist of four major and a large number of minor components. G.l.c. analysis of the mixture showed the presence of seven components. Before g.c.-m.s. facilities became available simplification of the mixture was attempted. In the belief that most of the products would contain a β-hydroxyether function and since there was the possibility of forming epimers at C-4 and C-5 (a total of four such products) we attempted to cleave the ether linkages to give the parent alcohols which could then be oxidised to the corresponding ketones. Treatment of the crude mixture successively with toluene-4-sulphonyl chloride, sodium iodide and zinc[45], and finally Jones Reagent gave a mixture, Figure (17), which was even



(8)



(9)

fig 17

1% OV-1 225°

(- 5min -)

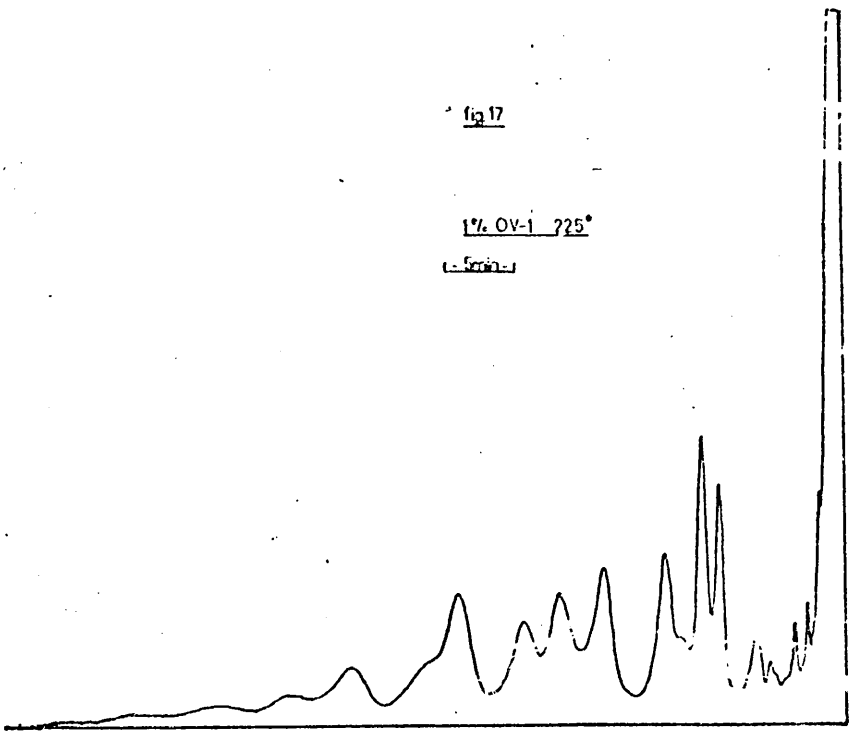


fig 18

1% OV-1 200°

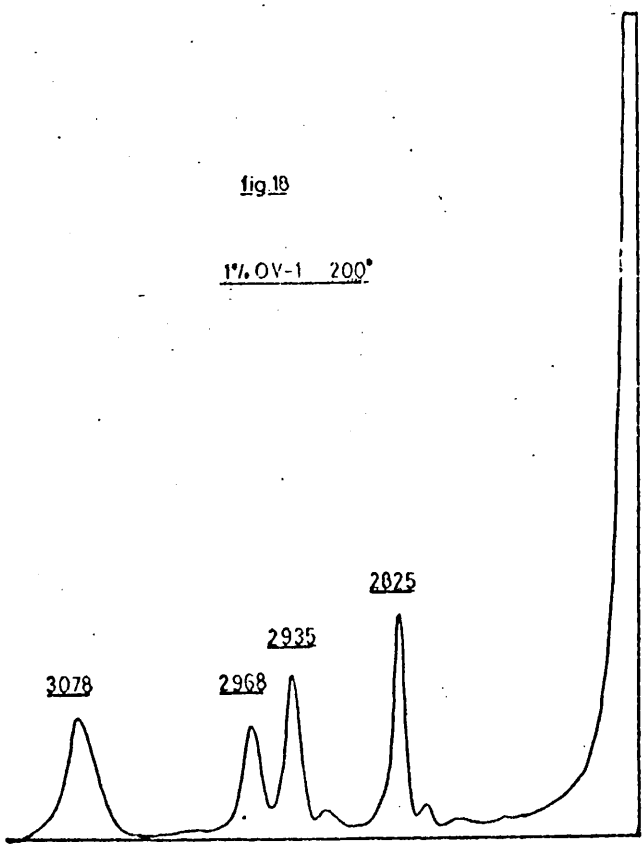


TABLE (1)

G.c. - m.s. data for Products obtained by treatment of the olefin-acetal (8) with $SnCl_4$ in nitromethane

Peak on g.l.c.	A	B	C	D	E	F	G
Carbon Number	2780	2800	2822	2870	2935	2966	3078
						430 (16)	428 (100)
m/e values						415 (6)	413 (28)
and relative				386 (40)	388 (62)	388 (20)	387 (34)
intensities	368 (41)	368 (100)	368 (100)	368 (100)	373 (44)	386 (14)	368 (37)
	353 (6)	353 (25)	353 (14)	353 (50)	370 (47)	368 (100)	
	298 (8)	281 (8)			355 (13)	353 (36)	
	270 (100)	270 (16)			353 (10)		288 (40)
	255 (10)	255 (26)	255 (38)	255 (38)	248 (48)	253 (40)	273 (32)
	217 (18)	213 (40)	247 (75)	247 (15)	233 (100)	233 (26)	227 (19)
		207 (20)	213 (22)	213 (37)	215 (58)	215 (22)	213 (34)
		201 (20)		201 (55)		201 (46)	207 (60)

TABLE (2)

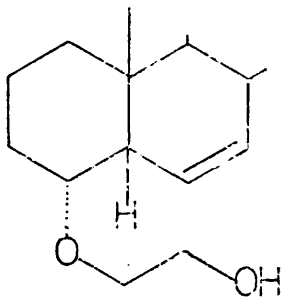
G.c. - m.s. data for the T.M.S. Ethers

Peak on g.l.c.	H	I	J	K
Retention time (min)	6.6	10.7	12.65	26.25
m/e values and			502 (4)	502 (7)
relative intensities	458 (5)	460 (11)	487 (2)	487 (2)
	443 (10)	445 (14)		
	368 (100)	370 (100)	368 (100)	368 (75)
	353 (26)	355 (24)	353 (19)	353 (29)
	313 (7)	315 (63)		273 (100)
	255 (25)	257 (30)	255 (22)	255 (20)
	229 (17)		247 (10)	
	213 (20)	215 (33)	229 (10)	
	201 (38)		213 (14)	213 (15)
			201 (32)	207 (15)
				201 (10)

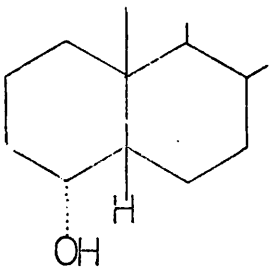
more complex than that initially produced by cyclisation of the olefin-acetal (8)!

On obtaining results of a g.c.- m.s. analysis of the cyclisation mixture, Table (1) it became evident that there was only one component of the mixture, formed in a minor amount, which was isomeric with the olefin-acetal. To obtain further information about the other components the crude mixture was treated with trimethylsilyl chloride so that any alcohols present could be characterised as the corresponding trimethylsilyl ethers. A g.c.-m.s. analysis of the silylated mixture was also performed and the results are listed in Table (2). Since it did not prove possible to isolate any pure compounds from the cyclisation mixture conditions were sought to optimise the proportion of material in the mixture isomeric with the olefin-acetal (8). It seemed possible that if the cyclisation was performed at lower temperatures then fewer products might result and a greater selectivity obtained. Solutions of the olefin-acetal in nitromethane could not be handled below 0° due to the limited solubility of the olefin-acetal and so methylene chloride in which it was freely soluble was used.

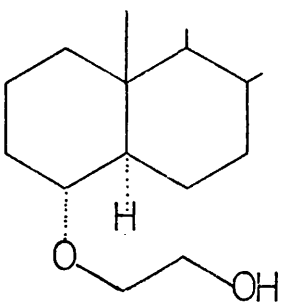
A preliminary experiment using this solvent at 20° resulted in principally aldehydic material and was not further investigated. However, a mixture of the olefin-acetal and stannic chloride (1:4) in methylene chloride at -78° resulted in a dramatic improvement and two components appeared sufficiently well separated on t.l.c. to allow isolation and g.l.c. showed the presence of only four major peaks, Figure (18). A preparative scale (100 mg) cyclisation was carried out using a ratio of (1:20) and after chromatography two pure components were isolated from the cyclisation mixture in yields of 15%. The more mobile band showed features characteristic of an unsaturated



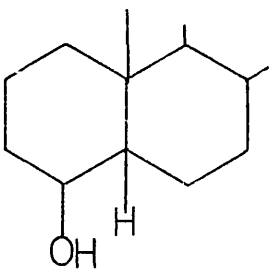
(38)



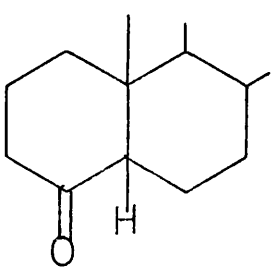
(39)



(40)



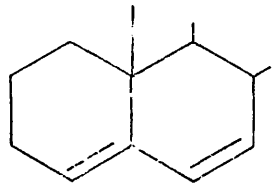
(41)



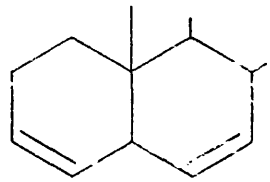
(16)

β -hydroxyether [ν_{max} 3530, 3010, 1645, 1098, and 1050 cm^{-1}, τ 4.38 (2H,q), 6.38 (4H,m) and 8.54 (1H,s)] and the m.s. is also consistent with such a structure. Further evidence that this compound was the desired hydroxy-ether (38) was obtained by degrading it to the saturated parent alcohol, which also established the stereochemistry at C-4 and C-5. Hydrogenation using diimide afforded the dihydro-compound which was not isolated but treated with toluene-4-sulphonyl chloride followed by sodium iodide and zinc. The saturated alcohol produced was shown to be identical (t.l.c., g.l.c., i.r. and m.s.) with authentic 4α -hydroxy-5β -cholestane (39). A previous attempt to hydrogenate the hydroxy-ether (38) using palladium/charcoal resulted in formation of two products, one corresponding on t.l.c. to that produced by diimide reduction, and the other probably resulting by epimerisation at C-5 on the catalyst to give the corresponding 5α -saturated hydroxyether (40). Thus the hydroxy-ether (38) produced in cyclisation was shown to be the 4α , 5β -isomer as indicated. The position of the double bond, Δ^6, was deduced from the n.m.r. spectrum which shows the presence of a two proton quartet in the olefin-region (τ 4.38), and this will be further substantiated by deuterium labelling studies (see below). The second component, also an alcohol, was shown to be identical (t.l.c., g.l.c., ii.r., n.m.r. and m.s.) with 4β -hydroxy-5β -cholestane (41) and could be oxidised to the known 5β -cholestan-4-one (17).

The remaining components in the mixture were not investigated due to difficulties in separation of pure components but some proposals about their identies can be made from their mass spectral and chromatographic properties. The first three peaks (A, B, and C), Table (1), are isomeric and from their retention times are probably hydrocarbons. These may well result from elimination of alcohols on the g.l.c. column since they are not present in the g.l.c. trace of the



(42)



(43)

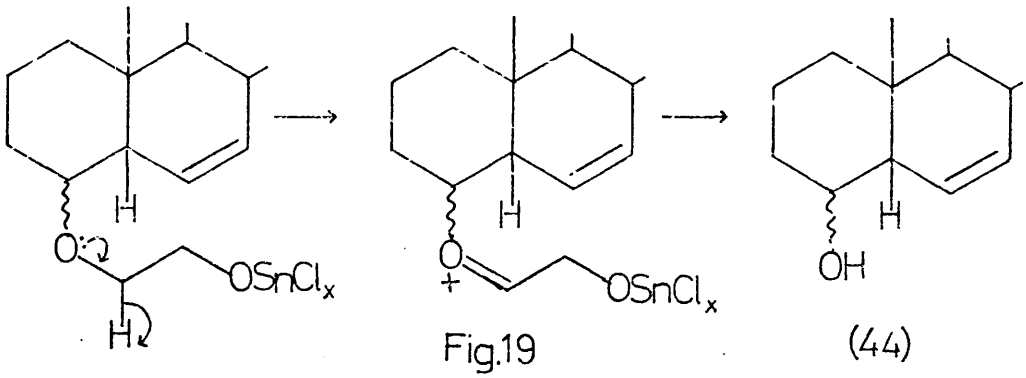


Fig.19

(44)

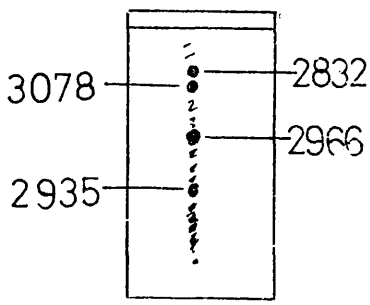


Fig.20

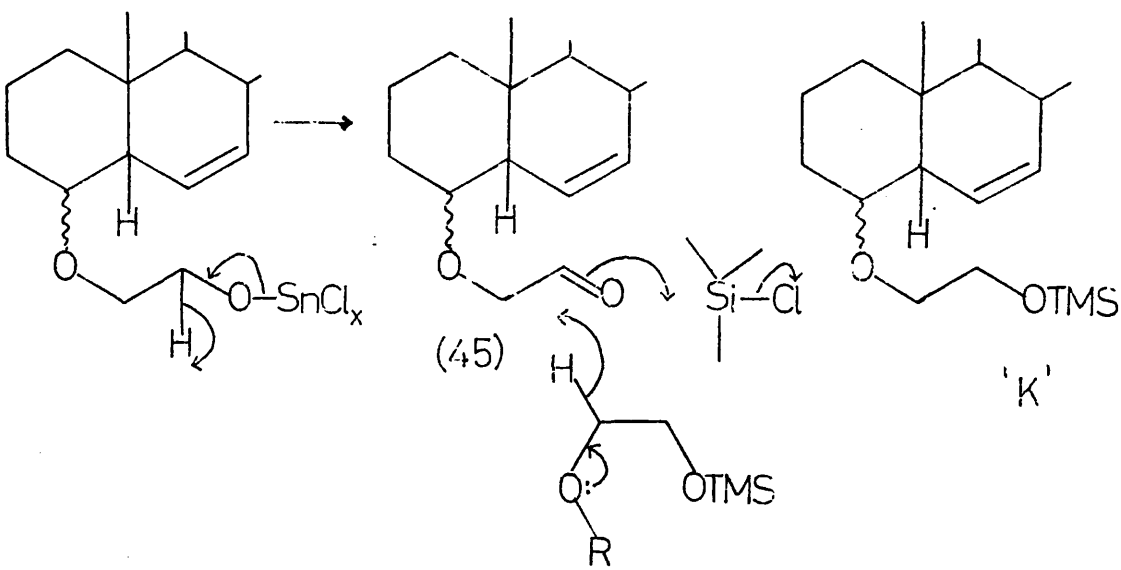


Fig.21

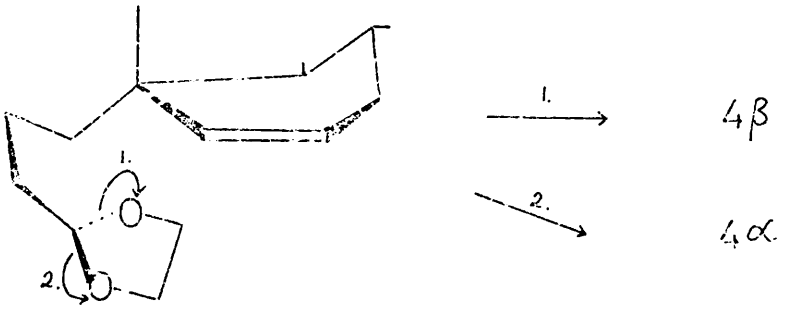
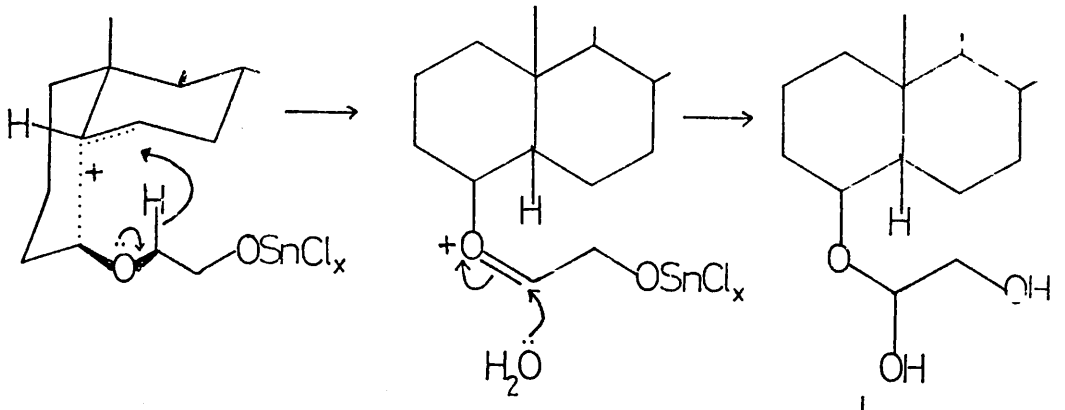
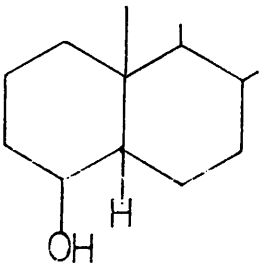


Fig. 22



(46) (47) (48)



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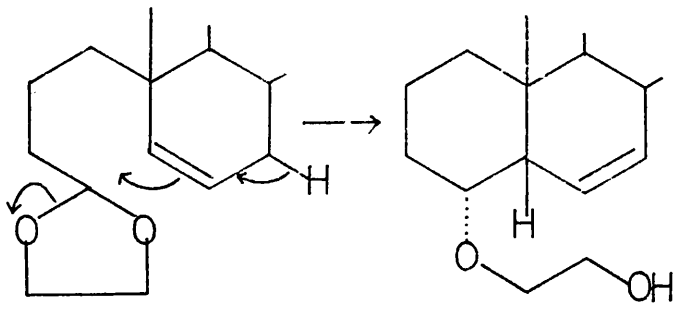


(8) (38)

corresponding trimethylsilyl ethers, and structures such as (42) and (43) therefore seem likely. The fourth peak, D, shows M^+ at 386 and a base peak at 368 and it forms a TMS ether, E. These features seem best accounted for by the unsaturated alcohol (44) which could arise by loss of the ether fragment by intermolecular hydride transfer. Figure (19). Peaks E and F correspond to (41) and (38) which give TMS ethers I and J respectively, but peak G is rather difficult to explain. It has been shown that this peak corresponds to a non-polar component on t.l.c., Figure (20), and yet it appears to give a TMS ether, K. It is possible that this compound is the aldehyde (45) which could arise by hydride transfer, but to form a TMS ether of this aldehyde would require a further hydride transfer. Although these ideas may appear unlikely it is possible that on silylation of the mixture hydride could be supplied as shown, Figure (21).

Our main concern, however, was to be able to account for formation of the products we had definitely characterised. The hydroxy-ether (38) is readily explained in terms of the desired S_E2' reaction, but the occurrence of the saturated alcohol (41) was at first perplexing. On constructing a model of the olefin-acetal (8), it became clear that cleavage of the acetal by stannic chloride was possible in two ways, Figure (22), and depending on which C-4/oxygen bond is cleaved, the product will contain the acetal residue in either the 4α - or 4β - configuration. However when C-O fission occurs to leave the acetal residue attached in a 4β - configuration, a hydride transfer is possible from the α -carbon of the β -hydroxyether to C-6 through a six-membered 'chair'-like transition state (46). This will afford the oxonium ion (47) which on quenching in water will hydrolyse to give the saturated alcohol (41) via the hemiacetal (48), and it is possible that the complex appearance of thin layer chromatograms resulted from

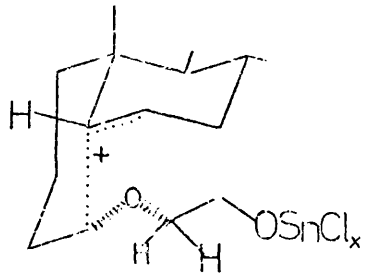
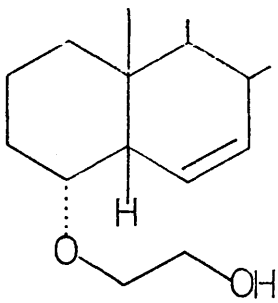
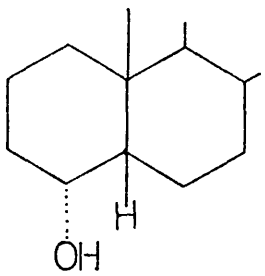


Fig.23



(38)



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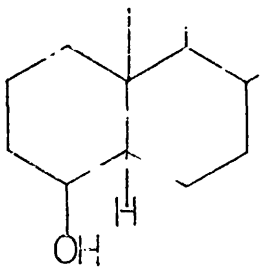
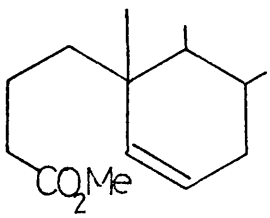


(41)



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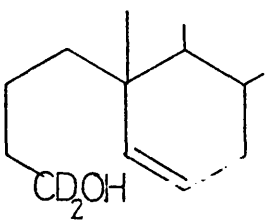

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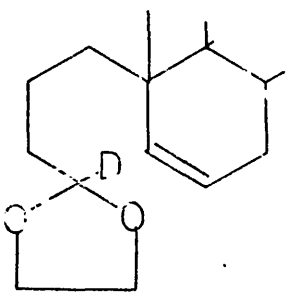

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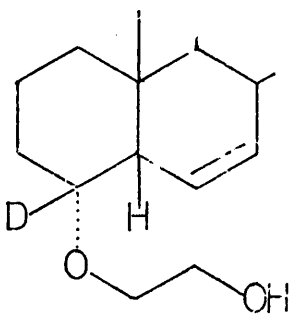

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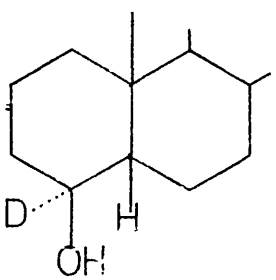

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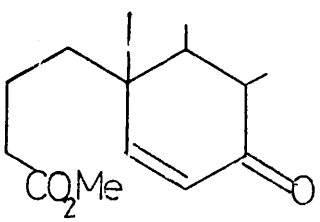


(54)

incomplete hydrolysis of this hemiacetal and its subsequent degradation on t.l.c.. A similar favoured transition state for hydride transfer is not available to the corresponding 4α -isomer, Figure (23), and consequently the hydroxy-ether (38) is formed by loss of a proton from C-7.

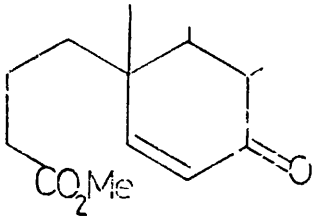
An interesting feature of the i.r. spectra of 'authentic' 4α -and 4β -hydroxy-5β -cholestane is the appearance of a shoulder on the 'free - OH' adsorption. The 4α -isomer (39) shows a shoulder to higher wave number (ν_{max} 3640 sh and 3630 cm^{-1}) while the converse is true for the 4β -isomer (41) (ν_{max} 3626 and 3604 sh cm^{-1}), and this may reflect the energies of different rotameric positions for the O-H bond in these compounds, but in any case it provided a quick method of differentiating between the two isomers.

Having established the conditions for cyclisation to give the desired hydroxy-ether (38) and having proved its structure, we then set out to demonstrate that (i) a hydrogen atom from C-7 was cleanly lost and (ii) that there was no scrambling of hydrogen during the reaction.

(iii) Deuterium Labelling Studies and Apparent Conclusions

Deuterium could be readily incorporated at C-4 by reduction of the olefin-ester (29) with lithium aluminium deuteride to give the dideuterio-hydroxy-olefin (49) which was elaborated to the 4-deuterio-olefin-acetal (51) as before. Cyclisation of this acetal (51) gave the deuterio-hydroxy-ether (52) and 4α -deuterio-4β -hydroxy-5β -cholestane (53) with complete retention of deuterium at C-4.

To label the 7-position it was our intention to reduce the corresponding enone-ester (54) with dichloroaluminium deuteride. Allylic oxidation of the olefin-ester (29) using N-bromosuccinimide in moist dioxan according to Thomson[46] resulted in the production of two compounds neither of which was an enone. The major component was identified as



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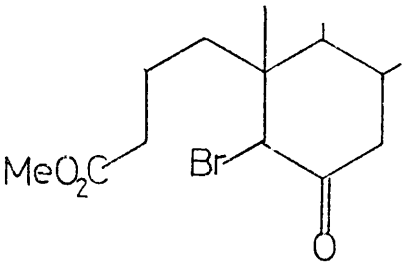

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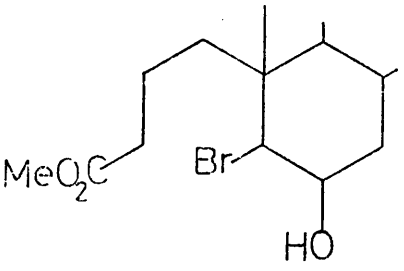

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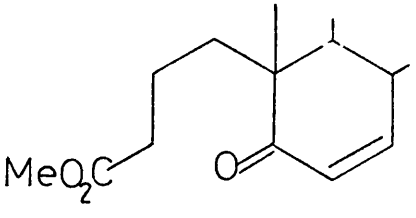
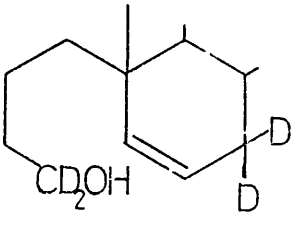


(57)



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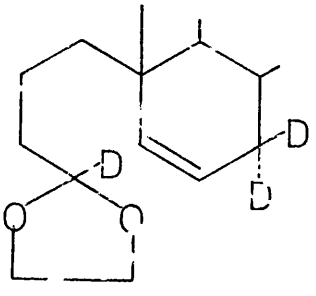

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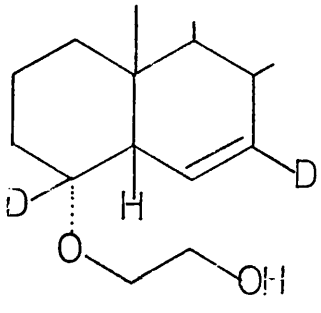

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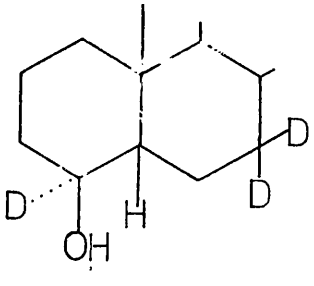

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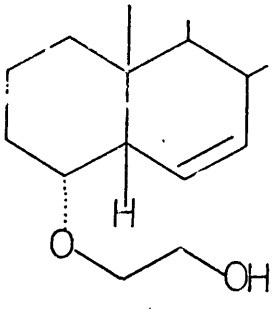


(38)

the bromo-keto-ester (55) [ν_{max} 1740, 1720 cm^{-1}, τ 6.10 (1H,s), 6.42 (3H,s), M^+ 512 and 510] and the minor component the corresponding alcohol (56). It is clear that under the conditions used bromohydrin formation had occurred, followed by oxidation of (56) by N-bromosuccinimide to give the bromo-keto-ester (55), and a more recent publication by Mazur[47] confirms this as being the more common course of reaction under these conditions. However use of anhydrous sodium chromate effected the desired transformation and gave a mixture (5:1) of the enone-ester (54) and an isomeric enone-ester (57) characterised by its n.m.r. spectrum which exhibited a small allylic coupling (3Hz) on the limbs of the quartet arising from the vinyl protons. The enone-ester (54), purified by preparative t.l.c. was reduced as described using dichloroaluminium deuteride to give the tetradeuterio-hydroxy-olefin (58) which was converted to the trideuterio-olefin-acetal (60) as described above. Cyclisation of this substrate (60) afforded a dideuterio-hydroxy-ether (61) which clearly showed the presence of a vinyl deuterium [ν_{max} 2235 cm^{-1}, τ 4.43 (1H,d)] and the complete loss of one deuterium atom in the cyclisation, which constitutes additional proof that the double bond in the hydroxy-ether (38) is Δ^6. The corresponding 4β-hydroxy-5β-cholestane (62) was shown to retain its deuterium completely and although it cannot be stated with certainty, it seems probable that it is retained at C-7 in accord with the mechanism advanced for its formation. Thus we had established that

(i) an S_E2' process occurs,

(ii) a proton is lost from C-7, and

(iii) the electrophilic group (C-4) becomes attached from the α-face, and it now remained to determine whether there was any stereoselectivity in the proton loss from C-7.

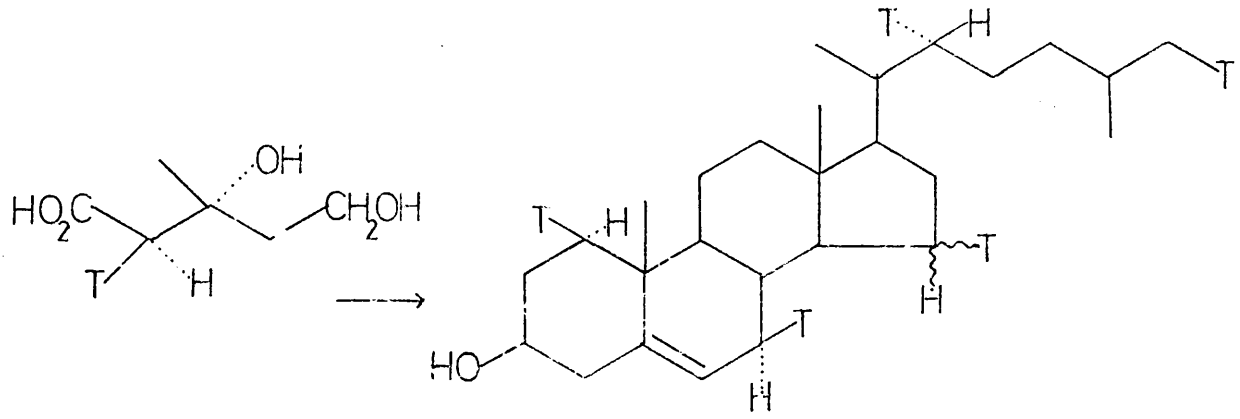


Fig.24

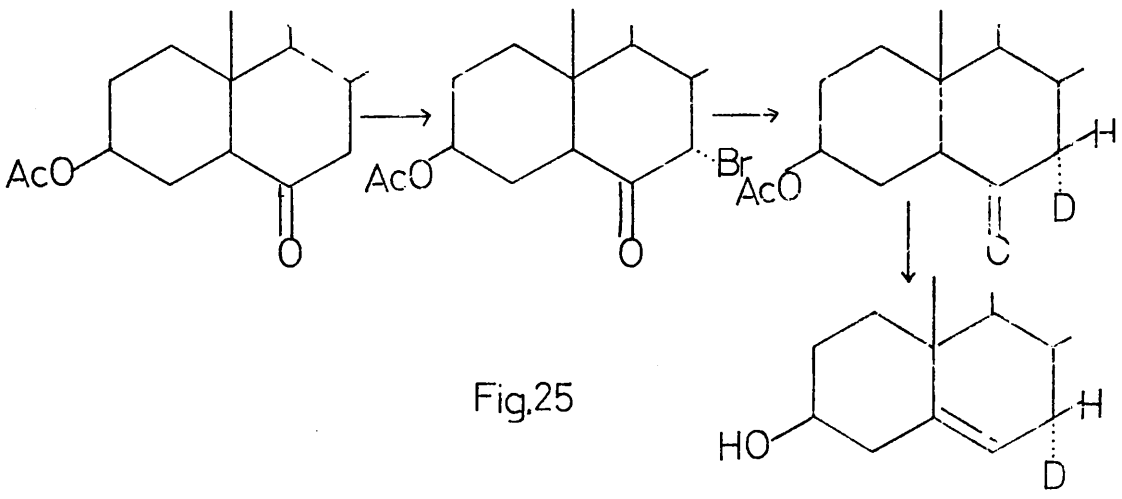
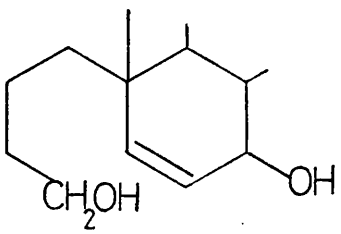


Fig.25



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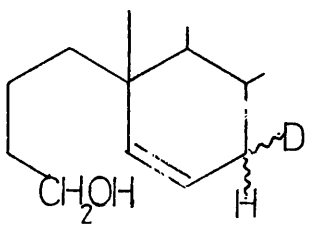

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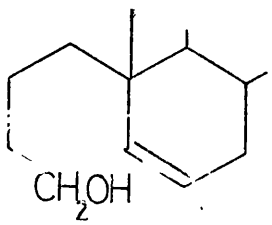

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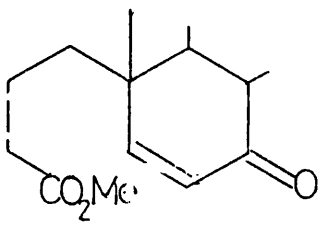

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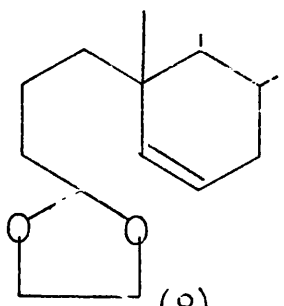

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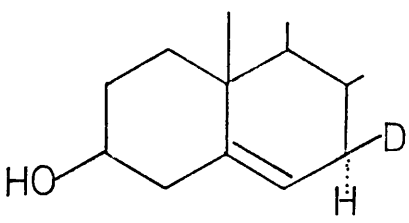

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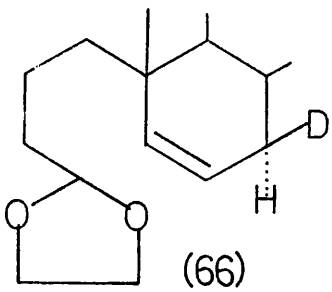
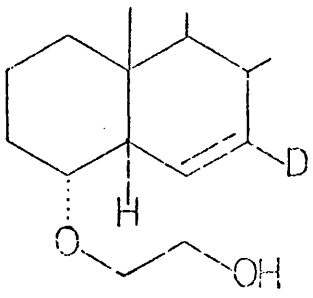


(66)

To complete the investigation two stereospecifically and isotopically C-7 labelled olefin-acetals were required. It was known that cholesterol could be stereospecifically labelled at C-7 by incorporation of 3R, 2R-2-$^{3}H_{1}$-mevalonic acid using a preparation of rat-liver slices[48], Figure (24), or by the method of Corey[26] involving protonation of the enol form of 3β-acetoxy-6-oxo-cholestane as outlined in Figure (25). However our experience in using dichloroaluminium hydride for the reduction of enones and the known preference for α-attack at C-7 in steroids prompted us to try using the corresponding deuterated reagent for reduction of the dihydroxy-olefin (63), readily available from the corresponding enone-ester (54) by lithium aluminium hydride reduction. Reduction of (63) did in fact produce a monodeuterio-hydroxy-olefin (64) but we were unable to determine precisely the distribution of deuterium label between the 7α-and 7β-positions. The n.m.r. spectrum of (64) showed the same basic pattern for the olefinic region as the unlabelled compound (32) with the exception that the lowfield limbs of the olefinic quartet were not so well resolved. An attempt to photo-oxygenate the olefin-acetal (8) to establish an analytical procedure for estimation of the 7α-proton failed and we then had to revert to cholesterol, photo-oxygenation of which is known to be specific for the 7α-proton[49], to introduce a label at C-7. A sterioselective introduction of deuterium at C-7 was achieved by reduction of 7β-hydroxycholesterol with dichloroaluminium deuteride, the procedure and deuterium analysis of these compounds are fully discussed in Part II.

The 7β-deuteriocholesterol (65) (97% 7β-d) was converted to the corresponding 7β-deuterio-olefin-acetal (66) by the route described, and the n.m.r. spectrum of this compound, Figure (26), clearly shows the removal of the 2.5 Hz vicinal coupling between the C-6-and 7β-hydrogens, and also that the C-5 proton retains an allylic



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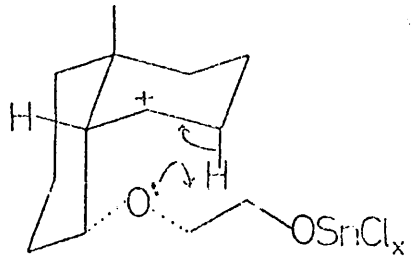

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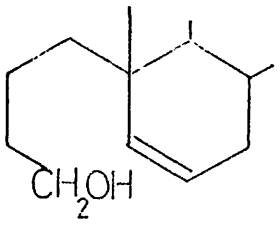

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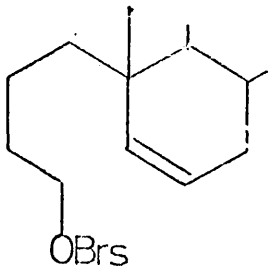

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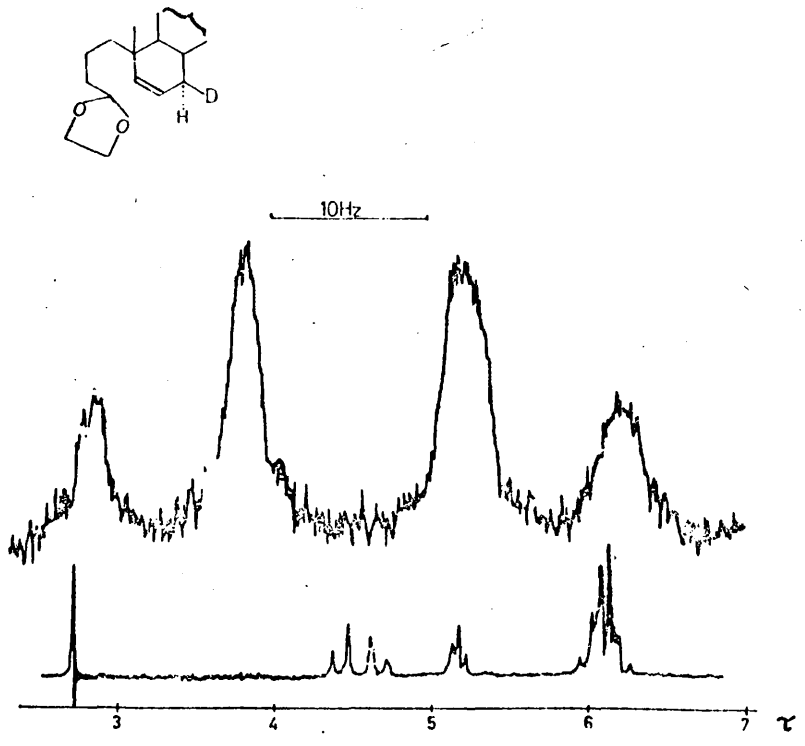
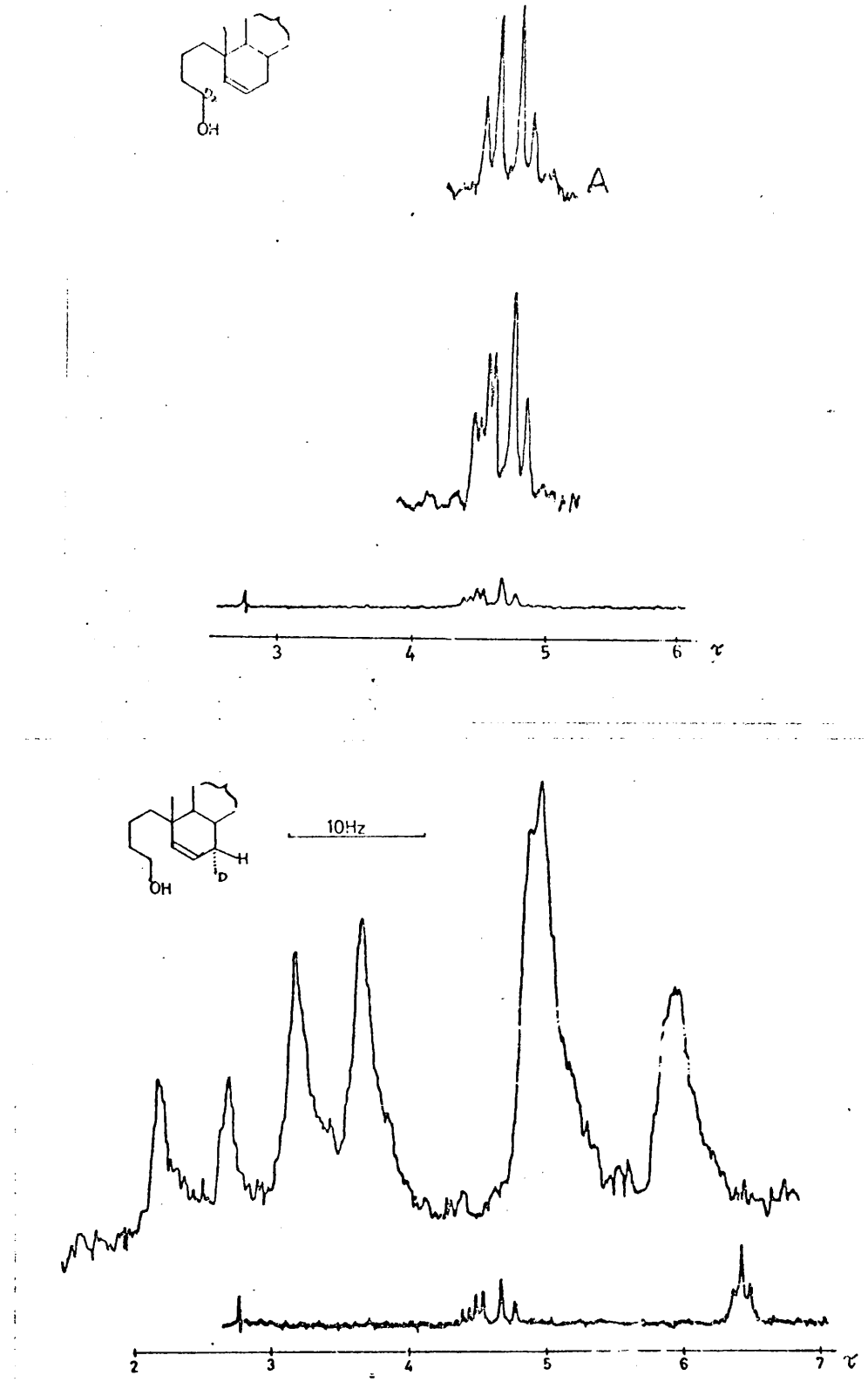


Fig.26



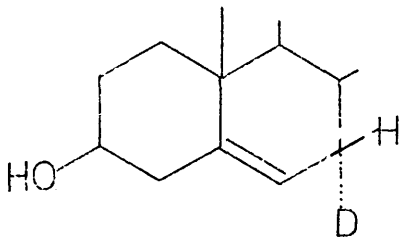
Labelled and Unlabelled hydroxy-olefins (72) and (32)
A = decoupling at 8.1τ.

Fig.27

coupling which the C-6 proton does not have, by comparison of the half-band width of the signals ($W_{\frac{1}{2}}$ 3Hz and 2Hz respectively). Cyclisation of this acetal afforded a hydroxy-ether (67) (> 96% - d_1) and which showed a vinyl deuterium (ν_{max} 2230 cm^{-1}).

Our immediate conclusion was therefore that this S_E2' reaction proceeds stereospecifically with syn stereochemistry, by loss of the 7α -hydrogen and formation of an α -C-4/C-5 bond. About this time, however, we became concerned lest the 7α -proton was being abstracted in an intramolecular process in which the oxygen atom of the potential β -hydroxy-ether might participate as shown (69). To allay these fears we determined to find conditions for cyclisation as different as possible from those described, to show that for a different leaving group, solvent, and initiator, the stereochemistry of the reaction remains constant.

The use of trifluoroethanol as a suitable solvent for cyclisation of acyclic olefinic-sulphonate esters has been described by Trahanovsky and Doyle[50]. Trifluoroethanol is a useful medium for such reactions since (i) it is a good ionising solvent thus promoting the cleavage of sulphonate esters to give carbonium ions, and (ii) it is non-nucleophilic and therefore allows the olefinic double bond to compete favourably with solvent for the carbonium ion centre. The p-bromobenzene sulphonate ester (brosylate) of the hydroxy-olefin (32) was therefore prepared and refluxed in trifluoroethanol for 3 days in the presence of urea (to neutralise any acid formed). A small yield of hydrocarbons was obtained consisting of one principal component, 5β -cholest-6-ene, identical with an authentic specimen on t.l.c., g.l.c. and m.s. The yield of hydrocarbon from solvolysis of the brosylate (70) was improved by conducting the reaction in a sealed tube at 105° for 40h, and again 5β -cholest-6-ene was the predominant



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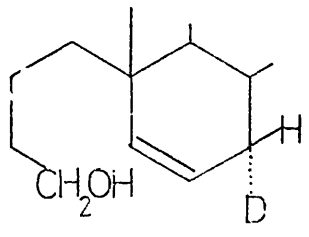

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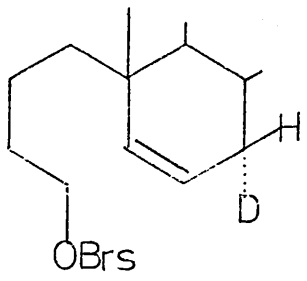

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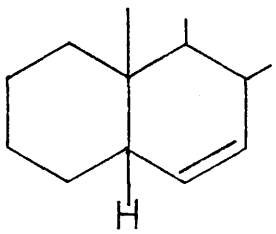

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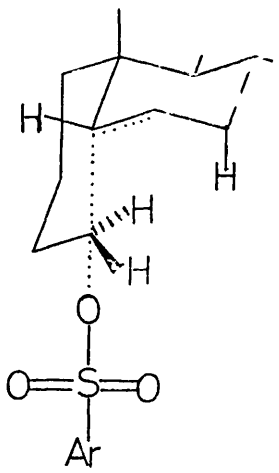
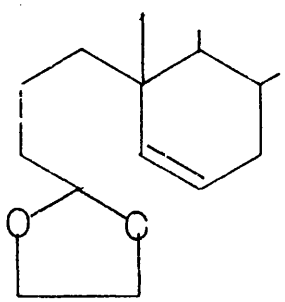
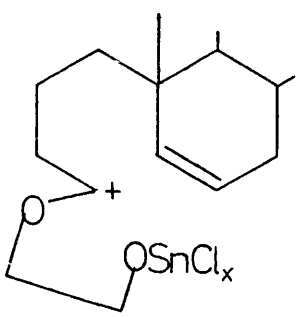


Fig.28



(8)



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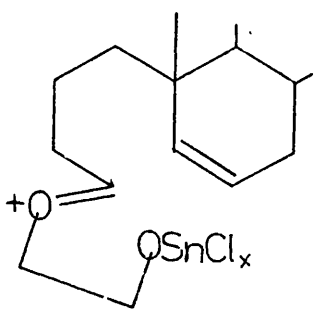
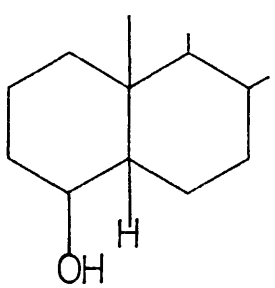


(76)

Fig.29



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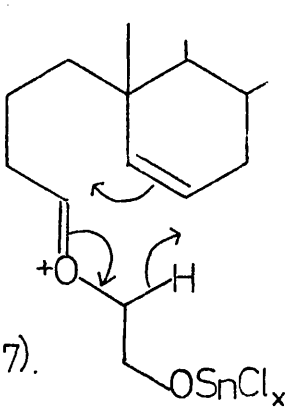


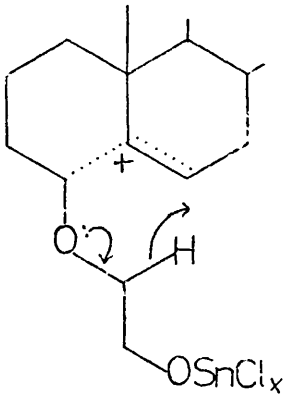
(77).

product in the hydrocarbon fraction.

The sample of 7α -deuteriocholesterol (71) (82.5% 7α -d, 17.5% 7β -d) was converted to the 7α -deuterio-hydroxy-olefin (72) by the usual route and the n.m.r. spectrum showed that C-7/C-6 coupling was still present as expected, Figure (27). Conversion of (72) to the corresponding brosylate (73) and cyclisation in a sealed tube afforded a hydrocarbon fraction, predominantly 5β -cholest-6-ene (74) which was 16.5% d_1, 83.5% d_0 by mass spectrometry.

Thus the stereochemistry of this S_E2' reaction is also <u>syn</u> and is less likely to involve internal abstraction of the C-7 proton by the brosylate group, since on cleavage to form a carbonium ion at C-4 the oxygen atoms will have moved away from the steroidal fragment and no transition state for proton removal in a cyclic process is available, Figure (28). Our initial conclusion was therefore reinforced by this second example.

Since theoreticians differ in their predictions for the stereochemistry of the S_E2' reaction depending on whether it is concerted or not, it is of interest to speculate on the timing of bond breaking and formation in the present examples. Cleavage of the acetal in (8) by stannic chloride, possibly assisted by the other oxygen atom will generate the initiating carbonium ion (75) or oxonium ion (76) as shown in Figure (29). Circumstantial evidence that the C-7/H and C-4/C-5 bonds do not cleave and form concertedly is available from the isolation of the saturated alcohol (41) as a cyclisation product. If, as suggested, this is produced by an internal hydride transfer process, then C-4/C-5 bond formation must have advanced considerably before this occurs. It is likely that the initial C-4/Oxygen cleavage can be assisted by participation of the second oxygen atom as mentioned above or by the formation of the new C-4/C-5 bond and concomitant development of positive charge at C-6. While it is possible to write an electrocyclic reaction for hydride transfer to C-6 (77) the geometry of the transition



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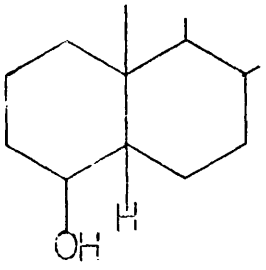

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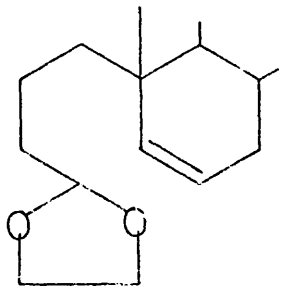

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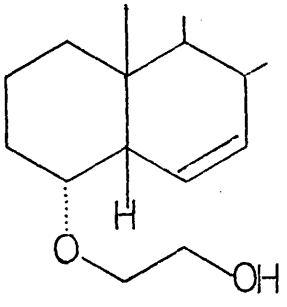

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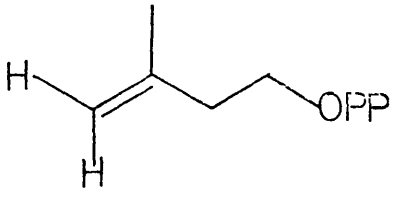


(4)

state is not so favourable as that in (78) where C-4/C-5 bond formation accompanies C-4/oxygen cleavage. Not only does this mechanism produce a better transition state geometry for hydride transfer, but it has the added advantage of positive character at C-6 and a lone pair of electrons on the oxygen atom available to assist transfer as previously indicated in (46). If as suggested formation of the C-4/C-5 bond precedes hydride transfer in formation of the alcohol (41) then it is tempting to suppose that in the cyclisation of the olefin-acetal (8) to produce the hydroxy-ether (38), a similar timing of events occurs and that the C-4/C-5 bond forms in advance of C-7/H cleavage. This need not necessarily be so, but in the light of arguments previously advanced[51] it is felt that the cyclisation is probably not - concerted.

If the above findings are generalised to include the enzymic condensation of C-5 units, then it is clear that an X-group mechanism is not required to explain the observed syn-stereochemistry, but that an alternative explanation is required to explain the observed anti-isomerisation of 3-methyl-3-butenyl-pyrophosphate (4).

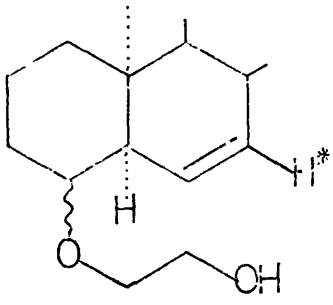
(iv) Limitations of the Model System and Attempts to Surmount Them

A more critical examination of the choice of a model system revealed two basic limitations;

(i) the 7α -and 7β -protons are not equally disposed with respect to the π-system, and

(ii) the side chain carrying the potential cationic site can only approach the olefin from the α-face due to steric restrictions.

This is not a satisfactory situation since it does not allow a choice in the mode of approach of the cation to the olefin, and because of the different angles the 7α -and 7β -protons make with



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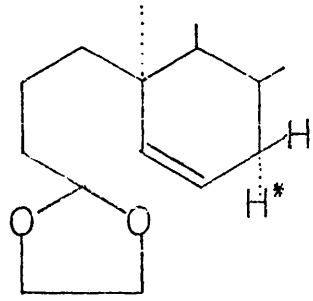

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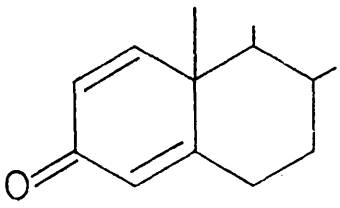
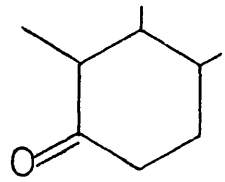


(81)



(82)

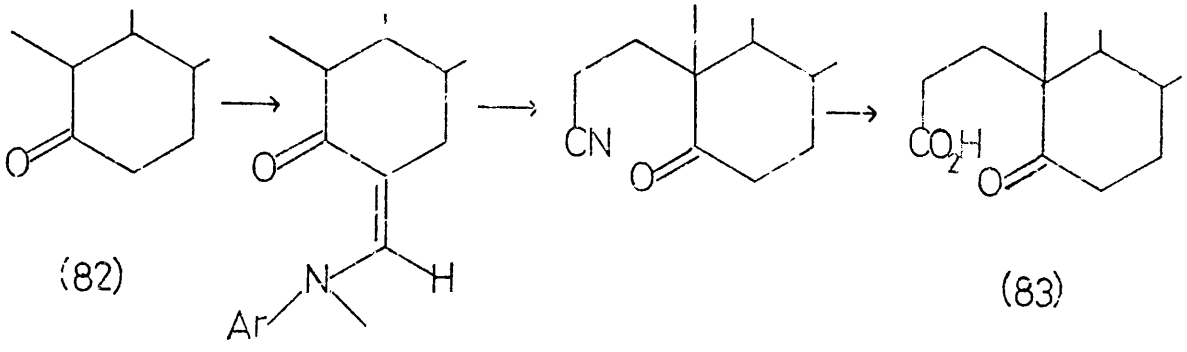
the plane of the lobes of the π-system (25° and 52°) it may well have a built-in discrimination in favour of loss of the 7α -proton due to the greater ability of the C-7/H_{α} bond to conjugate with the olefin. However if orbital symmetry is the controlling feature in the stereochemistry of cyclisation, and if the stereochemistry of S_E2' reactions is generally syn, then cyclisation of the analogous 10α -methyl-olefin-acetal (80), should result in loss of the 7β -hydrogen and formation of a new β - C-4/C-5 bond (79). As in the above model the side chain would again be constrained to approach the olefin in only one direction, but this time from the β -face. It was our intention therefore to synthesise this 10α -methyl-olefin-acetal (80) and attempt similar cyclisations.

The 10α -methyl steroids are synthetically more difficult to prepare than their 10β -isomers due principally to the eclipsing of the 9α -hydrogen and 10α -methyl groups. The plan was once more to use cholesterol as starting material and to invert the configuration at C-10. There are two obvious ways in which this might be accomplished;

(i) by removal and replacement of the 10β -methyl group, or

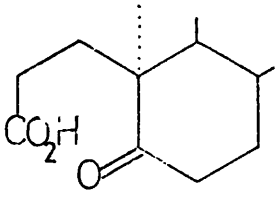
(ii) by removal and reconstitution of ring A,

and it was the latter process which was first investigated since our interest was in 4,5-seco-derivatives. Examination of the literature revealed that the degradation of ring A of cholesta-1,4-dienone (81) had been reported[52] and the product, the so-called Inhoffen Ketone (82), identified. The yield of this ketone (82) produced by ozonolysis of (81) was very small (< 10%) and subsequent investigations by Robinson[53] effected only a slight improvement.



(82) (84) (83)

Fig.30



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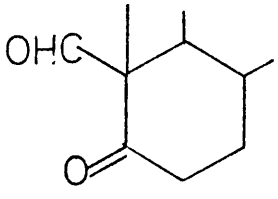

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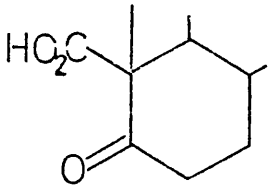
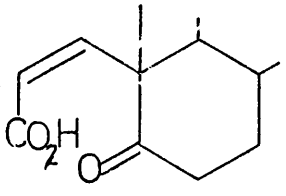


(87)



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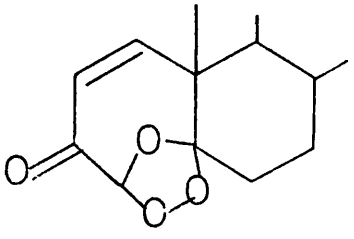

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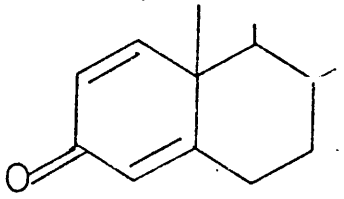
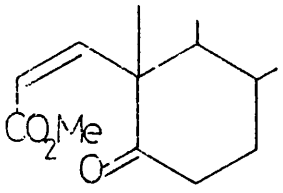


(81)

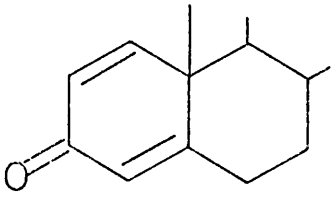
In the same publication Robinson reported on the synthesis of the keto-acid (83) from Inhoffen Ketone, Figure (30), and found that the 10 β -methyl-keto-acid (83) was the principal product formed on condensation of (84) with acrylonitrile followed by hydrolysis, with less than 15% of the reaction mixture being possibly ascribable to the 10 α -methyl isomer (85). If this route were to be of use in synthesis of 10 α -methyl derivatives then clearly better yields of the ketone (82) were required and modification of the condensation reaction was also necessary.

It was found that ozonolysis of cholest-1,4-dienone in various solvents at room temperature led to a number of products on oxidative work up. The keto-aldehyde (86), keto-acid (87) and the unsaturated keto-acid (88) were the most prominent but some Inhoffen Ketone was also detected among the several other minor products. In general this procedure proved unreliable and consistent yields of products could not be obtained. Ozonolysis of (81) at -78° resulted in rapid formation of the ozonide (89) identified by its spectral properties, [ν_{max} 3020, 1805, 1685, 1100, and 700 cm^{-1}, τ 4.06 (1H,s), 4.18 (1H,d), and 4.69 (1H,s)] and by its decomposition to give (88).. The n.m.r. spectrum shows a small coupling on the peak at 4.18 τ and this is most likely due to coupling across the keto-group and the resonance at 4.69 τ , although a singlet is broadened compared to that at 4.06 τ.

Attempts to induce retro-Aldolisation with the keto-aldehyde (86) or to decarboxylate the keto-acid (87) resulted in a poor recovery of Inhoffen Ketone along with much polar material, however the unsaturated keto-acid (88) could be obtained in high yield from the ozonide (89) by refluxing in acetic acid. Means were sought to effect degradation of (88) without success and further ozonolysis of the corresponding



(90)



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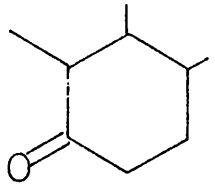

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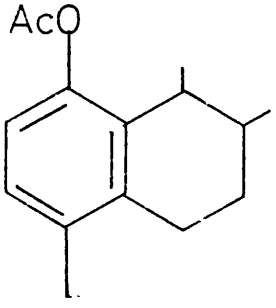

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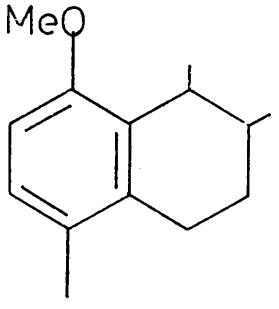

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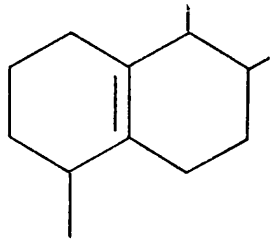

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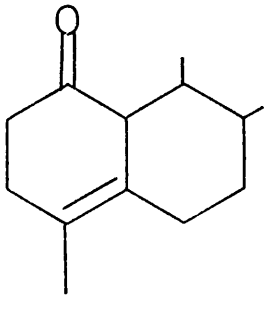

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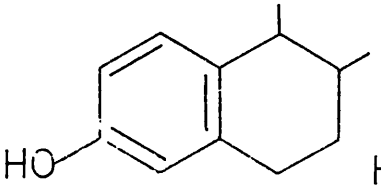

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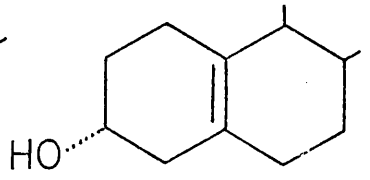
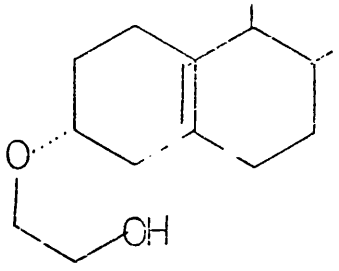


(97)

ester (90) proceeded slowly and resulted in mixtures. Osmylation of the double bond in (90) using catalytic quantities of osmium tetroxide in the presence of sodium periodate failed, but a paper by Caspi[54] gave hope that the dienone (81) might be degradable to the ketone (82) by use of ruthenium tetroxide. After experimentation with this oxidant yields of Inhoffen Ketone were no better than those achieved by ozonolysis and work on this scheme was discontinued.

Two methods for removal of the 10β -methyl group were investigated. The first, a dienone-phenol rearrangement[55,56] gave the acetate (91) from the dienone (81) in near quantitative yield, by treatment with concentrated sulphuric acid in acetic anhydride, and was readily converted to the methyl-ether (92). Reduction of this ether using lithium in ethylamine[57,58] afforded the $\Delta^{5,10}$ olefin (93) whereas lithium in ammonia gave no reaction. This olefin was of little use since it lacks suitable functionality to allow elaboration to the 10α-methyl series, while one of the expected products of Birch reduction, the ketone (95), could in theory be cleaved by ozonolysis and methylated at C-10 to give 4,5-seco-10α -compounds. However reductive elimination of C-19 is also possible by use of lithium and biphenyl in tetrahydrofuran[59]. Treatment of the dienone (81) with this reagent afforded the 3-phenol (96) which can be directly reduced to the 3α -hydroxy- $\Delta^{5,10}$-olefin (97) in high yield using a large excess of lithium in ammonia[60]. In a synthesis of 10α -testosterone, Ginsig and Cross[61,62] effected a stereospecific Simmons-Smith α -methylenation of a 3α -hydroxy- $\Delta^{5,10}$ olefin system in high yield. However this reaction involved the use of a sealed tube and high temperatures (100°) and in our hands reproducible conditions could not be found for the reaction of (97) with methylene iodide and zinc/copper couple although



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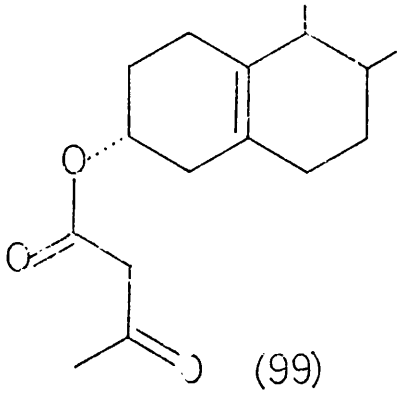

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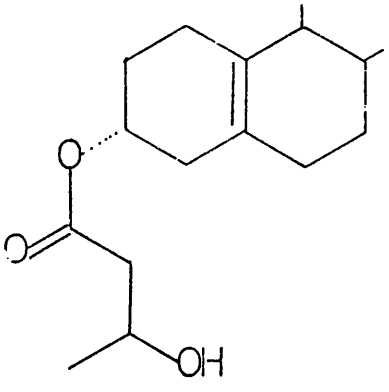

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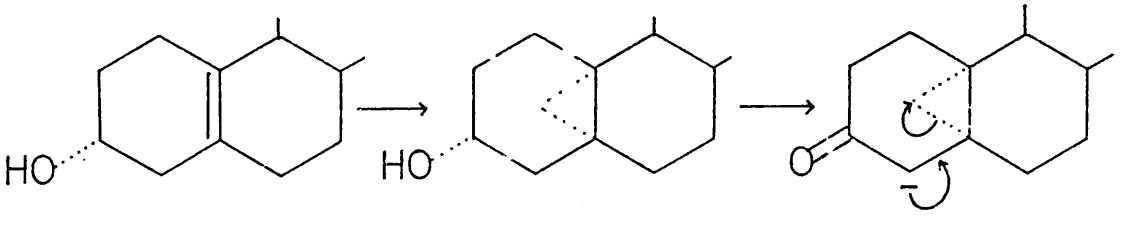
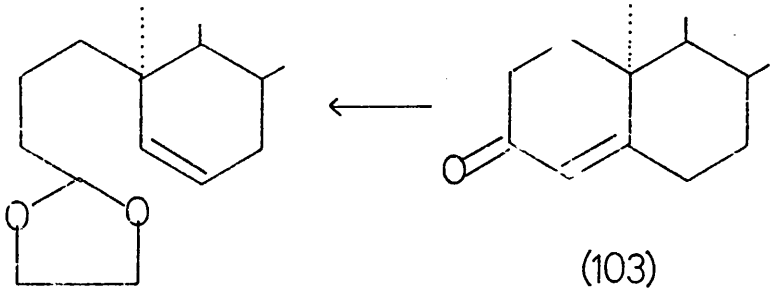


(97) (101) (102)



(103)

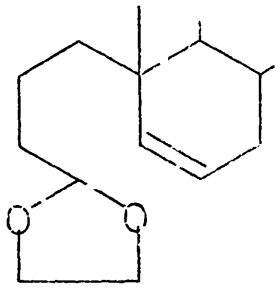
(80)

Fig.31

there was some evidence of cyclopropyl ring formation [τ 9.42 (d, J4Hz) and 9.71 (d, J4Hz)].

Since it is believed that the 3α -oxygen directs approach of the reagent to the double bond, but has in fact an equatorial configuration, the high temperature is probably required to change the conformation of ring A so that the hydroxyl becomes axial. To obviate the need for high temperature and pressure it was proposed to attach a small side chain to the 3α -hydroxyl group which might still direct the methylene reagent but at a lower temperature. A β -hydroxy-ether function (98) seemed best suited since it could be readily removed after reaction, but attempts to O-alkylate the 3-hydroxyl with ethylene oxide failed, as did an attempt using ethylene glycol-monotosylate-tetrahydropyranyl ether which probably resulted in tosylate elimination by the 3α - alkoxide. A further attempt using β -propiolactone also resulted in failure, again probably due to competing elimination but reaction with ketene-dimer gave the adduct (99) which was readily reduced to the hydroxy-ester (100). Unfortunately Simmons-Smith methylenation of this compound failed to give any cyclopropyl derivatives. The remainder of the proposed synthesis is outlined in Figure (31).

The failure to obtain a 10α -methyl derivative for cyclisation was disappointing, but about this time our theoretical studies of the S_E2' process were coming to fruition and they caused us to reconsider the reaction in a new light.



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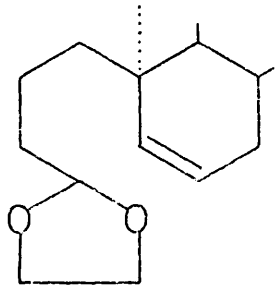


(80)

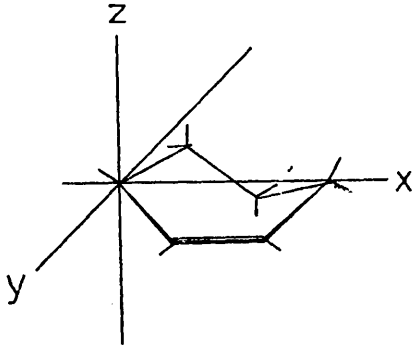


Fig. 32

(v) Molecular Orbital Calculations

With the advent of modern high speed computers in the late 1960's it became possible to calculate molecular orbitals for relatively complex molecules by use of various semi-empirical approaches, such as 'Complete Neglect of Differential Overlap' (CNDO) and 'Intermediate Neglect of Differential Overlap' (INDO). Since all previous ideas pertaining to the S_E2' and S_N2' reactions were advanced before this period the present study is by far the most reliable to date. The calculations, results of which are described below, were performed on an IBM 370/155 computer using a program made available by Pople[63] for calculation of molecular orbitals using the INDO parameterisation. This program allows up to 35 atoms to be included in a molecule and uses as a basis set the valence atomic orbitals of the component atoms, that is 2s, $2p_x$, $2p_y$, and $2p_z$ for carbon, nitrogen and oxygen and 1s for hydrogen. Fuller details of the calculation procedure are described in the appendix.

The failure to produce the 10α-methyl acetal (80) for cyclisation denied us the opportunity to test experimentally whether the S_E2' reaction would proceed with <u>syn</u> stereochemistry in spite of the fact that the allylic C-H bond to be broken was less favourably oriented with respect to the π-system (see above). Our initial objective therefore was to construct a model of the olefinic part of the acetal (8) and to discover what changes in bonding occurred in the allylic system on allowing a positive charge to approach perpendicular to the plane of the olefin. In all calculations in which a cyclohexene system was used the ring was placed in a coordinate system, Figure (32), such that the double bond lay in the x,y-plane with the p_z orbitals forming the π-system, and the ring was assumed to be in the half-chair

TABLE (3)

Bond Indices

Molecule	Bond	Index
CH_4	C - H	0.987
C_2H_6	C - H	0.981
C_2H_4	C - H	0.979
C_2H_2	C - H	0.964
HCHO	C - H	0.918
C_2H_6	C - C	1.023
C_2H_4	C = C	2.032
C_2H_2	C ≡ C	2.985
HCHO	C = O	1.997

Source Ref.64.

TABLE (4)

Approach of CH_3^+ to Trimethylethylcyclohexene

Table of Bond Indices

Bond	Distance of methyl group from C - 1 (Å)								
	∞	+3.0	-3.0	+2.5	-2.5	+2.0	-2.0	+1.535	-1.535
3 - 4	0.952	0.951	0.954	0.948	0.953	0.948	0.951	0.951	0.949
3 - 5	0.945	0.939	0.943	0.931	0.942	0.924	0.937	0.926	0.940
2 - 3	1.038	1.044	1.038	1.051	1.040	1.061	1.044	1.046	1.037
1 - 2	1.934	1.832	1.932	1.691	1.907	1.494	1.744	1.317	1.374
1 - 6	-	0.096	0.002	0.248	0.019	0.488	0.183	0.686	0.597

conformation with all bonds 'staggered' where possible. The basic approach used to calculate whether there is any preferential stereochemistry for the S_E2' and S_N2' reactions was to keep the geometry of the olefinic system fixed and to allow a group to approach the double bond at C-1 in the z-direction from either above or below the x,y-plane. The effect of allowing an electrophile or nucleophile to approach the double bond was monitored at various arbitrary distances ranging from 3.0 to 1.1 Å above and below the x,y-plane. A measure of bond weakening and hence chemical reactivity of the allylic hydrogen atoms was obtained by calculating the Bond Indices[64] from the density matrix. for example a bond index of 1 would correspond to a "pure covalent-two-electron-bond" and a value of 0 a "purely ionic bond". Examples of bond indices previously calculated for some small molecules are listed in Table (3). The model system is therefore also physically reasonable in that all that is assumed in the calculations is the geometry of the molecule which could be varied at will. In the calculations a positive charge is ascribed to the complete molecular system as necessary for approach of a methyl group or proton and it is a measure of the accuracy of the calculations that the major part of the charge is found to be located on these groups.

To simulate as closely as possible the olefin-acetal cyclisation a planar CH_3 group was allowed to approach C-1 of trimethylethylcyclohexene, Figure (33), from above or below the x,y-plane. The results from calculations on this system are listed in Table (4) which shows the bond indices for the two allylic C-H bonds, the olefinic double bond and the new bond which is forming between the incoming methyl group and C-1. From the Table it can be seen, in common with ideas about hyperconjugation[65], that in the isolated cyclohexene system the bond which is more nearly eclipsed with the p_z-orbitals, C-3/H-5, is weaker than its geminal partner C-3/H-4. On placing a planar CH_3 group either

TABLE (5)

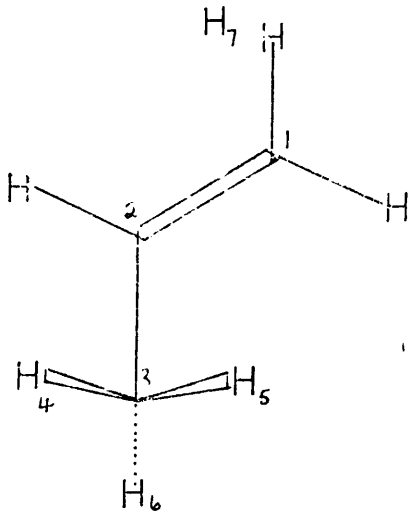
Approach of H^+ to Trimethylethylcyclohexene

Table of Bond Indices

Bond	Distance of H^+ from C - 3 (Å)							
	+2.5	-2.5	+1.5	-1.5	+1.3	-1.3	+1.1	-1.1
3 - 4	0.945	0.954	0.941	0.949	0.942	0.932	0.943	0.935
3 - 5	0.917	0.943	0.906	0.921	0.905	0.916	0.904	0.917
2 - 3	1.064	1.039	1.076	1.066	1.077	1.074	1.077	1.071
1 - 2	1.630	1.909	1.398	1.471	1.375	1.376	1.364	1.409
1 - 6	0.235	0.021	0.548	0.465	0.560	0.621	0.637	0.560

above or below the double bond in the plane of p_z at C-1 and giving the whole system a positive charge, it can be seen most markedly that it is the C-3/H-5 bond which weakens in both cases and this trend is continued as the methyl group is allowed to approach closer to C-1 whereas the C-3/H-4 bond index remains nearly constant. It is evident also that approach from above the plane would appear to cause bond weakening to a greater extent than from below, and at first glance this might then seem to be a case for proposing that anti substitution is favoured over syn. This cannot be deduced from this particular model since as a result of the geometry of the model system chosen there is severe interaction of the incoming methyl group with the ethyl-side chain, (for example at 2.5Å below the plane of the olefin, one of the hydrogen atoms of the methyl group gets to within 0.6Å of hydrogen atoms on the ethyl side chain) and since this would never occur in a reasonable physical situation, any calculations performed on this system will not apply to the real reaction since changes in conformation would result before the groups could become so close.

In an attempt to remove this criticism the calculations were repeated, only this time allowing a hydrogen atom to approach perpendicular to C-1. The effects of this change are twofold;
(i) there is more positive charge concentrated on the hydrogen atom than on the carbon atom of the methyl group used above, which could spread the charge over three hydrogen atoms, and therefore the bond indices are affected to a greater extent, Table (5);
(ii) although interaction with the ethyl side chain has been reduced there is still more steric hindrance from below the plane than from above. The general trend in bond index is however the same as for approach of a methyl group; viz. it is the C-H bond most nearly



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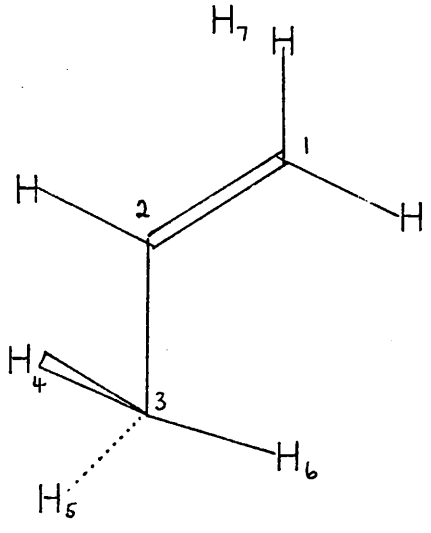


(106)

TABLE (6)

Approach of H^+ to Propene (i)

Table of Bond Indices

Bond	Distance of H^+ from C – 1 (Å)								
	∞	+3.0	-3.0	+2.0	-2.0	+1.5	-1.5	+1.1	-1.1
3 - 4	0.973	0.962	0.962	0.965	0.960	0.966	0.958	0.966	0.955
3 - 5	0.973	0.963	0.962	0.963	0.965	0.962	0.966	0.959	0.967
3 - 6	0.962	0.912	0.913	0.916	0.920	0.916	0.921	0.921	0.919
2 - 3	1.053	1.089	1.089	1.096	1.095	1.100	1.099	1.104	1.102
1 - 2	1.990	1.671	1.670	1.500	1.499	1.408	1.407	1.484	1.420
1 - 7	-	0.338	0.333	0.508	0.508	0.617	0.617	0.685	0.685

TABLE (7)

Approach of H^+ to Propene (ii)

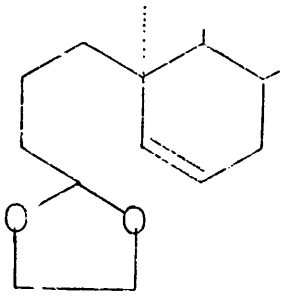
Table of Bond Indices

Bond	Distance of H^+ from C - 1 (Å)						
	∞	+3.0	+2.75	+2.5	+2.0	+1.5	+1.1
3 - 4	0.965	0.928	0.930	0.931	0.935	0.937	0.937
3 - 5	0.965	0.928	0.928	0.928	0.928	0.927	0.924
3 - 6	0.976	0.978	0.978	0.978	0.978	0.977	0.977
2 - 3	1.055	1.092	1.094	1.095	1.099	1.103	1.107
1 - 2	1.987	1.671	1.636	1.595	1.500	1.407	1.361
1 - 7	-	0.332	0.366	0.407	0.507	0.616	0.684

eclipsed with the π-system that weakens preferentially no matter whether the incoming group approaches C-1 from above or below.

To fully substantiate this trend by calculation requires a system in which there is no steric restrictions for approach of an electrophile to C-1. The simple propene model (105) therefore seemed to be ideal and calculation of the molecular orbitals for this system revealed that the C-3/H-6 bond, deliberately chosen to be in the plane of the p_z orbital on C-2, is weaker than the two other allylic C-H bonds which are equally disposed with respect to the p_z-orbital on C-2, Table (6). On placing a hydrogen atom at various distances perpendicular to C-1 and giving the whole system a positive charge it is strikingly obvious that the C-3/H-6 bond is weakened considerably whereas the other two allylic bonds weaken only slightly. Again it is very clear that the direction of approach of the electrophile to the olefin is unimportant, either resulting in the same degree of bond weakening in the C-3/H-6 bond.

It next seemed appropriate to investigate the situation (106) in which the two allylic C-H bonds were equally disposed with respect to the x,y-plane. In this situation calculations revealed, Table (7), that the bond index of the C-3/H-6 bond remains constant and that the other two allylic C-H bonds (C-3/H-4 and C-3/H-5) are weakened with a slight preferential weakening of that bond anti to the incoming group. This would be expected on simple electrostatic grounds, since in the absence of any preferential bonding with the π-system, maximum charge separation will lead to a more stable arrangement and thus an incoming positive charge at C-1 would create positive charge on the opposite side of the molecule at C-3.



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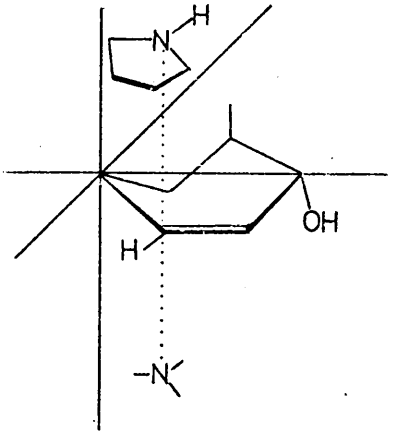


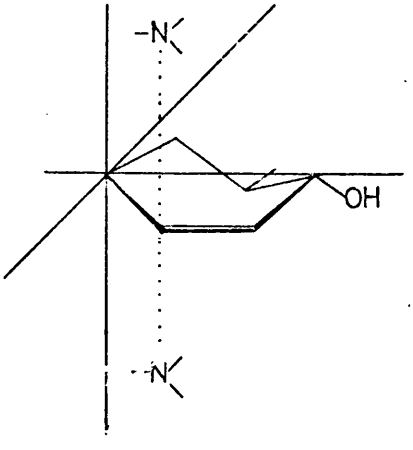


Fig.34

The conclusions from these calculations are that (i) the direction of approach of the electrophile to the olefin in an S_E2' reaction does not control the configuration from which the allylic substituent is lost except when the allylic groups are equally disposed about the plane of the double bond, and (ii) the allylic bond which is broken in an S_E2' process will be that which is more nearly eclipsed with the p_z-orbital of the neighbouring carbon atom. It therefore seems reasonable to suppose that it will be steric factors which will control the approach of the electrophile to the double bond, and consequently it is not surprising that in our own experimental examples of an S_E2' reaction syn stereochemistry was observed. The above proposals do allow a prediction to be made about the stereochemistry of cyclisation of the corresponding 10α -methyl-acetal (80) and on the above basis cyclisation should lead to an overall anti stereochemistry by formation of the C-4/C-5 bond on the β -face and loss of the 7α -proton, in contrast to our previous expectations.

Since the S_N2' reaction is superficially similar to the S_E2' reaction, it might be expected that the same factors would also control the stereochemistry of this reaction. To test this idea by calculation we chose to examine the example of Stork and White[17] in which piperidine was used to effect an S_N2' substitution on the dichlorobenzoate of trans-4-methylcyclohexen-3-ol. Since the program could only cope with 35 atoms we chose as a model the interaction of pyrrolidine with 4-methylcyclohexen-3-ol in which the nitrogen atom was allowed to approach perpendicular to C-1 in the plane of the π-system. Four cases were considered; approach from above and below to the trans-diequatorial form, and approach from above and below to the trans-diaxial form as in Figure (34). The results of INDO calculations on

TABLE (8)

Approach of Pyrrolidine to trans-4-methylcyclohexen-3-ol.

Table of Bond Indices

(i) Diaxial Conformation

Bond	Distance of nitrogen atom from C - 1 Å						
	∞	+2.5	-2.5	+2.0	-2.0	+1.5	-1.5
C - O	0.937	0.935	0.934	0.927	0.924	0.890	0.885
C - N	-	0.010	0.011	0.095	0.098	0.567	0.547

(ii) Diequatorial Conformation

C - O	0.946	0.945	0.945	0.940	0.940	0.920	0.922
C - N	-	0.010	0.010	0.090	0.087	0.527	0.529

TABLE (9)

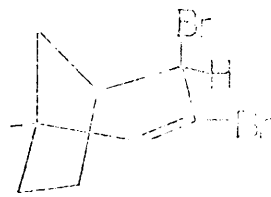
Comparison of Energetics of Approach of Pyrrolidine to trans-4-methylcyclohexen-3-ol

Diaxial Conformation

Approach from (Å)	Approach from below favoured by (Kcal/mol)
3.0	8.27
2.5	49.05
2.0	160.05
1.5	155.57

Diequatorial Conformation

Approach from (Å)	Approach from below favoured by (Kcal/mol)
3.0	0.11
2.5	0.09
2 0	0.48
1.5	4.86

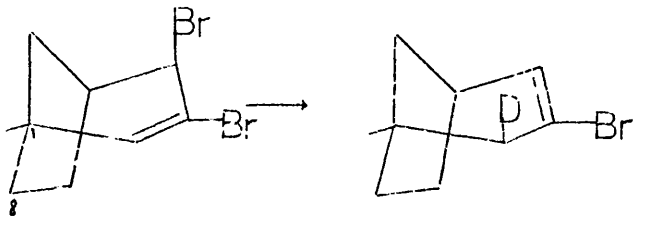


(108)

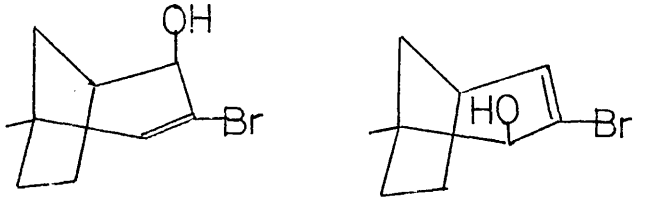
these four systems are listed in Table (8), from which it can be seen that for the trans-diaxial conformer equal C-O bond weakening is obtained on approach from above or below. A similar situation pertains in the diequatorial conformer but the extent of bond weakening obtained is less, in agreement with the view that a quasi-equatorial allylic group is not so well conjugated with the π-system as a quasi-axial group.

If a comparison of the total energies for these interacting groups is made, Table (9), then it becomes apparent that in the diaxial conformation approach of pyrrolidine is much more favoured from below, while the diequatorial conformer shows little preference for attack from either side. It is not difficult to suggest a plausible explanation for this situation. The most obvious cause for the higher energy of approach to the diaxial conformer from above is the steric interaction of the incoming pyrrolidine group with the axial 4-methyl group, and this is confirmed from the density matrix as being a major interaction. The stereochemistry of this S_N2' reaction can then be adequately explained as follows: (i) the cyclohexenol system will consist of a mixture of equilibrating trans-diaxial and diequatorial forms, and the most favoured conformation for C-O bond cleavage will be trans-diaxial; (ii) in this conformation approach of pyrrolidine is prevented from above due to steric interactions and therefore, as observed. the incoming nucleophile approaches from below and on the same side as the leaving group to give syn stereochemistry.

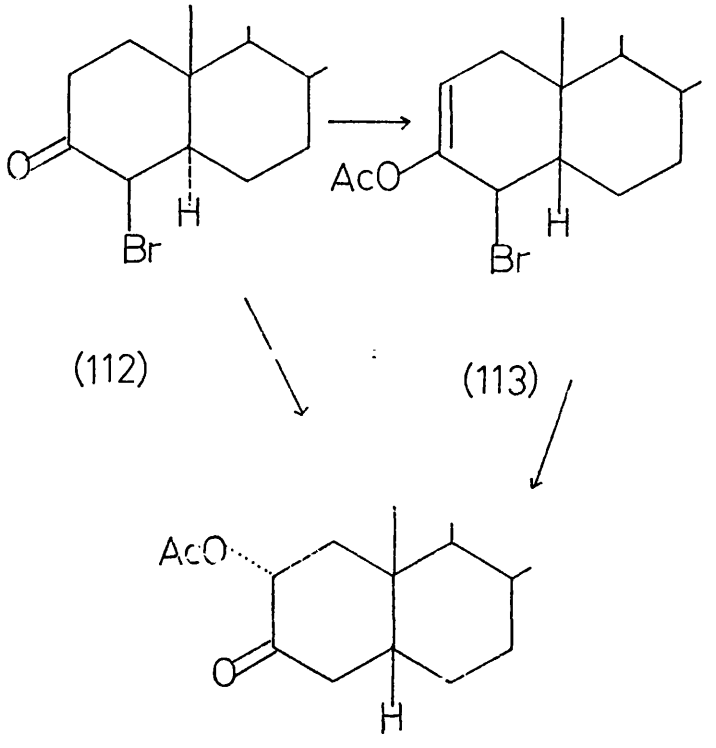
Jefford[66] has studied the stereochemistry of the reductive debromination of exo-1-methyl-3,4-dibromobicyclo [3,2,1] oct-2-ene (108) with lithium aluminium deuteride and established that allylic rearrangement takes place and that deuteride is supplied from the same



(108) (109)



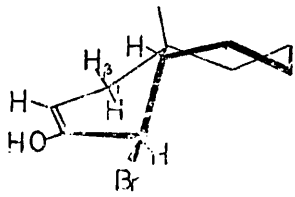
(110) (111)



(112) (113)

face as bromide is removed (109), and also that solvolysis of (108) with aqueous silver nitrate gives equal amounts of the 2 - and 4-hydroxy-compounds (110) and (111). He proposed that the equal formation of (110) and (111) on solvolysis implied intermediacy of the allylic carbonium ion and then stated that the complete exo-stereospecificity was explained by the preferential formation of quasi-axial bonds. He goes on to say that the S_N2' process is not affected by steric factors and that the geometry of the reaction is favoured on quantum mechanical grounds. These arguments are not satisfactory since, (i), if an allylic carbonium ion is formed in the solvolysis, then capture by water should be equally possible from both sides, and if there is complete exo-stereospecificity then there must be greater steric hindrance onthe endo face, which there is; (ii) it has just been demonstrated that the departure of a leaving group from an allylic position does not direct the approach of a nucleophile to the double bond, therefore in conjunction with (i) approach of hydride must be exo for steric reasons, or possibly by favourable interaction with the leaving group in a "cyclic" type pathway. In a later paper[67] Jefford admits to someof the above possibilities but makes no mention of what must be significant 1,2-torsional interaction between incoming hydride and the C-1/C-8 bond if there was to be endo attack, a feature not present for exo-attack.

A trans-S_N2' reaction has been proposed[68] to explain the acetolysis of 4 β -bromo-5 β -cholestan-3-one (112) by way of the corresponding enol form (113). This finding is entirely consistent with the proposals advanced above, but not with previous theories. The departure of bromide from C-4 will occur from a conformation in which



(113)

the C-Br bond is quasi-axial and consideration of torsional interactions with the incoming nucleophile reveals a 1,2-torsional interaction with the C_2/H_β bond for approach from the β-face but no such interaction from below. Hence the observed stereochemistry is anti. The authors do not refer to the theories extant at the time of publication but do make the point that the stereochemistry of the reaction depends on the stereochemistry of the substrate.

It is proper now to consider the question of "concertedness" with respect to the above calculations and in this context it is the timing of bond breaking and bond formation that is implied by the term. The above calculations and results should be equally valid whether bond making and bond breaking are synchronous or not since in both cases the incoming and outgoing groups are bonding in the transition state, though not necessarily to the same extent. Therefore by the term S_N2' or S_E2' as applied to the description of an allylic substitution reaction, no implication is made about the timing of bond formation or cleavage but rather only that the reaction (i) is bimolecular, (ii) is initiated by an anion or cation respectively and (iii) involves an allylic rearrangement. Since there is no discrimination electronically between syn and anti stereochemistry in these reactions, then the stereochemistry of the substrate, or bonding between the incoming and outgoing groups will control the course of the reaction.

The calculations described herein are the first to be performed on S_E2' and S_N2' reactions and in the light of these the former postulates should be examined. The first general criticism of previous work is that all the conclusions presented are based largely

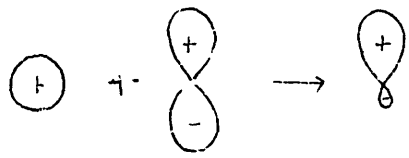


Fig.35

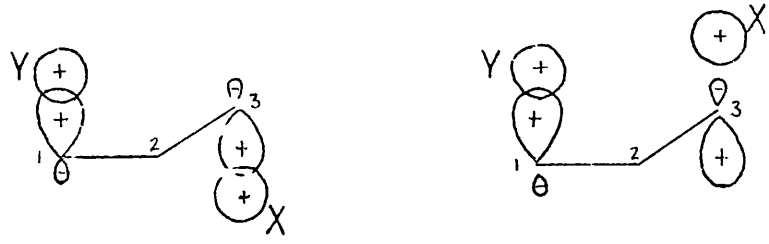


Fig.36

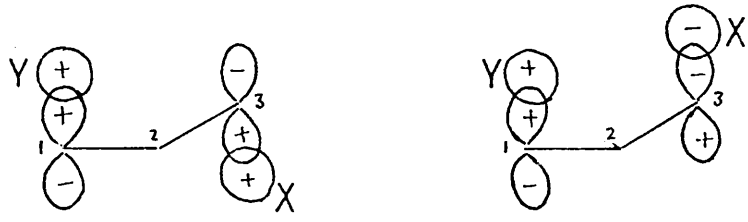
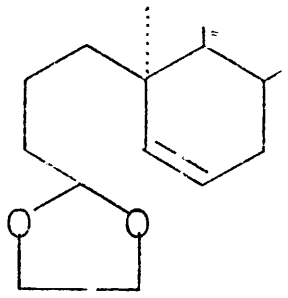


Fig.37

on analogies for the reactions and are not supported by detailed calculation. For example, Anh[15] considers the S_N2' reaction in terms of an interacting allylic cation and two anions (and correspondingly an S_E2' reaction is regarded as an allylic anion interacting with two cations). This is not a physically real situation since any such system of charged species will be inherently unstable; consequently predictions made from this model, although internally consistent, need not apply to the real reactions. Anh also presents orbital diagrams for the highest filled orbital of the allylic anion in support of his arguments in which he has taken s - p combinations to produce hybrid orbitals intermediate between p and sp^3 orbitals, Figure (35), and then considers two cases;

(i) Y - C-1 bond formation and X - C-3 bond breaking are synchronous in which case anti-stereochemistry is predicted;

(ii) X - C-3 bond cleavage precedes Y - C-1 bond formation in which case X becomes antibonding with respect to C-3 and syn - stereochemistry is predicted, Figure (36). In fact calculation shows that the carbon 2s and $2p_z$ atomic orbitals contribute largely to different molecular orbitals and that the major contribution to the highest filled molecular orbitals result from only the $2p_z$ atomic orbitals, Figure (37). From these orbital diagrams it can be seen that there is no preference for syn or anti-stereochemistry. The approaches of Fukui[14], Drenth[16], and Jefford[69] suffer from similar pitfalls and although the ideas presented are all "correct" they are not supported by calculation and therefore contain no measure of just how applicable they are to the real situation. It appears, moreover, that from the above results these approaches by the four theoreticians are not applicable to the S_E2' and S_N2' reactions and hence they have no predictive value.



(80)

(vi) Conclusions

(a) The S_N2' and S_E2' reactions may show either syn or anti stereochemistry depending on the stereochemistry of the substrate and the nature of the entering and leaving groups.

(b) In such reactions, the leaving group (X) will prefer to depart so that there is maximum bonding with the π-system, that is the allylic C X bond should be as nearly eclipsed as possible with the direction of the p-orbitals which constitute the π-system.

(c) The experimental model chosen to illustrate the stereochemistry of the S_E2' reaction has inbuilt bias as previously described, and can only result in a syn process though the corresponding 10α -methyl-acetal (80) should cyclise with anti-stereochemistry.

(d) The condensation of C-5 units observed experimentally to be syn and the isomerisation of 3-methyl-3-butenyl pyrophosphate observed to be anti are equally possible electronically and there is no reason therefore to invoke an X-group mechanism to account for the stereochemistry.

(e) There has been much confusion unnecessarily introduced into discussions on the stereochemistry of S_E2' and S_N2' reactions by the use of "intuitive arrow-pushing" ideas about electronic reorganisation during the reaction and by the use of unreal model systems.

The findings of this study clearly show the need for further work to establish experimentally the stereochemistry of S_N2' and S_E2' reactions in a variety of different systems, so that the generality of the above conclusions may be further tested. It is also suggested that similar detailed molecular orbital calculations be performed on the E_2' reaction to establish whether there is any electronically favoured stereochemical pathway for this process.

EXPERIMENTAL

EXPERIMENTAL

General Directions

All melting points (m.p.) were determined on a Kofler hot-stage apparatus and are uncorrected. Routine infra-red (i.r.) spectra were recorded, in carbon tetrachloride solution, on a Perkin-Elmer 257 spectrophotometer and high resolution spectra were recorded by Mrs. F. Lawrie and staff using a Unicam S.P.100 or a Perkin-Elmer 225 double beam spectrophotometer. Only the major peaks or those of diagnostic value are quoted. Nuclear magnetic resonance (n.m.r.) spectra were recorded in $CDCl_3$ using T.M.S. as an internal standard on a Varian T.60 or H.A.100 spectrometer by Mr. J. Gall and Mr. A. Haetzman. The n.m.r. data is recorded on the τ scale and only significant signals are reported. Mass spectra (m.s.) were routinely determined on an A.E.I. M.S.12 spectrometer, whilst mixtures were normally examined by means of an L.K.B.9000 gas-liquid chromatograph-mass spectrometer (g.c. - m.s.). High resolution spectra were obtained on an A.E.I. M.S.902 spectrometer and all spectra were recorded at 70eV unless otherwise stated. Analytical gas-liquid chromatography (g.l.c.) was performed on a Pye-Argon or Perkin-Elmer F.11 chromatograph and peaks are identified by either their retention time in minutes or by their carbon number. Merck Kieselgel HF_{254} was used for all thin layer chromatography (t.l.c.), analytical on 0.25mm plates and preparative on 1.0mm plates. 'Silver nitrate plates' were made using a slurry of 15% by weight of silver nitrate in silica gel G. Micro-analysis were performed by Mr. J.M.L. Cameron, B.Sc., and his staff. Petrol refers to the fraction b.p. 60-80°. All organic extracts were washed to neutrality by appropriate acid or base treatment and were dried over anhydrous magnesium sulphate.

SYNTHESIS AND CYCLISATION OF THE OLEFIN-ACETAL (8)

Cholestenone (14)

Oxidation of cholesterol by the method of Fieser[27] gave cholestenone (14) in yields of 65-70% with m.p. 79-80° (lit.,[27] 79.5-80.5°).

Cholest-4-ene (11)

Anhydrous aluminium chloride (48g, 0.36mol) was carefully added to dry ether (200ml) stirred at 0° under nitrogen. A slurry of lithium aluminium hydride (4.6g, 0.12mol) in dry ether (150ml) was cautiously added and the resulting mixture stirred for 10 min. A solution of cholestenone (23g, 0.06mol) in dry ether (200ml) precooled to 0° was added over 20 min and the mixture stirred for a further 30 min, when the reaction was quenched by dropwise addition of water till the inorganic salts coagulated. The ethereal solution was decanted, dried and evaporated to give cholest-4-ene, (19.1g, 86%) m.p. 80-81° (lit.,[29] 79-80 and 82-83°), $(\alpha)_D$ = + 68.5° (C 1.45), (lit.,[29] +65° and +76°), after crystallisation from ether-methanol.

Ozonolysis of Cholest-4-ene (11)

Ozone enriched oxygen was bubbled through a solution of cholest-4-ene (0.5g) in hexane (10ml) at -78° until an aliquot of the solution failed to decolourise a dilute solution of bromine in acetic acid. Dry nitrogen was then passed through the solution which was allowed to warm to 20°, when acetic acid (10ml) and zinc dust (0.5g) were added. The mixture was stirred for 20h, filtered and the product

(0.45g) isolated by extraction into ether. Analytical t.l.c. of the clear oil in ethyl acetate-petrol (1:3) showed the presence of one compound (Rf 0.6) with tailing to the base line. The crude oil (12) showed ν_{max} 2720w, 1735s, 1710s, 1290w, 1040w, and 950 cm^{-1}, τ 0.26 (1H,t,J3Hz). The sample could not be purified by t.l.c. due to its lability on the adsorbent and was immediately reacted further as below.

Attempted Selective Acetalisation of the Keto-aldehyde (12)

The oil (12) (400mg, ~1mmol) from the above ozonolysis, ethylene glycol (70mg, 1.1mmol) and toluene-p-sulphonic acid (5mg) in dry benzene (10ml) were refluxed for 1h with continuous water separation. The solution was quenched in aqueous sodium hydrogen carbonate and the products extracted into ether. Analytical t.l.c. of the product, a clear oil (380mg), revealed the presence of two incompletely separated components (Rf 0.5) and a preponderance of more polar material. Preparative t.l.c. in ethyl acetate-petrol (1:3) with multiple elution (X3) afforded 10mg of each compound contaminated with a little of the other. The more mobile band gave a keto-acetal (13) as an oil, ν_{max} 1712s, 1135s, 1095m, 1040m, and 945m, cm^{-1}, τ 5.15(1H,t,J5Hz) and 6.08br (4H,s), and the more polar band gave a clear oil, possibly the diacetal (15) with ν_{max} 1710vw, 1120s, 1040w, and 940w cm^{-1}, τ 5.15 (1H,t,J5Hz) and 6.10br (8H,s).

5α -Cholestan-4-one (16) and 5β -Cholestan-4-one (17)

Hydroboration of cholest-4-ene by the method of Jones[29] gave a mixture (1:1) of (16) and (17), after oxidation of the intermediate

alcohols with 8N Jones Reagent. The two isomeric ketones were separated by preparative t.l.c. in ethyl acetate-petrol (1:9). 5α-Cholestan-4-one showed ν_{max} 1715s, 1210m, 935m, and 905m cm^{-1}, τ 9.11, 9.17, 9.26 and 9.36, methyl resonances, g.l.c. 1% OV-1 at 225°, 2917 and 2975 (1:8). 5β-Cholestan-4-one had ν_{max} 1710s, 1180m, 1165m, and 930m cm^{-1}, τ 8.94, 9.15, 9.21, and 9.40, methyl resonances, g.l.c. 1% OV-1 at 225°, 2917 and 2975 (1:1). The above mixture of ketones (16) and (17) was equilibrated to give a mixture of (16) and (17), (9:1), by refluxing with 5% methanolic potassium hydroxide solution, and this mixture was used without further purification.

Baeyer-Villiger Oxidation of the Ketones (16) and (17)

(i) To trifluoroacetic anhydride (70mg, 0.3mmol) in dry methylene chloride (2ml) stirred at 0° under nitrogen was added two drops of 90% hydrogen peroxide. After stirring for 15 min a solution of the ketones (100mg, 0.26mmol) in methylene chloride (2ml) was added dropwise and stirring continued for a further 2h. The reaction mixture was diluted with methylene chloride and poured into aqueous sodium carbonate. Extraction into methylene chloride gave a clear oil (84mg) which showed ν_{max} 3600-2500br, 1785s, 1740s, 1260s, 1250s, and 1035m cm^{-1}. Analytical t.l.c. in ethyl acetate-petrol (1:3) showed the presence of two components Rf 0.5 and 0.05 in equal proportions.

(ii) A solution of peroxytrifluoroacetic acid was prepared by careful addition of trifluoroacetic anhydride (10.2ml, 0.07mol) to an ice-cooled suspension of 90% hydrogen peroxide (1.6ml, 0.06mol) in dry methylene chloride (10ml) under nitrogen. The resulting

solution was added over 30 min to a stirred suspension of anhydrous disodium hydrogen phosphate (16g) in dry methylene chloride (80ml) containing the ketones (15.5g, 0.04mol). The solutions were kept under nitrogen at 0° during addition and for a further 1h at this temperature before removing the ice bath and allowing to warm to 20° over 2h. The reaction was worked up as in (i) above to give a mixture (9:1) of the lactones (19) and (20) (14.5g). Preparative t.l.c. in ethyl acetate-petrol (1:3) afforded a pure sample of both. The more polar band gave 4,5-seco-cholestane-4,5β-carbolactone (19) m.p. 120-122° after sublimation at 180° and 0.7 torr, ν_{max} 1740s, 1292s, 1278s, 1190s, 1110m, and 1035s cm^{-1}, τ 5.87 (1H, q, limbs at ± 3 and ± 10Hz), (Found: C, 80.4; H,11.4. $C_{27}H_{46}O_2$ requires C, 80.55;H,11.5%). The less polar band yielded 4,5-seco-cholestane-4,5α-carbolactone (20), purified by sublimation at 170° and 0.1 torr, as a gum which showed ν_{max} 1755s, 1300s, 1290s, 1280m, 1265m, 1185s, and 1065s cm^{-1}, τ 5.36 (1H,q, limbs at ± 2 and ± 12Hz), (Found: C, 80.65; H,11.75. $C_{27}H_{46}O_2$ requires C, 80.55; H,11.5%).

Hydrolysis of the Lactones (19) and (20)

Hydrolysis of the lactones was effected by stirring the lactones in 5% methanolic potassium hydroxide solution for 16h at 20°. The hydroxy-acids (21) and (22) were isolated by extraction into ether and separated from any neutral components by partition into base followed by reacidification. The resulting sticky gum showed ν_{max} 3600-2500br, 1705s, 1110w, and 1075m cm^{-1}, τ 4.94br (2H,s, exchangeable with D_2O), and 6.40 (1H, unresolved multiplet).

Hydroxy-methyl esters (23) and (24)

The above gum was dissolved in ether and treated with excess ethereal diazomethane. The two components in the product were isolated by preparative t.l.c. in ethyl acetate-petrol (1:3) to give 4,5-seco-4-carbomethoxy-5β-hydroxycholestane (23) as a gum which had ν_{max} 3622w, 3550brw, 1738s, 1235s, 1195s, 1169s, 1080m, 1040m, and 980w cm^{-1}, τ 6.32 (3H,s) and 6.40 (1H,q, obscured by signal at 6.32 τ), M^+ 434 (Calc. for $C_{28}H_{50}O_3$: M, 434), and a more mobile component, 4,5-seco-4-carbomethoxy-5α-hydroxycholestane (24) as a gum with ν_{max} 3625w, 3540brw, 1735s, 1235s, 1200s, 1168s, 1055m and 995m cm^{-1}, τ 6.33br (4H,s), M^+ 434 (Calc. for $C_{28}H_{50}O_3$: M, 434).

4,5-Seco-4-carbomethoxy-5β-acetoxycholestane (25)

The hydroxy-methyl ester (23) (120mg, 0.27mmol) in anhydrous pyridine (2ml) and acetic anhydride (2ml) was kept at 20° for 17h. Evaporation of the solvent in vacuo and purification by preparative t.l.c. in ethyl acetate-petrol (3:7) yielded 4,5-seco-4-carbomethoxy-5β-acetoxycholestane (25) (95mg) as a gum which had ν_{max} 1740sh, 1730s, 1210brs, 1165s, 1020s, 1000sh, and 960m cm^{-1}, τ 5.27 (1H,q, limbs at ± 4 and ± 13 Hz), 6.34 (3H,s) and 7.98 (3H,s), (Found: C, 75.55; H 10.95. $C_{30}H_{52}O_4$ requires C, 75.6; H,11.0%).

4,5-Seco-4-carbomethoxy-5α-acetoxycholestane (26)

In a similar manner the hydroxy-ester (24) (52mg) was converted to the acetate (26), also a gum, which showed ν_{max} 1738s, 1240s, 1170m, 1015w, and 965w cm^{-1}, τ 5.20br (1H,s), 6.33 (3H,s), and 7.94 (3H,s), (Found: C, 75.7; H,11.0. $C_{30}H_{52}O_4$ requires C, 75.6; H, 11.0%).

The Tosylate (28)

The hydroxy-ester (23) (1g, 2.4mmol) and freshly crystallised toluene-4-sulphonyl chloride (0.95g, 5mmol) in the minimum of anhydrous pyridine were set aside at 20° for 65h. The mixture was poured on to iced-water and extracted with ether to give 4,5-seco-4-carbomethoxy-5β-tosyloxycholestane (28) (1.25g, 90%). A sample of the gummy tosylate, purified by preparative t.l.c. in ethyl acetate-petrol (1:3) showed ν_{max} 1740s, 1180s, 1170s, 1095w, 965w, 930m, 900m, and 890m cm^{-1}, τ 2.44 (4H, q, limbs at ± 16 and ± 33Hz), 5.50 (1H,q, limbs at ± 3 and ± 12 Hz), 6.32 (3H,s), and 7.56 (3H,s).

4,5-Seco-4-carbomethoxy-5α-tosyloxycholestane

By the above method, the hydroxy-ester (24) gave 4,5-seco-4-carbomethoxy-5α-tosyloxycholestane in 65% yield which showed ν_{max} 1740s, 1180s, 1170s, and 900m cm^{-1}, τ 2.50 (4H,q, limbs at ± 16 and ± 28 Hz), 5.35br (1H,s), 6.35(3H,s) and 7.33(3H,s).

Jones Oxidation of the Hydroxy-ester (23)

A solution of the hydroxy-ester (23) (100mg, 0.24mmol) in acetone (5ml) was treated dropwise with 8N Jones Reagent at 0° till a red colour persisted in solution. Methanol (1ml) was added, the mixture evaporated in vacuo, and the residue thoroughly extracted with ethyl acetate to give 4,5-seco-4-carbomethoxy-5-oxocholestane (27) (90mg, 90%) which was purified by preparative t.l.c. in ethyl acetate-petrol (1:6) and showed ν_{max} 1740s, 1710s, 1190s, 1165s, 1090m, and 950m cm^{-1}, τ 6.33 (3H,s), M^+ 432.35830 (Calc. for $C_{28}H_{48}O_3$: M, 432.36032).

ATTEMPTS TO FORM THE OLEFIN-ESTER (29)

(i) By Dehydration of the Hydroxy-acid (21)

The hydroxy-acid (21) (10mg, 0.025mmol) in anhydrous pyridine (1ml) at 0° was treated with phosphoryl chloride (1 drop) for 30 min. Quenching with water and extraction into ether gave a mixture (7mg) which was shown by t.l.c. to consist of two compounds identified as the lactone (19) and unreacted hydroxy-acid.

(ii) By Dehydration of the Hydroxy-ester (23)

The hydroxy-ester (23) (100mg) in anhydrous pyridine (3ml) was treated with phosphoryl chloride at 60° for 30 min. Work up as above gave a complex mixture from which was obtained an unidentified methyl ester (15mg) by preparative t.l.c. in ethyl acetate-petrol (1:9) which showed ν_{max} 1740s, 1165s, and 890w cm^{-1}, τ 6.34 (3H,s).

(iii) By Pyrolysis of the Acetate (25)

(a) A 2m narrow bore silica tube, loosely packed with glass wool, was heated in an oven to 570°. A slow bleed of helium was allowed through a septum at one end, while at the other was fitted a cooled U-tube receiver which was in turn attached to a vacuum pump which maintained the pressure in the tube at 0.1 torr. A solution of the acetate (25) (10mg) in chloroform (0.1ml) was injected and the material which collected in the receiver examined by t.l.c. in ethyl acetate-petrol (1:9). This was shown to be principally unchanged acetate (25) (by i.r. comparison) along with two more mobile compounds.

(b) A sample of the acetate (25) (50mg) was sublimed into the pyrolysis tube from a small side arm and the product examined on t.l.c.. The same two mobile components were present and preparative t.l.c. yielded a sample of both. The more mobile band gave a gum (5mg) which showed ν_{max} 3010w, 1640w, 910w, and 880m cm^{-1}, and the second component a gum (20mg) had ν_{max} 3010m, 1740s, 1640w, 1190m, 1165s, and 890w cm^{-1}, τ 4.53 (1.8H,q, limbs at $\pm$ 9 and $\pm$ 24 Hz, the lower field signals being broad and not well resolved), 5.30 (small signal not resolved), and 6.36 (3H,s), M^+ 416 (Calc. for $C_{28}H_{48}O_2$: M, 416), g.l.c. 1% OV-1 at 225°, 19.5 and 21.25sh min, ($C_{24,26,28,32}$ at 4.0, 7.25, 13.5, and 45 min respectively).

(c) The pyrolysis tube was clamped vertically and a solution of the acetate (25) (40mg) in benzene (1ml) introduced dropwise in a flow of dry nitrogen. The product was collected in a cooled receiver under the furnace which was maintained at 540°. After one pass through the tube the mixture was shown by t.l.c. to be similar to that obtained in (b) above. A further two passes of this mixture utilised all the acetate present but increased substantially the proportion of the very mobile band. Total recovery after three passes was 25mg.

(iv) By Elimination of the Tosylate (28)

(a) On Alumina

The tosylate (28) (250mg) was stirred with neutral alumina, grade I, (10g) in benzene-petrol (1:1) for 80h. Filtration and repeated extraction of the alumina with ethyl acetate gave unchanged tosylate (150mg) and 30mg of a component identical on t.l.c. with that obtained in (iii)(b) above.

(b) In Dimethyl Sulphoxide

The tosylate (28) (900mg, 1.5mmol) in dry dimethylsulphoxide (5ml) was maintained at 115° for 17h under nitrogen. Dilution with water and extraction into petrol gave a gum (560mg) which on examination by t.l.c. showed a band corresponding to the olefin-ester from acetate pyrolysis as the major product. This component was isolated by preparative t.l.c. in ethyl acetate-petrol (1:9) and on g.l.c., 1% OV-17 at 200°, showed peaks at 50.5, 54, and 58.5min (6:4:1). Analytical t.l.c. on silver nitrate plates in the same solvent showed three bands which were incompletely separated. Preparative t.l.c. on 0.5mm silver nitrate plates with multiple elution (X4) yielded a pure sample of the least mobile component, 4,5-seco-4-carbomethoxycholest-5-ene (29) as a gum which had ν_{max} 3010m, 1740s, 1640w, 1190m, 1165s, and 890w cm^{-1}, τ 4.55 (2H,m, limbs at ± 10 and ± 20 Hz, lowfield limbs further split by ± 3Hz), 6.36(3H,s), and 7.75(2H,t,J7Hz). Irradiation at 8.08 τ reduced the multiplet at 4.55 τ to a quartet with limbs at ± 9 and ± 20 Hz, g.l.c., 1% OV-17, 200°, 54 min, identical with the peak at 19.5 min from (iii)(b), and with the central peak in the above mixture by co-injection. (Found: C, 80.8; H, 11.6. $C_{28}H_{48}O_2$ requires C, 80.7; H, 11.6%).

The middle band yielded a gum (115), still a mixture, which showed ν_{max} 1740s, 1240m, 1190s, and 1165s cm^{-1}, τ 4.95 (0.75H,t, poorly resolved), 5.37 (0.25H,d,J8Hz), 6.37 (3H,s), 7.67 (3H,d,J3Hz) and 8.38br (2.5H,s). Irradiation at 7.67 τ resolved the signal at 4.95 τ into a broad singlet, and irradiation at 8.38 τ produced a sharper signal at 4.95 τ superimposed on a broad unresolved peak from 5.84 - 6.04 τ . Irradiation at 4.95 τ reduced the doublet at

7.67 τ to a singlet and sharpened the signal at 8.38 τ . G.l.c. on 1% OV-17 at 200° showed one peak at 50.5 min corresponding to the first peak in the mixture.

Elution of the most mobile band yielded a gum (116), also a mixture which showed ν_{max} 1740s, 1250m, 1185s, and 1165s cm^{-1}, τ 4.52br (0.8H, poorly resolved doublet?). 6.36 (3H,s) and 8.40br (3H,s), g.l.c. 1% OV-17, 200°, peaks at 50.5 and 58.5 min (5:1), identical with those in the mixture by co-injection.

(c) In Dimethyl Sulphoxide with Potassium t-butoxide

The tosylate (28) (10mg) and potassium t-butoxide (3mg) in dry dimethyl sulphoxide (2ml) were kept at 110° under nitrogen for 18h. Work up as above and analytical t.l.c. on silver nitrate plates showed the presence of two components corresponding to (29) and (115); g.l.c., 1% OV-17, 200°, peaks at 50.5, 54 and 58.5 min (2:3:1).

(d) In Pyridine

The tosylate (10mg) in dry pyridine (1ml) was refluxed for 6h. The product was isolated by extraction into ether and the olefinic band separated by preparative t.l.c.. Silver nitrate t.l.c. showed two bands corresponding to (29) and (115); g.l.c., 1% OV-17, 200°, peaks at 50.5, 54 and 58.5 min (1.5:3:1).

(e) With Sodium Acetate in Acetic Acid

The tosylate (10mg) and anhydrous sodium acetate (50mg) in acetic acid (1ml) were refluxed for 1.5h and the product isolated by ether extraction. Silver nitrate t.l.c. again showed (29) and (115) to be present; g.l.c., 1% OV-17, 200°, peaks at 50.5, 54, and 58.5 min (2.5:2:1).

(f) In Dimethylformamide

A solution of the tosylate (20mg) in dry dimethylformamide was maintained at 100° for 24h under nitrogen. Work up as in (iv)(b) above and silver nitrate t.l.c. of the olefinic fraction showed the same two bands as above and the same peaks on g.l.c. (2:3.5:1).

(g) In Dimethylformamide with Lithium Bromide and Lithium Carbonate

The tosylate (50mg, 0.085mmol), anhydrous lithium bromide (11mg, 0.128mmol) and anhydrous lithium carbonate (13mg, 0.19mmol) in dry dimethylformamide (5ml) were refluxed under nitrogen for 1h and worked up as before. Silver nitrate t.l.c. showed two bands as before; g.l.c., 1% OV-17, 200°, peaks at 50.5, 54, and 58.5 min (1.5:6:1).

(h) Repeat of (g)

The tosylate (10mg, 0.017mmol), anhydrous lithium bromide (11 mg, 0.128mmol) and anhydrous lithium carbonate (10mg, 0.135mmol) in dry dimethylformamide (1ml) were stored at 120° for 3.5h. Silver nitrate t.l.c. showed only one component identical with (29); g.l.c.,1% OV-17, 200°, peaks at 50.5, 54, and 58.5 min (1:8:1).

(i) Optimum Conditions for Elimination

The tosylate (28) (600mg, 1.02mmol), anhydrous lithium bromide (850mg, 10mmol) and anhydrous lithium carbonate (150mg, 2mmol) in dry dimethylformamide (12ml) were kept at 115° under nitrogen for 2h. The crude product was isolated by extraction into petrol and filtered through a short column of neutral alumina (grade III)

in benzene to give the desired olefin-ester (29) (250mg, 62%). G.l.c., 1% OV-17, 200°, peaks at 50.5 and 54 min (1:30) and homogeneous on silver nitrate t.l.c..

The Hydroxy-Olefin (32)

A solution of the olefin-ester (29) (140mg, 0.33mmol) in dry ether (4ml) was added dropwise to a stirred suspension of lithium aluminium hydride (80mg, 2.1mmol) in dry ether (5ml) stirred at 0° under nitrogen, and stirring continued for a further 30 min. Water was cautiously added till the inorganic salts coagulated, the ethereal layer decanted, and the salts washed with a small portion of ether. The combined ethereal extracts yielded 4,5-seco-4-hydroxycholest-5-ene (32) as a gum (122mg, 94%) which was purified by preparative t.l.c. in ethyl acetate-petrol (3:17) and showed ν_{max} 3636m, 3480brw, 3008m, 1652w, 1050brs, and 1030sh cm^{-1}, τ 4.54 (2H,m, limbs at ± 8 and ± 18Hz, lowfield limbs further split by 4Hz) and 6.38 (1h,t,J6Hz). (Found: C, 83.4;H,12.65. $C_{27}H_{48}O$ requires C, 83.45; H, 12.45%).

The Olefin-Aldehyde (33)

Oxidation of the hydroxy-olefin (32) was carried out using chromium trioxide in pyridine and methylene chloride according to Radcliffe and Rodehurst[70]. Yields were in the range 70-90% and the aldehyde, 4,5-seco-4-oxocholest-5-ene (33) purified by preparative t.l.c. in ethyl acetate-petrol (1:9) was isolated as a gum which had ν_{max} 3010m, 2710m, 1730s, and 1655w cm^{-1}, τ 0.3 (1H,t,J2Hz) and 4.54 (2H,m, limbs at ± 6 and ± 22 Hz, lowfield limbs further split by 4Hz). (Found: C, 83.95; H,12.2. $C_{27}H_{46}O$ requires C, 83.85; H, 12.0%).

Acetalisation of the Aldehyde (33)

The aldehyde (33) (1.13g, 2.92mmol), toluene-p-sulphonic acid (20mg) and excess ethylene glycol in benzene (25ml) were refluxed for 3h with continuous water separation. The solution was poured into aqueous sodium hydrogen carbonate and extracted with ether. A sample of the crude 4,5-seco-cholest-5-ene-4-ethylene acetal (8) was purified by preparative t.l.c. in ethyl acetate-petrol (1:9) and the gum showed ν_{max} 1652w, 1135sh,1128s, 1060brm, 955w, and 940m cm^{-1}, τ 4.57 (2H,m, limbs at $\pm$ 7 and $\pm$ 17 Hz, lowfield limbs further split by 5Hz). (Found: C, 80.8; H, 11.65. $C_{29}H_{50}O_2$ requires C, 80.9; H, 11.7%).

Rearranged Aldehyde (34)

In the synthesis of one batch of the olefin-acetal, analytical t.l.c. at the olefin-aldehyde stage revealed the presence of a second component not previously identified. Preparative t.l.c. of the aldehyde mixture in ethyl acetate-petrol (1:9) afforded a pure sample of the minor component, the oily aldehyde (34) which showed ν_{max} 3080w, 2705w, 1730s, 1630w, and 890m cm^{-1}, τ 0.22 (1H,t,J2Hz), 5.32 (1H,s), and 5.52 (1H,s). (Found: C, 83.85; H, 12.2. $C_{27}H_{46}O$ requires C, 83.85; H, 12.0%).

Osmylation of (34)

The aldehyde (34) (30mg, 0.075mmol) and osmium tetroxide (40mg, 0.16mmol) in dry benzene (3ml) were stored at 20° for 46h.

The solution was diluted with benzene and hydrogen sulphide bubbled through it for 5 min. Removal of osmium sulphide by filtration and evaporation of the solvent in vacuo left a clear gum, consisting of one major component. Preparative t.l.c. in ethyl acetate-petrol (1:9) afforded this component, homogeneous on t.l.c., which showed ν_{max} 1145m, 1120s, 1105s, 1085s, 1040m, 1025s, 1010m, 995m, 870m, and 840s cm^{-1}, τ 4.56 (1H,s,$w_{\frac{1}{2}}$ 4Hz), 5.71 (0.7H,d,J7Hz), 5.91 (0.3H,d,J7Hz), 6.74 (0.7H,d,J7Hz), and 6.87 (0.3H,d,J7Hz). Irradiation at 5.71 τ reduces to a singlet the doublet at 6.74 τ and similarly the doublet at 6.87 τ collapses to a singlet on irradiation at 5.91 τ. G.l.c., 1% OV-1, 225°, 2967:3006 = 2:5. G.c. - m.s. gives for the minor component, M^+ 402 (100), other peaks at 387 (10), 384 (15), 372 (70), 357 (15), 327 (55), 288 (40), 248 (58) and 247 (85) and for the major component M^+ 402 (83), other peaks at 387 (11), 372 (81), 357 (32), 343 (16), 327 (100), 314 (29), 301 (34), 247 (74) and 215 (61). (Calc. for $C_{27}H_{46}O_2$: M, 402).

Synthesis of the Olefin-acetal (8) from Cholesterol, General Procedure

Cholestenone (14) was prepared as described and purified by crystallisation from methanol. Reduction of (14) (23g) with dichloroaluminium hydride gave cholest-4-ene (11) (20.5g, crude). Without purification this was converted to a mixture of 5α-and 5β-cholestan-4-ones (16) and (17) (9:1) (17g, crude), which was oxidised to give the corresponding lactones (19) and (20) which in turn were opened by basic hydrolysis to afford a mixture of hydroxy-acids (21) and (22). These were separated from residual neutral material by partition between ether and aqueous sodium hydroxide and the resulting sticky gum methylated to give a mixture

of the hydroxy-esters (23) and (24) (12g). Tosylation of this mixture using a 2.5-fold excess of toluene-4-sulphonyl chloride in pyridine gave the corresponding tosylates and unreacted hydroxy-ester (24) (16g, crude). Elimination of the tosylates was effected as described and the crude product (9.5g) purified by filtration in benzene through a column of grade III neutral alumina to give the olefin-ester (29) (5.1g). Reduction of the olefin-ester (29) followed by oxidation and acetalisation gave the olefin-acetal (8) (2.6g, 10% from cholestenone, 6% from cholesterol).

CYCLISATION OF THE OLEFIN-ACETAL (8)

(i) In Benzene

A solution of the olefin-acetal (0.3ml of a 0.023M solution in benzene) and a solution of stannic chloride (0.3ml of a 0.094M solution in benzene) were mixed at 20° and kept for 3h. The reaction was quenched by pouring it into dilute aqueous sodium hydrogen carbonate and the product isolated by ether extraction. Analytical t.l.c. showed only the original acetal.

(ii) In Nitromethane

A 0.023M solution of the olefin-acetal (8) in freshly distilled nitromethane and a 0.09M solution of stannic chloride in nitromethane were prepared.

(a) Equal volumes (0.2ml) of each solution were mixed at 20° and kept for 40 min. Work up as above gave a complex mixture which was not further investigated.

(b) Admixture of the olefin-acetal solution (0.1ml) and stannic chloride solution (0.2ml) for 15 sec at 20° before quenching and work up gave a more promising mixture consisting of apparently four major and several minor components by analytical t.l.c.. G.l.c., 1% OV-1, 225°, showed the presence of seven peaks at 2780, 2800, 2832, 2870, 2935, 2966, and 3078 and g.c. - m.s. gave M^+ 368, 368, 368, 386, 388, 430 and 428 respectively, further details are listed in Table (1). The crude mixture was dissolved in dry pyridine (0.15ml) and treated with hexamethyldisilazane (50ml) and trichlorosilane (10ml) at 40° for 1h. The mixture of T.M.S. ethers was isolated by evaporation of the solvent in vacuo and extracting the residue with ether. Analytical t.l.c.in ethyl acetate-petrol (1:9) showed the presence of three major components and g.l.c., 1% OV-1, 225° showed peaks at 6.6, 10.7, 12.65, and 26.25 min which on g.c. - m.s. showed M^+ 458, 460, 502, and 502 respectively and full details are listed in Table (2).

(iii) In Methylene Chloride

Standard solutions of the olefin-acetal (8) and stannic chloride were made up in freshly distilled methylene chloride (0.023M and 0.09M respectively).

(a) At Room Temperature

Equal volumes of the standard solutions (0.5ml) were mixed at 20° for 1 min. Quenching and work up as before gave a mixture, with one major component as judged by t.l.c. and which showed aldehydic adsorption in the i.r. spectrum, but which was a complex mixture on g.l.c. analysis and was not further investigated.

(b) At -78°

Equal volumes of the standard solutions (0.5ml) were precooled to -78° before mixing for 1 min at -78°. The usual work up gave a mixture, which on analytical t.l.c. showed the presence of two components sufficiently separated from the rest to allow an attempt at isolation. G.l.c., 1% OV-1, 220°, showed peaks at 2832, 2870, 2935, 2966, and 3078 (1:3:4:3:2). This procedure was repeated on a larger scale (5ml of solution) and preparative t.l.c. in ethyl acetate-petrol (1:9) allowed the isolation of one component (4mg), slightly impure, which had ν_{max} 3540, 3010sh, 1050s, and 890m cm^{-1}, g.l.c., 2966, g.c. - m.s. gave M^+ 430 (15) other peaks at 415 (5), 368 (100), 353 (35), 255 (36), 229 (19) and 201 (55).

(c) At -78° with Different Stoichiometry

A solution of stannic chloride in methylene chloride (2 ml of a 0.045M solution) cooled to -78° was quickly added to a solution of the olefin-acetal (8) in methylene chloride (10ml of a 0.002M solution) at -78° and the reaction quenched as quickly as possible. G.l.c., 1% OV-1, 200°, showed peaks at 2825, 2935, 2968 and 3078 (1:1:1.3:2), Figure (18). By elution of the bands from analytical t.l.c. of this mixture a correlation was obtained between t.l.c. and g.l.c. as shown in Figure (20).

Attempted Simplification of the Cyclisation Mixture

The crude mixture from cyclisation (b) (40mg) was dissolved in dry pyridine (1ml) and toluene-4-sulphonyl chloride (60mg) added. After storage for 24h at 20° the crude product was isolated as previously described. The crude tosylates (40mg) and sodium iodide (100mg)

in dry dimethoxyethane (4ml) were refluxed for 30 min when freshly purified zinc powder (50mg) was added in small portions and the reflux continued for a further 2h. Filtration of the mixture followed by extraction into ether afforded a complex mixture which was oxidised with 8N Jones Reagent. Analytical t.l.c. of the product showed no predominant component, and there was a veritable plethora of peaks on g.l.c..

(d) Preparative Cyclisations

A solution of stannic chloride (0.5ml, 4.5mmol) in methylene chloride (10ml) at -78° was quickly added to a solution of the olefin-acetal (100mg, 0.23mmol) in methylene chloride (10ml) at -78°. After 30 sec the reaction was quenched and the product isolated as usual. Preparative t.l.c. in ethyl acetate-petrol (1:9) afforded two slightly impure components. Further purification of these samples by t.l.c. on 0.25mm plates gave a pure sample of each. The more mobile component, the hydroxy-ether (38), a gum (15mg) had ν_{max} 3530w, 3610m, 1645w, 1150w, 1098s, 1050s, 955m, and 890m cm^{-1}, τ 4.38 (2H,m, limbs at ± 4 and ± 14 Hz, upfield limbs further split by 4Hz), 6.38br (4H,m), 6.54br (1H,s), and 7.44 (1H,t,J6Hz, exchangeable with D_2O). Irradiation at 8.24 τ removes the 4Hz coupling from the upfield limbs of the multiplet at 4.38 τ. G.l.c., 1% OV-1, 200°, 2966, M^+ 430.3778 (Calc. for $C_{29}H_{50}O_2$: M, 430.3811) other peaks at 415, 368, 353, 273, 255, 229, 228, 213, and 201. The more polar component (16 mg) showed ν_{max} 3630w, 3610sh, 1050s, 1030sh, 1020s, 1010sh, and 930m cm^{-1}, τ 6.06br (1H,s,$w_{\frac{1}{2}}$ 20Hz), 9.04, 9.12, 9.18 and 9.36 (methyl resonances), g.l.c., 1% OV-1, 200°, 2936 identical by co-injection with authentic 4β-hydroxy-5β-cholestane (41),

M^+ 388.3700 (Calc. for $C_{27}H_{48}O$: M, 388.3705) other peaks at 373, 370, 355, 317, 287, 257, 248, 233, and 215.

Hydrogenation of the Hydroxy-ether (38)

(i) Palladium/Carbon/Hydrogen

The alcohol (38) (10mg) in dry ethyl acetate (2ml) and a dispersion of 10% palladium on charcoal (5mg) were stirred under an atmosphere of hydrogen for 2h. Filtration and evaporation of the solvent in vacuo afforded a gum (9mg). Analytical t.l.c. in ethyl acetate-petrol (1:9) showed the presence of two components (Rf 0.5 and 0.2) in approximately equal amounts which on g.l.c., 1% OV-1, 225° appeared as one peak, 3045.

(ii) Diimide

The alcohol (38) (5mg) and tosylhydrazine (40mg) in diglyme (1ml) were refluxed for 3h under nitrogen. Analytical t.l.c. of the product, isolated by extraction into ether, showed one component (Rf 0.5) g.l.c., 1% OV-1, 225° peaks at 2965, and 3045 (1:9).

Cleavage of the β-Hydroxy-ether

The saturated hydroxy-ether (5mg) produced by diimide reduction, above, was converted to the corresponding tosylate under standard conditions. The crude tosylate and sodium iodide (20mg) in dry dimethoxyethane were refluxed for 30 min when zinc dust (20mg) was added in portions over 1.5h. Filtration and extraction into ether gave an oil (2mg) which on analytical t.l.c. showed one major component. Purification on a 0.25mm t.l.c. plate afforded a pure sample which showed ν_{max} 3635sh, 3619w, 1215w, 1170m, 1005m, 968m,

940sh, 945s, and 900w cm^{-1}, g.l.c., 1% OV-1, 225°, 2910 identical by co-injection with a sample of 4α -hydroxy-5β -cholestane (39).

Oxidation of the Alcohol (41)

Standard Jones oxidation, as described previously, of the alcohol (41) (6mg) afforded a ketone (4mg) ν_{max} 1710s, 1180m, 1165m, 1080w, 1050w, 940sh, and 930m cm^{-1}, τ 8.90, 9.12, 9.18 and 9.36, (methyl resonances), g.l.c., 1% OV-1, 200°, peaks at 2937, and 3000 (1:1) identical with 5β -cholestan-4-one (17).

Lithium Aluminium Hydride Reduction of 5α -Cholestan-4-one (16)

Reduction, as previously described, afforded a two-component mixture which was separated by preparative t.l.c. in ethyl acetate-petrol (1:9). The more mobile band gave 4β -hydroxy-5α -cholestane (45mg) m.p. 128-130° (lit.,[71] 130-132°), ν_{max} 3640m, 1170m, 1120m, 1060m, 990m, 960m, 930m and 865w cm^{-1}, τ 6.20br (1H,s,$w_{\frac{1}{2}}$ 8Hz), 8.97, 9.08, 9.18, and 9.36, (methyl resonances), g.l.c., 1% OV-1, 200°, 2950. M^+ 388 (Calc. for $C_{27}H_{48}O$: M, 388). The more polar band yielded 4α -hydroxy-5α -cholestane (17mg) which had m.p. 185-187° (lit.,[71] 186-187°), ν_{max} 3640m, 1065m, 1035s, 1030sh, and 945w cm^{-1}, τ 6.38br (1H,s,$w_{\frac{1}{2}}$ 20Hz), 9.06, 9.18, and 9.36, (methyl resonances), g.l.c., 1% OV-1, 200°, 2945. M^+ 388 (Calc. for $C_{27}H_{48}O$: M, 388).

Lithium Aluminium Hydride Reduction of 5β -Cholestan-4-one (17)

In a like manner, 5β -cholestan-4-one (17) gave on reduction and separation of the mixture, a mobile component, 4α -hydroxy-5β -cholestane (39) (31mg) which showed ν_{max} 3640sh, 3630w, 1215m, 1170m, 1155sh, 1005m, 970m, and 945s cm^{-1}, τ 6.00br (1H,s,$w_{\frac{1}{2}}$ 8Hz),

9.06, 9.17, and 9.35 (methyl resonances), g.l.c., 1% OV-1, 200°, 2905. M^+ 388 (Calc. for $C_{27}H_{48}O$: M, 388), and a more polar component, 4β-hydroxy-5β-cholestane (41) (15mg) which showed ν_{max} 3628m, 3604sh, 1050m, 1030sh, 1020s, 1008m, and 920m cm^{-1}, τ 6.06br (1H, s, $w_{\frac{1}{2}}$ 20Hz), 9.03, 9.09, 9.19. and 9.38 (methyl resonances), g.l.c., 1% OV-1, 200°, 2935. M^+ 388 (Calc. for $C_{27}H_{48}O$: M, 388).

Lithium Aluminium Deuteride Reduction of the Olefin-ester (29)

Reduction of the olefin-ester (29) as previously described gave on purification 4,5-seco-4,4-dideuterio-4-hydroxycholest-5-ene (49) which had ν_{max} 3630m, 3005m, 2185brw, 2085brw, 1640w, 1100w, 960s and 890w cm^{-1}, τ 4.58 (2H,m, limbs at ± 8 and ± 18Hz, lowfield limbs further split by 4Hz) M^+ 390 (Calc. for $C_{27}H_{46}D_2O$: M, 390).

Oxidation of the Dideuterio-alcohol (49)

Oxidation as described for the unlabelled alcohol (32) gave a deuterio-aldehyde (50) with ν_{max} 3010m, 2060m, 1715s, 1650w, 1090m, and 955w cm^{-1}. τ 4.53 (2H,m, limbs at ± 7 and ± 24 Hz lowfield limbs further split by 8Hz), M^+ 387 (Calc. for $C_{27}H_{45}DO$: M, 387).

Acetalisation of the Deuterio-aldehyde (50)

Acetalisation under conditions described for the aldehyde (33) afforded 4,5-seco-4-deuteriocholest-5-ene-4-ethylene acetal (51) which had ν_{max} 3010m, 2090w, 1652w, 1205m, 1060s, and 940w cm^{-1}, τ 4.53 (2H,m, limbs at ± 6 and ± 23 Hz, lowfield limbs further split by 8Hz) and 6.09br (4H,m), M^+ 431 (Calc. for $C_{29}H_{49}DO_2$: M, 431; > 97% d_1 by comparison with unlabelled acetal (8)).

Cyclisation of the Deuterio-acetal (51)

Cyclisation of the 4-deuterio-acetal (51)(95mg) as above gave, after purification by t.l.c. a mobile component, the deuterio-hydroxy-ether (52) (10mg) as a gum with ν_{max} 3530m, 3010m, 2110w, 1645w, 1290m, 1160m, 1135m, 1115sh, 1094s, 1050s, 970w and 955w cm^{-1}, τ 4.34 (2H,m, limbs at ± 4 and ± 14Hz, highfield limbs further split by 4Hz), 6.14br (4H,m) and 7.42br (1H, unresolved, exchangeable with D_2O), M^+ 431 (Calc. for $C_{29}H_{49}DO_2$: M, 431; > 98% d_1 by comparison with unlabelled material (38)). The more polar component 4 α-deuterio-4 β-hydroxy-5 β-cholestane (53) (13mg) showed ν_{max} 3628w, 3605sh, 2140w, 1100m, 1070sh, 1055s, 1020m, 940sh, and 930s cm^{-1}, τ 9.05, 9.11, 9.17, and 9.35 (methyl resonances), M^+ 389 (Calc. for $C_{27}H_{47}DO$: M, 387; > 98% d_1 by comparison with authentic unlabelled material (41)).

Allylic Oxidation of the Olefin-Ester (29)

(i) With N-bromosuccinimide[46]

The olefin-ester (29) (22mg, 0.053mmol), N-bromosuccinimide (50mg) and calcium carbonate (20mg) in dioxan (2ml) containing water (2 drops) was irradiated for 4h at 20° with a standard 60W desk lamp, and the product isolated by extraction into ether. Analytical t.l.c. showed the presence of (29) and two more polar components in the mixture. Preparative t.l.c. in ethyl acetate-petrol (1:3) yielded a bromo-keto-ester (55) (10mg) which showed ν_{max} 1740s, 1720s, 1275s, 1255m, 1240m, 1190s, 1160s, and 925w cm^{-1}, τ 6.10br (1H,s), 6.42 (3H,s) and 7.76br (2H,d,J6Hz), M^+ 512 and 510, other peaks at 497, 495, 481, 479, 431, 411, 409, 399, 385, 331, and 277 (Calc. for $C_{28}H_{47}O_3Br$: M, 512 and 510). The second more polar component,

an alcohol (56) (4mg) showed ν_{max} 3620m, 3500brm, 1740s, 1275m, 1165s, 1035m, and 985m cm^{-1}, which on treatment with 8N Jones Reagent gave the bromo-keto-ester (55) as the only product.

(ii) With Anhydrous Sodium Chromate[72]

The olefin-ester (29) (400mg, 0.96mmol) and anhydrous sodium chromate (160mg, 1mmol) in a mixture of acetic acid (4ml) and acetic anhydride (2ml) were stored at 40° for 24h, when a further portion of sodium chromate (160mg) was added. After standing for a further 27h at this temperature the reaction was diluted with water and the product thoroughly extracted into ethyl acetate. Analytical t.l.c. of the resulting oil showed the presence of three components. Preparative t.l.c. of a sample of the oil in ethyl acetate-petrol (1:4) gave the olefin-ester (29) (10mg) as the most mobile component followed by the enone-ester (54) (58mg), an oil which showed ν_{max} 3020m, 1740s, 1680s, 1260brm, 1196s, and 1172s cm^{-1}, τ 3.49 (1H,d,J9Hz), 4.20 (1H,d,J10Hz), 6.38 (3H,s) and 7.76 (2H, unresolved), g.l.c., 1% OV-1, 225°, 317S. (Found: C, 77.9; H, 10.5. $C_{28}H_{46}O_3$ requires C, 78.1; H, 10.75%). Just separate from this band was the enone-ester (57)(12mg) which showed ν_{max} 3020m, 1740s, 1675s, 1270m, 1230s, 1190s, 1170s, and 1105m cm^{-1}, τ 3.21 (1H,d,J18Hz, each limb further split by 1.5Hz), 3.77 (1H,d,J18Hz, each limb further split by 3Hz), and 6.34 (3H,s), g.l.c., 1% OV-1, 225°, 3152 (Found: C, 77.9; H, 10.9. $C_{28}H_{46}O_3$ requires C, 78.1; H, 10.75%).

Deuterolysis of the Enone (54)

Treatment of the enone-ester (54) (90mg) with lithium aluminium deuteride and aluminium trichloride as described in Part II afforded 4,5-seco-4,4,7,7-tetradeuterio-4-hydroxycholest-5-ene (58) (70mg) which had ν_{max} 3640m, 3550-3150brw, 3010m, 2180w, 2085w, 1640w, 1165m, 1110m, 1090brm, 950s and 885m cm^{-1}, τ 4.51 (2H,q, limbs at $\pm$ 7 and $\pm$ 21Hz), M^+ 392 (Calc. for $C_{27}H_{44}D_4O$: M, 392; > 95% d_4 by comparison with unlabelled alcohol (32)).

Oxidation of the Tetradeuterio-hydroxy-olefin (58)

Oxidation as described for the unlabelled alcohol (32) gave a trideuterio-aldehyde (59) which had M^+ 389 (Calc. for $C_{27}H_{43}D_3O$: M, 389; > 95% d_3 by comparison with unlabelled aldehyde (33).

Acetalisation of the Trideuterio-Aldehyde (59)

Standard acetalisation afforded 4,5-seco-4,7,7-trideuteriocholest-5-ene-4-ethylene acetal (60) which showed ν_{max} 3010m, 2168w, 2085m, 1645w, 1265m, 1215w, 1200s, 1170m, 1060s, and 890m cm^{-1}, τ 4.54 (2H,q, limbs at $\pm$ 3 and $\pm$ 21Hz) and 6.09br (4H,m), M^+ 433 (Calc. for $C_{29}H_{47}D_3O_2$: M, 433; > 95% d_3 by comparison with unlabelled acetal (8)).

Cyclisation of the Trideuterio-olefin-acetal (60)

The acetal (60) (80mg) gave on preparative cyclisation as before a dideuterio-hydroxy-ether (61) (13mg), a gum which showed ν_{max} 3530m, 3012w, 2235w, 2110w, 1635w, 1290m, 1260w, 1160m, 1130m, 1120m, 1095s, 1055s, and 895m cm^{-1}, τ 4.43 (1H,d,J3Hz), 6.36br (4H,s), and 7.44br (1H, exchangeable with D_2O), M^+ 432 (Calc. for $C_{29}H_{48}D_2O_2$: M, 432; > 95% d_2 by comparison with unlabelled material (38)).

The more polar component, the trideuterio-alcohol (62) (11mg) showed ν_{max} 3625w, 3605sh, 2180w, 2135w, 2100w, 1165m, 1090w, 1075m, 1050sh, 1045s, 1020m, 1010m, and 940m cm^{-1}, τ 9.04, 9.11, 9.17, and 9.37 (methyl resonances), M^+ 391 (Calc. for $C_{27}H_{45}D_3O$: M, 391; > 96% d_3 by comparison with unlabelled alcohol (41)).

Lithium Aluminium Hydride Reduction of the Enone-ester (54)

Standard reducing conditions afforded a two component mixture in which one product predominated. Preparative t.l.c. in ethyl acetate-petrol (2:3) afforded a pure sample of the major component, 4,5-seco-4,7β -dihydroxycholest-5-ene (63) (65% yield from (54)), m.p. 121-123°, ν_{max} 3640m, 3608m, 3010w, and 1660w cm^{-1}, τ 4.51 (2H,q, limbs at ± 1 and ± 11Hz), 6.14 (1H,d, J6Hz), and 6.37 (2H,t,J6Hz), M^+ 404 (100), other peaks at 389 (16), 386 (36), 371 (16), 334 (27), 331 (60), 315 (22), 313 (63), 290 (23) and 219 (37) (Calc. for $C_{27}H_{48}O_2$: M, 404).

Deuterolysis of the Diol (63)

Deuterolysis of the diol (63) (78mg) as described in Part II afforded a deuterated-alcohol (64) (59mg) which showed ν_{max} 3638m, 3008m, 2155w, 2115w, 1650w, and 1120s cm^{-1}, τ 4.51 (2H,q, limbs at ± 8 and ± 18Hz, lowfield limbs broad), and 6.16 (2H,t,J6Hz), M^+ 389 (Calc. for $C_{27}H_{47}DO$: M, 389; > 98% d_1 by comparison with unlabelled alcohol (32)).

Attempted Photo-oxygenation of the Olefin-acetal (8)

The olefin-acetal (8) (50mg) and hematoporphyrin (4mg) in dry pyridine (5ml) were irradiated with a 60W desk lamp and oxygen

bubbled through the solution for 24h. The product was recovered by extraction into ether and shown to be unchanged olefin-acetal.

In another experiment a similar solution was irradiated with a high pressure Hanovia ultra-violet lamp but this resulted in decolourisation of the solution after 30 min and again the olefin-acetal was recovered unchanged.

7 α - and 7 β -Deuteriocholesterol (71) and (65)

Both compounds were prepared as described in Part II and the 7 α -deuteriocholesterol (71) (> 97% d_1) was shown to be 82.5% 7 α -d, 17.5% 7 β -d. The 7 β -deuteriocholesterol (65) (> 97% d_1) had 97% 7 β -d.

7 β -Deuterio-acetal (66)

This was prepared by synthesis from 7 β -deuteriocholesterol (65) by the route described for unlabelled acetal (8) and showed ν_{max} 3010m, 2155w, 1650w, 1140sh, 1130s, 1050sh, 1035s, and 940 cm^{-1}, τ 4.54 (2H,q, limbs at $\pm$ 7 and $\pm$ 17Hz, lowfield limbs $w_{\frac{1}{2}}$ = 2Hz, highfield limbs $w_{\frac{1}{2}}$ = 3Hz), 5.18 (1H,t,J4Hz), and 6.12br (4H,m), M^+ 431 (Calc. for $C_{29}H_{49}DO_2$: M, 431).

Cyclisation of the 7 β -Deuterio-acetal (66)

Under the previously described conditions the 7 β -deuterio-acetal (66) afforded a deuterated-hydroxy-olefin (67) which showed ν_{max} 3540m, 3010m, 2230w, 1630w, 1150m, 1095s, 1050s, 955m, and 900m cm^{-1}, τ 4.46br (1H,d,J4Hz), 6.40br (4H,m), 6.56br (1H,s) and 7.45br (1H,s, exchangeable with D_2O), M^+ 431 (Calc. for $C_{29}H_{49}DO_2$: M, 431; > 96% d_1 by comparison with unlabelled hydroxy-ether (38)).

Brosylation of the Hydroxy-olefin (32)

The hydroxy-olefin (32) (80mg, 0.21mmol) and 4-bromo-benzenesulphonyl chloride (100mg, 0.39mmol) in dry pyridine (3ml) were stored at 0° for 12h. The brosylate (70) (111mg, 87%) isolated by ether extraction and purified by t.l.c. in ethyl acetate-petrol (1:9) as a gum showed ν_{max} 3010m, 1820w, 1650w, 1270m, 1185s, 1175s, 1095s, 1068s, 1010s, 960s, and 940s cm^{-1}, τ 2.28 (4H,s), 4.59 (2H,m, limbs at ± 11 and ± 21Hz, lowfield limbs further split by 5Hz).

Solvolysis of the Brosylate (70)

(i) At reflux

The brosylate (70) (22mg) and urea (4mg) in 2,2,2-trifluoroethanol (4ml) under nitrogen were refluxed for 72h. Extraction into ether gave a gum which on t.l.c. in petrol was shown to consist of a very mobile component (Rf 0.9) and some much more polar components. Preparative t.l.c. afforded a sample (3mg) of the mobile band. G.l.c., 1% OV-1, 225°, of this sample showed peaks at 4.60 and 5.5 min (8:1) (c.f. cholestane, 5 α-cholest-6-ene, and 5 β -cholest-6-ene at 5.5, 5.5 and 4.65 min; $C_{24,26,28}$ at 1.9, 3.45 and 6.05 min respectively).

(ii) In a Sealed Tube

The brosylate (70) (42mg) and urea (8mg) in trifluoroethanol (5ml) were sealed in a glass tube and kept at 105 ±5° for 40h. Isolation of the product as above gave a sample of the mobile component (4mg). G.l.c., 1% OV-1, 225° showed peaks at 2.5, 3.2 and 4.6 min (1:1.5:16). The peak at 4.6 min was identified as 5 β -cholest-6-ene by co-injection with an authentic sample and by comparison of their mass spectra obtained by g.c. - m.s.

7α -Deuteriobrosylate (73)

The sample of 7α -deuteriocholesterol (71) was converted to 4,5-seco-7α -deuterio-4-hydroxycholest-5-ene (72) as previously described. The alcohol (72) had τ 4.58 (2H,m, limbs at ± 10 and ± 20Hz, lowfield limbs further split by 5Hz), M^+ 389 (Calc. for $C_{27}H_{47}DO$: M, 389; >97% d_1 by comparison with unlabelled alcohol (32)), and was converted to the 7α -deuterio-brosylate (73) as above.

Solvolysis of the 7α -deuteriobrosylate (73)

The 7α -deuterio-brosylate (73) (30mg) and urea (8mg) in trifluoroethanol (3.5ml) were sealed in a glass tube and kept at 105 ±5° for 40h. Work up as before and chromatography gave the hydrocarbon fraction (3mg), which on g.l.c., 1% OV-1, 200°, was one peak identical by co-injection with 5β -cholest-6-ene. G.c. - m.s. gave M^+ 370 (100) other peaks at 355 (42), 301 (8), 274 (60), 257 (66), 247 (26), and 215 (66), isotopic ratio 370:371 = 79:39 (average of 5 scans),

∴ Relative size of d_1 contribution = 39 - 29.7% of 79 = 15.5

∴ Ratio d_o:d_1 = 79:15.5, ∴ d_1 = 16.5%

Authentic 5β -cholest-6-ene showed M^+ 370 (100), 355 (44), 301 (6), 274 (62), 257 (68), 247 (30), and 215 (62).

ROUTES TO THE 10α-METHYL ACETAL (80)

Cholesta 1,4-dienone (81)

Cholestenone (15g, 4mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (12g, 5.2mmol) were refluxed in dry benzene (600ml) for 20h. Filtration of the crude solution and evaporation of the solvent *in vacuo* afforded a dark red gum from which was obtained cholesta-1,4-dienone (12.5g, 83%), m.p. 114-115° (lit.,[53] 111-112°) by filtration of the gum through grade H alumina in petrol-benzene (1:4).

Ozonolysis of Cholesta-1,4-dienone

(i) In Acetic acid/Ethyl acetate

Ozone-enriched oxygen was bubbled through a solution of the dienone (81) (300mg) in acetic acid (3ml) and ethyl acetate (3ml) at 0° for 1h, at which point t.l.c. showed the absence of starting material. Excess ozone was discharged from solution by passage of nitrogen, and the mixture poured into aqueous sodium hydroxide containing 30% hydrogen peroxide (5 drops). After stirring thoroughly for 15 min the neutral products (150mg) were extracted into ether. Acidification of the remaining aqueous solution and reextraction into ether afforded acidic products (142mg). Preparative t.l.c. of the neutral components in ethyl acetate-petrol (3:17) gave a keto-aldehyde (86) (72mg) m.p. 110-112° with softening at 103° which showed ν_{max} 2710w, 1738s, 1705s, 1315w, 1075m, and 940m cm^{-1}, τ 0.53 (1H,s), 8.70 (3H,s), 9.09, 9.18, and 9.24 (methyl resonances), M^+ 360 (Calc. for $C_{24}H_{40}O_2$: M, 360) other peaks at 345, 342, 327,

275, 247, and 229. Methylation of the acidic fraction with ethereal diazomethane gave after preparative t.l.c. in ethyl acetate-petrol (1:3) a sample of a keto-ester (118) (22mg) as a gum which had ν_{max} 1735-1715s, 1265s, 1240sh, 1205m, 1185m, 1160s, 1100s, 1015m, and 900w cm^{-1}, τ 6.33 (3H,s), 8.12 (3H,s), 9.09, 9.18, and 9.36 (methyl resonances), M^+ 390 (Calc. for $C_{25}H_{42}O_3$: M, 390) other peaks at 372, 359, 331, 313, 303, 302, 285, 260, and 247. A further two attempts to reproduce the yields in this reaction failed, although small amounts of (86) and (118) could be detected by t.l.c. of the mixtures obtained.

Attempted Deformylation of the Keto-aldehyde (86)

The keto-aldehyde (86) (50mg) was refluxed in 5% methanolic potassium hydroxide for 3h. The product was isolated by extraction into ether and preparative t.l.c. in ethyl acetate-petrol (1:9) afforded a sample of Inhoffen Ketone (82) (6mg) as a gum which crystallised slowly on standing at 0° and which had m.p. 44-48° (lit.,[53] 52°), ν_{max} 1712s, 1275w, 1180m, 1150m, 950w and 910w cm^{-1}, τ 8.94, 9.08. 9.17, and 9.23 (methyl resonances). The remainder of the product consisted of more polar material which was not investigated.

Attempted Decarboxylation of the Keto-acid (87)

The crude acidic fraction from ozonolysis of the dienone (81) was refluxed in 10% methanolic potassium hydroxide for 4h, but this resulted in formation of only a small quantity of Inhoffen Ketone as judged by t.l.c..

Ozonolysis of the Dienone (81)

(ii) In Ethyl acetate at -78°

Ozonolysis of (81) (100mg) in ethyl acetate (1ml) at -78° for 5 min led to formation of a mobile component on t.l.c. and complete consumption of (81). Excess ozone was discharged by passage of nitrogen and the solvent evaporated *in vacuo* to leave a semi-solid mass. Preparative t.l.c. in ethyl acetate-petrol (1:4) gave the ozonide (89) (20mg) m.p. 114-118° which showed ν_{max} 3020w, 1805m, 1685s, 1615m, 1315m, 1270m, 1190m, 1110sh, 1100s, 980w, 955w, and 915m cm^{-1}, ν_{max} (cyclohexane) 780s, 760sh cm^{-1}, τ 4.06 (1H,s), 4.18 (1H,d,J2Hz), 4.69 (1H,s), and 8.85 (3H,s), M^+ (20ev) 430 (Calc. for $C_{27}H_{42}O_4$: M, 430) other peaks at 414, 402, 384, 369, 332, 302, 271, 247, and 245.

Decomposition of the Ozonide (89)

The oxonide (89), obtained as above from the dienone (81) (100mg) was not isolated but dissolved directly in acetic acid and refluxed for 30 min. The crude product, isolated by extraction into ether was purified by crystallisation from ether-ethyl acetate to give the unsaturated keto-acid (88) (38mg) as white crystals m.p. 203-206° (lit., 206-207°) and showed ν_{max} 3580w, 3500-2500br, 1720-1700s, 1110m, and 965s cm^{-1}, τ 3.32 (1H,d, J10Hz), 4.05 (1H,d, J10Hz), and 6.15br (1H,s, exchangeable with D_2O). The corresponding ester (90) was obtained by methylation of the acid (88) with ethereal diazomethane and after purification by preparative t.l.c., the gummy solid showed ν_{max} 3015w, 1715brs, 1630m, 1200s, 1170s, 1080w, 1000w, and 950w cm^{-1}, τ 3.81 (1H,d, J12Hz), 4.09 (1H,d, J12Hz), and 6.35 (3H,s).

Ozonolysis of the Ester (90)

The ester (90) (10mg) in ethyl acetate (1ml) at -78° was ozonised as described above for 1h. Analytical t.l.c. of the product obtained by refluxing the crude ozonide in acetic acid, followed by methylation, showed the presence of the ester (90) as the principal product with trace amounts of Inhoffen Ketone (82) and keto-ester (118).

Attempted Osmylation of the Unsaturated Keto-ester (90)

In a typical experiment the ester (90) (120mg) and osmium tetroxide (25mg) in tetrahydrofuran (5ml) were stirred during addition of a solution of sodium periodate (220mg) in water (1ml). After setting aside for 24h the ester (90) was recovered unchanged.

Attempted Oxidation of the Dienone (81) with Ruthenium Tetroxide

The dienone (300mg) in acetone (20ml) was added to a suspension of ruthenium dioxide (20mg) in acetone (20ml) followed by a solution of sodium periodate (700mg) in water (2ml). A further 1.5g of sodium periodate was added as a solution in the minimum volume of acetone-water (1:1) over a period of 4h. Methanol was added and the solution filtered and concentrated in vacuo before isolating the product by extraction into ether. The crude product, principally acidic material, was refluxed in acetic acid containing a trace of phosphoric acid for 2h and Inhoffen Ketone (82) (25mg) recovered by preparative t.l.c. of the neutral fraction produced.

Rearrangement of the Dienone (81)

A solution of the dienone (81) (2.5g) and concentrated sulphuric acid (1g) in acetic anhydride (70ml) was kept at 20° for 3h. The product was isolated by carefully pouring the solution into 45% aqueous potassium hydroxide solution followed by ether extraction to give the acetate (91), which was not purified but dissolved in 5% methanolic potassium hydroxide and stirred for 17h. Extraction into ether and recrystallisation from methanol afforded the phenol (94) (2.0g), m.p. 144-146° (lit.,[55] 145-146°) which showed ν_{max} 3620s, 3080w, 1585m, 1300s, 1270m, 1260m, 1220m, 1180m, 1155s, 1025m, 960w, 940w and 850w cm^{-1}, τ 3.15 (1H,d, J4Hz), 3.55 (1H,d, J4Hz), 5.50 (1H,s), and 7.88 (3H,s).

Methylation of the Phenol (94)

To the phenol (94) (2g) in 95% ethanol (60ml) was added cautiously 60% aqueous sodium hydroxide (7ml) followed by dimethylsulphate (10ml). A further three portions of sodium hydroxide solution and dimethylsulphate were added alternately. The solution was diluted with water and extracted into ether to give the methyl-ether (92)(1.8g) m.p. 104-106° (lit.,[73] 104-105°) after recrystallisation from methanol.

Reduction of the Methyl-Ether (92)

(i) A solution of the methyl-ether (1g) in dry tetrahydrofuran (70ml) was slowly added to redistilled liquid ammonia (70ml). Portions of lithium metal (270mg in total) were added and the resultant deep blue solution stirred for 1.5h. The reaction was quenched by careful addition of absolute ethanol and the ammonia allowed to evaporate. Ethereal extraction of the residue afforded unchanged methyl-ether (92).

(ii) To a solution of the methyl-ether (92) (1g) in anhydrous ethylamine (100ml) and t-amyl alcohol (12ml) was added lithium (1.1g) in portions over 15 min. The reaction mixture was stirred for a further 1.5h and then quenched and worked up as in (i) to give, after filtration of the crude product in petrol through grade H alumina, the olefin (93) (780mg) as a semi-crystalline solid which showed ν_{max} 2950s, 2880s, 1470s, and 1380s cm^{-1}, τ 9.09, 9.18, and 9.33 (methyl resonances), M^+ 370 (Calc. for $C_{27}H_{46}$: M, 370).

Reductive Elimination of the Dienone (81)

To a refluxing solution of biphenyl (660mg) and diphenylmethane (365mg) in dry tetrahydrofuran (4ml) under nitrogen was added a pellet of lithium (50mg). The solution was stirred for 15 min when the lithium dissolved to produce a dark green solution to which was added dropwise a solution of the dienone (81) (750mg) in dry tetrahydrofuran at a rate such that the green colour of the solution was maintained. The solution was refluxed for a further 30 min, cooled, and methanol (1.5ml) followed by water (2ml) cautiously added. The product was isolated by extraction into ether and the phenol (96) (700mg) obtained pure by filtration through a short column of grade H alumina. The phenol had m.p. 119-120° (lit.,[74] 113-114°) and showed ν_{max} 3620s, 3060w, 1610w, 1500m, 1280m, 1240m, 1175s, 1150m, 925w, and 915w cm^{-1}, τ 2.87 (1H,d, J9Hz), 3.40 (1H,d, J9Hz, lowfield limb further split by 2Hz), 3.46 (1H,s, superimposed on highfield limb of doublet), 4.66br (1H, exchangeable with D_2O) and 7.23br (2H).

Reduction of the Phenol (96)

A solution of the phenol (96) (150mg, 0.4mmol) in dry tetrahydrofuran (15ml) was added to redistilled liquid ammonia (30ml) and lithium (1.25g, to give a 4.5M solution) added in small portions. A two-phase system (bronze and blue) developed and stirring was continued for a further 1h when absolute ethanol (5ml) was cautiously added. After a further 1h sufficient absolute ethanol was added to quench the reaction and the product isolated as before. The sole product, the hydroxy-olefin (97)(130mg) was purified by preparative t.l.c. to give a gum which slowly solidified and showed ν_{max} 3630m, 1040s, and 955w cm^{-1}, τ 6.35br (1H,s) and 8.12br (~7H, allylic -CH-), M^+ 372 (Calc. for $C_{26}H_{44}O$: M, 372) other peaks at 370, 354, 339, 294, 241, and 215.

Attempted Methylenation of the Hydroxy-olefin (97)

Following the method of Ginsig and Cross[61,62], methylene iodide (1.35g, 5.02mmol) was added to freshly prepared zinc/copper couple (0.42g, 6.4mmol) in dry ether (4ml) and the mixture refluxed for 10 min. The hydroxy-olefin (97) (100mg, 0.27mmol) in dry ether (1ml) was added dropwise and the mixture refluxed for a further 30 min. The entire mixture was transferred to a thick-walled glass tube, distilled to half-volume, and a fresh portion of ether (1ml) added. The tube was sealed and maintained at 95° for 3h. Isolation of the product by extraction into ether gave a complex mixture on t.l.c.. Elution of a band corresponding to (97) on preparative t.l.c. was shown to be a mixture of two closely spaced components on reexamination by analytical t.l.c.. Separation of these components could not be

achieved by further t.l.c. but the mixture showed ν_{max} 3620m, 3050m, 1040s, and 940m cm^{-1}, τ 6.40br (1H,s), 9.42 (0.4H,d, J4Hz), and 9.71 (0.4H,d, J4Hz). A further six attempts to repeat the yields obtained by Ginsig and Cross failed to improve the product ratio obtained above.

Attempted o-Alkylation of the Hydroxy-olefin (97)

(i) The hydroxy-olefin (97)(1.1g, 3mmol) and sodium hydride (80mg, 3.3mmol) in dry ether (10ml) were stirred at 20° for 17h while ethylene oxide was allowed to bubble through the solution. No reaction was detectable by t.l.c..

(ii) The hydroxy-olefin (370mg, 1mmol) and sodium hydride (27mg. 1.1mmol) in dry dimethoxyethane (10ml) were refluxed for 1h. A solution of the dihydropyranyl ether of ethylene glycol monotosylate (320mg, 1.1mmole) in dry dimethoxyethane (10ml) was added dropwise over 5 min and the solution refluxed for 17h, and again no reaction ensued. A similar experiment at 20° also showed no products other than (97).

(iii) The hydroxy-olefin (40mg, 0.1mmol) and sodium hydride (10mg, 0.5mmol) in dry dimethoxyethane (3ml) were treated with β-propiolactone (10mg, 0.15mmol) at 20° for 1h. No simple new products were discernible but polymeric material was produced.

(iv) To a solution of the hydroxy-olefin (97) (1.1g, 3mmol) in toluene (12ml) at reflux was added triethylamine (2 drops) followed by dropwise addition of freshly distilled diketene (0.3ml) and the reaction maintained at reflux for 1.5h. Isolation of the crude

product by ether extraction gave an oily β-keto-ester (99) (1.4g) as the sole product ν_{max} (film) 1740s, 1720s, 1650m, 1325m, 1245s, 1160s, 1040s, 1000m, and 750m cm^{-1}. The crude β-keto-ester (99)(1.4g) was dissolved in ethanol-ether (1:1) (15ml) and added to a suspension of sodium borohydride (100mg) in ethanol (10ml). The mixture was stirred for 10 min, and the excess borohydride decomposed with dilute mineral acid before isolating the gummy hydroxy-ester (100) (1.25g) by extraction into ether. The hydroxy-ester showed ν_{max} (film) 3600 - 2600brs, 1730s, 1190s, 1085s, 1005s, 950m, and 740w cm^{-1}.

Attempted Simmons-Smith Methylenation of the Hydroxy-ester (100)

The hydroxy-ester (100) (130mg, 0.33mmol) was treated with zinc/copper couple (340mg, 5mmol) and methylene iodide (670mg, 2.5mmol) according to Rawson and Harrison[75]. After reflux for 17h no reaction had ensued and the hydroxy-ester was recovered.

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PART II

Reductions with Chloroaluminium Hydrides

INTRODUCTION AND DISCUSSION

Since its introduction in 1947[1] as a reducing agent for organic compounds, lithium aluminium hydride has proved to have widespread utility. Over the past twenty-six years various means have been sought to alter the selectivity and steriospecificity of the reagent. One of the most common has been by reaction with various alcohols or esters to produce lithium alkoxyaluminium hydrides of differing stoichiometries and these have found application in improving the sterioselectivity of ketone reductions and in syntheses of compounds which would normally react further with lithium aluminium hydride, as in the formation of aldehydes from acid chlorides[2] or from nitriles[3].

A particularly fruitful modification of the reagent has been achieved by reaction with group IIIb metal halides and in particular with boron trifluoride and aluminium trichloride. The former reaction provides a convenient means of generating diborane and the latter combination, of much synthetic utility, has found application in the hydrogenolysis of acetals, ethers, and allylic alcohols, and in the epimerisation of alcohols, and has been usefully employed in the present work to stereoselectively label allylic methylene groups with deuterium.

The nature of the reagent formed when lithium aluminium hydride is mixed with aluminium trichloride is still not completely resolved. Conductivity studies of ethereal solutions of lithium aluminium hydride and aluminium trichloride led Evans[4] to suggest the following species in solution:-

$$LiAlH_4 + 2AlCl_3 \longrightarrow LiCl + Al_2Cl_5^+ + AlH_4^-,$$

$$LiAlH_4 + Al_2Cl_5^+ + AlH_4^- \longrightarrow LiCl + 4AlH_2Cl,$$

$$LiAlH_4 + AlH_2Cl \longrightarrow LiCl + 2AlH_3.$$

The presence of dichloroaluminium hydride in solution was discounted by Evans but Ashby and Prather[5] have demonstrated aluminium hydride, dichloroaluminium hydride, and monochloroaluminium hydride in such solutions by infra-red analysis, and isolated their corresponding triethylamine complexes. Since lithium chloride does not precipitate from solution it is suggested that $LiAlHCl_3$ and $LiAlH_2Cl_2$ may also be present in solution, and their presence may in part explain its conductivity which in itself is very small. The stoichiometry of the reaction appears to be best defined as follows:-

$$LiAlH_4 + 3AlCl_3 \longrightarrow 4AlHCl_2 + LiCl = 3AlHCl_2 + LiAlHCl_3,$$

$$LiAlH_4 + AlCl_3 \longrightarrow 2AlH_2Cl + LiCl = AlH_2Cl + LiAlH_2Cl_2,$$

$$3LiAlH_4 + AlCl_3 \longrightarrow 4AlH_3 + LiCl.$$

The most commonly used ratio, $LiAlH_4$: $AlCl_3$ = 1:4, has been shown[5] to result in production of dichloroaluminium hydride and excess aluminium chloride remains in solution, viz.

$$LiAlH_4 + 4AlCl_3 \longrightarrow 3AlHCl_2 + LiAlHCl_3 + AlCl_3 \quad \ldots\ldots \quad (1)$$

The effect of adding a Lewis acid to lithium aluminium hydride is twofold,

(i) the nucleophilicity of the reagent is reduced and correspondingly its electrophilic properties are enhanced, and

(ii) the overall direct reducing power is diminished.

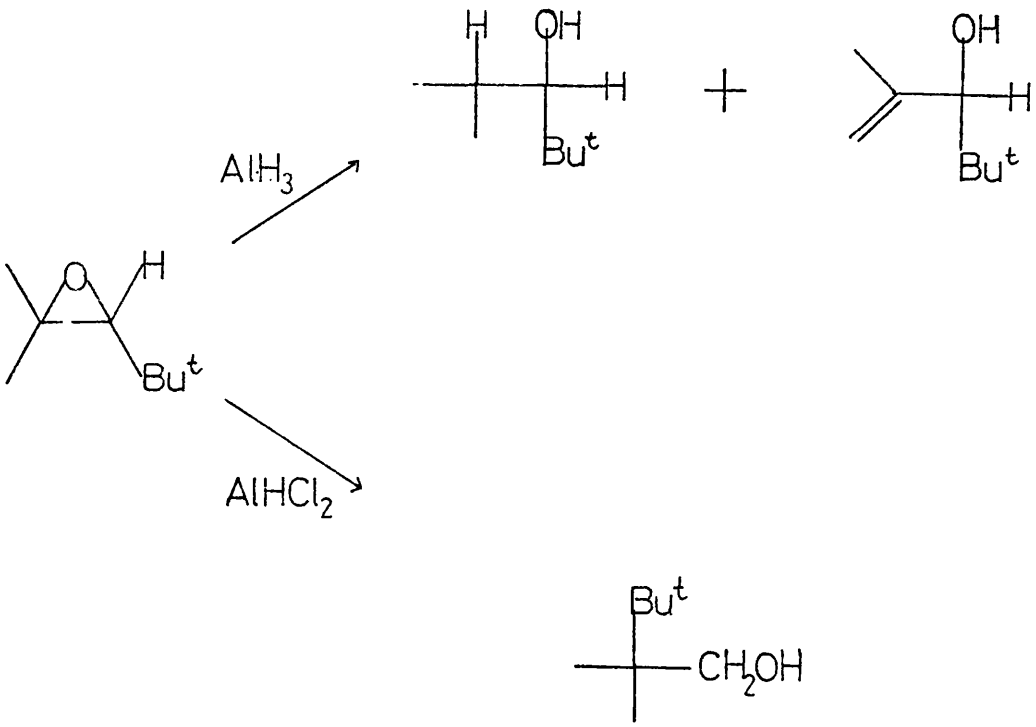
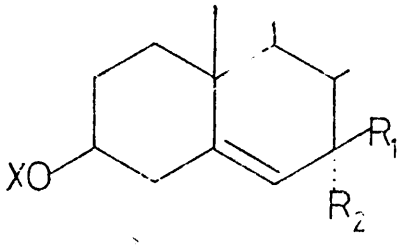
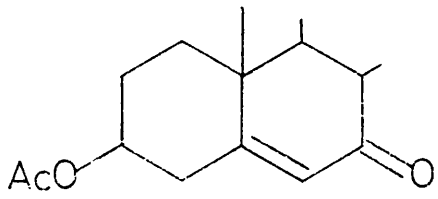


Fig.1

On the basis of elegant work on epoxide opening with differing 'mixed hydride' reagents[6], and by using lithium aluminium deuteride in combination with aluminium trichloride, the products arising from these reactions have been rationalised in terms of the properties of the 'mixed hydride' used. A marked contrast is evident in the properties of aluminium hydride, favouring direct reduction, or opening to the allylic alcohol, and dichloroaluminium hydride giving largely rearranged products via carbonium ion intermediates, Figure (1). Participation by $LiAlHCl_3$ in the reduction is shown by comparing product ratios obtained using $LiAlH_4$: $AlCl_3$ = 1:3 and pure dichloroaluminium hydride; this shows that $LiAlHCl_3$ is a better hydride donor than dichloroaluminium hydride and that it gives more direct reduction at the expense of rearrangement.

The present studies on the hydrogenolysis of allylic alcohols stemmed from the requirement to stereoselectively label the 7 α - or 7 β - positions in cholesterol with either deuterium or tritium. Two methods for achieving this have already been mentioned[7], but our attention was drawn to the possibility of using chloroaluminium hydrides by our previous use of the reagent in the reduction of cholestenone to cholest-4-ene. It seemed likely that this reduction proceeded through the allylic alcohol, or a complexed form thereof, and since it is well known that in displacement reactions at C-7 in steroids the incoming group approaches selectively from the α -face because of steric hindrance to β -approach, the chances that a steriospecific introduction of hydride or deuteride could be achieved seemed good. These assumptions received support from a previous extensive study by Brewster and Bayer[8] on the hydrogenolysis of enones and allylic alcohols.



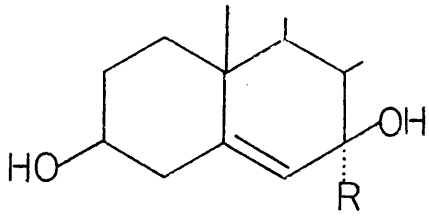


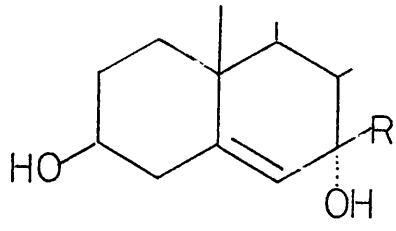
(1) $R_1 = R_2 = X = H$.

(2) $R_1 = R_2 = H$: $X = Ac$.

(3) $R_1 = R_2 = D$: $X = H$.

(6)



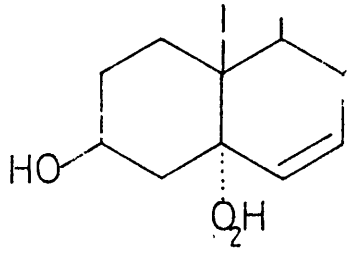


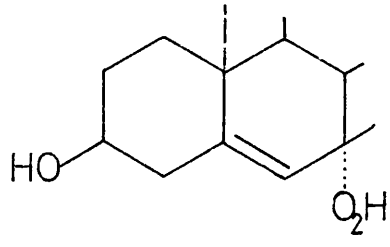
(7) R=H

(8) R=D

(9) R=H

(10) R=D





(11)

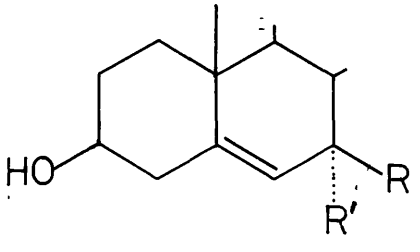
(12)

Synthesis of 7β -hydroxycholesterol (7) from cholesterol (1) was accomplished in high yield as follows; (a) allylic oxidation of the acetate (2) with anhydrous sodium chromate in acetic acid and acetic anhydride[9]; (b) carefully controlled reduction of the resulting 7-oxocholesteryl acetate (6) using lithium aluminium hydride. Similar reduction of (6) with lithium aluminium deuteride afforded 7α -deuterio - 7β -hydroxycholesterol (8). The corresponding 7α -hydroxycholesterol (9) was produced by photooxygenation of cholesterol to give in the first instance 5α -hydroperoxy-3β -hydroxycholest-6-ene (11), which when stirred in chloroform rearranged to give 7α -hydroperoxycholesterol (12) and this was reduced to 7α -hydroxycholesterol (9) with sodium borohydride. A sample of 7β -deuterio-7α -hydroxycholesterol (10) was produced in a like manner by photooxygenation of 7,7-dideuteriocholesterol (3).

Hydrogenolysis of the allylic alcohols was performed in a standard manner. Dichloroaluminium hydride was prepared at 0° under a nitrogen atmosphere by adding a suspension of lithium aluminium hydride in ether to a solution of anhydrous aluminium chloride in ether. After 15 min the allylic alcohol was introduced dropwise as an ethereal solution; reduction was essentially complete after 5 min but stirring was usually continued for 30 min, when the mixture was quenched with water and the product isolated. In all cases a ratio of four parts aluminium chloride to one part lithium aluminium hydride was used which will generate dichloroaluminium hydride as shown in equation (1), and this reagent was used in four-fold excess in reduction of the alcohols since it has been shown[8] that this improved both the rate and yield of hydrogenolysis.

TABLE (1)

Substrate	Reducing Agent	Products %				Products
		4	5	7	9	
7	$AlDCl_2$	97	3			Cholesterol (A)
8	$AlHCl_2$	17.5	82.5			Cholesterol (B)
9	$AlDCl_2$	91	9			Cholesterol (C)
10	$AlHCl_2$	12	88			Cholesterol (D)
6	$LiAlH_4$			78	22	



(4) R=H R′=D

(5) R=D R′=H

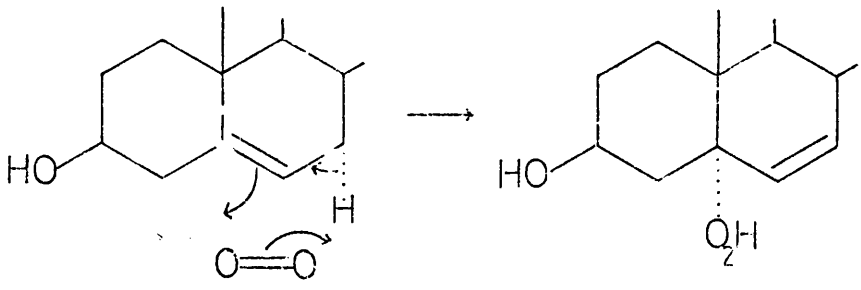
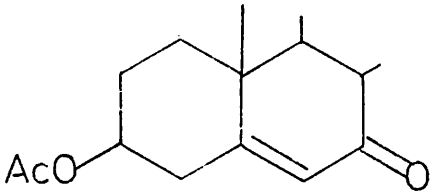
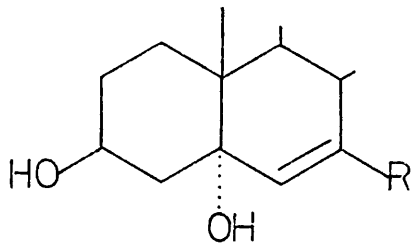


Fig.2

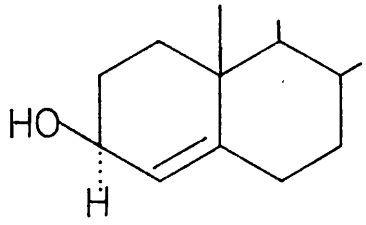


(6)



(13) R=H

(14) R=D



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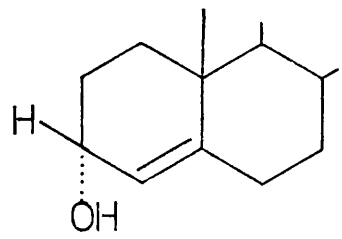

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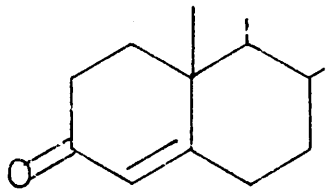
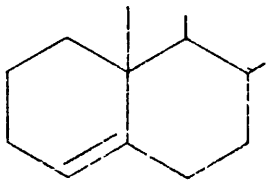


(17)

To establish the stereochemical result of hydrogenolysis a reliable means of estimating the amounts of α - and β - deuterium at the allylic position was required. While the n.m.r. of the products gave a useful indication from the structure of the olefinic proton at 4.65 τ (singlet for 7 β - deuteriocholesterol, doublet for 7 α - deuteriocholesterol), this could not provide the quantitative estimate required for the project in hand[7]. Photooxygenation, however, is known[10] to react stereospecifically on cholesterol by abstraction of the 7 α - proton, Figure (2), and so by treating the 7-deuterated cholesterols in this way and by obtaining the mass spectrum of the resultant 3 β , 5 α - dihydroxycholest-6-ene (13) a reliable and accurate analytical procedure was established.

The results obtained, shown in Table (1), indicate that the reduction is usefully stereoselective with hydride or deuteride entering predominantly from the α -face. For comparison, the reduction of 7 -oxocholesteryl acetate (6) with lithium aluminium hydride at 0° was performed and the proportions of 7 α - and 7 β - hydroxycholesterol in the mixture measured by integration of the relevant n.m.r. peaks. Having established the selectivity obtainable in a sterically hindered situation, it seemed relevant to examine the reaction of a less hindered allylic alcohol. The most accessible system satisfying this condition appeared to be the 3 α - and 3 β - hydroxycholest-4-enes in which, moreover, the ring incorporating the alcohol is conformationally more mobile.

Synthesis of 3 β - hydroxycholest-4-ene (15) and 3 α - hydroxycholest-4-ene (16) was readily accomplished by reduction of cholestenone (17) with lithium aluminium hydride, and separation of the resulting alcohols by acetylation of the mixture and crystallisation



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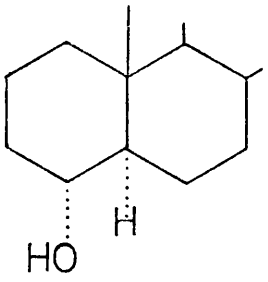

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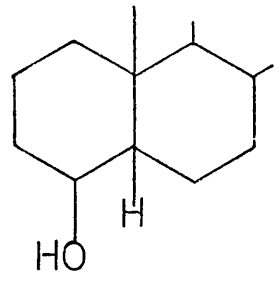


(22)

TABLE (2)

4β -Hydroxy-5β -cholestane (22) + 0.4 equivalents of $Eu(dpm)_3$

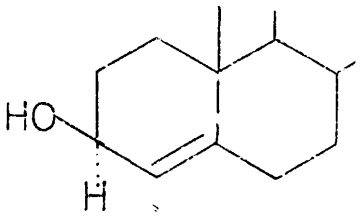
4β -Hydroxy-5β -cholestane (22) + 0.4 equivalents of $Eu(dpm)_3$

Peak	Position*	Multiplicity	Effect of Decoupling at Peak	Assignment
1	1530	br s	(4) becomes s, (3) sharpens	4α
2	1010	d, J13Hz	(5) becomes d, J 12Hz	6α
3	910	br m, (2H)	(1) sharpens	3α+ 3β
4	890	d, J13Hz	(1) sharpens	5β
5	500	t, J13Hz	(2) becomes s	6β
6	280	s, (3H)		C-10 Methyl

* In p.p.m. downfield from T.M.S.

of 3β -acetoxycholest-4-ene, followed by preparative t.l.c. of the material from mother liquors to obtain the 3α - isomer. The alcohols were regenerated from their acetates by reduction with lithium aluminium hydride. Deuterolysis of these substrates was effected as before to produce 3-deuteriocholest-4-enes. Location of the introduced deuterium by photooxygenation was not possible in this case since it has been shown[11] that the process is not specific for the 3α - proton, and that abstraction of the 3β - proton occurs because of the more flexible ring A.

Accordingly cholest-4-ene (18) was hydroborated and oxidised to give a mixture of 4α -hydroxy-5α -cholestane (21) and 4β -hydroxy-5β -cholestane (22) which were separable by preparative t.l.c.. These compounds were then analysed by n.m.r. using lanthanide shift reagents, in the hope that the resonances due to the 3α - and 3β - protons might be distinct and identifiable. By similar examination of the deuterated samples the incorporation of deuterium in both the 3α - and 3β - positions might then be determined. Use of 0.4 equivalents of tris - [dipivaloylmethanato] - europium (III), Eu $(dpm)_3$, with 4β -hydroxy-5β -cholestane (22) in deuteriochloroform shifted the 4α - proton signal to lowest field (excepting the hydroxyl proton) followed by the 6α, 3α and 3β , 5β and 6β signals. These assignments were made on the basis of decoupling experiments details of which are recorded in Table (2). Unfortunately the 3α - and 3β - resonances did not separate at this concentration of shift reagent and higher concentrations could not be used because of its limited solubility. Similar difficulties were met in the analysis of 4α -hydroxy-5α -cholestane (21), so the more soluble shift reagent, tris - [hexafluoro-octanedionato] - europium (III), Eu$(fod)_3$, was employed. Using 0.45 equivalents of Eu$(fod)_3$ the 100MHz spectrum of



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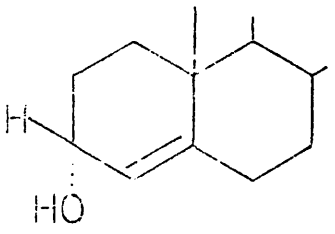

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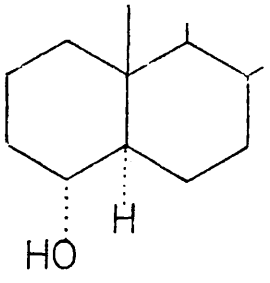

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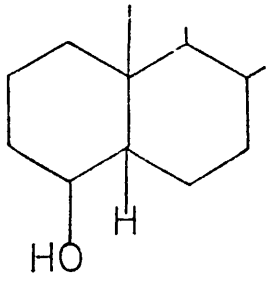

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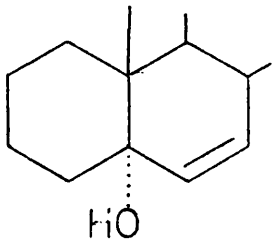


(23)

TABLE (3)

4β -Hydroxy-5β.-cholestane (22) + C.45 equivalents of $Eu(fod)_3$

Peak	Position*	Multiplicity	Effect of Decoupling at Peak	Assignment
1	1518	br s	(5) becomes s, (3) and (4) sharpen.	4α
2	939	d, J14Hz	(6) becomes d, J14Hz.	6α
3	912	d, J12Hz	(1) sharpens.	3α
4	868	t, J11Hz	(1) sharpens.	3β
5	806	d, J9Hz	(1) sharpens.	5β
6	486	t, J14Hz	(2) becomes s.	6β
7	430	q,	(8) becomes s.	7α?
8	290	d, J10Hz		7β?
9	266	s, (3H)		C-10 Methyl

* In p.p.m. downfield from T.M.S.

TABLE (4)

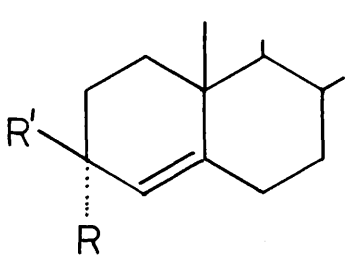
4 α -Hydroxy, 5 α -cholestane(21) + 0.8 equivalents of $Eu(fod)_3$

Peak	Position*	Multiplicity	Effect of Decoupling at Peak	Assignment
1	> 2000		(5) becomes d J10Hz, (3) and (4) sharpen.	4 β
2	1352	d	(6) becomes t, (5) sharpens.	6 α
3	1262	br m		3 α
4	1198	d,		3 β
5	1174	t,	(6) becomes t.	5 α
6	684	q		6 β

* In p.p.m. downfield from T.M.S.

TABLE (5)

Substrate	Reducing Agent	Products % 19	20	15	16	Products	Reference
15	$AlDCl_2$	49	48			Cholest-4-ene(E)	This work
15	$AlDCl_2$	65	35				12
15	AlD_2Cl	35	65				12
16	$AlDCl_2$	12	84			Cholest-4-ene(F)	This work
16	$AlDCl_2$	15	85				12
16	AlD_2Cl	15	85				12
17	$LiAlH_4$			89	11		This work



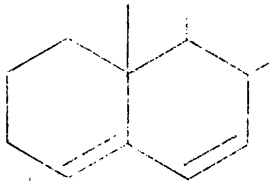
(19) R = H R' = D

(20) R = D R' = H

4 β -hydroxy-5 β -cholestane (22) was analysed and resonances assigned to specific proton configurations on the basis of chemical shift, coupling constants and decoupling experiments. At this concentration the 3 α - and 3 β - protons were separated and identifiable, and results are listed in Table (3). Analysis of 4 α -hydroxy-5 α -cholestane (21) required the use of 0.8 equivalents of $Eu(fod)_3$ before assignments could be made and all the relevant peaks resolved; Table (4) contains the details.

Having established this technique, the 3-deuteriocholest-4-enes were hydroborated and europium-shifted 100MHz n.m.r. spectra of the two alcohols from each sample were analysed. Repeated integration over the resonances corresponding to the 3 α - and 3 β - protons and internal standards, usually the 5 α - or 5 β - proton, provided a reliable estimate of the deuterium incorporation in each position. A summary of the results obtained from deuterolysis of both 3-hydroxycholest-4-enes along with those obtained by other workers[12] after the completion of this work is given in Table (5). For comparison cholestenone was reduced with lithium aluminium hydride and the proportions of the two alcohols (15) and (16) in the mixture measured by integration over the relevant n.m.r. peaks.

Carbonium ions have been invoked[8] to explain the products from hydrogenolysis of allylic alcohols. Both 7-hydroxycholesterol and 3-hydroxycholest-4-ene give only products in which the double bond has not migrated (see above), but it seemed probable that hydrogenolysis of 5 α -hydroxycholest-6-ene (23) might result in formation of cholest-5-ene by allylic rearrangement of the initially formed carbonium ion. To determine the stereochemistry of hydride addition at C-7, deuterolysis of 5 α -hydroxycholest-6-ene (23) was performed under standard conditions, however dehydration occurred



(24)

to give cholesta-4,6-diene (24) as the only product.

The mechanism of hydrogenolysis of allylic alcohols has received some attention[8] and it is postulated that the first step is formation of a chloroalkoxyaluminium compound thus:-

$$R - OH + AlHCl_2 \longrightarrow ROAlCl_2 + H_2 \quad \ldots\ (2)$$

which occurs rapidly on mixing of the reagents. On the basis of a study of benzylic, allylic, and saturated alcohols the next step is presumed to be the slower formation of a carbonium ion by alkyl-oxygen fission. It has been demonstrated[8] that the rate of reaction decreases with dilution and hence the slow step is not a simple heterolysis but may involve additional chloroaluminium hydride, or aluminium chloride further complexing with the oxygen atom:-

$$ROAlCl_2 + AlCl_3 \longrightarrow \begin{array}{ccccc} Cl & & Cl & & Cl \\ & \diagdown & & \diagup & \\ & & Al & & \\ & \diagup & & \diagdown & \\ Cl & & O & & Cl \\ & & | & & \\ & & R & & \end{array} \longrightarrow R^+ + Al_2Cl_5O^- \quad \ldots\ (3)$$

In their study of the hydrogenolysis of 3-hydroxycholest-4-enes, Romeo et al.[12] assert that the reduction of the 3α - and 3β - alcohols cannot both involve an S_N1 mechanism since, contrary to observation, both compounds would then be expected to give the same carbonium ion, and they conclude that the 3α - alcohol is reduced by an S_N1 mechanism, but that reduction of the 3β - alcohol proceeds only partly by such a mechanism. No alternative mechanism is suggested.

Such an explanation of the results is less than satisfactory and no rationalisation of the observations was attempted in terms of

the previously demonstrated reactivity of the chloroaluminium hydrides. The allylic alcohols examined may be divided into two classes, those (1) with a quasi-axial hydroxyl group, and those (2) with a quasi-equatorial hydroxyl group. In class (1) the result of the reaction is seen to be one of predominant retention of configuration at the allylic position with either little or no variation in the product ratios on changing from formally dichloroaluminium hydride to monochloroaluminium hydride. The results with quasi-equatorial alcohols, class (2), may not seem at first sight to show any obvious consistencies, but a rationalisation of the results is possible as follows.

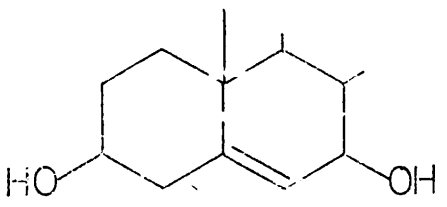
It is assumed that the first step in all these reactions is the rapid formation of an alkoxyaluminium chloride according to equation (2). The rate of the next proposed step, equation (3), the heterolysis to give a carbonium ion, will depend on two factors; (i) the electrophilicity of the aluminium group attached to the oxygen atom will affect the rate of reaction, dichloroaluminium hydride being more efficient than monochloroaluminium hydride; (ii) the orientation of the carbon-oxygen bond with respect to the double bond should be important; it is anticipated that the most favoured orientation for fission is that in which the bond to be broken is axial and in the plane of the π - system since in this orientation maximum bonding is achieved. Thus one would predict that, other things being equal, the quasi-axial alcohol should cleave more readily than the quasi-equatorial.

The next feature to be considered in the overall process is the nucleophilicity of the hydride donor, and in this respect monochloroaluminium hydride is more effective than dichloroaluminium hydride. Also, since lithium chloride remains in solution, species such as $LiAlHCl_3$ and $LiAlH_2Cl_2$ should also be considered as reacting entities although they will be present at only about one third of the concentration of the

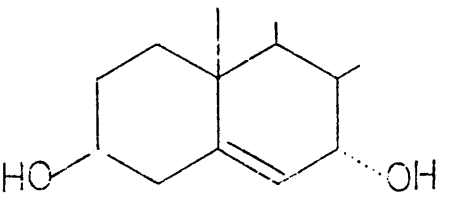
Fig. 3

uncomplexed forms. A very important factor in these reductions is the geometry of the transient carbonium ion[13] and its stereochemical surroundings. π- Assisted fission of quasi-axial C-O bonds ($\sim 25^{\circ}$ out of plane with the π- system) requires little change in the geometry of the ring so that the predominant features controlling the approach of hydride to the resulting carbonium ion will be torsional strain involving partial bonds in the transition state[14]. If one considers approach of a hydride donor perpendicular and from the top face to such carbonium ions, then in both the case of the 3 α - and 7 α - alcohols there is an axial hydrogen on the β -face adjacent to the carbonium ion (at C-2 and C-8 respectively), in addition to the bulk of the 10 β - methyl group. No such direct 1,2-interactions exist for approach from below and as a consequence the predominant course is one of α -attack.

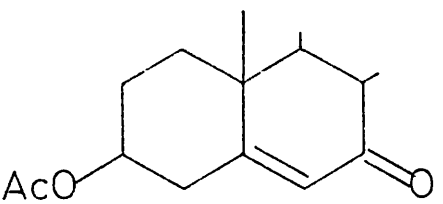
If the heterolysis of the quasi-equatorial C-O bonds ($\sim 52^{\circ}$ out of plane with the π- system) prefers to occur when they are most nearly eclipsed with the π- system, then considerable rotation about the adjacent C-C bonds is necessary, Figure (3). The consequence of this will be that where such rotation can occur, the initially formed carbonium ion will differ markedly in conformation at the adjacent methylene group from that which results from an axial alcohol. From examination of models it is evident that the major torsional interaction of the adjacent axial C-H bond is reduced and so more β -attack of hydride might be expected. This is indeed observed in the hydrogenolysis of the 3 β - alcohol where ring A still retains some flexibility and the proportion of product arising from β -attack is seen to increase on using a more nucleophilic hydride donor, monochloroaluminium hydride, which would be expected to capture the transient carbonium ion more rapidly before equilibration occurs.



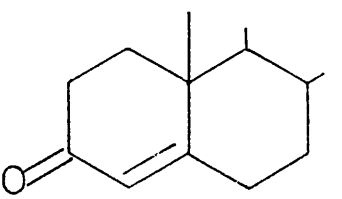
(7)



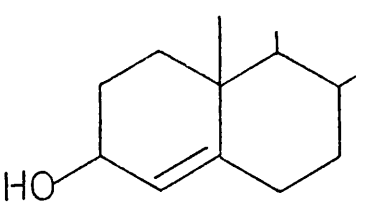
(9)



(6)



(17)



(15)

Hydrogenolysis of 7β -hydroxycholesterol (7) gives however substantially the same products as its 7α - isomer (9). The reason for this lies in the fact that the necessary deformation of ring B cannot be obtained due to its rigidity, although a twisting is possible such that the 7β - oxygen will depart in a more nearly axial orientation, it will be the π- system and ring A which will move rather than the rigid B/C ring fusion. Thus the hindrance of the 8β - axial hydrogen is not diminished and the same product ratio is obtained from both the 7α - and 7β - hydroxycholesterols. It is evident from the results listed, that the stereochemical course of hydride reduction of the corresponding enones, (6) and (17), mirrors very closely that of hydrogenolysis of the quasi-axial alcohols. This is only to be expected since the major torsional interactions are similar for both the enone system and the allylic carbonium ion produced from the - alcohols and such differences as do exist in product ratios may well reflect the change in the nature and size of the hydride donor, and in the centre being attacked.

From the Tables it is evident also that there is some discrepancy in the ratio of products obtained by us and the Italian group from hydrogenolysis of the 3β - alcohol (15). The lack of experimental details available for the work of Romeo[12] precludes an exact comparison, but no mention is made of the temperature at which reduction was performed. However, it seems possible that if the reaction was carried out at room temperature rather than at 0° then in the presence of the relatively poor nucleophile, dichloroaluminium hydride, more equilibration of the carbonium ion will have occurred prior to capture, thus resulting in more α -attack as observed.

Further studies in related systems will be necessary to fully test the proposals advanced here in explanation of the experimental observations.

EXPERIMENTAL

For general experimental directions see Part I.

Cholesteryl Acetate (2)

Cholesterol (10g), purified via the dibromide[15] was dissolved in dry pyridine (100ml) and treated with excess acetic anhydride. The resulting solution was stirred at 60° for 2h, allowed to cool, and poured onto crushed ice. The crude acetate was isolated by filtration and recrystallised from methanol to yield 9.4g and had m.p. 111-113° (lit.,[16] 111-116°).

7-Oxocholesteryl Acetate (6)

To a solution of cholesteryl acetate (2) (9g, 21mmol) in acetic acid (70ml) and acetic anhydride (30ml) stirred at 60° under nitrogen was added anhydrous sodium chromate[9] (7.3g, 45mmol). The mixture was stirred for 20h, cooled, and cautiously poured on to iced water (600 ml) and stirred vigorously to induce crystallisation. The resulting mixture was filtered through glass fibre paper and the crude product washed with a little cold acetic acid. The pale green solid, recrystallised from hot acetic acid yielded 7-oxocholesteryl acetate (7.1g, 76%) as a white crystalline solid m.p. 159-160° (lit.,[17] 158-159°)

Reduction of 7-oxocholesteryl acetate (6)

(a) At 0°

Normal lithium aluminium hydride reduction as previously described[18] gave a white amorphous solid shown by t.l.c. to consist of

one major product, and just separated from it but more polar, a much smaller amount of a second component. Preparative t.l.c. in ethyl acetate-petrol (1:1) yielded a pure sample of both components. The major component, 7β -hydroxycholesterol (7) had m.p. 172-174° (lit.,[19,20] 172-176°), ν_{max} 3615, and 1040 cm^{-1}, τ 4.74br(1H,s), 6.20(1H,d,J6Hz), and 6.40(1H,m), M^+402 (Calc. for $C_{27}H_{46}O_2$: M,402). The minor component, 7α -hydroxycholesterol (9) had m.p. 181-183° (lit.,[19,20] 183-184°), ν_{max} 3615, 1050 and 935 cm^{-1}, τ 4.42 (1H,d,J6Hz), 6.16 (1H,d,J6Hz), and 6.44(1H,m). M^+402 (Calc. for $C_{27}H_{46}O_2$: M,402).

The 100MHz n.m.r. spectrum of the original reduction mixture made it possible to estimate the proportions of (7) and (9). Expansion (X20) and repeated integration over the region 4.35-4.80 τ indicated the presence of 7β -hydroxycholesterol (7)(78%) and 7α -hydroxycholesterol (9)(22%).

(b) <u>At -20°</u>

To a stirred suspension of lithium aluminium hydride (0.85g, 22.5mmol) in dry ether (200ml) under nitrogen and cooled to -20°, was added a solution of the keto-acetate (6)(10g, 22.5mmol) in dry ether (200ml) over a period of 30 min. The mixture was stirred at -20° for a further 15 min and then allowed to warm to room temperature and stirred for a further 30 min. Usual work up gave 7β -hydroxycholesterol(7)(8.1g, 90%), which was purified by one recrystallisation from methanol.

(c) <u>At -20° with lithium aluminium deuteride</u>

Reduction of the keto-acetate (6) using lithium aluminium deuteride as in (6) above afforded 7α -deuterio-7β -hydroxycholesterol (8), ν_{max} 3515, 2120, and 1040 cm^{-1}, τ 4.72(1H,s) and 6.40(2H,m), M^+403

(Calc. for $C_{27}H_{45}DO_2$: M,403), isotopic ratios,
402:403:404:405 = 28.5:100:30.9:7,(Calc. for $C_{27}H_{45}DO_2$,
403:404:405 = 100:29.7:2).

Reductions with Dichloroaluminium Hydride, General Procedure

Anhydrous aluminium chloride (8mmol) was carefully added to dry ether (40ml) stirred at 0° under nitrogen. A suspension of lithium aluminium hydride (2mmol) in dry ether was carefully added and the resulting mixture allowed to stir for 10 min. To this was added a solution of the allylic alcohol (1mmol) in dry ether and stirring continued for 15 min, when the reaction was carefully quenched by dropwise addition of water and the products isolated in the usual manner. This procedure was applied in all hydrogenolysis reactions with minor variations in stoichiometries and substitution of lithium aluminium hydride by lithium aluminium deuteride as required. Yields were generally in the range 75-90%.

Deuterolysis of 7β -hydroxycholesterol (7)

On reduction with dichloroaluminium deuteride, 7β -hydroxycholesterol gave a deuterated cholesterol (A) with ν_{max} 3625, 3013, 2120, 2098, 1048, and 948 cm^{-1}, τ 4.65(1H,d,J5Hz) and 6.48(1H,m), M^+387 (Calc. for $C_{27}H_{45}DO$:M, 387), isotopic ratios, 386:387:388:389 = 7:100:31:4 (Calc. for $C_{27}H_{45}DO$, 387:388:389 = 100:29.7:2), other peaks at 372, 369, 354, 302, 275, 274, 256, 247, 232, 214, and 200.

Hydrogenolysis of 7α -deuterio-7β -hydroxycholesterol(8)

Reduction of (8) with dichloroaluminium hydride gave a deuterated cholesterol (B) with ν_{max} 3621, 3027, 2156, 2144, 1047 and 949 cm^{-1}, τ 4.67(1H,s) and 6.50(1H,m), M^+387 (Calc. for $C_{27}H_{45}DO$:M, 387),

isotopic ratios, 366:387:388:389 = 8:100:30:4 (Calc. for $C_{27}H_{45}DO$, 387:388:389 = 100:29.7:2), other peaks at 372, 369, 354, 302, 275, 274, 256, 247, 232, 214, and 200.

3β, 5α -Dihydroxycholest-6-ene(13)[21]

Cholesterol (300mg, 0.8mmol) and hematoporphyrin (2mg) were dissolved in dry pyridine (3ml) and oxygen was bubbled through the clear red solution while it was illuminated using a 60W desk lamp. The reaction was terminated after 6h and the hydroperoxide (11) formed, reduced to the corresponding alcohol by pouring the pyridine solution into a suspension of sodium borohydride in methanol, stirring for 15 min and then working up as usual to give a mixture of cholesterol and (13). The latter component, constituting 75% of the mixture, was isolated by preparative t.l.c. with ethyl acetate-petrol (2:3) and had m.p. 180-181° (lit.,[21] 181°), ν_{max} 3622, 3015, 1635, 1037, 1018, 950, and 862 cm^{-1}, τ 4.40(2H,s) and 5.90(1H,m), M^+402 (Calc. for $C_{27}H_{46}O_2$:M, 402), isotopic ratios, 401:402:403:404 = 15.5:100:31:8 and 384:385:386 = 100:32:6.5, other peaks at 384, 366, 355, 301, 247, and 213. These isotopic ratios were used in calculating the deuterium content of labelled 3β,5α -dihydroxycholest-6-enes, since by obtaining the mass spectra under standard conditions due allowance can be made for the $(M-1)^+$ ion.

Photooxygenation of Cholesterol (A)

By the above procedure, cholesterol (A) yielded on photooxygenation and purification a 3β,5α -dihydroxycholest-6-ene with ν_{max} 3622, 3015, 1635, 1037, 1019, 949 and 862 cm^{-1}, τ 4.40(2H,s) and 5.90(1H,m), M^+402, other peaks at 384, 366, 351, 301, 247 and 213,

isotopic ratios 402:403:404 = 100:52:13.5 (Unlabelled 401:402:403:404 = 15.5:100:31:14), ∴ contribution from d_1 species in 403 = 21, but this will give an $(M-1)^+$ of relative size 3.5, and thus adjusted ratios $d_0:d_1$ = 96.5:21, and hence deuterium content = 17.5% d_1.

Photooxygenation of Cholesterol (B)

Photooxygenation of cholesterol (B) gave a 3β,5α-dihydroxycholesterol which showed ν_{max} 3621, 3015, 2233, 1625, 1036, 1028, 952 and 865 cm^{-1}, τ 4.42(1H,d,J2Hz) and 5.88(1H,m), M^+403 other peaks at 385, 384, 367, 352 and 247, isotopic ratios 402:403:404:405 = 19:100:30.5:6, and a similar calculation gives a deuterium content = 97% d_1

7α-Hydroxycholesterol (9)

Photooxygenation of cholesterol (1g) was performed as previously described for 24h. The red solution was then poured on to iced water and the product isolated by filtration. The crude solid was then taken up in ether and washed with cold dilute hydrochloric acid (3x5ml), water (1x5ml), dilute sodium bicarbonate (1x5ml) and brine, dried, and the ether replaced by chloroform. This solution was stirred at 20° for 24h, and the hydroperoxide (12) reduced with sodium borohydride to give (9). Recrystallisation from methanol gave material m.p. 182-183°, (0.46g, 45%).

Deuterolysis of the Keto-acetate (6)

Reduction of (6) was performed in the standard way using 6 equivalents of dichloroaluminium deuteride per mole of substrate. The product, 7,7-dideuteriocholesterol (3) was purified by crystallisation from ether-methanol and had ν_{max} 3620, 2170. 2080, 1655, 1040. and 945 cm^{-1}, τ 4.67(1H,s) and 6.48(1H,m), M^+388, other peaks at

373, 370, 355, 303, 276, 275, 257, 247, 232, 214 and 200, isotopic ratios 388:389:390 = 100:29:5 (Calc. for $C_{27}H_{44}D_2O$:M, 388).

7-Deuterio-3β,5α-dihydroxycholest-6-ene (14)

This was prepared by photooxygenation of (3) as above and showed ν_{max} 3620, 3015, 2230, 1622, 1035, 1025, 950, and 862 cm^{-1}, τ 4.42(1H,d,J2Hz), and 5.90(1H,m), M^+ 403, other peaks at 385, 384, 370, 369, 367, 352 and 247 (Calc. for $C_{27}H_{45}DO_2$:M, 403)

7β-Deuterio-7α-hydroxycholesterol (10)

This was prepared from (3) following the procedure used in the synthesis of (9) above, and had ν_{max} 3620, 2130, 1655, 1115, 1052, and 1040 cm^{-1}, τ 4.40(1H,s) and 6.45(1H,m), M^+ 403, other peaks at 386, 385, 367, 352, 254, 247, and 212 (Calc. for $C_{27}H_{45}DO_2$:M, 403).

Deuterolysis of 7α-hydroxycholesterol (9)

A deuterated cholesterol (C) was obtained on treatment of (9) with dichloroaluminium deuteride under standard conditions and showed ν_{max} 3622, 2110, 2090, 1660, 1110, 1045, 1020, and 945 cm^{-1}, τ 4.63(1H,d,J6Hz) and 6.45(1H,m), M^+ 387, other peaks at 372, 369, 354, 302, 275, 274, 256, 247, 232, 214 and 200, isotopic ratios 387:388:389 = 100:29:5 (Calc. for $C_{27}H_{45}DO$:M, 387).

Hydrogenolysis of 7β-deuterio-7α-hydroxycholesterol (10)

Standard reduction of (10) with dichloroaluminium hydride gave a deuterated cholesterol (D) which showed ν_{max} 3622, 2150, 2137, 1660, 1110, 1045, 1035, and 943 cm^{-1}, τ 4.63(1H,s) and 6.45(1H,m), M^+ 387, other peaks at 372, 369, 354, 302, 275, 274, 256, 247, 232, 214 and 200,

isotopic ratios 387:388:389 = 100:30.5:5 (Calc. for $C_{27}H_{45}DO$:M, 387).

Photooxygenation of Cholesterol (C)

Standard photooxygenation of cholesterol (C) afforded a 3β,5α-dihydroxycholest-6-ene which showed ν_{max} 3622, 3015, 1635, 1037, 1018, 950, and 862 cm^{-1}, τ 4.40(2H,s), and 5.90(1H,m), M^+ 402, other peaks at 384, 366, 351, 301, 247, and 213, isotopic ratios 384:385:386 = 100:42:10 and calculation gives a deuterium content = 9% d_1.

Photooxygenation of Cholesterol (D)

Analytical data for the 3β,5α-dihydroxycholest-6-ene isolated from standard photooxygenation of cholesterol (D) are ν_{max} 3618, 3015, 2230, 1622, 1035, 1023, 947, and 861 cm^{-1}, τ 4.42(1H,d,J2Hz), and 5.90(1H,m), M^+ 403, other peaks at 385, 384, 367, 352, and 247, isotopic ratios 384:385:386:387 = 13:100:29:7 and hence a deuterium content = 88% d_1

Lithium Aluminium Hydride Reduction of Cholestenone (17)

Standard conditions[18] gave a white solid which was shown to consist of two components by t.l.c.. Preparative t.l.c. in ethyl acetate-petrol (1:4) with repeated elution (X3) gave two closely spaced bands, the more polar of which gave on recovery 3β-hydroxycholest-4-ene (15) m.p. 131-133° (lit.,[22] 130-132), ν_{max} 3621, 3604, 1655, 1110, 1027, 968, 918, 849, and 841 cm^{-1}, τ 4.76(1H,d,J1Hz), and 5.88(1H,m). The less polar band yielded 3α-hydroxycholest-4-ene(16) m.p. 82-84° (lit.,[22] 83-84°), ν_{max} 3618, 1655,1016, 985, 895, 858, and 849 cm^{-1}, τ 4.55(1H,d,J5Hz), and 5.94(1H,m).

The relative proportions of (15) and (16) in the reduction mixture were estimated from the 100MHz n.m.r. spectrum by integration over the signals at 4.55 and 4.76 τ respectively, which showed the presence of 3β -hydroxycholest-4-ene (15)(89%) and 3α -hydroxycholest-4-ene (16)(11%), [lit.,[23] 70% (15) and 24% (16)]. Large scale separation of these alcohols (15) and (16) was not possible by crystallisation since they form a molecular complex. The corresponding acetates can be crystallised[23] however and the pure alcohols regenerated from them by reduction with lithium aluminium hydride.

Deuterolysis of 3β -hydroxycholest-4-ene (15)

On reduction with dichloroaluminium deuteride, (15) gave a deuteriocholest-4-ene (E) which showed ν_{max} 2125, 1658, 1440, 1372, and 933 cm^{-1}, τ 4.72 (1H,d, partly resolved), M^+ 371 (Calc. for $C_{27}H_{45}D$:M, 371).

Deuterolysis of 3α -hydroxycholest-4-ene (16)

Treatment of (16) with dichloroaluminium deuteride under standard conditions gave a deuteriocholest-4-ene (F) which showed ν_{max} 2120, 1655, 1465, 1440, 1372, and 933 cm^{-1}, τ 4.71(1H,d,J5Hz), M^+ 371 (Calc. for $C_{27}H_{45}D$:M, 371).

Hydroboration of Cholest-4-ene (18)

Hydroboration of cholest-4-ene (18) (1.5g, 4mmol) by the method of Bull. Jones and Meakins[24] afforded a mixture of alcohols (1.3g) which were separated by preparative t.l.c. in ethyl acetate-petrol (3:17) after repeated elution (X4) to give a less polar component,

TABLE (6)

Analysis of alcohols (G) and (H) from deuterolysis of 3β-hydroxy cholest-4-ene (15) followed by hydroboration of the resulting 3-deuterio cholest-4-ene (E).

Alcohol (G) + 0.8 equivalents of $Eu(fod)_3$

Peak	Position	Multiplicity	Integration	% age Deuterium
6α	1262	d	1.00	
3α	1178	m	0.50	50
3β	1125	m	0.54	46
5β	1094	m	1.00	

Alcohol (H) + 0.45 equivalents of $Eu(fod)_3$

Peak	Position	Multiplicity	Integration	% age Deuterium
6α	920	d	1.00	
3α	872	m	0.54	46
3β	836	t	0.48	52
5β	778	d	1.00	

Distribution of deuterium from deuterolysis of (15) is

3α = 48% d

3β = 49% d

TABLE (7)

Analysis of alcohols (I) and (J) resulting from deuterolysis of 3α-hydroxy cholest-4-ene (16) followed by hydroboration of the resulting 3-deuteriocholest-4-ene (F).

Alcohol (I) + 0.8 equivalents of $Eu(fod)_3$

Peak	Position	Multiplicity	Integration	% age Deuterium
6α	1357	d	1.00	
3α	1267	m	0.19	81
3β	1214	m	1.85	15
5α	1187	t		

Alcohol (J) + 0.4 equivalents of $Eu(fod)_3$

Peak	Position	Multiplicity	Integration	% age Deuterium
6α	882	d	1.13	
3α	856			87
3β	806	t	0.91	9
5β	750	d	1.00	

Distribution of deuterium from deuterolysis of (16) is

3α = 84% d.

3β = 12% d.

4β -hydroxy-5β -cholestane (22), identical with the material previously prepared[18] by reduction of 5β -cholestan-4-one and the more polar band afforded 4α -hydroxy-5α -cholestane (21), identical with a specimen prepared by reduction of 5α -cholestan-4-one[18].

Hydroboration of Deuteriocholest-4-ene (E)

By the above procedure (E) was converted to give a 3-deuterio-4α -hydroxy-5α -cholestane (G) and a 3-deuterio-4β -hydroxy-5β -cholestane (H).

Hydroboration of Deuteriocholest-4-ene (F)

Similar conversion of (F) gave a 3-deuterio-4α -hydroxy-5α -cholestane (I) and a 3-deuterio-4β -hydroxy-5β -cholestane (J).

N.M.R. Analysis of the alcohols (G), (H), (I) and (J)

The 100MHz n.m.r. spectra of these alcohols were recorded with added europium-shift reagent, $Eu(fod)_3$, such that the signals from the 3α - and 3β -protons were distinct. The proportion of shift reagent necessary was determined by examining the spectra resulting from the unlabelled 4α - and 4β -alcohols (21) and (22); the results of these experiments are shown in Tables (3) and (4). The deuterium content in either the 3α - or 3β -position was estimated by repeated integration of these signals and comparison with an internal standard. The results of the deuterium analysis of the four alcohols are listed in Tables (6) and (7).

Deuterolysis of 5α-hydroxy-cholest-6-ene (23)

The normal deuterolysis procedure gave a white crystalline compound in 96% yield which was identified as cholesta-4,6-diene (24) and had m.p. 92-93° (lit.,[25] 88-92°), ν_{max} 3015, 1640, 930, 880, and 860 cm^{-1}, τ 4.03, 4.15, 4.42, 4.60(3H,m), M^+ 368 (Calc. for $C_{27}H_{44}$:M, 368). The multiplet in the n.m.r. spectrum probably results from superposition of a doublet due to the C-4 proton on to one limb of a quartet arising from the vinyl protons at C-6 and C-7.

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APPENDIX

TABLE (10)

Bond Lengths and Angles in Cyclohexene

Bond Lengths	
C-1/C-2	1.340 Å
C-2/C-3 and C-1/C-6	1.504 Å
Other C-C	1.535 Å
C-1/H and C-2/H	1.085 Å
Other C-H	1.108 Å
Bond Angles	
C-1/C-2/C-3 + C-6/C-1/C-2	122° 30'
C-2/C-3/C-4 + C-1/C-6/C-5	111° 17'
C-3/C-4/C-5 + C-2/C-3/C-4	107° 43'
Others	109° 28'

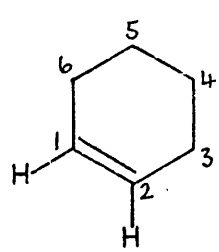

TABLE (11)

Atomic Coordinates of Cyclohexene Systems

(i) Trimethylethylcyclohexene

Carbon Atoms

0.0000;	0.0000;	0.0000	-0.0385;	1.2901;	-2.3974
0.8410;	-1.2120;	-0.4206	-0.0358;	-0.4941;	-2.6743
2.1140;	-1.2120;	0.4206	-1.8025;	1.0255;	-0.6457
2.9550;	0.0000;	0.0000	-1.8077;	-0.7701;	-0.9258
2.1470;	1.2690;	0.0000	-0.1864;	-2.5779;	0.9200
0.8070;	1.2690;	0.0000	-0.8905;	-2.4819;	-0.7431
2.9050;	-2.5010;	0.1591	0.6555;	-3.3748;	-0.4683
0.0500;	-2.5010;	-0.1597	1.1034;	-1.1268;	-1.4936
-0.5807;	-0.2384;	1.3990	1.8520;	-1.1268;	1.4936
-1.1936;	0.1518;	-0.9516	3.1414;	-2.5779;	-0.9206
-0.6578;	0.3718;	-2.3729	3.8455;	-2.4819;	0.7425
			2.2995;	-4.3748;	0.4677

Hydrogen Atoms

			3.8166;	0.1096;	0.6069
			3.3742;	-0.1721;	-1.0098
0.2450;	-0.3434;	2.1303	2.6890;	2.2070;	0.0000
-1.1901;	-1.1637;	1.3947	0.2640;	2.2070;	0.0000
-1.2211;	0.6191;	1.6856			
-1.5062;	0.4797;	-3.0760			

(ii) Trans-diaxial-4-methylcyclohexen-3-ol

Carbon Atoms					
0.0000;	0.0000;	0.0000	2.6850;	-2.1425;	0.2323
0.8410;	-1.2120;	-0.4206	1.1777;	-0.1606;	2.0753
2.1140;	-1.2120;	0.4206	1.1430;	-1.9695;	2.3104
2.9550;	0.0000;	0.0000	2.6752;	-1.1130;	2.5172
2.1470;	1.2690;	0.0000	3.8166;	0.1096;	0.6869
0.8070;	1.2690;	0.0000	2.6890;	2.2070;	0.0000
1.7505;	-1.0940;	1.9071	0.2640;	2.2070;	0.0000
Hydrogen Atoms					
-0.4192;	-0.1721;	1.0098	4.0780;	-1.1358;	-1.3793
-0.8616;	0.1096;	-0.6869	Oxygen Atom		
1.1034;	-1.1268;	-1.4936			
0.2700;	-2.1425;	-0.2323	3.5033;	-0.2952;	-1.3702

(iii) Trans-diequatorial-4-methylcyclohexen-3-ol

Carbon Atoms

0.0000;	0.0000;	0.0000	3.1414;	-2.5779;	0.9200
0.8410;	-1.2120;	0.4206	2.2995;	-3.3748;	-0.4683
2.1140;	-1.2120;	-0.4206	3.8455;	-2.4819;	-0.7431
2.9550;	0.0000;	0.0000	1.8516;	-1.1266;	-1.4936
2.1470;	1.2690;	0.0000	3.3742;	-0.1721;	1.0093
0.8070;	1.2690;	0.0000	3.7388;	0.2929;	-1.9190
2.9050;	-2.5010;	-0.1597	2.6890;	2.2070;	0.0000

Hydrogen Atoms

			0.2640;	2.2070;	0.0000
-0.4192;	-0.1721;	-1.0098			
-0.8616;	0.1096;	0.6869			
0.2700;	-2.1425;	0.2323			
1.1034;	-1.1266;	1.4936			

Oxygen Atom

4.0981;	0.1454;	-0.9113

TABLE (12)

Atomic Coordinates for Pyrrolidine

Pyrollidine is orientated such that the N-H bond bisects angle C-6/C-1/C-2, in cyclohexenol and values are quoted for the case in which the nitrogen atom is in the x,y-plane and coincident with C-1.

Nitrogen Atom		
0.8070; 1.2690; 0.0000	-0.7536; 0.3808; -1.1059	
Carbon Atoms	-1.5745; 3.1346; -0.8889	
-0.5787; 1.2577; -0.4898	-1.0026; 2.2134; -2.3727	
1.5097; 2.4635; -0.4898	0.3820; 4.2642; -0.8889	
0.5223; 3.2921; -1.3433	0.9139; 3.3430; -2.3727	
-0.8027; 2.5271; -1.3433	1.8401; 3.0709; 0.3655	
Hydrogen Atoms	2.3565; 2.1765; -1.1059	
-1.2700; 1.2752; 0.3655	1.3137; 0.3914; -0.3433	

Bond Angles: CNC = 110° 14'; CCN = 108° 08'; CCC = 106° 45'

Bond Lengths: C-N = 1.47 Å; C-C = 1.53 Å; C-H = 1.1 Å

TABLE (13)

Atomic Coordinates for Propene

(i)

Carbon Atoms					
0.0000;	0.0000;	0.0000	-0.9397;	-0.5425;	0.0000
1.1610;	-0.6700;	0.0000	0.0000;	1.8731;	-1.0440
0.0000;	1.5040;	0.0000	-0.8991;	1.8820;	0.5257
Hydrogen Atoms			0.8991;	1.8820;	0.5257
1.1610;	-1.7550;	0.0000			
2.1007;	-0.1275;	0.0000			

(ii)

Carbon Atoms					
As above			-0.9397;	-0.5425;	0.0000
Hydrogen Atoms			1.0440;	1.8731;	0.0000
1.1610;	-1.7550;	0.0000	-0.5257;	1.8820;	0.8991
2.1007;	-0.1275;	0.0000	-0.5257;	1.8820;	-0.8991

APPENDIX

(i) Calculation of Atomic Coordinates

Published bond lengths and angles for cyclohexene[76] were used, Table (10),. and a standard half-chair conformation assumed. The molecule was placed in a suitable system of axes and the atomic coordinates calculated by vector algebra[77]. These values, listed in Table (11), were verified by use of a computer program (BONDLA[78]) which calculates bond angles and lengths from a given input of coordinates. Pyrrolidine was assumed to be planar and the bond lengths and angles used to calculate the coordinates are given in Table (12). Coordinates for the atoms in the two propene models are listed in Table (13).

(ii) Molecular Orbital Calculations

For a full account of the underlying theory ard methods used in the calculations the book by Pople and Beveridge[63] should be consulted; only a brief description is presented below.

Self Consistent Field Molecular Orbital Theory (SCF)

To simplify solution of the Schroedinger equation for a many electron system it is usual to make many approximations. A general feature of approximate solutions is an attempt to construct a satisfactory many-electron wave function from a combination of functions each dependent upon the coordinates of one electron only.

For an n-electron system the simplest way to do this is to associate the n-electrons with n one-electron functions, thus:-

$$\Psi(1,2,\ldots\ldots n) = \chi_1(1)\chi_2(2)\ldots\ldots\chi_n(n),$$

and such one-electron functions χ_i are called <u>orbitals</u>.

The electronic hamiltonian operator for a many electron system has the general form

$$H = -\sum_p \tfrac{1}{2}\nabla_p^2 - \sum_A \sum_p Z_A r_{Ap}^{-1} + \sum_{p<q} r_{pq}^{-1} .$$

This expression may be separated into one - and two - electron parts, H_1 and H_2. Thus $H = H_1 + H_2$,

$$H_1 = \sum_p H^{core}(p) = -\sum_p \tfrac{1}{2}\nabla_p^2 - \sum_A \sum_p Z_A r_{Ap}^{-1} ,$$

and $$H_2 = \sum_{p<q} r_{pq}$$

The quantity H^{core} is the one - electron hamiltonian corresponding to the motion of an electron in the field of bare nuclei (A) of charge Z_A. After much simplification the expression for the total energy becomes

$$\mathcal{E} = \langle \Psi | H | \Psi \rangle = \langle \Psi | H_1 | \Psi \rangle + \langle \Psi | H_2 | \Psi \rangle$$

$$= 2\sum_i^n H_{ii} + \sum_i^n \sum_j^n (2J_{ij} - K_{ij}),$$

where H_{ii} is the expectation value of the one - electron core hamiltonian corresponding to the molecular orbital

$$H_{ii} = \int \chi_i(1)^* H^{core} \chi_i(1)\, d\tau ,$$

and J_{ij} and K_{ij} are the <u>Coulomb</u> and <u>Exchange</u> integrals respectively.

The coulomb integrals give the value that the total electron-electron repulsion would have if all the electrons moved independently in the orbitals to which they are assigned. The exchange integrals 'reduce' the energy of interaction between electrons with parallel spins in different orbitals.

If the many-electron function Ψ is adjusted to minimise the energy of the system then the accurate solution of the many-electron wave equation $H\Psi = E\Psi$ will be approached. The best molecular orbitals are obtained by varying all the contributing one electron functions (χ_i) until the energy is minimised, and these are then known as the <u>self-consistent</u> or <u>Hartree-Fock</u> molecular orbitals.

The problem then is to find a stationary value of the energy given by $\langle \Psi | H | \Psi \rangle$. This leads to the set of differential equations

$$\left[H^{core} + \sum_j \left(2J_j - k_j \right) \right] \chi_i = \sum_j \varepsilon_{ij} \chi_i$$

i.e.

$$F \chi_i = \sum_j \varepsilon_{ij} \chi_i$$

where F is the Fock hamiltonian operator. These equations each have a whole set of constants ε_{ij} embodied in them instead of a single eigenvalue. However this set of constants form a matrix and it is possible to multiply a matrix by a suitable transform matrix such that the product is diagonalised, i.e. $\varepsilon_{ij} = 0$ unless $i = j$, without affecting the value of the determinant. Thus after diagonalisation we have,

$$F \chi_i = \varepsilon_i \chi_i$$

The general procedure for solution of these equations is to assume a set of trial functions which allows calculation of the Coulomb and Exchange operators and hence a first approximation to the Fock operator. The eigenfunctions of this operator are used as a second trial function and the procedure is repeated till the orbital is invariant (within given limits) on further iteration. These orbitals are then self consistent.

Linear Combination of Atomic Orbitals (LCAO)

For molecular systems of any size direct solution of the above differential equations is impractical and more approximate methods are required. One of the best methods so far has been to approximate Hartree-Fock orbitals by a linear combination of atomic orbitals, viz.,

$$\psi_i = \sum_{\mu} c_{\mu i} \phi_{\mu} ,$$

where ϕ_{μ} are atomic orbitals. It is possible to define a Bond Index B_{AB}, between two atoms A and B such that

$$B_{AB} = \sum_{\mu \text{ on } A} \sum_{\nu \text{ on } B} P_{\mu\nu}^2 ,$$

and $\sum_{B \neq A} B_{AB} = V_A$ where V_A is the Valency of the atom, and $P_{\mu\nu}$ is the matrix of terms known as the Density Matrix and is given by

$$P_{\mu\nu} = 2 \sum_{i}^{occ} c_{\mu i} c_{\nu i} .$$

By a similar procedure to that described above the best molecular orbitals are obtained by finding optimum values for the coefficients $c_{\mu i}$ which give the lowest energy, and the final expression reduces to

$$\sum_{\nu} \left(F_{\mu\nu} - \varepsilon_i S_{\mu\nu} \right) c_{\nu i} = 0 \qquad \ldots\ldots \text{(ii)}$$

where the matrix elements of the Fock hamiltonian operator F are given

by,

$$F_{\mu\nu} = H_{\mu\nu} + \sum_{\lambda\sigma} P_{\lambda\sigma}\left[(\mu\nu|\lambda\sigma) - \tfrac{1}{2}(\mu\lambda|\nu\sigma)\right],$$

and the expression (ii) is known as the Roothaan equations, and $S_{\mu\nu}$ are the overlap integrals.

Approximate Molecular Orbital Theories

The most difficult and time-consuming part of LCAO-SCF molecular orbital calculations is the evaluation and handling of a large number of electron repulsion integrals. Many of these terms have values near zero especially those involving the overlap distribution with $\mu \neq \nu$. A useful approach is therefore to neglect such terms and under the zero-differential overlap approximation $(\mu\nu|\lambda\sigma) = (\mu\mu|\lambda\lambda)\delta_{\mu\nu}\delta_{\lambda\sigma}$ where δ_{ij} is the Kronecker Delta. The various levels of approximate self consistent field theory differ mainly in the extent to which the zero-differential overlap approximation is invoked in electron repulsion integrals.

The basic approximations of the CNDO method are:-

(i) Replacing the overlap matrix by the unit matrix in the Roothaan equations.

(ii) Neglecting differential overlap in all two-electron integrals so that

$$(\mu\nu|\lambda\sigma) = (\mu\mu|\lambda\lambda)\delta_{\mu\nu}\delta_{\lambda\sigma}$$

(iii) Reducing the remaining set of coulomb-type integrals to one value per atom pair,

$$(\mu\mu|\lambda\lambda) = \gamma_{AB} \quad \phi_\mu \text{ on } A,\ \phi_\lambda \text{ on } B$$

(iv) Neglecting monatomic differential overlap in the interaction integrals involving the cores of other atoms. i.e. $(\mu|V_B|\nu)$

(v) Taking diatomic off-diagonal core matrix elements to be proportional to the corresponding overlap integrals, i.e.

$$H_{\mu\nu} = \beta_{AB} S_{\mu\nu}$$

Thus in (iv) above terms such as $(\mu|V_B|\nu)$ are taken to be zero for ϕ_μ and ϕ_ν on A $(\mu\neq\nu)$ but in the INDO method monatomic differential overlap is retained.

The calculation is executed as follows:- Slater atomic orbitals are used to calculate the overlap integrals and the electron repulsion integral γ_{AB} is calculated as the two centre coulomb integral involving s functions. A zero-differential overlap, extended Huckel-type approximation to the Fock matrix is then effected with diagonal elements formed from combinations of ionisation potentials and electron affinities, and off diagonal terms from the product of the resonance integral β and the overlap integrals $S_{\mu\nu}$. This matrix is then diagonalised and an initial density matrix constructed. Corrections are then added to the hamiltonian for INDO calculations. Using the initial density matrix and the INDO modifications the Fock matrix is formed, diagonalised, and a new density matrix produced which in turn is used to construct a new Fock matrix. This procedure is repeated till the electronic energy converges to 10^{-6}. At this point, the Fock matrix is printed, then diagonalised once more and, the resulting eigenvectors are printed. The electronic energy is computed after each new Fock matrix is formed. The Bond Indices were calculated from the final density matrix.