REARRANGEMENTS OF UNSATURATED STEROID ALCOHOLS

by

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Where two page numbers are given, the first refers to the discussion and the second to the corresponding experimental details.

NOTE

The configuration of the hydrogen atoms at the ring junctions will be 5α, 8β, 9α and 14α, unless otherwise specified.
In a compound name where no specific configuration for carbon atom 5 is stated, it will be assumed that the A-B ring junction is trans
In the diagrams the configurations are shown as follows:

A solid line indicates a β-configuration

A broken line indicates an α-configuration

A wavy line indicates an unknown configuration or a mixture of isomers

A dotted line indicates a partially formed bond.

INTRODUCTION

The first rearrangement of an unsaturated steroid alcohol was unwittingly carried out by Stoll in 1932,[1] when in an attempt to epimerise cholesterol, he prepared the methyl ether of i-cholesterol (3α,5-cyclo-5α-cholestan-6β-ol). In the years following, the structure of this i-steroid was elucidated, and the i-steroid rearrangement identified as being the solvolysis of 5,6-unsaturated 3β-substituted steroids, where the substituent on carbon 3 is normally a good leaving group, such as toluene-p-sulphonyl, methanesulphonyl or a halogen. In recent years, investigations have concentrated largely on the determination of steric requirements necessary for the syntheses of these compounds and on a more precise definition of the non-classical carbonium ion intermediate in their formation.

Recently investigations have been carried out on hydrolysis of benzoate esters of cholest-1-en-3β-ol and cholest-4-en-3β-ol, the products of these reactions being cholestadienes and epimeric alcohols.[59,60,61]

This thesis describes the solvolysis of p-toluenesulphonate esters of cholest-1-en-3β-ol and cholest-4-en-3β-ol. In the first case a cyclo-steroid is formed and in the second, a diene, as the product of elimination of the ester group.

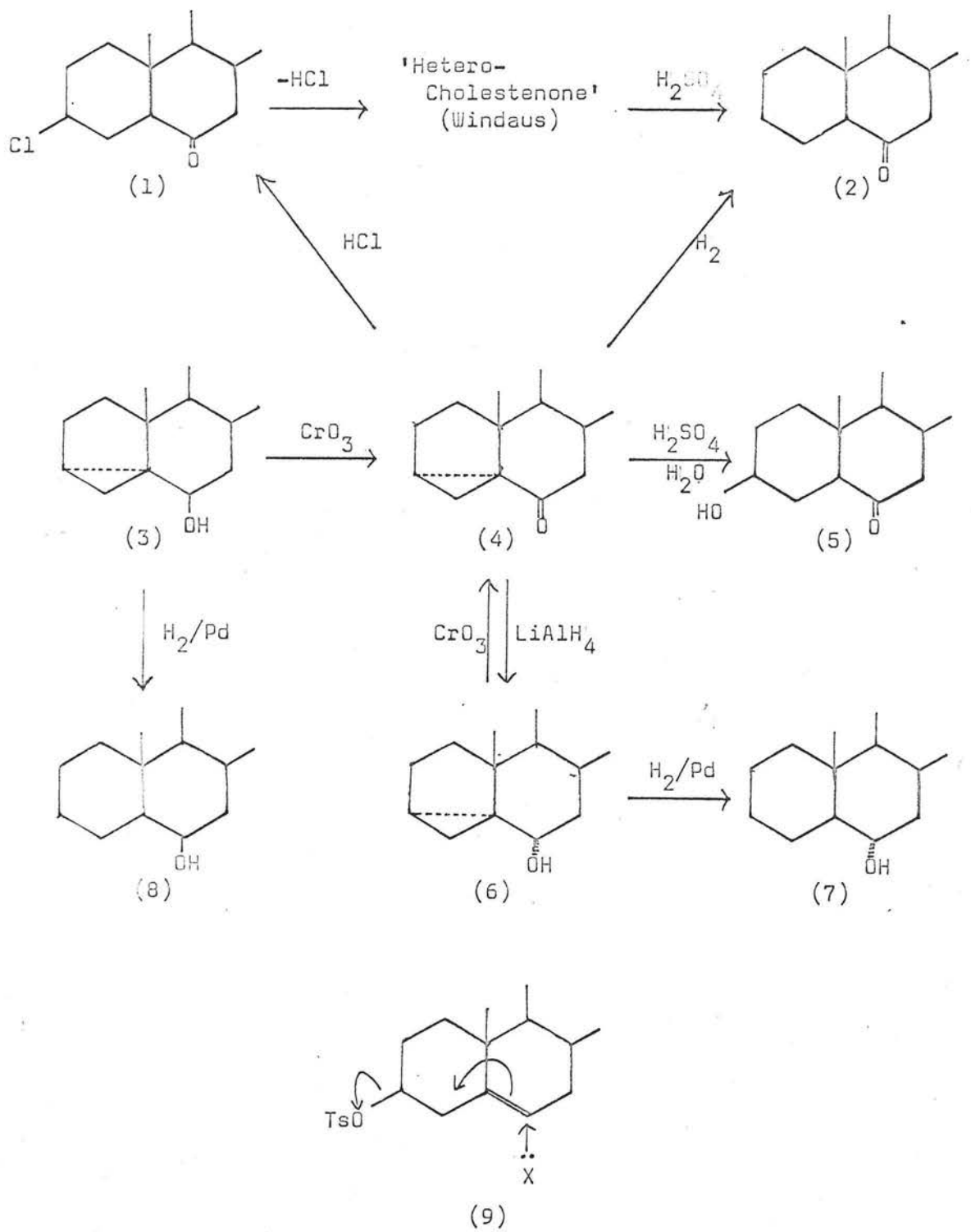
In view of the isolation of a steroid containing a cyclopropane ring system within the A-ring, there follows a review of the i-steroid transformation and other synthetic routes to cyclosteroids.

Until comparatively recently, knowledge of the chemistry and biological activity of steroids containing a cyclopropane ring was limited to the i-steroids, 16,17-exocyclic methylene compounds and some photochemically induced rearrangement products. Recently other syntheses have been evolved. Internal displacement of an electronegative substituent from the γ carbon atom of a keto steroid by an anion generated at the α carbon atom has been utilized to synthesize 9β,19-,[2] 12β,18-,[3] 5α,7α-,[4] and 17β,18-[5] cyclosteroids, while solvolysis of 19-substituted Δ5-steroids leads to 5β,19-cyclosteroids.[6] Introduction of a methylene group to produce exocyclic cyclopropyl steroids has been achieved with diazomethane,[7] halocarbenes,[8] ylides[9] and the Simmons-Smith reagent.[10] Further photochemical rearrangements leading to 1,5-[11] and 5β,19-[12] cyclosteroids have been reported.

Relatively few 3α,5-cyclosteroids have been examined for biological activity. 6β-Hydroxy-3α,5-cyclo-androstane, substituted at carbon 16 by hydroxyl or carbonyl is active in lowering blood pressure,[13] and 17β-hydroxy-3α,5-cycloandrost-6-ene shows anabolic action as well as exhibiting hypotensive properties.[14] Several i-steroids have been shown to have an effect on the central nervous system; 3α,5-cyclo-18,20-epoxy-20-hydroxypregnan-6-one is useful in prolonging barbiturate hypnosis,[15] while 6β-hydroxy-3α,5-cyclo-pregnan-20-one has been used as a tranquilizer.[16]

In 1948, 3α,5-cyclo-androstan-6β-ol-17-one was isolated from

the urine of a girl with adenoma of the adrenal cortex; this was the first report of an i-steroid prepared in vivo.[17] It is probable that previously any i-steroids present would have been isomerised into the corresponding Δ5-3β-ol by the acid hydrolysis normally used in the extraction of steroids from these sources.


Cl
O
(1)
-HCl
'Hetero-
Cholestenone'
(Windaus)
H_2SO_4
(2)
HCl
H_2
OH
(3)
CrO_3
(4)
H_2SO_4
H_2O
HO
(5)
H_2/Pd
CrO_3
$LiAlH_4$
(8)
(6)
H_2/Pd
(7)
TsO
X
(9)

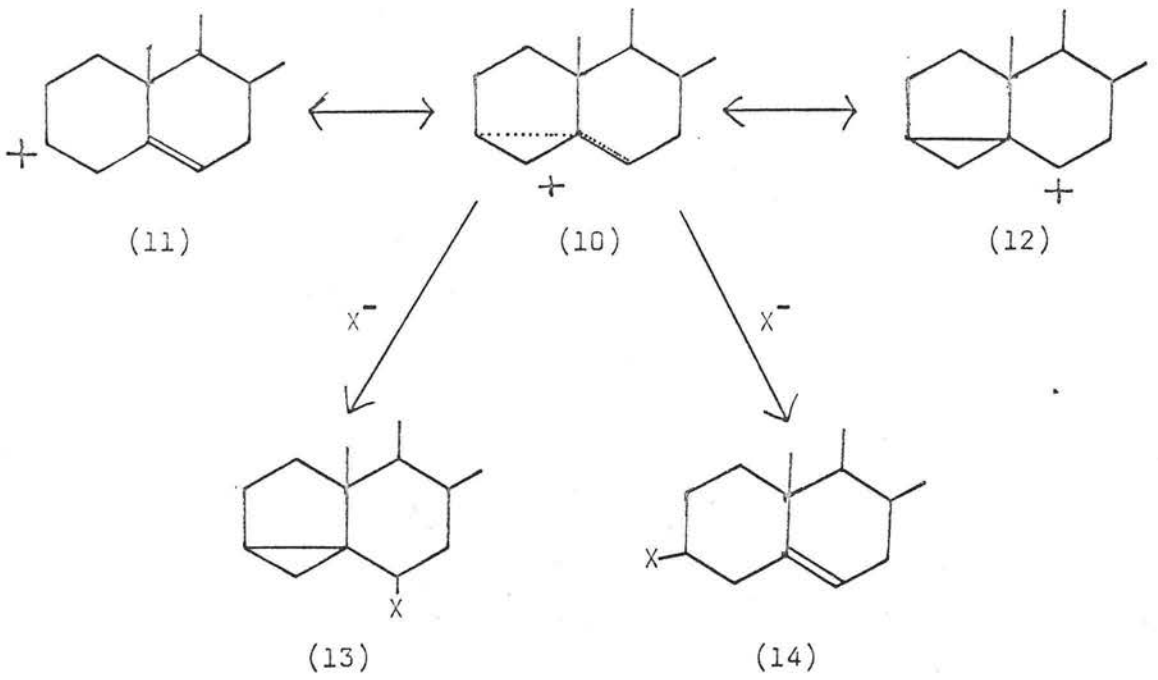
The i-Steroid Rearrangement

The first cyclosteroid was prepared by Windaus and Dalmer in 1919;[18] this product, obtained by elimination of hydrogen chloride on vacuum distillation of 3β-chloro-cholestan-6-one (1), was assumed to be an unsaturated ketone and was characterised by hydrogenation to cholestan-6-one (2). It was only recognised that this compound was structurally unusual almost twenty years later, after a series of investigations initiated by the results of Stoll[1] in 1932.

In an attempt to produce the C_3 epimer of cholesterol from the highly reactive cholesteryl tosylate (toluene-p-sulphonate), Stoll found that the ester is converted into cholesteryl methyl ether by boiling in methanol, while solvolysis of the ester with potassium acetate buffered methanol gives an abnormal ether. This is dextrorotatory ($[\alpha]_D$+55°) while the normal ether is laevorotatory ($[\alpha]_D$-46°), and has since been identified as the methyl ether of 3α,5-cyclo-5α-cholestan-6β-ol (3), (i-cholesterol). Wallis, Fernholz and Gephart[19] found that cholesteryl tosylate reacts with potassium acetate in acetic anhydride to give a dextrorotatory acetate; hydrolysis of this yields i-cholesterol. Similarly, methanolysis and acetolysis of cholesteryl halides gives two isomeric derivatives depending on the presence or absence of a buffer.[20] These abnormal ethers were, to begin with, regarded as cholest-5-en-3α-ol ethers, but with the preparation of an authentic sample of this compound,[21] this was shown to be incorrect.

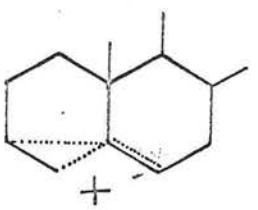
No reaction takes place between i-cholesteryl acetate and perbenzoic acid or ozone; it does not decolourise dilute bromine solution, and it is resistant to hydrogenation under conditions which reduce cholesteryl acetate. Wallis et al.[19] inferred from this lack of olefinic unsaturation that solvolysis of the tosylate involves closing of a cyclopropane ring at C_3-C_5 and migration of the oxygen function to C_6. Oxidation of i-cholesterol (3) with chromic acid gave i-cholestanone (4) which was found[22] to be identical to Windaus' heterocholestenone, obtained from 3β-chloro-cholestan-6-one (1) both by pyrolysis and reaction with alcoholic alkali. Synthesis of authentic 'heterocholestenone' (cholest-4-en-6-one),[23] proved that the unsaturated ketone isolated by Windaus was not this. Acid hydrolysis of the ketone (4) gives cholestan-3β-ol-6-one (5). Addition of hydrogen chloride to the cyclopropyl ketone (4) gives 3β-chloro-cholestan-6-one (1), identical to that prepared from cholesteryl chloride by Windaus.[18] Alkylation of i-cholesterol affords the abnormal ether of Stoll. Reduction of the cyclopropyl ketone (4) with lithium aluminium hydride gives a new alcohol (6),[24] shown to be 3α,5-cyclo-cholestan-6α-ol by catalytic reduction to cholestan-6α-ol (7)[25] and corresponding reduction of i-cholesterol gave cholestan-6β-ol (8).

Thus 'i-cholesterol' is shown to be 3α,5-cyclo-5α-cholestan-6β-ol (3), and its relationship with the compounds of Windaus and Stoll are established.



(11) (10) (12)

(13) (14)



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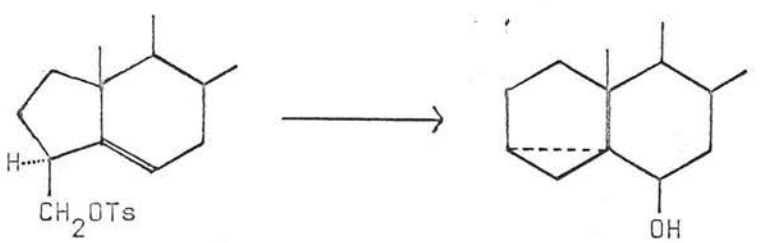


(16) (3)

Kinetic studies on the acetolysis of cholesteryl tosylate by Winstein[26] proved the reaction to be first order. The rate of reaction is markedly increased on the addition of sodium acetate; this however is an ionic strength effect. Increasing the concentration of sodium acetate from 0.01 to 0.02M gives no further increase in rate, a result expected for ionic strength effects in a low dielectric constant solvent. This indicates that the reaction must be unimolecular, and a concerted-type mechanism (9) is not important.

The rate controlling step in this solvolysis is unimolecular and therefore probably consists of ionisation to a non-classical ion (10). Cholesteryl derivatives have an enhanced rate of ionisation ascribed to delocalisation of the π electrons of the 5-6 double bond in the rate determining step. This participation of π electrons is made evident by the solvolysis rates for the tosylates of cholestanol, cholesterol and 7-dehydrocholesterol which are in the ratio 1:100:3000.[27] This non-classical ion (10) can be thought of as a hybrid of canonical forms (11 & 12). The intermediate (10) reacts with nucleophilic species at C_6 to yield 3,5-cyclo steroids or at C_3 to yield cholesteryl derivatives, with overall retention of configuration.

Substitution reactions at C_3 in Δ 5 steroids occur with retention of configuration,[28] indicating that the nucleophile attacks the carbonium ion preferentially from the side originally occupied by the displaced group. This probably occurs from solvation of the carbonium ion on the α side and so protects

it from nucleophilic attack on this side. At the same time, this solvation will increase the life time of the carbonium ion, allowing sufficient time for interaction between the electrons of the C_5-C_6 double bond and the carbonium ion at C_3, before the reaction is completed.

Another possible ionic intermediate has been recently postulated.[29] This 'symmetrical ion' (15) implies delocalisation of the electrons of the 4-5 bond as well as the π electrons of the double bond as in the 'unsymmetrical ion' (10). Solvolysis of 3β-hydroxymethyl-A-nor-cholest-5-ene tosylate (16) in buffered aqueous acetone gives 3α,5-cyclo-5α-cholestan-6β-ol (3) (82%) and cholesterol (18%).[30] It would therefore seem probable from this result that both the solvolysis of (16) and that of cholesteryl tosylate would proceed by the same intermediate. It is probable that the intermediate would involve distortion of the orbitals of all the bonds between carbon atoms 3,4,5 and 6 to some extent.

Since in buffered solutions, where acid cleavage of either methyl ether (13, 14, X=OCH_3) is eliminated, the product is primarily the i-ether; attack at C_6 of the ion (10) must predominate over attack at C_3. In buffered solution the reaction goes no further and the cyclo-steroid is the predominant product. In acid solution, however, acid cleavage of the cyclo ether can take place, since this system is analogous to an allylic ether, to produce ion (10), which would again be susceptible to attack at C_3 and C_6. The normal ether is stable

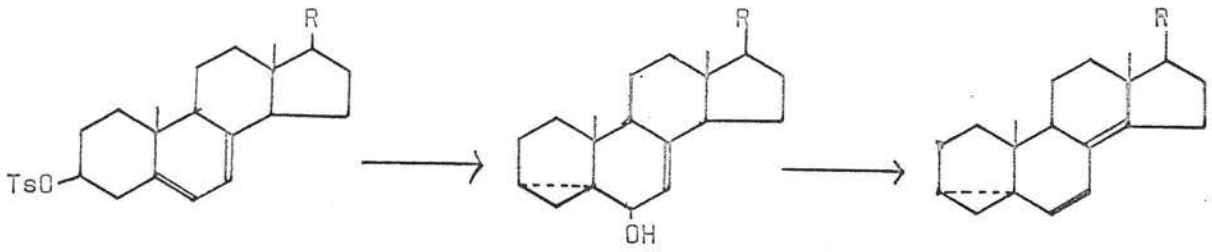
to acids, and therefore, although attack at C_6 predominates over C_3 attack, the normal ether (14) would be the only product isolated from acid solution.[31]

The postulation of ion (10) as an intermediate in the acid catalysed rearrangement of a 3,5-cyclo compound to a normal one, has been verified by the isolation of i-cholesteryl ethyl ether in the reaction of i-cholesteryl methyl ether with ethanol.

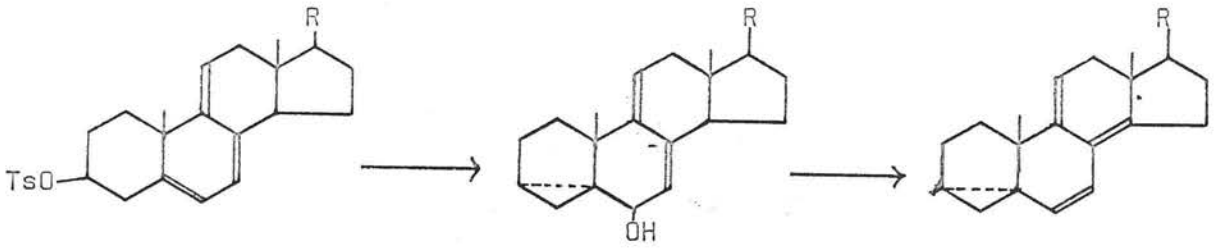
Solvolysis studies of i-cholesteryl acetate where the acetate is isotopically labelled with ^{14}C, in acetic acid of normal isotopic abundance, show that rate of loss of tracer is ten times faster than the rate of rearrangement to the cholesteryl isomer.[32] From this it has been deduced that both exchange and rearrangement proceed through a common intermediate, which reacts with acetic acid to regenerate i-cholesteryl acetate with loss of tracer nine times faster than it reacts with acetic acid to produce the rearranged normal cholesteryl acetate. From this it would appear that the thermodynamic product of the i-steroid rearrangement is cholesterol and the kinetic product is i-cholesterol.

Cyclosteroid formation is stereoselective in that the group eliminated from C_3 whether tosylate or halide is β orientated. Solvolysis of cholest-5-en-3α-ol tosylate gives a 94% yield of cholesta-3,5-diene by β elimination.

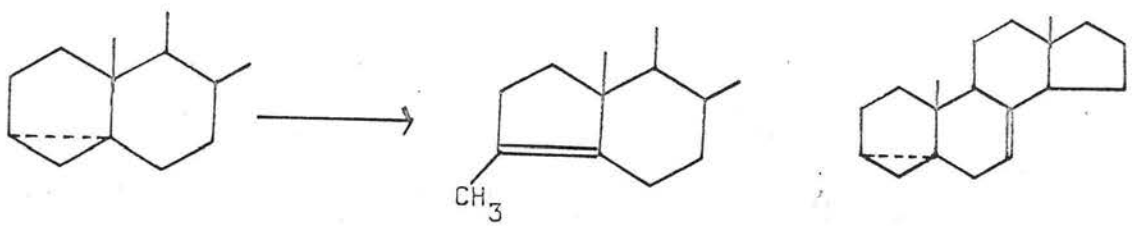
In solvents of low dielectric constant, and with a nucleophile of greater power than, for example, acetate, the



(17) (19) (21)



(18) (20) (22)



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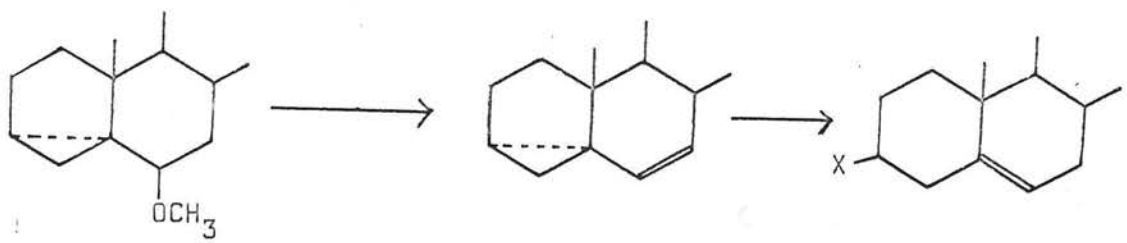
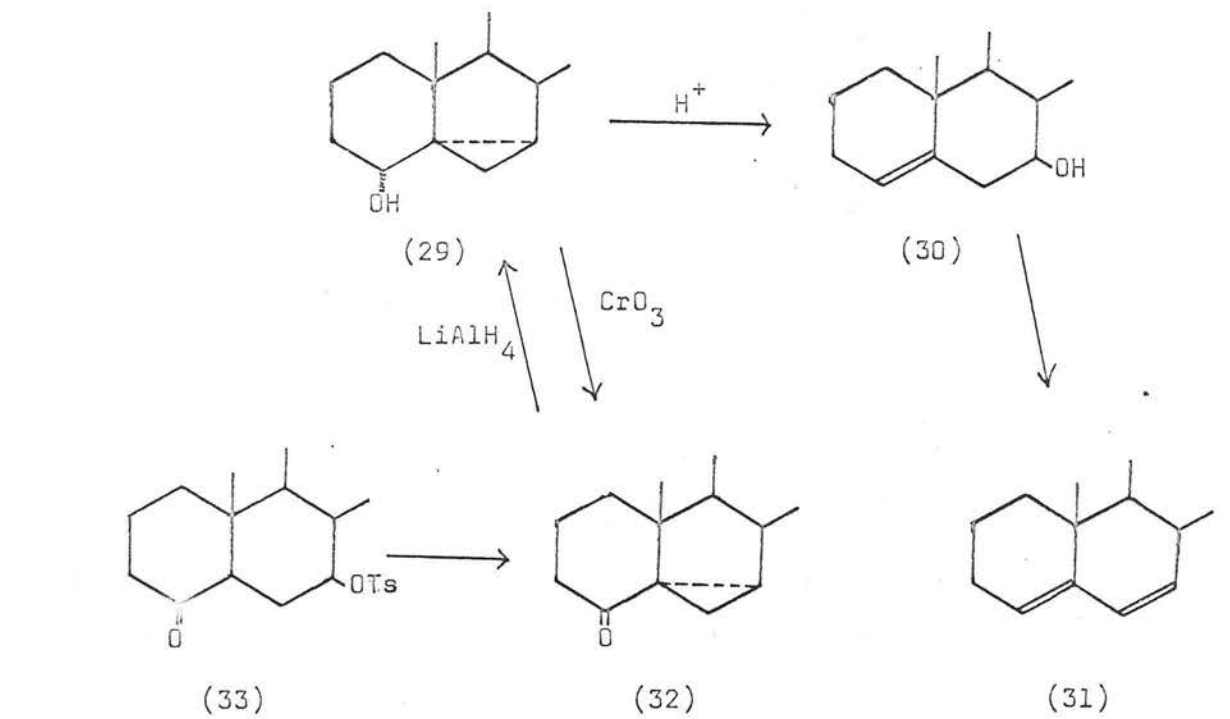


(27) (26) (28)

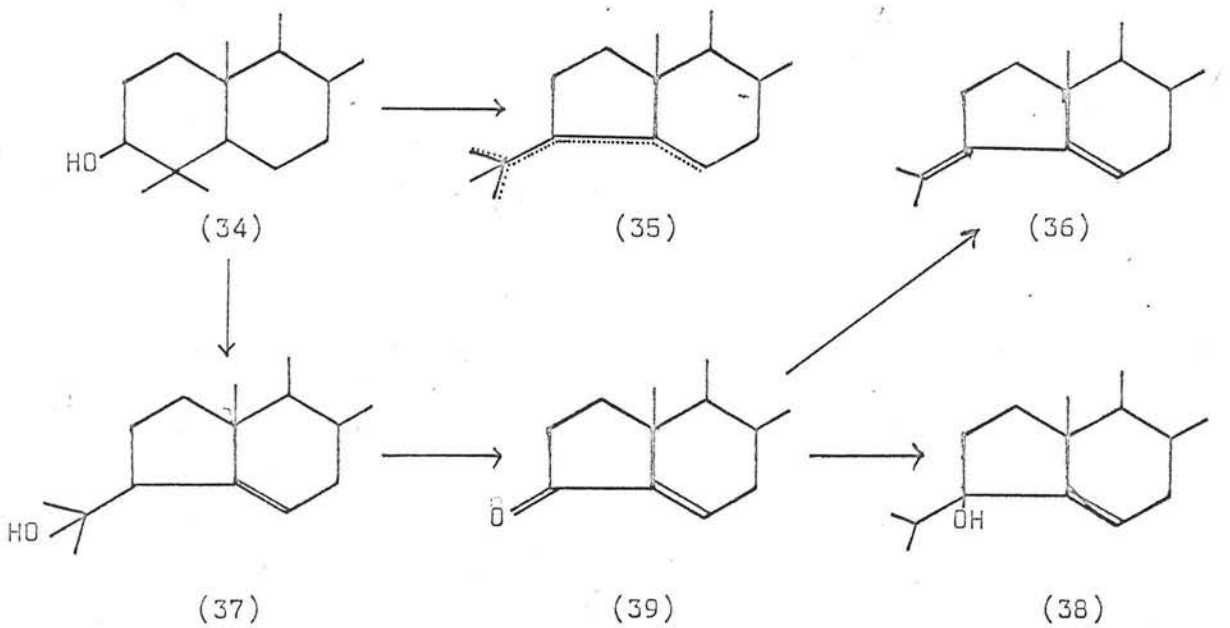
unimolecular solvolysis of 3β substituted Δ 5 steroids may be accompanied or largely superceded by a bimolecular substitution (S_N2), with inversion of configuration at C_3, in which the π electrons of the 5-6 double bond do not participate.[33]

Ergosterol and dehydroergosterol tosylates (17, 18) have been rearranged to the corresponding i-steroid isomers (19, 20) with potassium bicarbonate in aqueous acetone.[34,35,36] The initially formed i-steroids are converted to the respective unsaturated steroids (21, 22) by increasing the time of reaction. The i-steroid hydrocarbon from ergosterol (21) had been prepared by Rygh in 1929, while attempting to prepare the phosphate ester of ergosterol with phosphorous oxychloride.[37] The rate of solvolysis of ergosterol tosylate is greater than that of dehydroergosterol tosylate.[35] This would infer that the 9,11 double bond does not participate in the solvolysis reaction, and therefore the product is the 6-hydroxy rather than the 11-substituted isomer.

Reduction of cholesteryl tosylate with lithium aluminium hydride gives a mixture of cholest-5-ene and an isomeric hydrocarbon, i-cholestane[38] (23). This is identical to the hydrocarbon isolated from a Wolff-Kishner reduction of i-cholestanone (4).[40] i-Cholestane is rearranged by acid to an unsaturated hydrocarbon (24) whose structure has been confirmed by synthesis.[39] Reduction of 7-dehydrocholesterol tosylate with lithium aluminium hydride gives 3α,5-cyclo-cholest-7-ene (25).



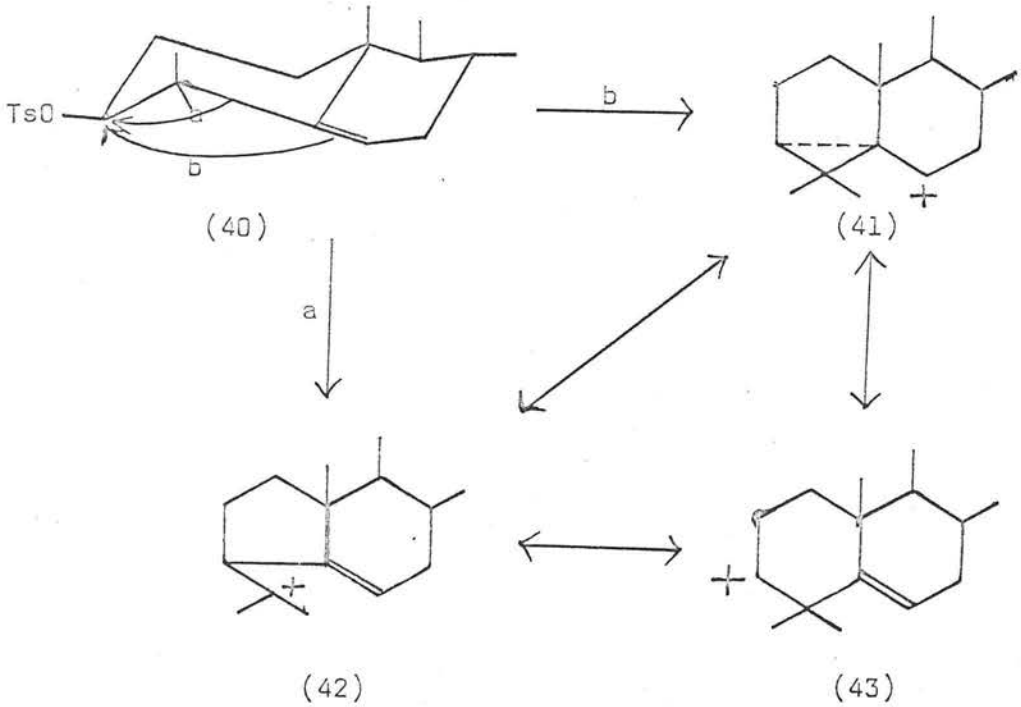
(29) (30) (33) (32) (31)



(34) (35) (36) (37) (39) (38)

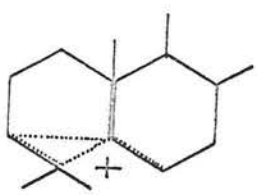
The conjugated i-cholestadiene (26), obtained by Riegel[41] by treating i-cholesteryl methyl ether (27) with alumina in boiling xylene, is the more interesting compound. Acid catalysed addition of water to this i-diene gives cholesterol (28, X=OH) and similarly addition of methanol or acetic acid give cholesteryl methyl ether (28, $X=OCH_3$) or cholesteryl acetate (28, $X=OCOCH_3$). Addition of hydrogen halides in acetone solution give cholesteryl halides (28, X=Cl,Br). One mole of perbenzoic acid reacts during titration, and similarly one mole of bromine adds rapidly. On standing however, with an excess of bromine two moles were consumed. Catalytic reduction, with palladium-charcoal catalyst at room temperature, of the i-diene produced cholestane, with the consumption of two moles of hydrogen.[41]

Attempts to prepare 5α,7α-cyclo-cholestan-4α-ol (29) by solvolysis of cholest-4-en-7β-ol tosylate (30) failed. Methanolysis with or without the addition of potassium acetate produced cholesta-4,6-diene (31) and cholest-4-en-7β-ol methyl ether.[42,43] Peterson has since prepared 5α,7α-cyclo-cholestan-4-one (32) by the action of alcoholic alkali on cholestan-7β-ol-4-one tosylate (33).[4] Reduction of this with lithium aluminium hydride produces 5α,7α-cyclo-cholestan-4α-ol (29); chromic acid oxidation brings about the reverse reaction. The reaction of this 5α,7α-cyclocholestan-4α-ol with acid causes a typical i-steroid rearrangement to cholest-4-en-7β-ol (30).

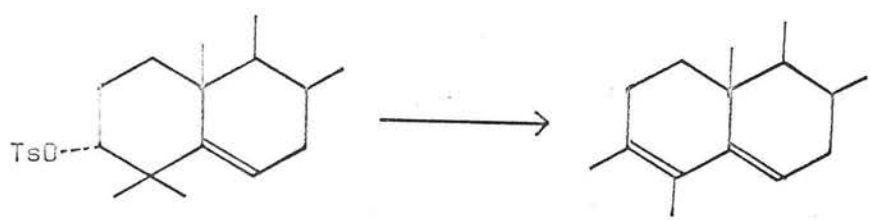


(40) (41)

(42) (43)



(40A)

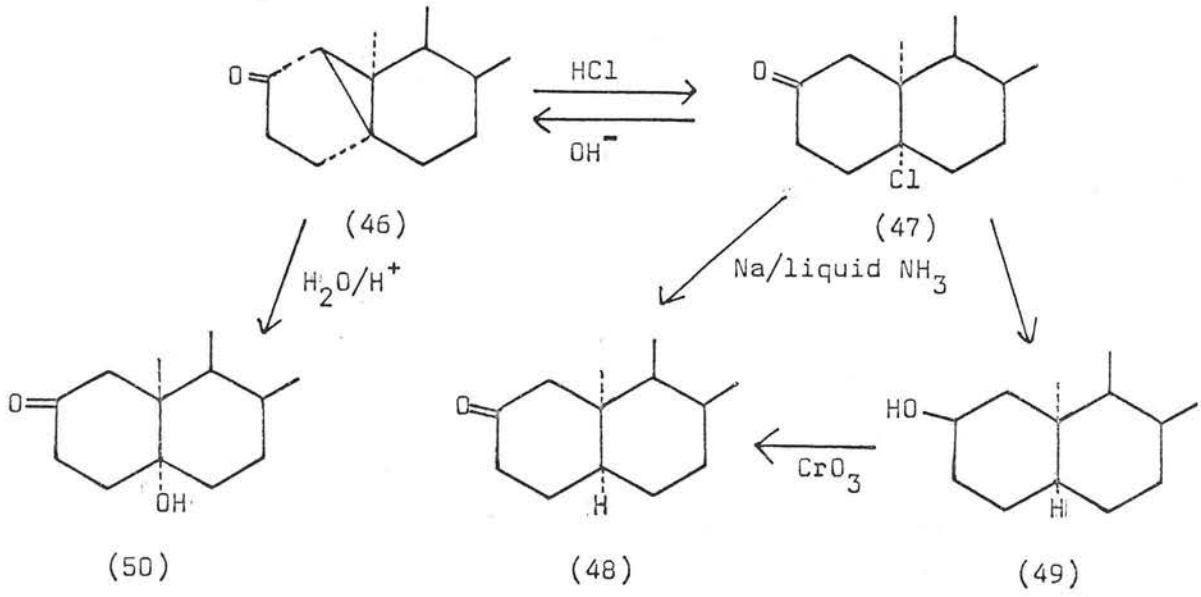


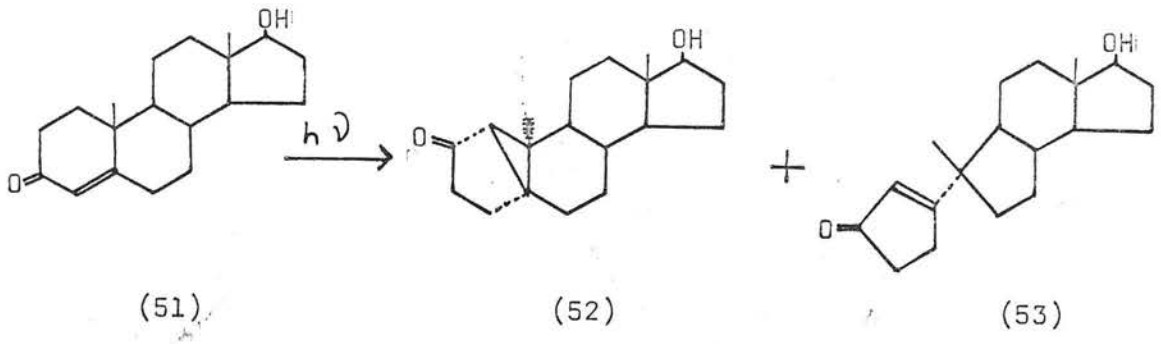
(44) (45)

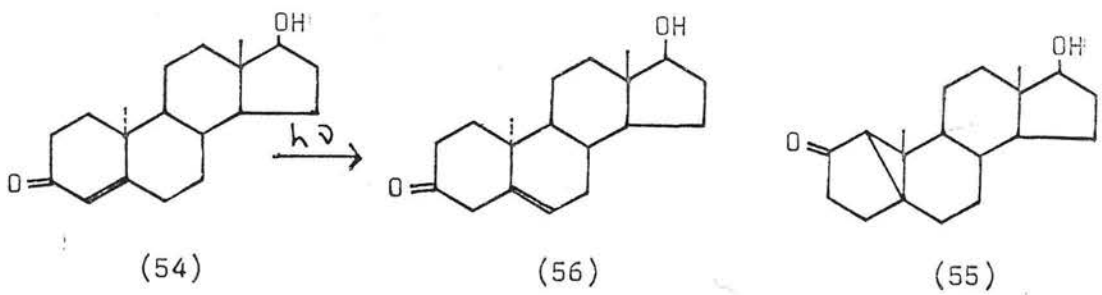
Solvolysis of 4,4-dimethylcholesteryl tosylate (34) with potassium acetate in aqueous acetone does not give rise to an i-steroid. The major product (70%) is a mixture of dienes, of general structure (35), occurring by a nuclear rearrangement.[44,45] The major component of this mixture is 3-isopropylidene-A -nor-cholest-5-ene (36). The other reaction product is an A -nor alcohol whose structure has been given as (37) but might equally well be the isomer where the hydroxyl group is attached to the cyclopentane ring (38). This alcohol on dehydration yields the diene (36) and on oxidation with chromic acid affords A -nor-cholest-5-en-3-one (39). Reaction of isopropyl magnesium iodide with the A-nor ketone gives diene (36) and an alcohol, formulated as 3β-isopropyl-A-nor-cholest-5-en-3α-ol, although this was not purified, but dehydrated to the diene (36) with phosphorus oxychloride. It remains to be decided therefore, which alcohol (37) or (38) is formed in this reaction.

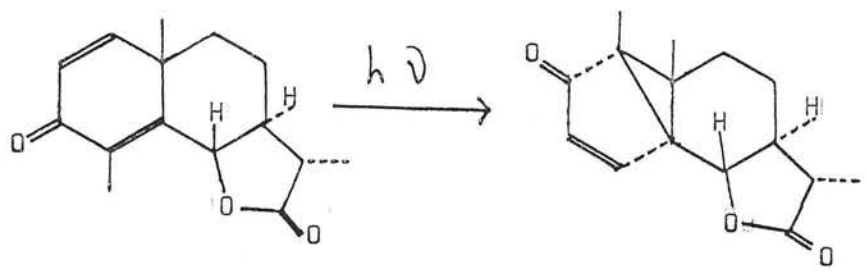
There are two possible pathways for this rearrangement. Wagner rearrangement (route a) should be sterically assisted by the relief of non-bonded interaction between the axial methyl groups at C_4 and C_{10}. In contrast, route b, involving homo-allylic participation of the 5,6 double bond, will not be so assisted. Reaction of the homo-allylically bridged ion at C_4 to give an A-nor alcohol is favoured over reaction at C_6 to give an i-steroid, because of possible unfavourable interactions between axial substituents on carbon atoms 4,6 and 10. In

either case, both reaction pathways lead to common intermediate (40A) [canonical structures (41,42,43)]. On solvolysis 4,4-dimethyl-cholest-5-en-3α-ol tosylate (44) undergoes methyl migration to give 3,4-dimethyl-cholesta-3,5-diene (45).


HCl
OH⁻
Cl
(46)
(47)
H₂O/H⁺
Na/liquid NH₃
HO
CrO₃
OH
H
(50)
(48)
(49)


OH
hν
(51)
(52)
(53)


OH
hν
(54)
(56)
(55)

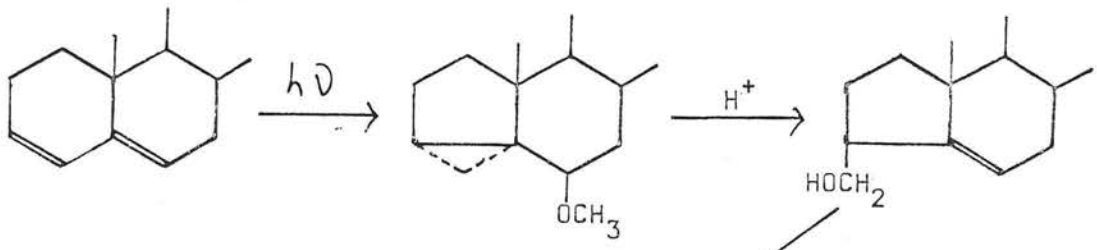

hν
H
O
(57)
(58)

Photochemically Induced Syntheses of Cyclo-Steroids

Photochemical rearrangements of conjugated steroidal unsaturated ketones have given rise to a considerable number of cyclosteroids. Ultra-violet irradiation of alkylated cyclohexenones in dilute t-butanol solution gives rise to molecular rearrangements of low efficiency. However, the occurrence of bimolecular photoreactions, such as dimerization, reduction and solvent addition are largely suppressed. Under these reaction conditions, cholest-4-en-3-one gives a bicyclo [3.1.0]hexanone derivative, 1β,5-cyclo-5β,10α-cholestan-2-one (46) in 25% yield.[11]

Reaction of this compound with hydrogen chloride in acetic acid gave a chloroketone (47) in which there was no longer a cyclopropane ring; the cyclopropyl ketone could be reconstituted from this by reaction with ethanolic alkali. Reduction of the chloroketone with sodium in liquid ammonia gave a ketone (48) and an alcohol (49). Mild oxidation of this alcohol gave the ketone (48) 5α,10α-cholestan-2-one. This reduction proceeds through a radical and/or carbanion intermediate and therefore the product (48) is likely to be the one which has the least strain energy. Since the 5β,10α configuration would force the B ring into a boat form, the product would be less stable than the 5α,10α configuration, where rings A,B and C are all in the chair form. Hydrolysis of lumicholestenone (46) catalysed by acid gives a ketol (50).

Irradiation of testosterone (51) produced two ketones



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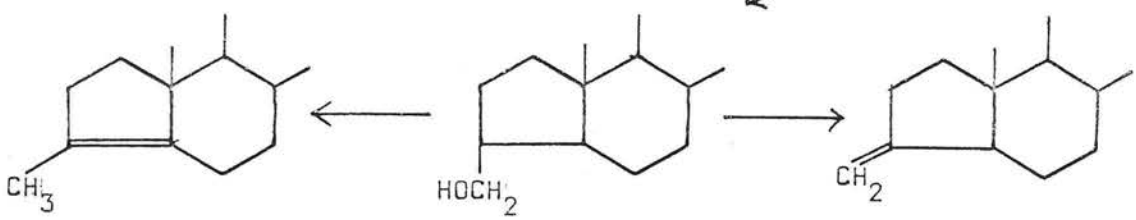

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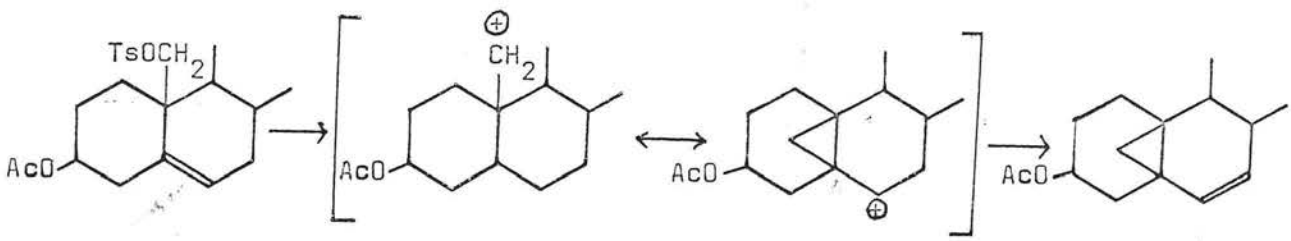

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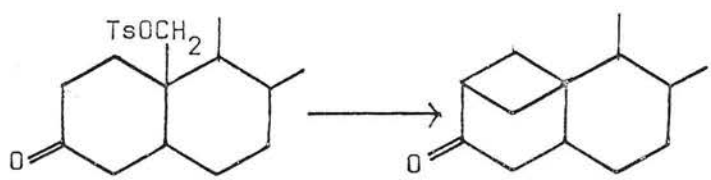

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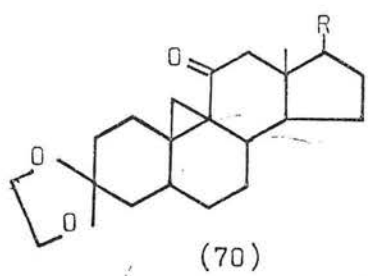


(70)

(52,53) in high yields.[46,47] Further irradiation of (52) produced (53) with traces of testosterone also present. Under the same conditions for the photochemical rearrangement of testosterone, 10α-testosterone (54) shows no tendency to an analagous rearrangement, that is to (55), but the deconjugated Δ 5-3-ketone (56) is formed.[48]

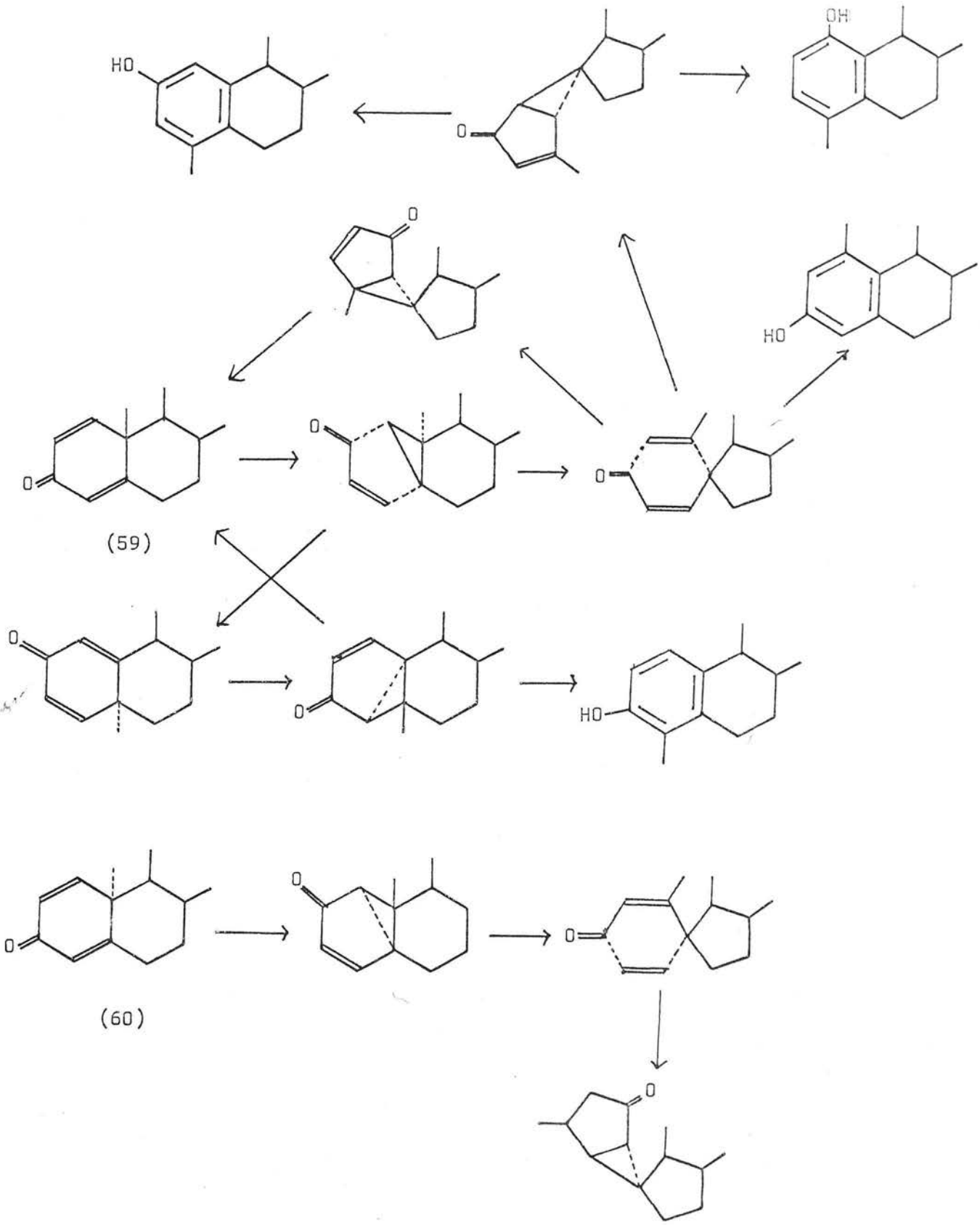
Cross-conjugated ketones also produce cyclopropyl ketones on irradiation. The sesquiterpene santonin (57) isomerises to lumisantonin (58).[49] However, more complex products are obtained by irradiation of 1-dehydrotestosterone acetate (59).[50,51] These products, together with the photoproducts of 1-dehydro-10α-testosterone acetate (60),[52] are summarised in Chart I.

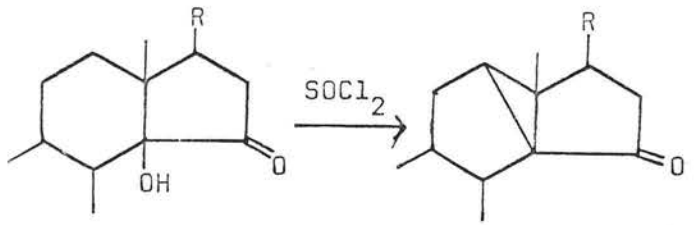
Irradiation of cholesta-3,5-diene (61) affords a 3,5-cyclo-cholesteryl ether (62).[53] Acid catalysed rearrangement of this ether does not give the expected cholesteryl derivative, but a homo-allylic alcohol (63) is produced. The dihydro derivative of this (64), undergoes dehydration to produce two olefins (65, 66). There are no chemical syntheses for this 3β,5-cyclo-5β-cholestane system.

While most cyclo-steroids have been synthesised by the i-steroid transformation or by photochemical reactions, trans-annular-bonded steroids have recently been prepared by several methods.

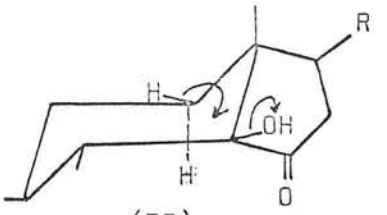
Solvolysis of the tosyl esters of 19-alcohols generally causes formation of a bond between C_{19} and either C_2, C_5 or C_9 depending on the presence of activating substituents.

Chart I





(71) (72)



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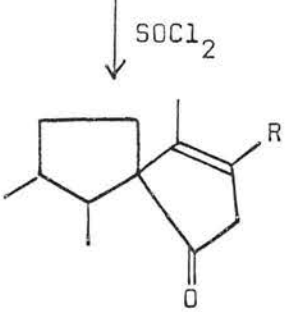

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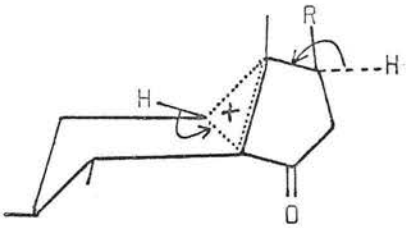
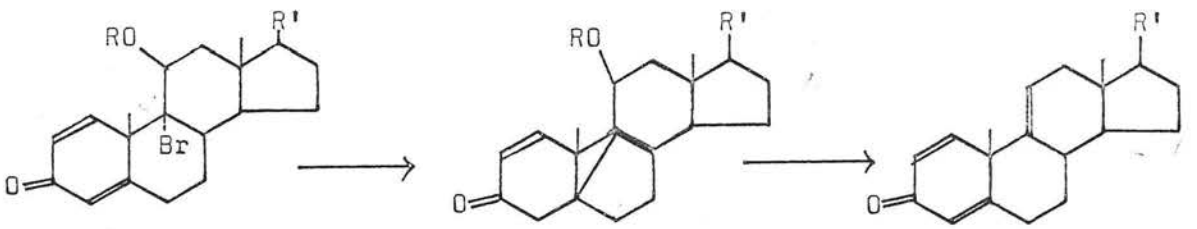
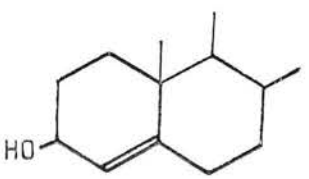


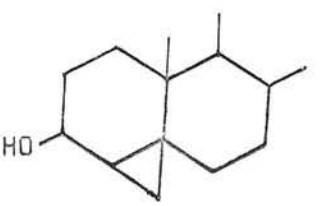
(75)



(76) (77) (78)



(79)



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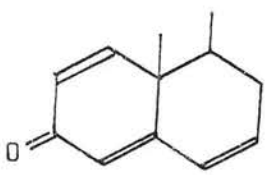

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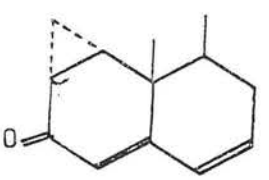


(82)

Hydrolysis of a 19-tosylate with a 5-6 double bond present affords a 5β,19-cyclosteroid (67).[54] This solvolysis reaction can be rationalised as proceeding through a rate controlled unimolecular reaction to a homoallylic bridged ion, which is stabilized by the distribution of the charge between C_{19} and C_6. Presence of a carbonyl group at the 3 position activates the methylene group C_2, and solvolysis of (68) results in formation of the cyclobutane system (69).[55] The presence of an 11-carbonyl activates position 9, and solvolysis of the 19 tosylate in this case affords a 9β,19-cyclo-steroid (70).[2]

A synthesis of a 12,14 cyclosteroid has been reported by dehydration of a tertiary hydroxyl at C_{14} with thionyl chloride.[56,57] The reaction of thionyl chloride with 3β,20-diacetoxy-14β-hydroxy-21-nor-5β-pregnan-15-one (71) gave the 12α,14α-cyclo compound (72) in 22% yield, by a concerted reaction represented in (73). The major product of this reaction was the spiroketone (74), formed through the carbonium ion (75) in 38% yield.

Chromous chloride reduction of 9α-bromo-$\Delta^{1,4}$-3-keto steroids (76) and their 1,2 dihydro analogues has been shown to give 5,9-cyclo steroids (77),[58] the reaction being a development of the olefin synthesis by reduction of halohydrins with chromous chloride. Reaction of these cyclosteroids with hydrochloric acid gives a trienone system (78).

Steroidal cyclopropyl ketones formed, not transannularly, but on the outside of the ring system by the addition of a

methylene group have been formed by two reactions. 4β,5-Methylene-cholestan-3β-ol (80) was obtained from cholest-4-en-3β-ol (79) in 62% yield by the action of the Simmons-Smith reagent,[10] methylene iodide and a zinc-copper couple. Similarly cholest-4-en-3α-ol gave the corresponding 4α,5-methylene compound. The second reaction involves the action of an ylide with an unsaturated ketone.[9] The trienone (81) reacts with dimethyl sulphoxonium methylide to give the 1α,2α-methylene derivative (82). Under the same conditions, a Δ4-3-ketone did not react, however 19-nor-Δ4-3-ketones react with the ylide to form a 4,5-methylene derivative.

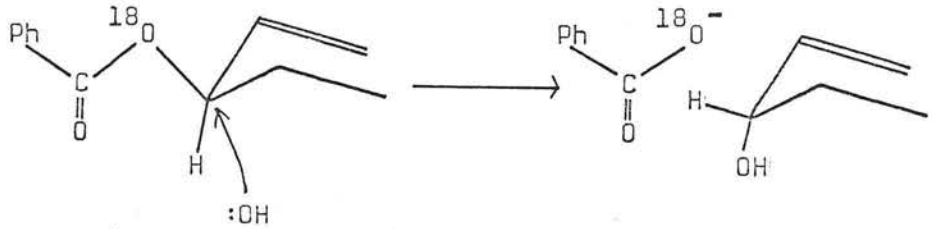
Table I

Starting Material	Product by Elimination	Yield %	Product by Substitution	Yield %
Cholestan-3β-ol benzoate	Cholest-2-ene	3	cholestan-3β-ol benzoate	70
			cholestan-3β-ol	10
cholestan-3α-ol benzoate	cholest-2-ene	13	cholestan-3α-ol benzoate	60
			cholestan-3α-ol	15
cholest-1-en-3β-ol benzoate	cholesta-1,3-diene	4.3	cholest-1-en-3β-ol benzoate	9
			cholest-1-en-3α-ol	34
			crystalline mixture	16
cholest-1-en-3α-ol benzoate			cholest-1-en-3β-ol	48
cholest-2-en-1β-ol benzoate	cholest-1,3-diene	10	cholest-2-en-1α-ol	22
			cholest-1-en-3-one	30
cholest-2-en-1α-ol benzoate	oil (cholesta-1,3-diene ?)	5	cholest-1-en-3-one	28
			cholestan-3β-ol	32
cholest-4-en-3β-ol benzoate	cholesta-3,5-diene	30	cholest-4-en-3β-ol benzoate	1
			cholest-4-en-3β-ol	28
			cholest-4-en-3α-ol	28
cholest-5-en-3β-ol benzoate	cholesta-3,5-diene	6	cholest-5-en-3β-ol benzoate	63
			cholest-5-en-3β-ol	13
			cholest-5-en-3α-ol	5
cholest-5-en-3α-ol benzoate	cholesta-3,5-diene	20	cholest-5-en-3α-ol benzoate	58
			cholest-5-en-3α- and 3β-ols	19
3α,5-cyclo-cholestan-6β-ol benzoate	3α,5-cyclo-cholest-6-ene	20	cholest-5-en-3β-ol	38
			3α,5-cyclo-cholestan-6β-ol benzoate	7
			3α,5-cyclo-cholestan-6β-ol	2
			3α,5-cyclo-cholestan-6α-ol	4

Substitution and Elimination on Alumina

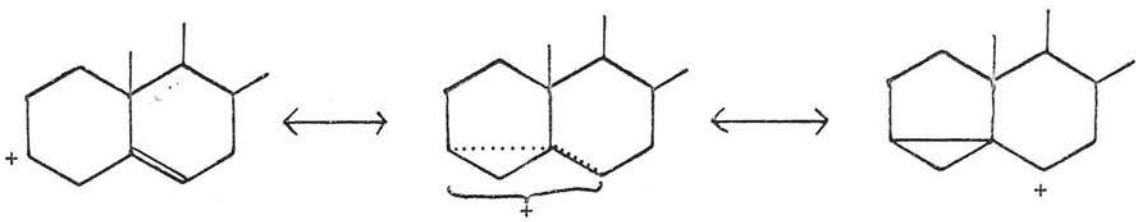
Hydrolysis of benzoate esters of both saturated and unsaturated steroid alcohols on activated alumina have been studied by Tamm.[59,60,61] These results are summarised in Table I. These however show largely elimination and substitution reactions without any change in the position of the original unsaturation. Similarly, no change in position of the oxygenated function is seen except changes in orientation. In only two cases is rearrangement seen. The first is the hydrolysis of cholest-2-en-1-ol benzoates to give cholest-1-en-3-one; the second is a reverse i-steroid transformation, similar to that already discussed.

Cholestan-3β-ol benzoate is recovered unchanged in 70% yield from alumina. Hydrolysis occurs to produce the free alcohol and to a certain extent elimination produces cholest-2-ene. Similarly cholestan-3α-ol benzoate is largely unchanged, although some hydrolysis and elimination do occur. Cholestan-3β-ol tosylate, on the other hand, is completely decomposed on alumina, to give cholest-2-ene 70% by elimination and cholestan-3α-ol by substitution.[62] The action of alumina on cholest-1-en-3β-ol benzoate in petrol is more complicated. Elimination of the ester group affords cholest-1,3-diene, which is also formed during pyrolysis of the same ester at 300°. The major product of hydrolysis is cholest-1-en-3α-ol. By ^{18}O labelling, it was shown[61] that this occurred by O-alkyl fission, producing the axial alcohol (83) free from labelled oxygen. In addition

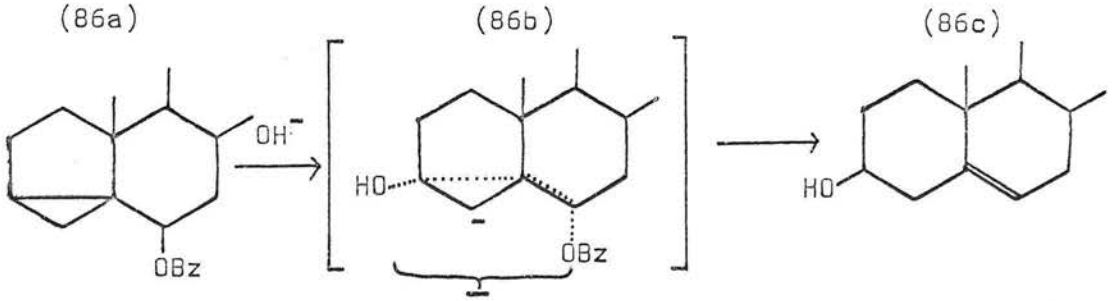


(83)

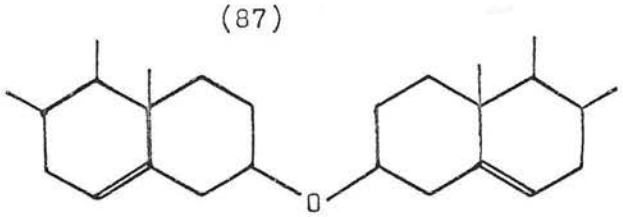
(84) (85)



(86a) (86b) (86c)



(87)



(88)

to starting material, a sharp melting crystalline product was isolated. This was a molecular compound of cholest-1-en-3α-ol (67%) and cholest-1-en-3β-ol (33%). Analagous treatment of 3β-chloro-cholest-1-ene with alumina gives the same mixture of allylic alcohols as substitution products, and cholest-1,3-diene as elimination product in equal amounts. Hydrolysis with inversion of configuration occurs in the reaction of cholest-1-en-3α-ol benzoate; the product being cholest-1-en-3β-ol.

Reaction of cholest-2-en-1β-ol benzoate with alumina affords three main products. Elimination gives cholesta-1,3-diene and substitution gives an alcohol, identified as cholest-2-en-1α-ol. The third product was cholest-1-en-3-one. Under the same conditions, cholest-2-en-1α-ol benzoate gives cholesta-1,3-diene, cholest-1-en-3-one and cholestan-3β-ol.

In the above cases, hydrolysis accompanied by inversion of configuration can be explained in terms of a bimolecular nucleophilic substitution [S_N2] reaction. However, the production of cholest-1-en-3-one suggests that in addition to this one step mechanism, there is a two step process, through a carbonium ion intermediate. These results could also be rationalised by an [S_N1] reaction with fixed ion pairs. In all instances in which allyl rearrangement is observed, the three substituent occurs in preference to the substituent at C_1, probably for steric reasons.

Cholest-4-en-3β-ol benzoate reacts on alumina to give equal proportions of cholesta-3,5-diene, cholest-4-en-3β-ol and cholest-

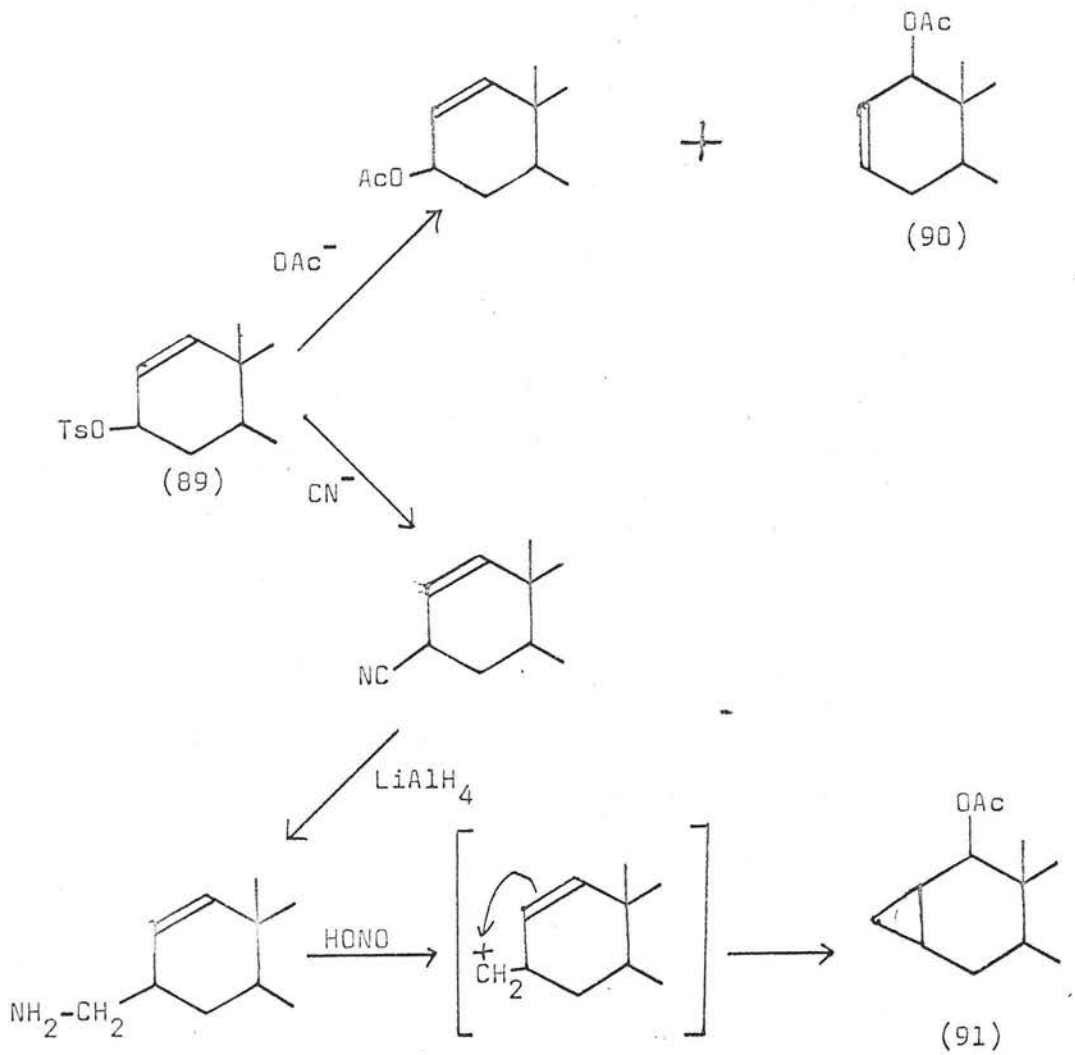
4-en-3α-ol, together with a small amount of unchanged cholest-4-en-3β-ol benzoate. It appears that this reaction occurs via a resonance stabilized carbonium ion (84). The products of hydrolysis of ^{18}O labelled cholest-4-en-3β-ol benzoate are free from ^{18}O, in agreement with this mechanism. Similarly, hydrolysis of 3α and 3β benzoyloxy cholest-5-enes proceed by [S_N1] mechanism through a carbonium ion (85). In this case there is, in addition to the expected products, small amounts (less than 5%) of 3α,5-cyclo-cholestan-6-ols.

The reaction on alumina of 3α,5-cyclo-cholestan-6β-ol benzoate produces by elimination 3α,5-cyclo-cholest-6-ene. The other major product is cholest-5-en-3β-ol. In addition small quantities of unreacted benzoate and 3α,5-cyclo-cholestan-6α- and 6β-ols were produced. On alumina there is a strong tendency for a resonance stabilised ion to be produced (86). While the existence of the free carbonium ion (86b) is probable, there is however the possibility of a [illegible] transitional intermediate arising by a concerted reaction (87). 3α,5-Cyclo-cholestan-6β-ol is rather more unstable on alumina, and produces 3α,5-cyclo-cholest-6-ene and cholesterol. In addition, however, a dicholesteryl ether is formed (88). This has previously been synthesised by dehydration of cholesterol with iodine.[63]

There is a considerable difference in the reactivity of benzoate and toluene-p-sulphonate esters of both saturated and unsaturated alcohols. The benzoate ester of cholestanol is recovered from alumina largely unchanged (70%), with 10% hydrolysis

and 3% elimination. The corresponding tosyl ester, however, affords on alumina, 70% elimination product, cholest-2-ene and 25% of the epimerised alcohol, cholestan-3α-ol. Similarly cholesteryl tosylate on alumina affords elimination products in 61% yield; under the same conditions, the corresponding benzoate yields 6% elimination products and 63% unreacted ester, as opposed to 1% unreacted tosylate. It is apparent therefore that the tosylate group forms an anion on attack by hydroxyl ion much more readily than does the benzoate group and will therefore very easily induce the formation of a carbonium ion.

Chart II



Two compounds are however missing from the series of ester reactions discussed above. The tosylates of cholest-1-en-3β-ol and cholest-4-en-3β-ol have not been synthesised. The initial object of this research was therefore to synthesize the former tosylate (89) and from it to obtain one-substituted steroids (e.g. 90,91) by the methods indicated in Chart II. The attempted syntheses and the resulting chemistry are discussed in the following pages.

EXPERIMENTAL RESULTS AND DISCUSSION

Cholest-1-en-3β-ol Toluene-p-sulphonate

Preparation of cholest-1-en-3β-ol.

Cholestan-3-one was prepared from cholesterol by catalytic reduction over a 10% palladium on charcoal catalyst using perchloric acid as promoter,[64] followed by oxidation of the resulting cholestan-3β-ol with 8N chromic acid (Jones reagent).[65] Addition of one mole of bromine to this saturated ketone afforded 2α-bromo-cholestan-3-one.[66,67] Dehydrobromination of this compound with lithium carbonate in refluxing dimethylformamide[68] [69,70] to give cholest-1-en-3-one, always produced some cholestan-3-one along with the allylic product. This could not be removed either by crystallisation or alumina chromatography. Careful purification of the bromoketone did not improve the quality of cholestenone, nor did variations in the conditions of dehydrobromination. The cholest-1-en-3-one was purified by reducing the mixture with lithium aluminium hydride to cholest-1-en-3β-ol and cholestan-3β-ol then oxidising the allylic alcohol selectively with manganese dioxide.[71] The cholest-1-en-3-one produced was isolated by crystallisation. An ultraviolet (u.v.) spectrum showed that the product did not contain any cholest-4-en-3-one.[69]

If 2α-bromo-cholestan-3-one is treated with semicarbazide hydrochloride in acetic acid, the product is cholest-1-en-3-one semicarbazone,[72] dehydrobromination occurring simultaneously

with semicarbazone formation. Cleavage of this compound should yield cholest-1-en-3-one in high purity. An exchange reaction between this semicarbazone and pyruvic acid[73] produced cholest-1-en-3-one on a small scale, but on increasing the quantity of steroid, no cholestenone could be isolated from the resulting tar. However cleavage of the semicarbazone with a 1:1 mixture of dioxan and 43% sulphuric acid[72] resulted in an 85% yield of cholest-1-en-3-one, the product being much cleaner than that from the pyruvic acid reaction. Unsuccessful attempts were made to introduce a 1,2-double bond by the acid catalysed reaction of dichloro-dicyano-benzoquinone on cholestanone. Using the same conditions, Ringold and Turner[74] report the preparation of 17β-hydroxy-5α-androst-1-en-3-one from the corresponding saturated compound. The difference in reaction between cholestan-3-one and 17β-hydroxyandrostan-3-one may well arise from the long range effects of the 17-hydroxyl grouping.

Reduction of cholest-1-en-3-one with lithium aluminium hydride proceeds stereospecifically to give cholest-1-en-3β-ol.[75]

Cholest-1-en-3β-ol Toluene-p-sulphonate.

In the normal procedure for making tosyl esters of steroid alcohols,[62,76] the alcohol and an equimolar amount of toluene-p-sulphonyl chloride are each dissolved in pyridine and the solutions mixed at 0°C. After 24 hours, ice is added, the precipitated steroid is extracted into ether and this solution

is washed free of acidic and basic material before removal of the solvent.

When this procedure was applied to cholest-1-en-3β-ol, a brown gum resulted which could not be crystallized. A proton magnetic resonance (p.m.r.) spectrum of this material, showed the presence of aromatic protons, which could be attributed to the presence of pyridine and p-substituted toluene, in addition to the normal methyl and methylene absorptions of a cholestane at τ 8.5 to 9.3. By comparison of the infra-red (i.r.) and p.m.r. spectra of this product with those of cholestan-3β-ol and cholest-5-en-3β-ol tosylates, prepared by the above method, it was apparent that this was not the expected tosylate. An attempt was made to purify this material by chromatography on alumina, however only part of this material was soluble in petrol, leaving a white amorphous solid. This was isolated and recrystallised from benzene. The petrol soluble fraction was adsorbed on alumina and elution with petrol afforded a hydrocarbon 'H', recrystallised from acetone. Further elution with petrol-benzene mixtures gave cholest-1-en-3β-ol. Continued elution with increasingly polar solvents afforded no material until a steroid was eluted with 5% methanol in chloroform. This steroid, 'S' was recrystallised from benzene and was found to be identical with the petrol-insoluble material isolated before chromatography. The reaction was reproducible, and in a typical case, reaction of cholest-1-en-3β-ol (2.0 g.) with toluene-p-sulphonyl chloride in pyridine afforded hydrocarbon

'H' (0.424 g.) 22%, cholest-1-en-3β-ol (0.617 g.) 33% and steroid 'S' (0.830 g.) 44%. The hydrolysis of tosylates on alumina to give olefins takes place easily.[62] In order to ensure that the compounds isolated were in fact reaction products and not artifacts brought about by reaction on alumina, or by the alkali on it, the crude reaction product in one case was chromatographed on Florisil. The products isolated were as before, and in the same ratios. A tosyl ester could therefore not have formed and then been hydrolysed during chromatography.

Dehydration of cholest-1-en-3β-ol with phosphorus oxychloride also gave, after alumina chromatography, hydrocarbon 'H' in 51% yield. Dehydration of 4,4-dimethylcholestan-3β-ol with phosphorus oxychloride in pyridine gives 4,4-dimethylcholest-2-ene (31%) and 3α-methyl-4-methylene-cholestane (10%); the same products were prepared by heating the corresponding tosylate in pyridine.[77]

Identification of Steroid 'S'

Steroid 'S' from the tosylation reaction, sparingly soluble in petrol, was recrystallised from benzene to give white plates, melting point 220-222°. The p.m.r. spectrum clearly showed nine aromatic protons between τ 0.7 and 3.0, together with two olefinic protons at τ 4.4, a methyl group at τ 7.68 and skeletal methyl groups at τ 9.09, 9.18 and 9.31. A sodium fusion test showed both nitrogen and sulphur to be present in the molecule; elemental analysis was consistent with the empirical formula $C_{39}H_{57}O_3SN$.

The infra red spectrum of steroid 'S' showed, in addition to a complicated pattern below 1300 $cm.^{-1}$, a broad hydroxyl band at 3450 $cm.^{-1}$. Treatment of this compound with benzoyl chloride in pyridine in an attempt to form a benzoate ester, produced material whose infra red spectrum showed similarities to that of a benzoate; the hydroxyl peak had diminished slightly in intensity. The product could not however be recrystallised, and, in an attempt to regenerate the parent hydroxy compound, it was treated with lithium aluminium hydride. The infra red spectrum of the gum produced did not bear much resemblance to that of steroid 'S'; the product was not identified. Treatment of steroid 'S' with an ethereal solution of diazomethane did not effect a methylation. The compound would therefore not appear to be either an alcohol or an acid.

Cholesteryl tosylate has been reported as reacting with

pyridine to form cholesteryl pyridinium tosylate.[79] Alkyl esters may be used as alkylating agents in the synthesis of quaternary pyridine compounds where the anion of a strong acid such as sulphate or toluene-p-sulphonate is formed. From the very polar nature of the compound, assessed from the difficulty of its elution from alumina, it would seem reasonable to suggest that steroid 'S' is in fact a salt of a quaternary pyridine compound, and it may be formulated as N-(cholest-1-en-3α-yl)-pyridinium tosylate. Generally when quaternisation takes place at an asymmetric centre, the configuration is inverted.[80] The p.m.r. spectrum of this compound agrees with the proposed structure. On treating the salt with sodium iodide in ethanol, a yellow precipitate of the corresponding pyridinium iodide is formed. The p.m.r. spectrum of this salt shows only five aromatic protons, τ 0.6 to 1.9 resulting from the pyridine nucleus.

Table II

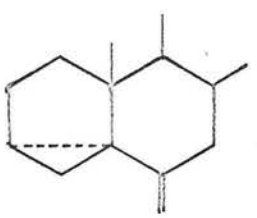
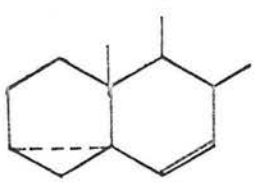
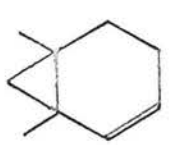
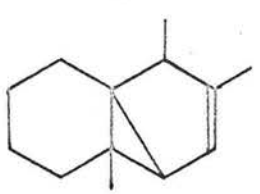
Cholestenes and Cholestadienes

	M.P.	$[\alpha]_D$	λ max	∈max
1. Cholesta-1,3-diene	60°	+73°	262	5,500
2. Cholesta-2,4-diene	68.5°	+169°	267	6,300
3. Cholesta-3,5-diene	80°	-123°	234	20,000
4. Cholesta-4,6-diene	92°	+4°	238	18,000
5. Cholesta-5,7-diene	89°	-127°	273	11,900
6. Cholest-1-ene	70°	+13°	<186	8,600*
7. Cholest-2-ene	75°	+70°	<186	5,900*
8. Cholest-3-ene	73°	+57°	<186	8,900*
9. Cholest-4-ene	83°	+76°	192	10,900
10. Cholest-5-ene	95°	-56°	190	8,400

(*= ∈ values for 190mμ)

Table III

Alkenylcyclopropanes: Ultra violet Spectra

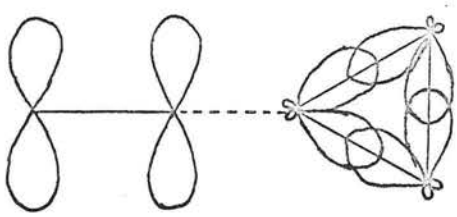
	λ max	∈max
	205	5,000
	197	12,200
	210	11,700
	210	6,000
	222	6,800

Identification of Hydrocarbon 'H'

The hydrocarbon 'H' was further purified by filtering in petrol through neutral alumina, activity I[81] and was then recrystallised from acetone. The hydrocarbon had a sharp melting point at 71-72°C, which was depressed on admixture with compounds 1,3,6 and 7 of Table II. The compound was homogeneous on gas-liquid chromatography. Gas-liquid chromatography of steroid hydrocarbons will be considered more fully in a later section. There were no strong absorption peaks in the infra red spectrum, confirming the conclusion drawn from the behaviour on alumina that no polar substituents were present. The strongest absorption in the ultra-violet was at 212 mμ (ε7,500); less intense maxima were at 237 mμ (ε3,500), 246 mμ (ε2,900) and 263 mμ (ε2,200).

The expected product of elimination of the ester group from cholest-1-en-3β-ol tosylate would be a diene, most probably cholesta-1,3-diene, in a reaction analagous to the alkaline hydrolysis on cholest-1-en-3β-ol benzoate.[59,60,61] However there is no absorption of sufficient intensity at the wavelengths predicted by Fieser,[82] 234 mμ or 263 mμ, for it to be either a heteroannular or a homoannular diene. Typical u.v. absorption maxima for dienes are quoted in Table 11.[82] It is mechanistically unlikely that mono-olefin would be formed in this reaction, and indeed the u.v. spectrum would preclude this.

It is possible, though unlikely, that cholesta-1,3-diene could be initially formed in this reaction, and under the same



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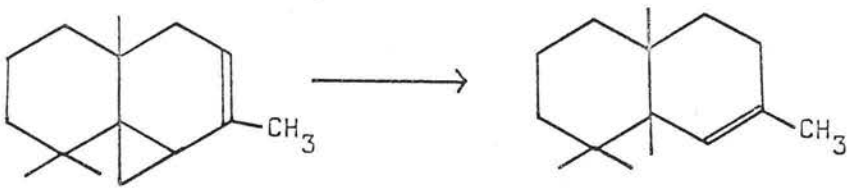

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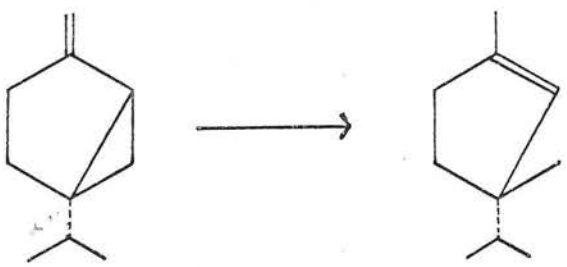

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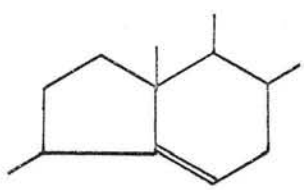

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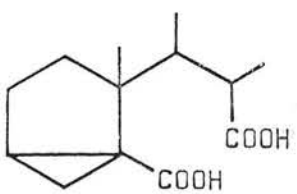

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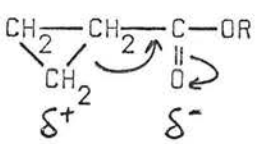


(97)

conditions could be rearranged to the hydrocarbon 'H'. Cholesta-1,3-diene, prepared by hydrolysis of cholest-1-en-3β-ol benzoate, was refluxed in pyridine with an excess of toluene-p-sulphonyl chloride; the diene was recovered quantitatively unchanged, and cannot therefore be a precursor of the hydrocarbon 'H'.

The ultra violet spectrum would point to an intermediate structure between that of an olefin and a diene. The u.v. absorption of an alkenylcyclopropane system is at approximately the same wavelength as hydrocarbon 'H' and is of the same order of magnitude. Isolated olefins generally have an absorption in the ultra violet at about 190 mμ corresponding to a $\pi \rightarrow \pi^*$ transition; increased conjugation moves the absorption into longer wavelengths. This bathochromic shift is observed with a cyclopropyl ring as an olefinic substituent, indicating that the ring has a system of weakly bound electrons similar in behaviour to the π-electrons of an ethylenic bond. The extent of conjugation between an olefin and a cyclopropane ring depends on the extent of overlap of the orbitals of the double bond and the sp^2 orbitals of the cyclopropane ring (92).[83] Maximum conjugation will therefore result if these π orbitals are in the plane of the ring, that is with the plane of the double bond at right angles to the ring. Table III summarises some ultra violet absorptions for steroid and terpenoid systems.

Thus the ultra violet spectrum identifies this hydrocarbon 'H' as having an alkenylcyclopropane system. In agreement with

this, a mass spectrum indicated a molecular weight of 368, that of a cholestadiene. On quantitative microhydrogenation,[84] with a palladium on charcoal catalyst, the hydrocarbon rapidly took up two moles of hydrogen and the product isolated was 5α-cholestane. This indicates that no skeletal rearrangements have occurred in the tosylation experiment. The ease of conjugate addition of hydrogen to a cyclopropyl olefin has been reported both in the terpene and steroid series;[85] the terpenes thujopsene (93) and sabinene (94) are both reduced smoothly, and under the same conditions 3,5-cyclo-cholest-6-ene is smoothly converted to cholestane. This conjugate addition of hydrogen is stereospecific,[85] which accounts for the non-formation of the A-nor compound (95) in the hydrogenation of 3α,5-cyclo-cholest-6-ene. In the case of 3α,5-cyclo-cholest-6-ene, cholest-5-ene is presumably formed, and is then reduced by a second mole of hydrogen to form cholestane. This two stage reduction no doubt occurs in the unknown hydrocarbon as well. In addition the geometry of the hydrocarbon must be such that cholestane, and not a nor-steroid with exocyclic methyl group is formed during the hydrogenation.

In order to ascertain the number and position of double bonds, hydrocarbon 'H' was oxidised. A specific reagent for the oxidative fission of olefinic double bonds has been developed by Lemieux and von Rudloff.[87] This reagent consists of aqueous sodium periodate and potassium permanganate under slightly

alkaline conditions, with a sixty fold excess of periodate over permanganate. The diol produced by permanganate oxidation of the olefin is cleaved by periodate, the intermediate aldehydes being further oxidised by permanganate to acids which, with ketones are the oxidation products. At the pH of the reaction (7.7) periodate also reoxidises Mn^{V} to Mn^{VII}. The reaction sequence has found uses in steroid chemistry despite difficulties in finding a suitable co-solvent.[88,89] This oxidation procedure was applied to the hydrocarbon 'H', and the product was a diacid, a conclusion arrived at by examination of a p.m.r. spectrum of the product of methylation of this acid with diazomethane. From the integral, there were clearly two methyl ester groups present at τ6.33. An infra red spectrum of the diester showed no carbonyl absorption associated with a ketone. It follows therefore that the olefinic double bond in the hydrocarbon was not more than disubstituted: it cannot be at a ring junction. Control experiments were carried out on cholest-1-ene and cholest-2-ene by the same reaction sequence, and the dimethyl esters of 1,2-seco-cholestan-1,2-dioic acid and 2,3-seco-cholestan-2,3-dioic acid were produced.

In order to establish finally the position of the double bond, the cyclopropane ring in the diacid produced above would have to be removed, and the resulting diacid identified. This however proved difficult since cyclopropane carboxylic acids are considerably more stable than other cyclopropyl derivatives.[90] Ladenburg, Chakravorty and Wallis[91] obtained a

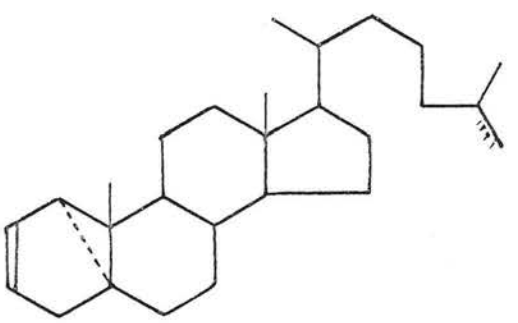
6,7-seco diacid (96) from the action of potassium hypobromite on 3α,5-cyclo-cholestan-6-one. In contrast to the parent ketone, this acid did not readily add bromine or hydrogen halides. From this they deduced that in this cyclopropyl carboxylic acid the cyclopropane ring showed greater stability. This diminished reactivity could be due to the electron withdrawing nature of the carboxyl group, drawing the loosely bonded electrons away from the ring (97).

In agreement with this decreased reactivity, catalytic reduction of the diacid derived from the hydrocarbon 'H' proved difficult. Attempted reduction of the dimethyl ester at atmospheric pressure and room temperature in glacial acetic acid with 10% palladium on charcoal or platinum oxide catalyst was unsuccessful; repeating the experiments at 100°C did not effect a reduction. Use of a 'high active' platinum catalyst[92] still did not reduce the methyl ester. Reduction of the dimethyl ester in benzene-methanol with Adams platinum oxide catalyst at room temperature and 90 atmospheres pressure of hydrogen for 22 hours did however proceed. The resulting product was chromatographed on alumina and elution with petrol-benzene gave a dimethyl ester, 85% of the product, with a similar infra red spectrum to that of the starting material. A p.m.r. spectrum no longer had a peak atτ 8.75 assigned to the C-19 methyl group attached to a cyclopropane ring (the assignment of this peak to the C-19 methyl group is discussed later). Examination of the reduction product on gas liquid

Table IV

Seco Cholestane Diacids

	M.P.	$[\alpha]_D$	G.L.C. Retention time
1,2-seco-cholestane-1,2-dioic acid	179°	-14°	
dimethyl ester	60°	-6°	2.66
2,3-seco-cholestane-2,3-dioic acid	196°	+31°	
dimethyl ester	60°	+23°	2.73
Dimethyl ester from hydrocarbon 'H'			
before reduction	73-75°	+46°	2.05
after reduction	59-60°	+22°	2.72



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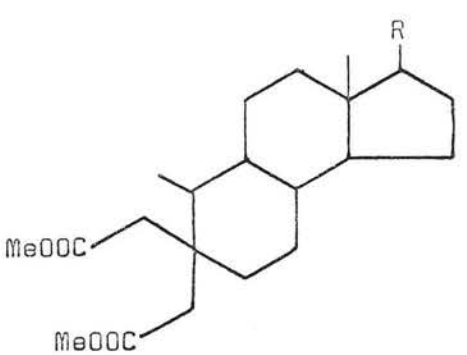


(99)

chromatography showed three main constituents, with the following retention times relative to cholestane: 1.56 (20%), 2.05 (20%) and 2.72 (60%). Under the same conditions 1,2-seco-cholestane-1,2-dioic acid dimethyl ester and 2,3-seco-cholestan-2,3-dioic acid dimethyl ester had retention times of 2.66 and 2.73 respectively. The material with retention time 2.05 is unreduced cyclopropyl diester. The reduction was repeated on a larger scale and after alumina chromatography a dimethyl ester was crystallised. This ester had melting point 59-60°C and specific rotation +22°. The i.r. and p.m.r. spectra were identical to those of authentic samples of the dimethyl ester of 2,3-seco-cholestane-2,3-dioic acid. The physical constants of various diacids are compared in table IV. The reduced product is therefore 2,3-seco-cholestane-2,3-dioic acid dimethyl ester.

It therefore follows that the hydrocarbon 'H' has an olefinic bond between C-2 and C-3. Reduction has shown that no migration of the C-19 angular methyl group has taken place and that the tetracyclic skeleton is unchanged. If the hydrocarbon is a conjugated alkenyl cyclopropane, as inferred from the ultra violet spectra and confirmed by reactions, then the only structure satisfying these conditions is that of 1α,5-cyclo-5α-cholest-2-ene (98).

The third component with retention time 1.56, of the reduction product of the diester derived from this hydrocarbon, can be tentatively given the structure (99) resulting from

fission of the 1-10 bond of the cyclopropyl diacid.

The infra red spectrum of hydrocarbon 'H' gives little information to confirm structure (98) mainly because, in spite of a great number of publications dealing with i.r. spectra of cyclopropyl compounds, the efficient use of i.r. in determining the presence of a cyclopropane ring in a molecule still remains open.[93]

The main absorptions in hydrocarbon 'H' are 3025, 1340, 1030, 965, 883, 775, 740 and 703 $cm.^{-1}$; all these bands are weak compared to those at 2940 $cm.^{-1}$ (strong) and 1380 $cm.^{-1}$ (medium) due to the cholestane skeleton. Absorption by cyclopropyl compounds in the 3000 to 3100 $cm.^{-1}$ region is due to the symmetrical vibration of the unsubstituted methylene groups in the ring.[83] In this case there is no such unsubstituted group present; the absorption at 3025 $cm.^{-1}$ must therefore be due to the ethylenic C-H stretching. The only consistent band in cyclopropyl compounds occurs around 1026 $cm.^{-1}$ due to ring deformation;[94-97] a medium intensity peak appears at 1030 $cm.^{-1}$ in hydrocarbon 'H'. It is not possible to assign specifically the remaining peaks in the spectrum.

The hydrocarbon 'H' gave a strong yellow colour with tetranitromethane in carbon tetrachloride solution. Since it has been reported[25,98] that cyclopropane rings give colourations with tetranitromethane, a quantitative relationship, based on that of Heilbronner[99] for differentiation between substituted olefins, is proposed, allowing the presence of a cyclopropane

ring to be detected by means of the intensity of the coloured complex formed with tetranitromethane.

Cyclopropane - Tetranitromethane Coloured Complexes

The coloured complex formed between tetranitromethane (TNM) and compounds containing an ethylenic double bond was first noticed by Ostromisslensky[100] in 1911. When a dilute solution of TNM in chloroform or carbon tetrachloride is added to a solution of an unsaturated compound, a yellow to red colour is produced. The simpler olefins and acetylenes give a yellow colour, the tetra-alkylated olefins and simple conjugated dienes give orange to light red colours and alkyl substituted dienes give a deep red colour. Aromatic unsaturation also gives a deep red complex with tetranitromethane. Even unreactive double bonds that do not react with bromine or undergo catalytic reduction give a colour with TNM, but α,β-unsaturated carbonyl compounds do not generally respond to the test. The test is also negative for allylic alcohols, acetates and ethers.

Since the cyclopropane ring is, in its chemistry, somewhat similar to a double bond, it might be expected to give a colour with TNM. In 1951, Barton[98] postulated the presence of a cyclopropane ring in the triterpene cycloartenol (9,19-cyclo-lanost-24-en-3β-ol). This terpene gave a strong yellow colour with TNM, and on catalytic reduction of the side-chain unsaturation to give cycloartanol a pale yellow colour was produced. At first this was attributed to a strongly sterically hindered ethylenic linkage, but this was precluded by the

reactions of cycloartanol. This compound failed to react with hot peracetic acid and it was resistant to oxidation with selenium dioxide and chromic acid. While an absorption maximum at 198 mμ in the ultra violet spectrum of cycloartenol was present, attributable to the isopropylidine group in the side-chain, on hydrogenation there was no uv absorption above 195 mμ. These facts were best explained by the presence of a cyclopropane ring in the molecule.

Shoppee[25] reports that carefully purified 3α,5-cyclocholestane, prepared from i-cholestanone, gives a pale but distinct yellow colour with TNM. It would therefore appear that the presence of a cyclopropane ring in a compound can, in the absence of any unsaturation, be inferred from TNM coloured complexes.

In 1953, Heilbronner[99] introduced a technique enabling double bond types to be differentiated by the colour of their TNM complexes. This involves measuring the wavelength of absorption of the TNM complex at a standard extinction coefficient, or extrapolation to this value. The extent of formation of the complex between cyclohexene and TNM in carbon tetrachloride can be shown experimentally to follow equation 1,

$$[C.TNM] = P\,[C]_o\,[TNM]_o \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 1$$

where [C.TNM] is the concentration of the complex in moles per litre, $[C]_o$ is the initial concentration of cyclohexene, $[TNM]_o$ is the initial concentration of TNM and P is a proportionality constant. This relationship is valid provided

$[C]_o$ = 0.0 to 0.1 m/l and $[TNM]_o$ = 0.0 to 3.0 m/l.

or $[C]_o$ = 0.0 to 3.0 m/l and $[TNM]_o$ = 0.0 to 0.1 m/l.

[C.TNM] is proportional to the optical density $D(\lambda)$ at the wavelengths where TNM shows no absorption of its own. For the absorption coefficient, $E(\lambda)$, of the complex C.TNM we have in the usual way,

$$E(\lambda) = \frac{D(\lambda)}{[C.TNM].l} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2$$

where l = path length. From equations 1 and 2,

$$\log E^*(\lambda) = \log E(\lambda) - \log P$$

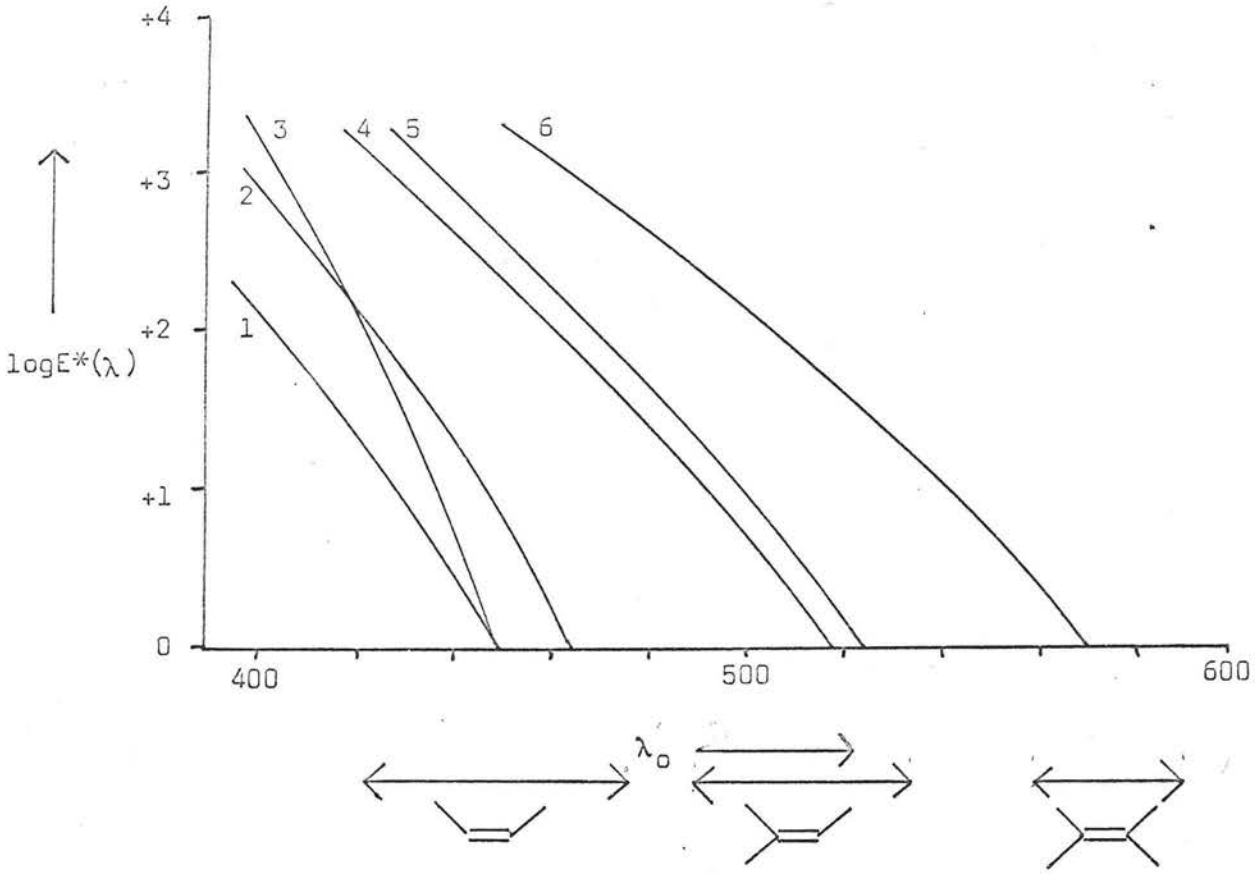
$$= \log D(\lambda) - \log l - \log [C]_o - \log [TNM]_o \quad \ldots 3$$

Log $E^*(\lambda)$ can therefore be calculated from equation 3.

Values of log $E^*(\lambda)$ are plotted against λ for a given complex and from this curve two quantities may be determined; λ_o is the wavelength where the curve cuts the axis of log $E^*(\lambda) = 0$ and $S(\lambda_o)$ is the gradient of the absorption curve from the point λ_o to log $E^*(\lambda) = 1$, in mμ.

Heilbronner plotted log $E^*(\lambda)$ against λ curves for olefins with 2,3 and 4 alkyl substituents, taking examples from cyclopentenes, cyclohexenes and a few cholestanes. From these he deduced that with an increase in the number of alkylated substituents, there is an increase in λ_o of about 56 mμ per alkyl substituent, to longer wavelength. This gives an indication of the possible position of olefinic substitution in an alicyclic ring system. Results for a series of steroid olefins are shown in Table V and figure I.

Figure I

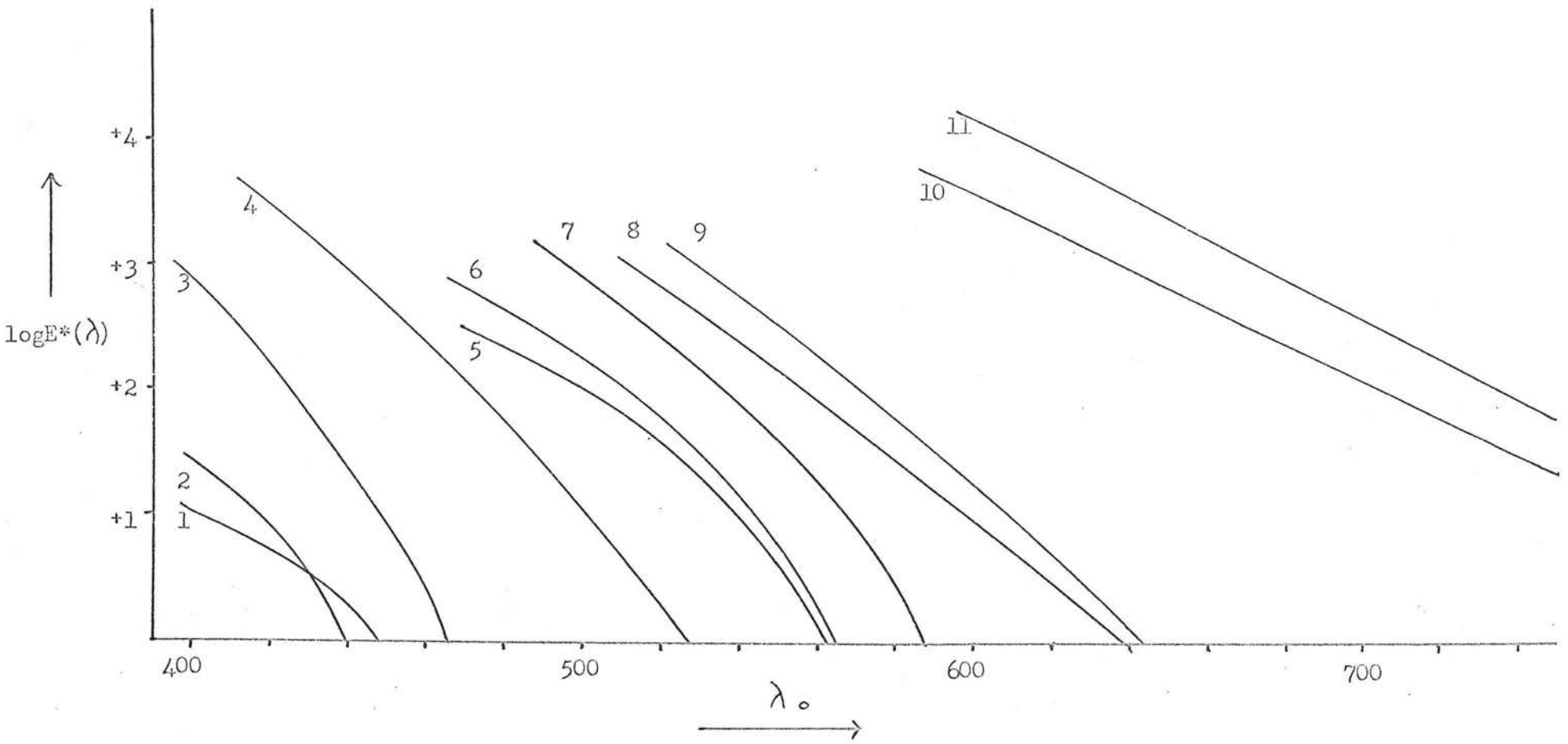


1 Cholest-1-ene
2 Cholest-2-ene
3 3-methylene-cholestane
4 3-methyl-cholest-2-ene
5 Cholest-5-ene
6 2,3-dimethyl-cholest-2-ene

Generally, the value of $S(\lambda_o)$ decreases with an increase in number of alkyl substituents,[99] although this was clear cut in the cases of cyclohexene and methyl and dimethyl cyclohexenes, some values for cholestenes did not fit this pattern. While in table V this trend is seen, the values for $S(\lambda_o)$ do not clearly fall into groups. It is however, possible to differentiate substituted steroid olefins by comparing the absorption maximum of the TNM complex at a standard extinction coefficient, (λ_o).

Since the intensity of absorption of a TNM complex of a diene is greater and at a higher wavelength, than that of a single double bond and also since the absorption of a cyclopropane complex is of lesser intensity than an olefin, it should be possible to evaluate the TNM complex absorption at standard extinction coefficient (λ_o) for a series of steroid hydrocarbons, given in order of increasing λ_o: cyclopropane, olefin, cyclopropyl olefin, diene, cyclopropyl diene, triene. Plots of log $E^*(\lambda)$ against λ are given in figure II for such a series of steroids. Values of λ_o for these compounds are given in table VI. Cholestane gave no absorption at all with TNM in carbon tetrachloride; the intensity of absorption for 3α,5-cyclocholestadiene and for cholestetriene are comparatively large, and the values of λ_o derived from figure II for these compounds are therefore approximate. The highest value of λ that could be recorded was 750 mμ, it was therefore not possible to obtain values of log $E^*(\lambda)$ below +2 in the latter cases.

Figure II



Curve numbers correspond to compounds in Table VI

Steroid Olefin - Tetranitromethane Complexes

Table V

		λ_o	$S(\lambda_o)$
Group II	Cholest-1-ene	450	-4.5×10^{-2}
(dialkyl)	Cholest-2-ene	466	-5.0×10^{-2}
	3-methylene-cholestane	452	-7.7×10^{-2}
Group III	Cholest-5-ene	528	-4.1×10^{-2}
(trialkyl)	3-methyl-cholest-2-ene	522	-3.6×10^{-2}
Group IV	2,3-dimethyl-cholest-2-ene	572	-3.2×10^{-2}
(tetra alkyl)		(values from figure I)	

Table VI

	λ_o	$S(\lambda_o)$
1. 1α,5-cyclo-cholestane	448	-2.3×10^{-2}
2. 3α,5-cyclo-cholestane	440	-4.8×10^{-2}
3. Cholest-2-ene	466	-5.0×10^{-2}
4. Cholest-5-ene	528	-4.1×10^{-2}
5. 1α,5-cyclo-cholest-2-ene ('H')	564	-4.6×10^{-2}
6. 17β-methoxy-1α,5-cyclo-androst-2-ene	566	-4.8×10^{-2}
7. 3α,5-cyclo-cholest-6-ene	588	-4.9×10^{-2}
8. Cholesta-1,3-diene	639	-2.7×10^{-2}
9. Cholesta-3,5-diene	645	-2.8×10^{-2}
10. 3α,5-cyclo-cholesta-6,8(14)-diene	835	
11. Cholesta-3,5,7-triene	860	
	(values from figure II)	

From figure II, it can be seen that the values of λ_o increase with increasing double bond character. λ_o is smallest for compounds containing only a cyclopropane ring, followed closely by compounds containing an ethylenic bond. The compounds containing a cyclopropane ring in conjugation with a double bond have values of λ_o greater than those for olefinic compounds, although some are in the region for tetrasubstituted olefins. λ_o for dienes is somewhat above the olefin values, being around 650 mμ. The values for trienes and cyclopropyl dienes are too inaccurate to be considered, but appear to follow the general trend. It is probable that differing amounts of alkyl substitution influences the value of λ_o in all these groups as it does with the mono-olefins. 1α,5-Cyclo-cholestane is more alkylated (three substituents) than the 3α,5-cyclo-cholestane (two alkyl substituents) and has a slightly higher value of λ_o. Likewise, 3α,5-cyclo-cholest-6-ene is more alkylated (three substituents) than 1α,5-cyclo-cholest-2-ene (two alkyl substituents) and has a higher λ_o value; cholesta-3,5-diene is more alkylated (three substituents) and has a higher λ_o than cholesta-1,3-diene (two alkyl substituents).

Obviously for the full effect of the alkyl substituents to be rationalised, considerably more compounds must be examined. Similarly, the effects of conjugation between participating groups on the tetranitromethane complex absorption would have to be looked at further.

It appears therefore, that the examination of TNM complexes

of cyclopropyl steroids can offer a clue to their identity. While a number of other functional groups are reported[101] to give coloured complexes with TNM, the ether and ester groups do not and therefore compounds containing these can be examined and compared with other possibly unsubstituted olefins.

In the case of hydrocarbon 'H', the results substantiate the conclusion that it is an alkenyl cyclopropane derivative.

Synthesis of Compounds in Tables V and VI.

Syntheses of the 1α,5-cyclo steroids, hydrocarbon 'H' and its derivatives are described elsewhere in this thesis. 3α,5-Cyclo-cholest-6-ene was prepared by acetolysis of cholesteryl tosylate[19] followed by alumina chromatography. The catalytic reduction of this to give 3α,5-cyclo-cholestane will be described later. Cholesta-1,3-diene was prepared by hydrolysis of cholest-1-en-3β-ol benzoate on alumina,[59] and cholesta-3,5-diene by dehydration of cholesterol.[102] 3α,5-Cyclo-cholesta-6,8(14)-diene was prepared by refluxing 7-dehydro-cholesterol in pyridine with toluene-p-sulphonyl chloride, purified by filtering the product through grade I alumina.[34,35,36] Cholesta-3,5,7-triene was synthesised by hydrolysis of 7-dehydro-cholesterol benzoate on alkaline alumina.[103]

Proton Magnetic Resonance Spectrum of Hydrocarbon 'H'

The p.m.r. spectrum at 60mc/s of the hydrocarbon had a peak corresponding to two olefinic protons at τ 4.36 with a shoulder

at τ 4.40. The following peaks occurred at higher field: τ 8.21, 8.75, 9.10, 9.19, 9.25 and 9.35. There was no absorption in the region of τ 9.75, often characteristic of the methylene group of a cyclopropane ring. The only cyclopropyl proton, on carbon 1, would however be expected to be deshielded slightly because of the conjugation effects of the 2,3-double bond. It would therefore resonate at a frequency within the methylene envelope. The usual absorption associated with tertiary allylic cyclopropyl protons is τ 8.50 to 8.75.[104]

It is probable that the absorption at τ 8.75 is due to the angular methyl group on C-10. From the proposed formula for this hydrocarbon (98), the C-19 methyl group will be on a cyclopropane ring, which is in conjugation with a double bond. The effect of this would be to deshield the methyl protons, causing them to resonate at a lower field. Because the various methyl groups in the cholestane side chain give rise to three superimposed doublets, normally absorbing at τ 9.05 to 9.25,[105] it is difficult to differentiate between these and the C-19 methyl group. By repeating the attempted tosylation on a Δ^1-3β-alcohol with no alkyl side chain, it should be possible to identify the absorption of the C-18 and C-19 angular methyl groups. The tosylation experiment was therefore carried out on androst-1-en-3β,17β-diol, androst-1-en-3β-ol, and 17β-methoxy-androst-1-en-3β-ol.

Synthesis of Starting Materials

Androst-1-ene-3β,17β-diol.

Testosterone (17β-hydroxyandrost-4-en-3-one) was reduced with hydrogen over a palladium on charcoal catalyst.[106] The product was crystallised from acetone to give 17β-hydroxy-5α-androstan-3-one (45%), m.p. 177-180°C. A second crop melted at 110-142° and gave two equal peaks on g.l.c. Separation of these two C-5-isomers through their semicarbazones was not successful. Stereospecific reduction of testosterone was achieved using lithium in liquid ammonia;[107] the product had m.p. 197-199°, 93% yield. This ketone was brominated in acetic acid[108] to give 2α-bromo-17β-hydroxyandrostan-3-one. Dehydrobromination[68-70,109] with lithium carbonate in refluxing dimethylformamide gave a mixture of saturated and unsaturated ketones. In contrast to the corresponding mixture of cholestane ketones, these were easily separated by alumina chromatography, to give 17β-hydroxyandrost-1-en-3-one (55%) and the saturated ketone (20%). Reduction of this enone with lithium aluminium hydride proceeded stereospecifically to give androst-1-ene-3β,17β-diol.

Androst-1-en-3β-ol.

The carbonyl group at C-17 on 3β-hydroxyandrost-5-en-17-one was reduced to methylene by the method of Huang-Minlong;[110] Cram's room-temperature modification of this reaction was not successful in this case.[111] The resulting androst-5-en-3β-ol was catalytically reduced using the same conditions as for cholesterol,[64]

and a stereospecific product androstan-3β-ol, was obtained. Chromic acid oxidation[65] of the resulting alcohol gave the 3-ketone.[112,113] Androst-1-en-3β-ol was synthesised from this by the same reaction sequence as above.

17β-Methoxy-androst-1-en-3β-ol.

In order to give slightly more polarity to the androst-1-en-3β-ol molecule, to facilitate chromatography of reaction products discussed later, attempts were made to introduce an unreactive substituent at C_{17}, by protecting the hydroxyl group. Attempts to introduce either a trityl ether[114] or a tetrahydropyranyl ether[115] failed. No reaction was detected after attempts to methylate the C_{17} alcohol with diazomethane,[116] alkaline dimethyl sulphate[117] or methyl iodide and sodium hydride.[118] 17β-Hydroxyandrostan-3-one was however methylated with methyl iodide and silver oxide.[119,120] Preparation of 17β-methoxy-androst-1-en-3β-ol from this was carried out by the same procedure as above.

Tosylation of 17-Substituted Androst-1-en-3β-ols

17β-Hydroxyandrost-1-en-3β-ol was treated with toluene-p-sulphonyl chloride in pyridine at 0° as in the previous cholestane experiments; the product isolated was a brown gum. Chromatography of this on alumina afforded two products. The first, non-crystalline, eluted with benzene appeared from its p.m.r. spectrum to be a monotosylate - probably esterified at the 17-hydroxyl group. There were three sharp signals in the p.m.r.

spectrum identified as three methyl groups: τ 7.56 (aryl methyl group of the tosyl ester), 8.74 (C-19 methyl group) and 9.20 (C-18 methyl group). The second product, eluted with 5% methanol in chloroform, would not crystallise and from spectra could be a 17-tosylate with a pyridinium tosylate grouping at C_3. Obviously the unknown effects of the 17-tosylate complicate this experiment and it would be simpler with no 17-substituent. Androstan-17β-ol tosylate is in fact eliminated by alkaline hydrolysis on alumina to give androst-16-ene. The product from this reaction would therefore be expected to be a hydrocarbon.

Androst-1-en-3β-ol, on attempted tosylation by the usual method, gave a pale brown gum, which was chromatographed on alumina. Elution with petrol gave a clear glass and from an infra red spectrum, this appeared to be a hydrocarbon. A p.m.r. spectrum showed three methyl groups: τ 8.75, 9.23 and 9.30, the 9.30 peak being of rather greater intensity than the other two. There were several small broad peaks in the olefinic proton region, 3.5 to 4τ. No further material was eluted, until with 5% methanol in chloroform, a brown material, assumed from spectra to be a pyridinium salt, was eluted.

A u.v. spectrum of the hydrocarbon fraction had maxima at 212 mμ (ε 6,300) and 261 mμ (ε 4,100). Analytical g.l.c. showed that this hydrocarbon had two components, whose retention times relative to 5α-androstane, were 0.89 (60%) and 1.14 (40%). Reduction of this mixture over palladium on charcoal catalyst at 35°C gave a glass whose p.m.r. spectrum and g.l.c. retention

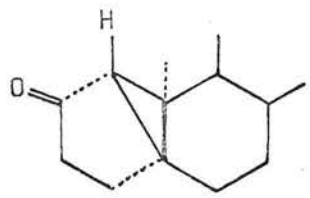
time were identical with those of 5α-androstane. It was not possible to separate the hydrocarbon mixture on an alumina column. A synthesis of androsta-1,3-diene by hydrolysis of androst-1-en-3β-ol benzoate on alumina was carried out, and this had retention time 1.13 on g.l.c., under the same conditions as those for the constituent hydrocarbons. The two hydrocarbons were separated on a small scale by preparative gas chromatography, sufficient material was collected of both components to enable u.v. and p.m.r. spectra to be recorded, the latter using a computer for averaging transients. The less polar material had λ max 212 mμ (ϵ7,100) in the u.v. and its p.m.r. spectrum showed two equal peaks at τ 8.74 and 9.30, the C-19 and C-18 methyl groups respectively. The spectrum was not sufficiently intense to enable any olefin protons to be observed. The second component had a u.v. maximum at 262 mμ (ϵ6,100). This is in agreement with Fieser's rules for a homoannular diene, and is at the same wavelength as the maximum of cholesta-1,3-diene.

The separation of these two hydrocarbons on alumina should be made possible by incorporation of a more polar yet unreactive substituent in the molecule. A protecting group on the C_{17} hydroxyl, which is unreactive under the conditions of these tosylations, should add sufficiently to the polarity of the molecule. As previously recorded, attempts to make trityl and tetrahydropyranyl ethers were unsuccessful, but a methyl ether was prepared. 17β-Methoxyandrost-1-en-3β-ol treated with toluene-p-sulphonyl chloride in pyridine at 0°C, produced the

usual brown glass on work-up. On thin layer chromatography this material showed eight spots. The least polar spot (R_f 0.68), representing about 75% of the total material, may be a hydrocarbon with a 17-methoxyl group. Other spots on the t.l.c. were at R_f 0.59, 0.47, 0.38, 0.28 and 0.18; the spot R_f 0.28 has the same R_f as the starting material, and the spot R_f 0.18 is probably androst-1-en-3β,17β-diol which was possibly present in the starting material. Chromatography on alumina of the crude product afforded, on elution with petrol, a clear glass (80%), whose i.r. spectrum showed it to have a methoxyl group; no other strong absorption was present. On g.l.c. this hydrocarbon was found to have two constituents. Further elution gave traces of material which were not identified.

Rechromatography of the hydrocarbon fraction on alumina afforded, on careful elution with small volumes of petrol, two hydrocarbons; the less polar material (70%) had a maximum in its ultraviolet spectrum at 212 mμ (ϵ 7,000), showing it to be an alkenylcyclopropane compound, as previously isolated. The second, more polar component (30%), had λ max 264 mμ (ϵ 5,600); this material would therefore appear to be a 1,3-diene. Neither fraction was crystallisable.

A p.m.r. spectrum of the methoxy hydrocarbon showed it to have three main peaks; τ 6.70 (methyl group of the ether), 8.75 and 9.25 (C-19 and C-18 methyl groups). A peak corresponding to two olefinic protons was present at 4.36τ, and had the same shape as that of the hydrocarbon 'H' in the cholestane experiment. This material was reduced over a palladium on charcoal



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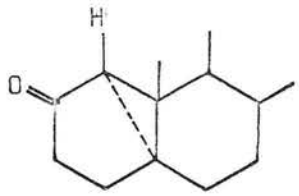

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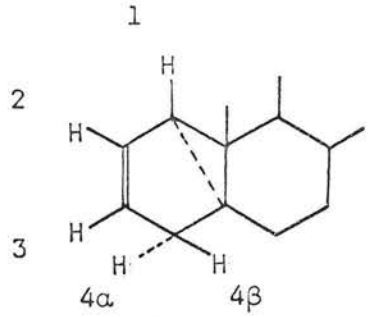

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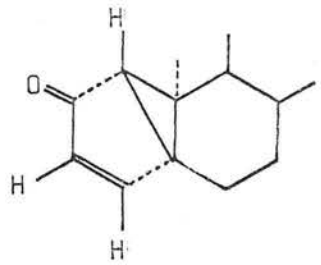

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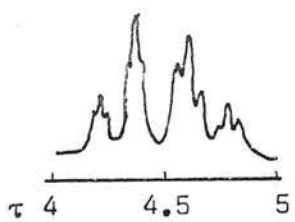

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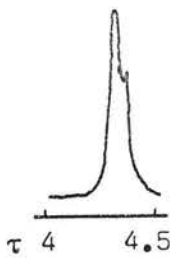

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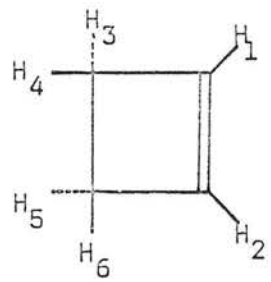


(106)

catalyst, taking up two moles of hydrogen to give 17β-methoxy-5α-androstane.

From these three experiments, it can be concluded that androst-1-en-3β-ol and its 17β-methoxyl and hydroxyl derivatives, on treatment with toluene-p-sulphonyl chloride in pyridine, give an alkenylcyclopropane derivative, as in the case of cholest-1-en-3β-ol. In each of the androstane experiments the p.m.r. spectrum has shown the C-19 methyl group to be at or near τ 8.75. It would therefore appear that the peak at this value in the spectrum of the cholestane hydrocarbon 'H' is due to the 19-methyl group.

The identification of the τ 8.75 peak with the C-19 methyl group - a methyl group on a cyclopropane ring - is further justified by comparison with p.m.r. spectra of other cyclopropyl steroids and terpenes. Lumicholestenone (100)[11] is reported to have 'significant singlets' at τ 8.50 and 8.84. The former (1 proton) was assigned to the lone cyclopropyl proton on C_1 and the latter (3 protons) to the 19-methyl group. Lumicholestenone was prepared by the same procedure as that of Shoulders,[11] and the resulting product had identical m.p., u.v. and i.r. spectra to the reported photoisomer. A p.m.r. spectrum, however, while showing a sharp signal at τ 8.83, had no outstanding absorption above τ 8. A broad multiplet, centered at τ 7.91 was presumably due to the protons on C_3, α to the carbonyl, since this feature disappeared on deuteration. It was not possible

to identify the cyclopropyl proton on this spectrum, run under the same conditions as those of the 1α,5-cyclo steroids prepared above. A similar product (100), obtained by irradiation of testosterone acetate,[51] had a p.m.r. absorption for the C_{19} methyl group at τ 8.82. No position was given for the C_1 cyclopropyl proton in this case.

The p.m.r. spectrum of the photoproduct (101) from 10α-testosterone acetate has a peak at τ 8.85 ascribed to the C-19 methyl group, again no assignment was made for the C-1 cyclopropyl proton.[52]

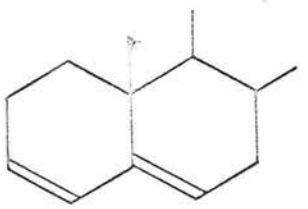
In normal 5α-steroids, the effect of introducing a 2-3 double bond on the C-19 methyl group proton resonance is nil; the effect of introducing a C-2 carbonyl group is to cause the resonance of the C-19 methyl group to move upfield by 1.5 c.p.s.[121] It is probable that the shielding effect on the C-19 methyl group in the case of this 1α,5-cyclosteroid would be similar, and therefore the C-19 methyl group resonance of hydrocarbon 'H' should be further downfield than these examples. Its occurrence at τ 8.75 would substantiate this.

From an initial consideration of the structure of hydrocarbon 'H' (102) the olefinic protons should appear as a far more complicated multiplet than they in fact are, because of coupling with the cyclopropyl proton, with the two protons on C-4, and with each other. The coupling with the cyclopropyl proton will be very small, probably in the order of 1 c.p.s. The coupling between the cyclopropyl proton and the olefinic protons of (103), a photoproduct from dehydrotestosterone acetate, is less than

1 c.p.s.[52]

The p.m.r. spectra of the various A-ring cholestenes have been examined by Meakins et al.[122] Apart from cholest-2-ene, the olefinic signals can be interpreted and the individual peaks assigned from the relative sizes of the coupling constants between the olefinic protons and protons on carbon atoms adjacent to the double bond, and their dependance on the dihedral angle of the protons concerned. In these spectra, the olefinic protons appear as complex multiplets, for example cholest-1-ene (104), but in the spectrum of cholest-2-ene (105) only one peak (τ 4.34) with a shoulder (τ 4.39) is seen. This latter spectrum is very similar to that of hydrocarbon 'H'. From the spectrum of cholest-2-ene, it would appear that the vicinal couplings are very weak, but the simplicity of this spectrum may have a complicated origin, like that of cyclobutene (106), which has two sharp peaks at τ 4.05 and 7.43.[123] The simplicity of this spectrum has led to the assertion that the coupling between the allylic and olefinic protons is zero. By analysis of the ^{13}C satellite spectra, Borcic and Roberts[123] have deduced coupling constants, $J_{1,3} = -0.80$ c.p.s. and $J_{1,5} = +1.55$ c.p.s. for cyclopentene; the vinylic coupling constant $J_{1,2}$ is -2.70 c.p.s. The various coupling constants calculated indicate that cyclobutene should have the two sharp peaks observed.

The situation in cholest-2-ene and in 1α,5-cyclo-cholest-2-ene may well be very similar. In the cyclo-steroid it would appear that the various coupling constants are small, and in order that a comparatively simple spectrum may result, the signs of these constants must be such that they will largely nullify each other.



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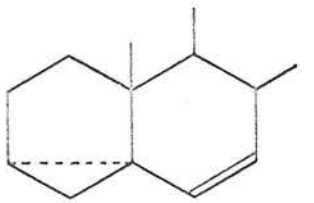

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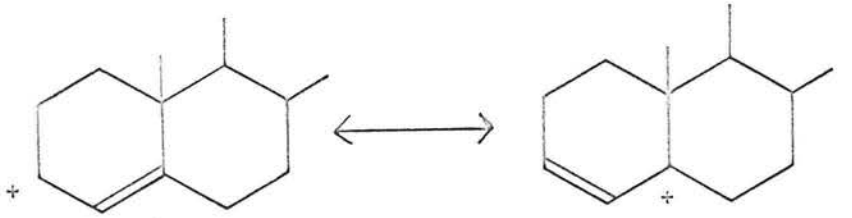

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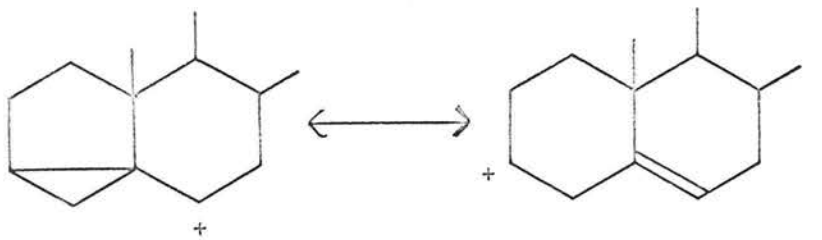


(111) (112)

Cholest-4-en-3β-ol Toluene-p-Sulphonate

Preparation of Cholest-4-en-3β-ol

Cholest-4-en-3-one was prepared from cholesterol by the four-stage route of Fieser.[124] Reduction of this ketone with lithium aluminium hydride gave cholest-4-en-3β-ol, which, after two crystallisations from methanol - acetone, had m.p. 130-132° and $[\alpha]_D$ +46°, (72% yield).[125] There was no evidence of the 1:1 molecular compound of cholest-4-en-3α-ol and cholest-4-en-3β-ol reported by McKennis and Gaffney,[126] m.p. 141, $[\alpha]_D$ +85°, from this solvent.

Cholest-4-en-3β-ol Tosylate

In view of the non-formation of cholest-1-en-3β-ol tosylate, it is to be expected that cholest-4-en-3β-ol will not form a tosylate either. Attempted synthesis, by mixing pyridine solutions of equimolar quantities of steroid and toluene-p-sulphonyl chloride at 0°C and standing for 24 hours at room temperature, did not produce an ester. Chromatography of the brown reaction product on alumina gave three products as in the case of cholest-1-en-3β-ol. Elution with petrol gave a hydrocarbon (27%), with petrol-benzene gave unchanged cholest-4-en-3β-ol (44%) and as before, elution with 5% methanol in chloroform gave a brown gum (28%). The hydrocarbon was crystallised from acetone and examination of its u.v., i.r., and p.m.r. spectra proved it to be cholesta-3,5-diene (107). This was confirmed by its melting point; a mixed melting point with an authentic

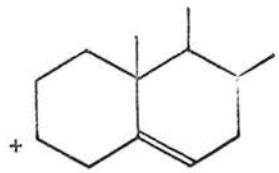
sample[102] showed no depression. While the crystalline material was homogeneous on g.l.c., examination of the mother liquors from the crystallisation showed the presence of two hydrocarbons, the minor component being 12% of the original reaction mixture. Comparison of the retention times indicated that this minor product was 3α,5-cyclo-cholest-6-ene (108). An i.r. spectrum of material collected after gas chromatography was identical with that of an authentic sample.[19]

The highly polar material from the alumina column was recrystallised from benzene. Its i.r. and p.m.r. spectra were very similar to those of the salt from the cholest-1-en-3β-ol reaction. It can therefore be concluded that this material is N-(cholest-4-en-3α-yl)-pyridinium tosylate.

Solvolysis of the tosylate of cholest-4-en-3β-ol, if this is momentarily formed, or elimination of hydroxyl ion from the alcohol, would lead to the carbonium ion (109). After initial ionisation, it is probable that this would rearrange to the more stable ion (110). Loss of a proton from these ions would lead to formation of cholesta-2,4-diene and cholesta-3,5-diene, respectively. Since no homoannular diene is isolated from the reaction, the ion (109) must rearrange rapidly to (110) and not itself lose a proton.

The postulation of these two ions in the formation of cholesta-3,5-diene does not, however, account for the formation of a cyclosteroid and indeed it is difficult to postulate the formation of an ion involving carbon-6 (e.g. 111) from either

(109) or (110) by a movement of electrons. The formation of the cyclosteroid (108) in 3% yield may arise from an initial shift of the double bond from Δ^4 to Δ^5. The ion (112) could be formed, either from the alcohol before ionisation or from the ion (109) by movement of a hydrogen atom. The cyclopropyl ion (111) can arise from (112) by an electron shift from the 5,6-double bond. Loss of a proton from (112) gives rise to cholesta-3,5-diene; from (111) the same process gives rise to 3α,5-cyclo-5α-cholest-6-ene.



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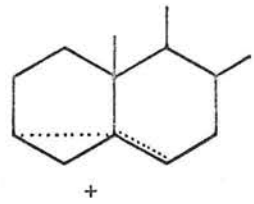

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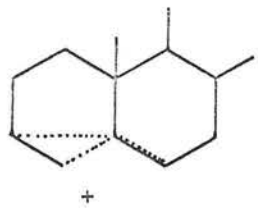

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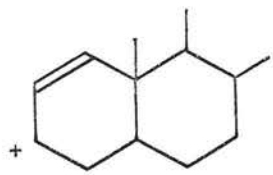

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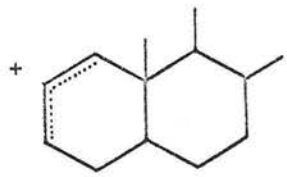

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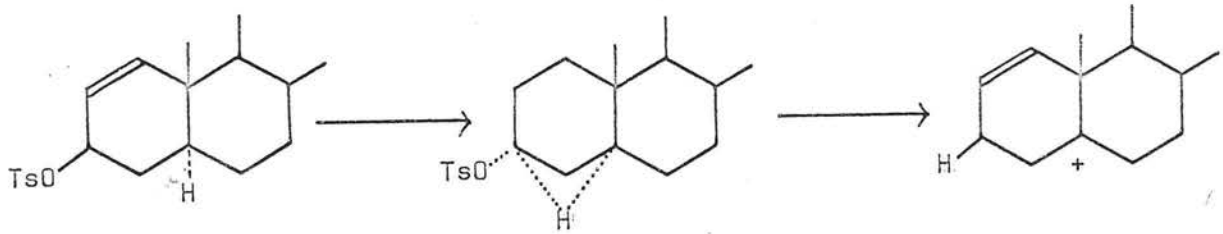

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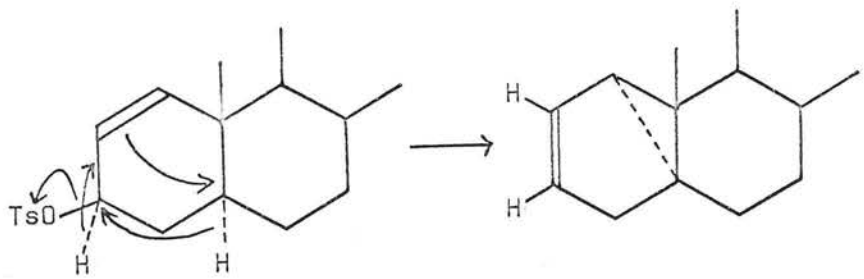


(120) (98)

Mechanism of Formation of 1α,5-cyclo-5α-cholest-2-ene

In the i-steroid transformation, the rate controlling step is unimolecular, and therefore probably consists of ionisation initially at C_3 (113), however, delocalisation of the π electrons give an enhanced rate of ionisation, and a non-classical carbonium ion (114) results.[26] This intermediate ion may well involve delocalisation of the 4,5-bond also (115).[29] Nucleophilic attack on these ions (114,115) gives rise to i-cholesteryl or cholesteryl derivatives as previously described.

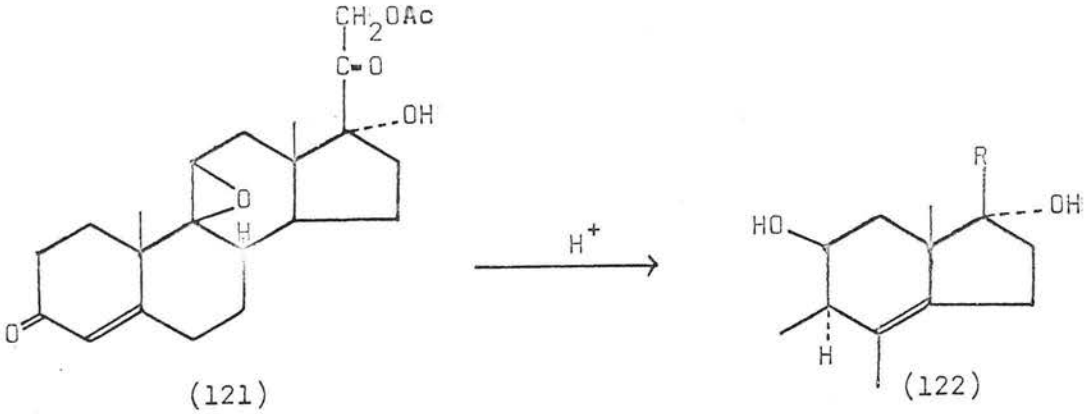
The initial step in the formation of 1α,5-cyclo-cholest-2-ene will be formation of the carbonium ion at C_3 (116). It is however difficult to determine whether or not a tosyl ester is formed briefly during this reaction or not. It would seem probable that it does in fact form, since merely refluxing cholest-1-en-3β-ol in pyridine is not sufficient to cause any reaction. The base strength of pyridine (pK 5.34) must be sufficiently great to cause solvolysis of the tosyl ester as soon as it is formed. Attempts to synthesise cholest-1-en-3β-ol tosylate using a slightly weaker base, o-toluidine (pK 4.39) and using acetone with sodium bicarbonate suspended in it, as solvents also failed. o-Toluidine could still be too strong a base for the tosylate to be stable in since the 1,5-cyclo steroid was formed as before. The unsaturated alcohol was recovered unchanged from the acetone experiment, probably not suffiently basic to form the tosylate.

Assuming that the tosylate forms momentarily, elimination

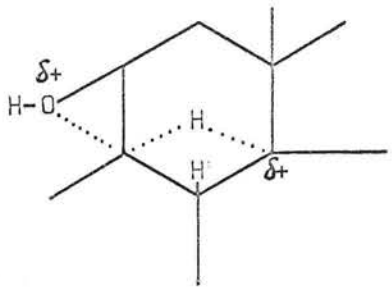
of the ester group will form a carbonium ion, whose positive charge will be located at C-3 (116). Simultaneously with this ionisation, participation of the π electrons of the 1,2 double bond would be expected, giving a non-classical carbonium ion (117) where the charge is spread over carbon atoms 1,2, and 3. Loss of a proton from either this ion (117) or from the ion (116) would result in the expected product, cholesta-1,3-diene (118). While no 1,3-diene was isolated in the cholestane experiments, the reaction with androst-1-en-3β-ol afforded 40% diene and with a 17β-methoxyl substituent, 30% diene was isolated.

Formation of the 1α,5-cyclo-steroid must involve the participation of the tertiary carbon atom C_5 in a reaction intermediate. Such a tertiary carbonium ion as (118) would be more stable than (116), but its formation from (116) with no participation of the π electrons of the double bond is difficult to imagine. It may, however, be possible to form the ion (118) by a transannular hydrogen shift from C-5 to C-3 (119). It seems more likely that there may not be any formation of a carbonium ion. The non-participation of the π electrons of the double bond in this process could be explained by considering the entire reaction to proceed through a concerted mechanism, illustrated as (120). On loss of the tosyl group, the 5α-proton moves to the C-3 position, the π electrons of the double bond form the new transannular 1-5 bond and the electron pair from the original C_3α-bond form the new π bond.

This entire reaction sequence, involving four almost



(121) (122)



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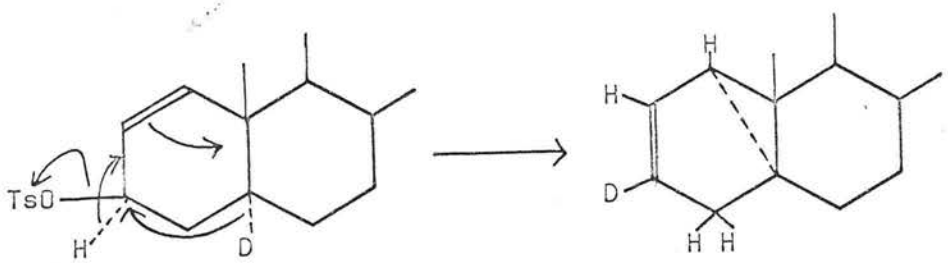


(124) (125)

(126) (127)

simultaneous processes, will have occurred in the time taken to produce an ion such as (117), which will itself have a finite lifetime because of solvation. The preferred formation of a cyclosteroid to a diene, arises therefore because of this comparatively greater reaction rate. It is rather more difficult to account for the formation of diene in the androstane reactions; it can only be assumed that there are long range effects of the C-17 substituents. Absence of the cholestane side chain could have a considerable effect on the ease of solvation of a steroid, rendering any ionic structures more stable.

Transannular hydrogen transfer in medium sized rings (rings with 8-12 members) was first reported in 1952[127] and four years later, a transannular transfer of hydrogen in a steroid was observed.[128,129] Treatment of the 9β,11β-epoxy pregnane (121) with 60% perchloric acid gives, as the major product, 17α-hydroxy$\Delta^{8(14)}$-dehydrocorticosterone-21-acetate (122).[129] It is sterically unlikely for the C-8β-proton to move from C-8 to C-9 in a 1,2-Wagner shift, since there is an adverse cis relationship in moving the C-8 proton and the C-9 oxygen departing; the product of Wagner shift would in fact be the C-9 epimer of (122). It was therefore concluded,[129] that the acid catalysed transformation (121) to (122) proceeds from a protonated species (e.g. 123) by way of a transannular migration of hydrogen with its bonding pair, from C-14α to C-9α with attending extrusion of the C-8β proton.

The hydrogen transfer in the formation of the 1,5-cyclo-

steroid is a similar process, between centres of identical stereochemistry. In the case of the cyclosteroid, hydrogen transfer is followed by migration of the C-1, C-2π electrons, rather than elimination of the C-4β proton to form a 4-5 double bond, to exactly parallel the above example. Both reactions involve extrusion of a proton as the ultimate step.

This postulated mechanism for the formation of hydrocarbon 'H', can be verified in two ways. By labelling the 5α-proton - replacing it with deuterium - in cholest-1-en-3β-ol, the hydrocarbon produced should have a deuterium atom on C_3, at one end of the double bond, and therefore it should be capable of easy identification from spectra. By blocking the 5α-position with a group not capable of undergoing transannular movement, such as a methyl group or fluorine, this reaction will be unable to proceed by the proposed mechanism and diene formation should result.

Synthesis of 5α-substituted cholest-1-en-3β-ols

Cholestan-3β-ol acetate labelled with deuterium at C-5α and C-6 was prepared by reduction of cholesteryl acetate in deuterated acetic acid[130] with deuterium, using platinum oxide catalyst at 70°.[131] The resulting product would be expected to contain 2.55 D atoms per molecule:[131] 0.87 D at C_5, 1.27 D at C_6 and 0.4 D at C_7. There was no obvious difference between the p.m.r. spectra of deuterated and non-deuterated cholestan-3β-ol acetate, since the 5 and 6 proton resonances are normally obscured in the

methylene envelope. An infra-red spectrum, in carbon tetrachloride, showed a triplet of low intensity due to the C-D stretching vibration at 2130, 2165 and 2205 cm.$^{-1}$.[132] Hydrolysis of this acetate with potassium hydroxide in methanol gave deuterated cholestan-3β-ol. This was converted into cholest-1-en-3β-ol as described previously for the non-deuterated material. At each stage the characteristic triplet absorption was present in the i.r. spectrum.

Present reactions leading to 5α-methylated steroids generally afford a very low overall yield, largely since the starting materials are difficult to prepare. A recent synthesis[151] involves reduction with lithium in liquid ammonia of a 4α,5-methylene steroid prepared by action of the Simmons Smith reagent on cholest-4-en-3α-ol:[10] this alcohol is obtained in about 10% yield by reduction of the corresponding ketone, along with the epimeric alcohol, from which its separation is difficult.[10]

Fluorine substituted at C-5α in cholest-1-en-3β-ol would not undergo transannular migration. 5α-Fluorocholestan-3β-ol acetate was prepared by the addition of anhydrous hydrogen fluoride to cholesterol acetate[133] and this was transformed to 5α-fluorocholestan-3-one. Direct bromination of this ketone in acetic acid gives the 2β-bromo-ketone, but a significant amount of dehydrofluorination takes place at the same time.[134] 5α-Fluorocholestan-3-one was enolacetylated[135] and this enol acetate was brominated to give 2β-bromo-5α-fluorocholestan-3-one. Since lithium carbonate - dimethylformamide dehydrobromination would also dehydrofluorinate, dehydrobromination was achieved by making

the semicarbazone and cleaving that with dioxan - sulphuric acid.[72] Reduction of this fluoro-ketone with lithium aluminium hydride gave 5α-fluorocholest-1-en-3β-ol. It was not possible to crystallise this alcohol, but its infra-red spectrum indicated the presence of fluorine by an absorption at 1143 $cm.^{-1}$. A chemical test for fluorine[136] was positive at each stage.

Tosylation Reaction on 5α-substituted Cholest-1-en-3β-ols

Reactions with both 5α-deutero- and 5α-fluorocholest-1-en-3β-ol were carried out as with the non-substituted material, except that chromatography of the fluorinated material was carried out on deactivated neutral alumina to prevent dehydrofluorination. The hydrocarbon product from the deuterated compound had comparable i.r. and p.m.r. spectra to the non-deuterated material, but there were however significant differences. The infra red spectrum (in CCl_4) had peaks at 2135 and 2170 $cm.^{-1}$ as in the spectra of the previous deuterated material. An additional peak was however present at 2240 $cm.^{-1}$, corresponding to a C-D stretching vibration on an olefinic bond.[132]

From the mechanism proposed above (124) the deuterated hydrocarbon produced will have a deuterium on C_3 (125). It is improbable that this deuterium atom will be on C_2. The p.m.r. spectrum of this product had the same pattern of signals due to the various methyl groups as the non-deuterated material. The intensity of the olefinic signal at τ 4.42 corresponded to only one proton, as predicted by the mechanism.

Since there are no protons on carbon atoms adjacent to C-4

(C-6 has two deuterium substituents[131]), the protons on C-4 should appear as a doublet, split by the C_2 olefinic proton. As this olefinic proton appears as a slightly broadened singlet, the coupling constant must be very small, and the doublet of the C-4 protons may be unresolved. These protons may be coupled to the cyclopropyl proton as well. A broad peak at τ 8.45 could represent these C-4 protons, but, because it is on the methylene envelope, it cannot be accurately integrated. This small peak could alternatively be assigned to the lone cyclopropyl proton on C-1.

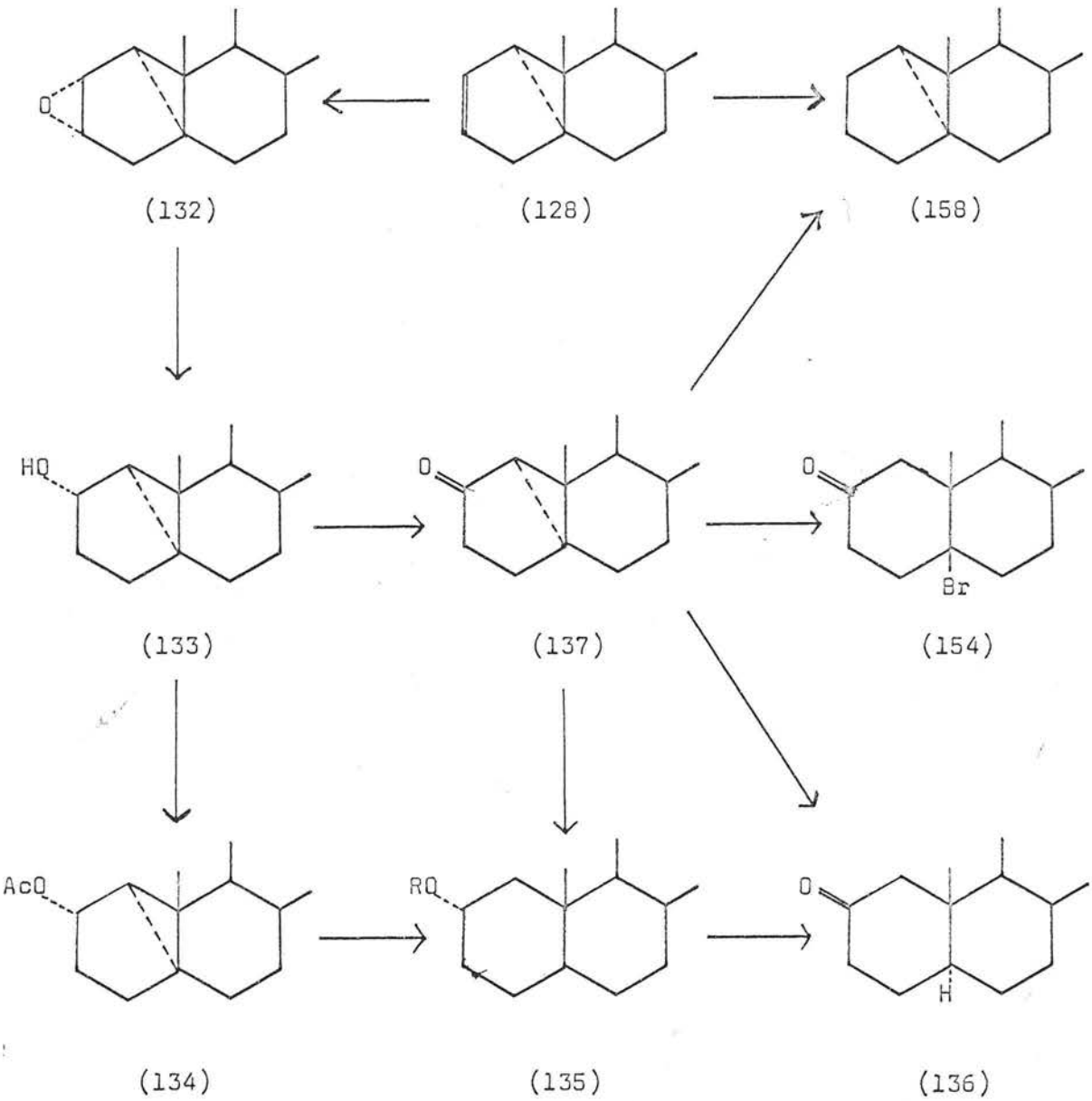
However, the spectrum establishes that there is a deuterium atom on C-3, in support of the proposed reaction mechanism.

This mechanism is also supported by the hydrocarbon product of the tosylation of the 5α-fluorinated alcohol. An ultra violet spectrum indicated that the product formed was not an alkenylcyclopropane, but a homoannular diene, 5α-fluorocholesta-1,3-diene (126). The material could not be induced to crystallise, but the intensity of its u.v. spectrum, ϵ 5,300, at λ max 263 mu indicates that it must be homogeneous, the corresponding intensity of cholesta-1,3-diene being ϵ 5,500 (Table II).

In this case the transannular migration of the 5α-substituent is either not possible, or slower than the rate of formation of the carbonium ion (127), incorporating the electrons of the olefinic bond. The major hydrocarbon product is therefore the 1,3-diene, and no cyclo-steroid is formed.

These two reactions support the concerted reaction mechanism proposed for the formation of 1α-5-cyclo-5α-cholest-2-ene from cholest-1-en-3β-ol.

Chart III



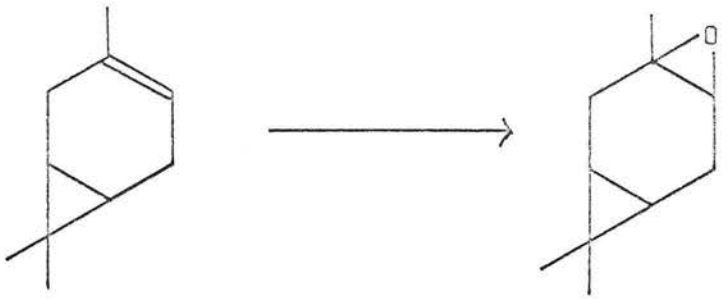
Reactions of 1α,5-cyclo-5α-cholest-2-ene

In a previous section, the structure of hydrocarbon 'H' was deduced as 1α,5-cyclo-5α-cholest-2-ene (128). In that section, the catalytic reduction to 5α-cholestane, using a conventional palladium on charcoal catalyst, was discussed. The position of the double bond was determined by oxidising with permanganate-periodate to a diacid and reducing this catalytically to 2,3-seco-cholestane-2,3-dioic acid. In the following description of further reactions of the hydrocarbon, the structure (128) has been assumed: the reactions confirm the proposed 1,5-cyclo structure. Some of these reactions are outlined in Chart III.

Reaction of 1α,5-cyclo-5α-cholest-2-ene with Peracid

Oxidation of ethylenic compounds with organic peracids generally results in epoxide formation.[137] Peracids however do not react with the cyclopropane ring system. Reaction of Δ^3-carene (129) with perbenzoic acid is reported to give an epoxide (130) with the cyclopropane ring unreacted,[138] similarly no reaction of the cyclopropane ring is recorded in the oxidation of umbellulone (131).[139] The action of perbenzoic acid on 1-methyl-cyclopent-1-ene gives only one of the isomeric oxides in 75% yield.[140]

It is therefore expected that the action of an organic peracid on hydrocarbon 'H' will afford stereospecifically one oxide at C-2 and C-3 without reacting with the cyclopropane ring. Reaction of one mole of p-nitro-perbenzoic acid[141] gave a product,



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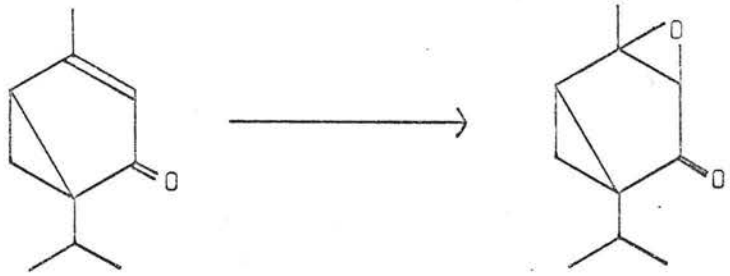

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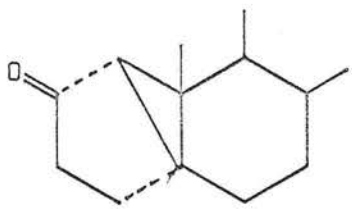

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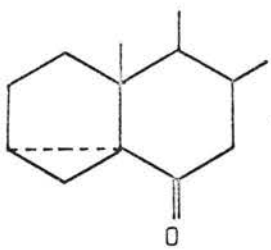
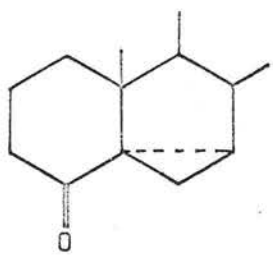




(139) (140)

readily crystallised from acetone. Micro analysis for carbon and hydrogen content corresponded to an empirical formula $C_{27}H_{44}O$ and determination of oxirane oxygen,[142] by reaction with a known excess of hydrogen chloride, showed that there was only one epoxide oxygen atom in the product. No reaction with the cyclopropane ring had occurred. Prolonged reaction with an excess (three moles) of peracid gave the same product. The infra-red spectrum of this compound had a peak at 820 $cm.^{-1}$, due to the C-O stretching vibration of the oxide group. The p.m.r. spectrum of this oxide had methyl group absorptions at τ8.77 (C_{19}), 9.12, 9.21, 9.27 and 9.38 (C_{18}). A peak at τ6.85 (two protons) was due to the protons on the epoxide ring. A small peak at τ8.17 could be the lone cyclopropyl proton. It is not possible to determine the configuration of this oxide either from its physical properties or from consideration of the mechanism of formation. Dreiding models of the two possible isomers show that in the case of the β-isomer, there will probably be strong steric interaction between the epoxide oxygen and the C-19 methyl group. This will not occur in the α-isomer, which is therefore likely to be the more stable. It should be possible to determine the configuration from the chemistry of this oxide.

Reaction of oxides with lithium aluminium hydride produces, in cyclohexane ring systems, an axial alcohol.[189] The epoxide (132) from the cyclosteroid, afforded quantitatively an alcohol (133) on reduction with lithium aluminium hydride;

recrystallisation from acetone gave needles with a sharp melting point 161-2°C. This alcohol had the following peaks in its p.m.r. spectrum due to methyl groups: τ 8.75 (C_{19}), 9.10, 9.19, 9.22 and 9.34 (C_{18}). A peak at τ5.95 disappeared on shaking the sample with deuterium oxide, and can therefore be assigned to the hydroxyl proton. This alcohol was acetylated to give a crystalline acetate (134) m.p. 101-2°C. The p.m.r. spectrum of this acetate had the following peaks due to methyl groups: τ7.96 (acetate), 8.75 (C_{19}), 9.09, 9.18, and 9.34 (C_{18}).

Wallis, Fernholz and Gephart[19] attempted catalytic reduction of i-cholesteryl acetate. With palladium on charcoal catalyst they observed no reaction, but using Adams platinum oxide catalyst in acetic acid, reduction occurred quickly to give cholestan-3β-ol acetate. This apparently facile reduction is presumably an acid catalysed rearrangement of the cyclosteroid to cholesteryl acetate followed by reduction of the C-5, C-6 double bond. Reduction of the acetate (134) with Adams catalyst at atmospheric pressure did not proceed at all during twenty-four hours. Repetition of the experiment at 120 atmospheres and 100°C for 3 hours brought about a reduction and a crystalline acetate was isolated in 65% yield. This was identified by its melting point (87-89°C) and rotation ([α] =-2°) as 5α-cholestan-2α-ol acetate (135,R= CH_3CO) (lit.[143] m.p. 90°C, $[\alpha]_D$=-1°). Hydrolysis followed by oxidation gave cholestan-2-one (136). Since the acetate (134) was recovered unchanged from the room temperature experiment, no acid catalysed rearrangement can occur

under these conditions, and both acetates (134 and 135) are C-2 substituted.

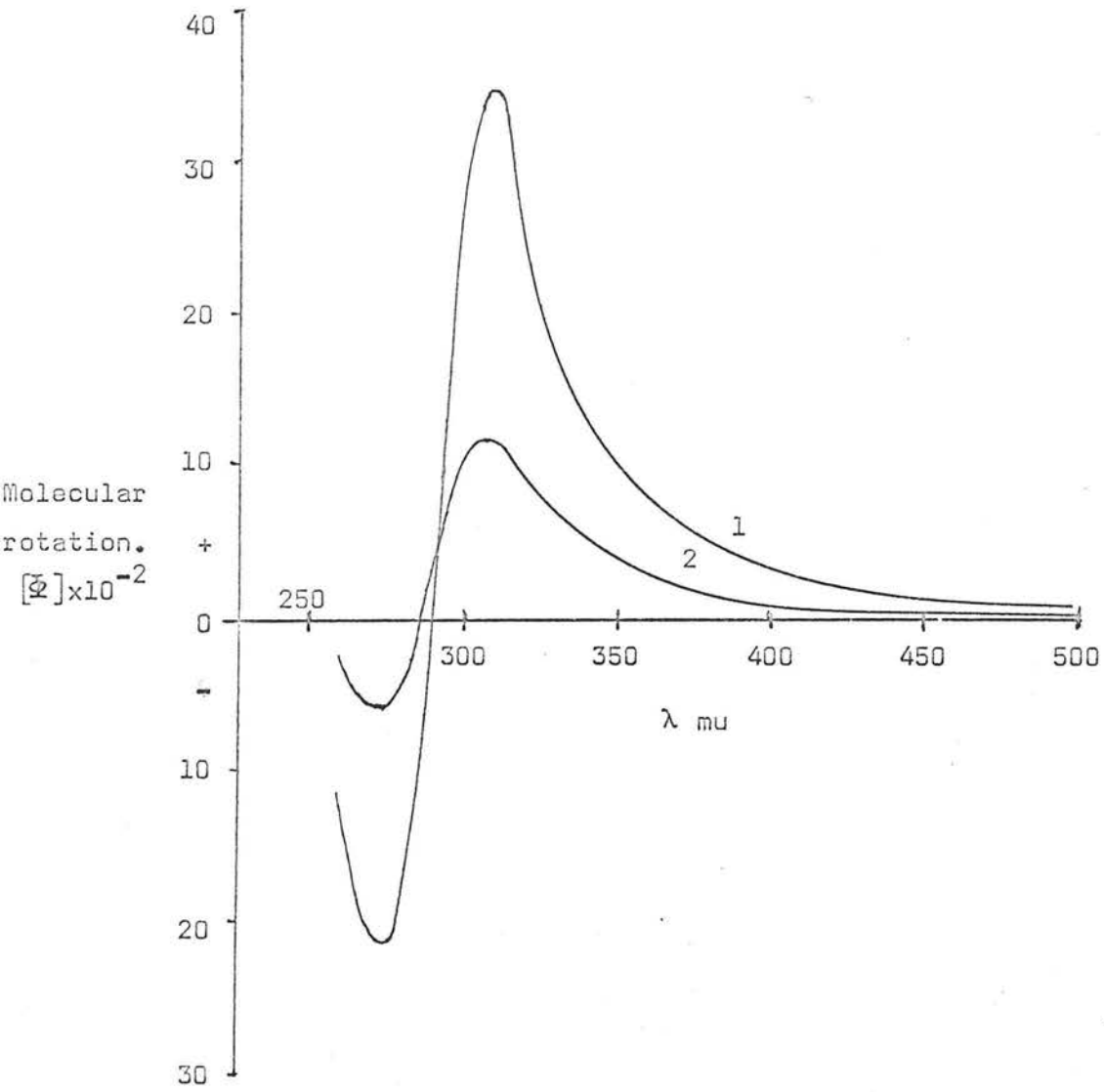
Since the product of hydrogenation of the cyclopropyl acetate (134) is the 2α-acetate, it is probable that the configuration of the acetate (134) and therefore of the alcohol (133) is α also. There is the possibility that cholestan-2β-ol acetate may be formed in the reduction if the acetate (134) is β, and then under the reaction conditions epimerised to the more stable equatorial 2α-acetate. This is unlikely, since the more probable affect of those rather drastic reduction conditions would be hydrogenolysis, and the isolation of cholestane as the reaction product. From the configuration of the alcohol (133) it immediately follows that the oxide (132) will have the 2α,3α configuration, in agreement with the prediction from models.

Oxidation[65] of the cyclopropyl alcohol (133) with chromic acid gave a ketone (137), the u.v. spectrum of which showed λ_{max} 215 mμ (ε7,800) with a smaller peak at 327 mμ (ε630). This u.v. absorption maximum is similar to that of lumi-cholestenone[11] (138) which has λ_{max} 212 mμ (ε7,900), typical of a cyclopropyl ketone.[144] The absorption of the carbonyl group in the infra-red at 1710 $cm.^{-1}$ is characteristic of a cyclopentanone where the carbonyl group is also conjugated to a cyclopropane ring. Similar values are found in the spectra of the photoproducts reported by Dutler et al.[51] where 17β-acetoxy-1β,5-cyclo-5β,10α-androstan-2-one had a carbonyl absorption at 1714 $cm.^{-1}$ In the 3α,5- and 5α,7α-cyclo steroids conjugated to a carbonyl (139,140)

the characteristic cyclohexanone absorption at 1720 $cm.^{-1}$ is brought down to 1690 $cm.^{-1}$ by conjugation with the cyclopropane ring.[83]

In the i.r. spectrum of the cyclopropyl ketone (137), there was a slight shoulder visible at 3018 $cm.^{-1}$ Although it has been pointed out earlier that absorption in the 3000-3100 $cm.^{-1}$ region only occurs when a cyclopropyl CH_2 group is in the molecule,[83] lumicholestenone[11](138) has a band at 3025 $cm.^{-1}$ in the i.r. Allen, Davis, Humphlett and Stewart[97] have reported that for cyclopropanes containing an unsubstituted CH_2 group, there are two bands at 3096 and 3012 $cm.^{-1}$ These are very close to the fundamental frequencies of liquid cyclopropane (3090 and 3019 $cm.^{-1}$) and it would appear that the same parallel and perpendicular modes of vibration of the ring CH_2 groups are involved. It would therefore be unrealistic to expect both bands to appear in spectra of compounds containing only ring CHR or CR_2 groups. Compounds containing cyclopropyl CH_2 groups sometimes show two, one or even no bands in the 3000 to 3100 $cm.^{-1}$ region; in some cases where there is no CH_2 group a band is observed. The inherent weakness of the C-H stretch absorption in cyclopropanes, as compared with that of larger ring compounds, would make any identification of this band in the infra-red difficult. The shoulder at 3018 $cm.^{-1}$ in the ketone (137) is therefore probably due to the C-H stretch of the lone cyclopropyl proton. The corresponding absorption in the i.r. spectrum of the parent hydrocarbon (128) could be obscured by the aliphatic C-H stretch absorption, since it would

Figure III



Rotatory Dispersion Curves

1 Cholestan-3-one a = + 57

2 Cyclopropyl ketone a = + 18

be expected at slightly lower frequency than when it is in conjugation with the carbonyl group of (137).

The proton magnetic resonance spectrum of the cyclopropyl ketone (137) had the following methyl absorption bands τ 8.79 (C_{19}), 9.01, 9.10, 9.20 and 9.32 (C_{18}); there were small peaks at τ 7.58, 7.69, 7.78 and 7.90, probably due to the two protons on carbon 3. On treatment of the ketone with monodeuteromethanol and sodium methoxide, these four bands were largely removed. The absorption of the cyclopropyl proton is presumably a multiplet within the methylene envelope, because of coupling with the four protons of C-3 and C-4. Replacement of all four of these protons with deuterium possibly would enable the resulting singlet from the cyclopropyl proton to be seen; this is not however possible.

The rotatory dispersion curve of the cyclopropyl ketone (137) has a positive cotton effect, with a molecular amplitude considerably less than that of cholestan-3-one; +18 compared with +57. The curve is slightly displaced to shorter wavelength. The two curves are recorded in figure III.

In the case of most steroid ketones, the Octant Rule[145] predicts the sign and magnitude of the cotton effects observed in rotatory dispersion. Cyclopropyl and epoxy ketones however do not generally follow this relationship. Examination of the rotatory dispersion curves of several of these compounds has shown that they follow a 'reversed' octant rule - the cotton effect is opposite to that predicted by the octant rule.[146]

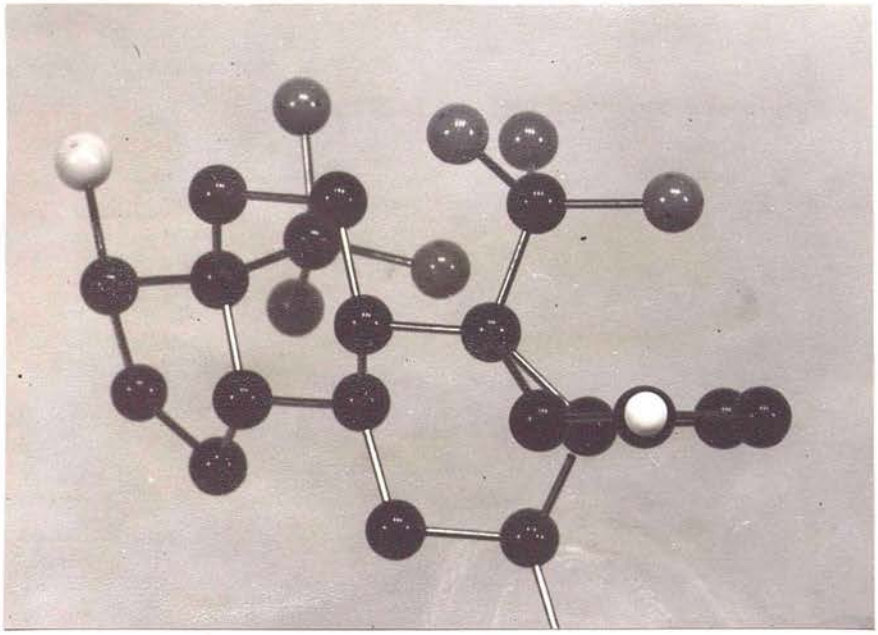
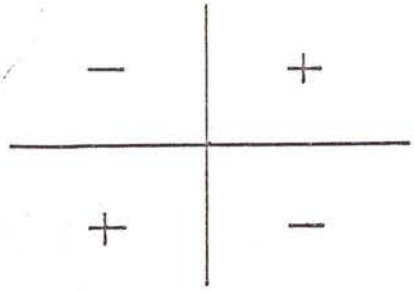
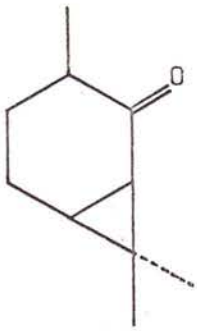


Figure IV



Signs for cyclopropyl and oxirane substituents in back octants: The reversed octant rule



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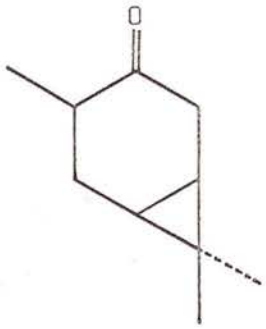


(142)

The reversed octant rule predicts (figure IV) that the cyclopropyl ketone should have a negative cotton effect and comparison with various similar compounds[146] suggests a high amplitude, in the region of 200. The number of available examples used in the compilation of the reversed octant rule were limited and there are some exceptions. Carone (141) for which a negative cotton effect is predicted, has a positive cotton effect, attributed to a strong contribution from the geminal dimethyl groups.[146] A similar situation occurs in 1α,5-cyclocholestan-2-one, where the cyclopropyl ring has instead of a gem-dimethyl group, one methyl group and an alkyl substituent, C-9, on the corresponding carbon atom of the ring. This could cause a reversal of the cotton effect from that predicted.

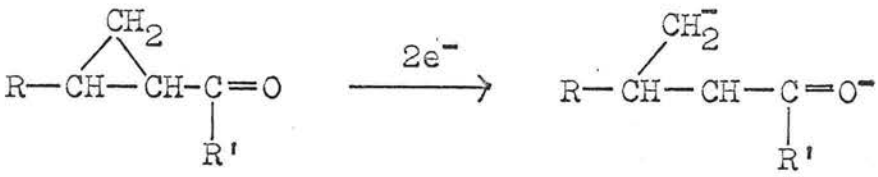
It has already been established that the carbonyl group in compound (137) is at position 2 and not 3 by reduction of the cyclopropyl ring. If it was in fact at 3, the octant rule (used since the ring is no longer conjugated to the chromophore[146]) predicts a negative cotton effect, although the homo-allylically conjugated cyclopropyl ketone, 4-iso-caranone (142) has a cotton effect opposite to that predicted by the octant rule.[147]

The rotatory dispersion curve does not confirm the structure (137) assigned on the basis of chemical transformations. It would seem therefore that this compound, although a conjugated cyclopropyl ketone, is an exception to the reversed octant rule.

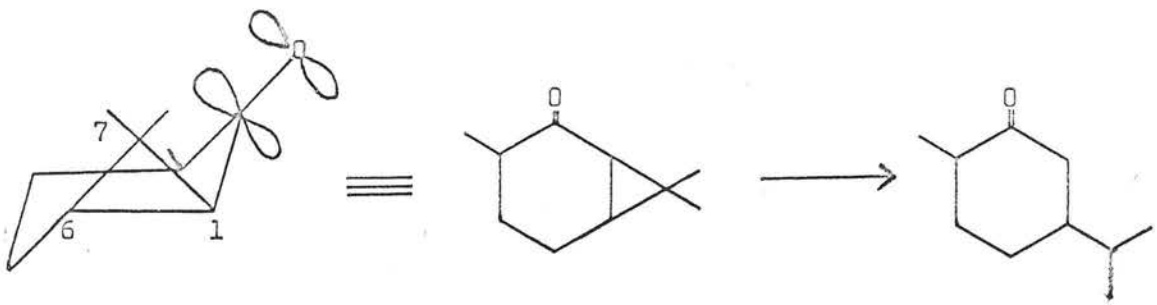
Further confirmation of the position of the oxygenated function of the cyclopropyl ketone was obtained by catalytic

Table VII

	m.p.	$[\alpha]_D$	Amplitude of rotatory dispersion curves, in MeOH	in MeOH/HCl
Cholestan-2-one	131°	+50°	+121	+112
Cholestan-3-one	129°	+41°	+57	+11
Cyclopropyl ketone (137)	143°	+37°	+18	+17
Reduced ketone (136)	130°	+47°	+117	+104



(143)



(144) (145)

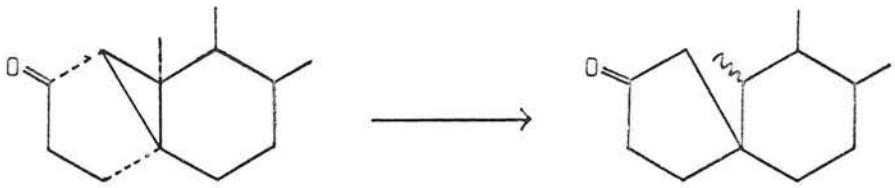
reduction. Despite the ease of hydrogenation of 5α,7α-cyclocholestan-4-one to 5α-cholestan-4-one by platinum in acetic acid at room temperature and one atmosphere reported by Davis and Summers,[148] no reaction was detected on attempted reduction of the cyclopropyl ketone (137) under the same conditions. Reduction was effected however with platinum oxide at 120 atmospheres, at room temperature, using acetic acid as solvent. The product isolated was a mixture of cholestan-2α-ol acetate and cholestan-2-one, readily separated by alumina chromatography. The combined yield of these products was 70%, and no appreciable quantity of other material was eluted. Hydrogenolysis of the 1,5-bond must have taken place preferentially, though no doubt fission of the 1-10 and 5-10 bonds did occur to a small extent. Hydrolysis and oxidation of the acetate gave cholestan-2-one.

Differentiation between cholestan-2-one and cholestan-3-one is somewhat difficult by physical techniques. The melting point and optical rotation are very similar, but careful comparison of the fingerprint region of the i.r. spectrum showed that the reduction product was cholestan-2-one. This was verified by rotatory dispersion measurements. These two compounds exhibit a positive cotton effect curve, but have very different amplitudes.[149] These are recorded in table VII. Further differentiation can be achieved by formation of a hemi-ketal of the carbonyl group.[150] Formation of hemi-ketals is very sensitive to steric effects, and can be readily observed from the rotatory dispersion curves of the ketone in methanol to which

a drop of hydrochloric acid has been added to catalyse the reaction. When hemi-ketal formation takes place, the amplitude of the curve decreases in proportion to the extent of reaction. In the hemi-ketal of a 2-keto steroid, there is a 1,3-diaxial interaction between the oxygenated C-2 substituent and the C-19 angular methyl group, so that ketal - ketone equilibrium lies well on the side of the ketone, with little reduction in the amplitude of the rotatory dispersion curve. With a 3-keto steroid, however, such steric interactions are not present, and 70% hemi-ketal formation is seen by a corresponding reduction in amplitude. Examination of the rotatory dispersion curve of the reduced cyclopropyl ketone (136) in methanol showed an amplitude of +117, which was reduced to +104 on addition of a drop of hydrochloric acid. This confirms cholestan-2-one as the product of reduction.

Reduction of the Cyclopropyl Ketone with Lithium in liquid Ammonia

Reductive opening of a cyclopropyl ketone with lithium in liquid ammonia is reported to proceed stereospecifically.[151] The bond in the cyclopropane ring which is cleaved by this reduction is that which overlaps the π bond system of the adjacent carbonyl group best. This reduction involves the addition of two electrons to yield a carbanion - enolate ion system (143). The bond of the cyclopropane ring which breaks should be that leading to the thermodynamically more stable carbanion, i.e. with the charge on the least substituted carbon atom. It has been found however,[151] that the bond cleaved is



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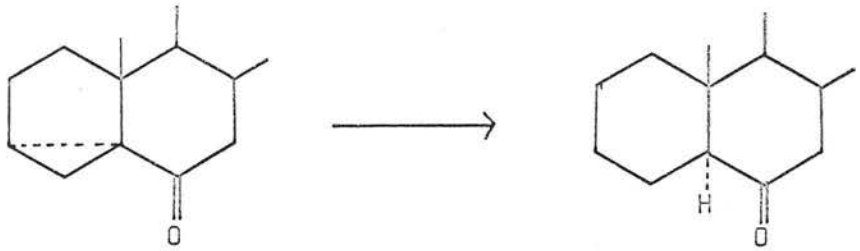
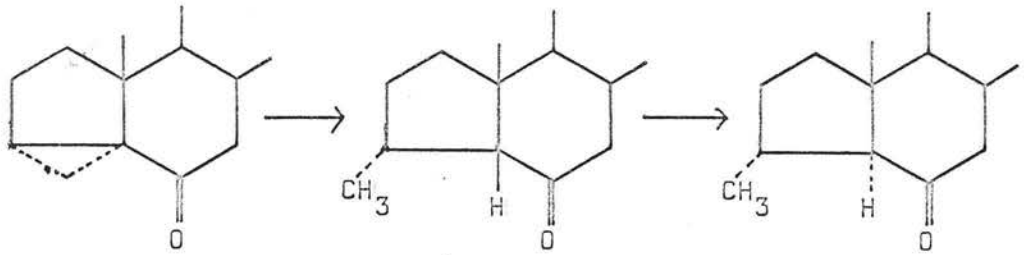
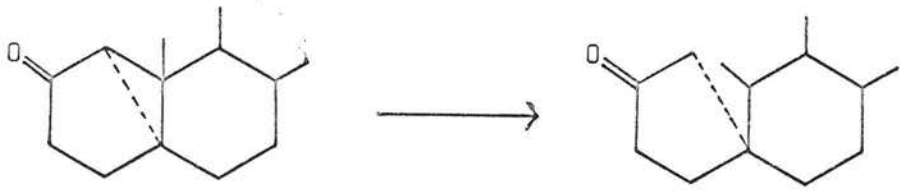


(148) (150)



(149) (151) (152)



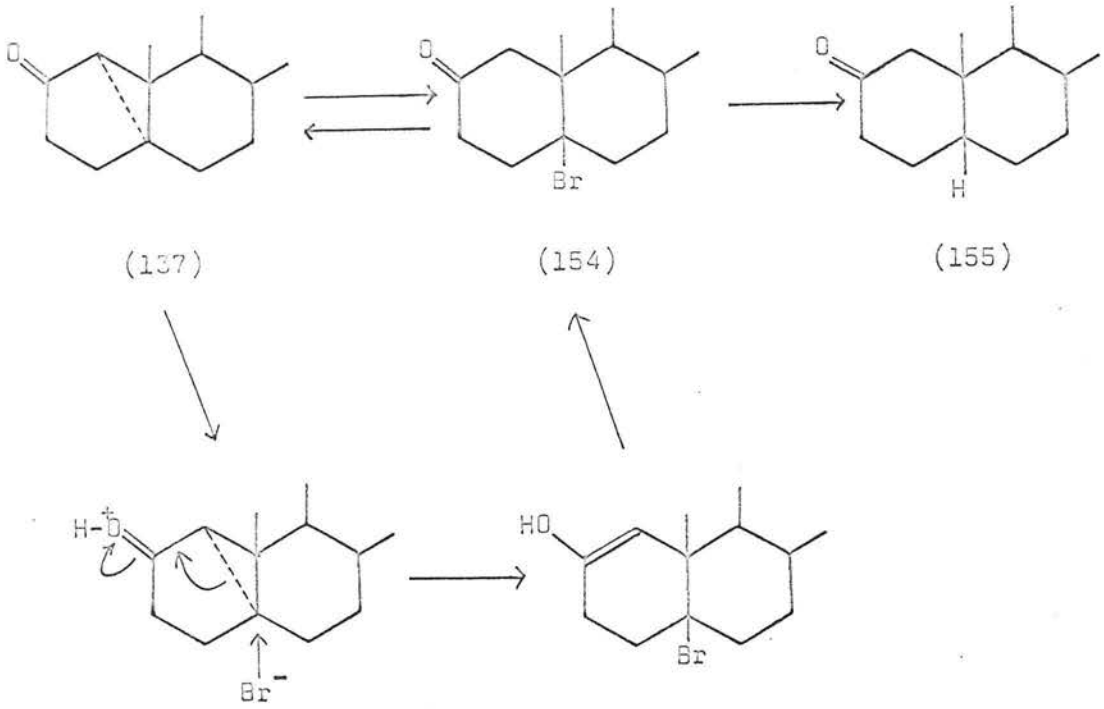
(137) (153)

that possessing maximum orbital overlap with the π electron system of the carbonyl, regardless of the relative stability of the carbanions which could have been formed.

Carone (144)[151,152] has been reduced to carvomenthone (145) by breaking the external 1,7-bond. Breaking the internal 1,6-bond, which does not overlap the π electron system of the carbonyl, would lead to the more stable secondary carbanion, while cleavage of the 1,7-bond, well overlapping the carbonyl π orbitals, would give a less stable tertiary carbanion. Similar preference for kinetic control of the reaction in preferance to thermodynamic control is found in the reduction of various cyclosteroids. Lumicholestenone (146) is reduced by lithium in liquid ammonia to give the spiro-ketone (147).[151] In this case, the bond broken, C-1, C-10, has the best overlap with the π electron system of the carbonyl group. Similarly, the isomeric 3α,5- and 3β,5-cyclo-cholestan-6-ones (148 and 149) are reduced to ketones (150, 151 and 152).[151] In the 3α,5-cyclo isomer the 3,5-bond has the better overlap with the carbonyl π electrons, and is cleaved in the reduction to give cholestan-6-one (150); no other products arising from cleavage of the other two cyclopropyl bonds could be detected. The 3β,5β-isomer was reduced to the A-nor ketone (151), which on alumina chromatography isomerised to (152). The bond cleaved in this case, 4,5 is overlapping the $\pi\pi$ electrons of the carbonyl group.

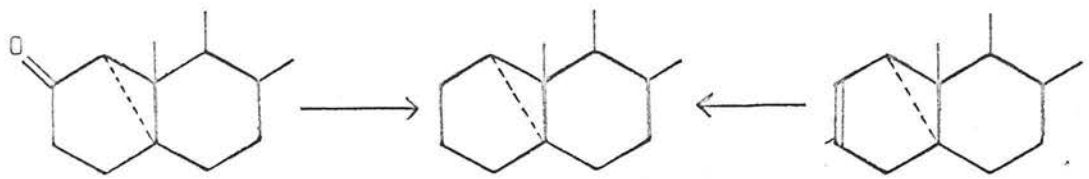
Reduction of the cyclopropyl ketone (137) derived from hydrocarbon 'H' (128), with lithium in liquid ammonia and t-butanol as co-solvent, gave a ketonic product which, from g.l.c.

analysis, and two components, only just separable. An infra-red spectrum of this mixture showed carbonyl absorption at 1715 and 1745 cm.$^{-1}$. One component, 20% by g.l.c., would appear to be 5α-cholestan-2-one from i.r. (1715 cm.$^{-1}$) and from g.l.c. retention time; the carbonyl absorption at 1745 cm.$^{-1}$ in the i.r. is characteristic of a cyclopentanone. The mixture was chromatographed on alumina, but separation proved difficult. Fractions containing varying amounts of the two ketones were eluted, but only a small amount of the cyclopentanone was obtained free from the cholestan-2-one. This could not be induced to crystallise. A p.m.r. spectrum of the clear glass, which was homogeneous to g.l.c., had two peaks at τ 8.98 and 9.03 probably the C-10β methyl group, split by the C-10α proton, peaks at τ 9.09, 9.20, and 9.32 attributable to the side-chain and C-18 methyl groups. Therefore the main product (80%) of lithium in liquid ammonia reduction of 1α,5-cyclo-cholestan-2-one (137) appears to be mainly 1(10→5α)-abeo-cholestan-2-one (153), formed by cleavage of the 1,10-bond, the delocalised electron orbitals of which lie in the same plane as the π electrons of the carbonyl group. By comparison with the previous examples, the spiro-ketone should have been the only reduction product, but cholestan-2-one was also isolated, presumably by fission of the 1,5-bond. In the previous examples the bond cleaved has been exactly in line with the π orbitals of the carbonyl (144). In this case, there is an angle of ca.30° between the bond and the π orbitals. This could account for conjugation to a small extent of the 1,10-bond with the



(137) (154) (155)

(156) (157)



(137) (158) (128)

carbonyl, resulting in cleavage of that bond also.

Addition of Hydrogen Bromide to the Cyclopropyl Ketone (137)

Addition of one mole of hydrogen bromide, in acetic acid solution, to the cyclopropyl ketone afforded a bromo-ketone (154). The infra-red spectrum of this had a carbonyl absorption at 1720 $cm.^{-1}$, typical of a cyclohexanone, however, had this compound been an α-bromo ketone, the carbonyl absorption would have been at a higher frequency. The p.m.r. spectrum of (154) had absorption due to the C-19 methyl group at τ 8.90. Removal of the 5β-bromine by reduction with sodium in liquid ammonia, produced 5β-cholestan-2-one, identified by its melting point and optical rotation;[153] the p.m.r. absorption of the C-19 methyl group of this ketone occurred at τ8.97. The C-19 methyl group resonance of the bromo-ketone occurring 0.07 p.p.m. lower than the corresponding signal of the unsubstituted ketone would establish the bromine atom to be at least one atom removed from C-10.[154] Absence of any absorption in the τ 5-6 region characteristic of protons on the same carbon atom as a halogen, would indicate that this bromine is tertiary, and therefore most likely a C-5 substituent.

The probable mechanism of this reaction would be a 1,4 addition to the cyclopropyl ketone group. Protonation of the carbonyl (156), movement of the positive charge to C-5 followed by attack there by bromide ion, to give the enol (157) and hence the bromoketone (154). Treatment of the bromoketone with ethanolic sodium hydroxide regenerated the cyclopropyl ketone

(137), this reaction being a convenient synthesis of cyclopropyl compounds.[155]

Formation of 1α,5-cyclo-5α-cholestane

Removal of the carbonyl group of the cyclopropyl ketone (137) by a modified Wolff-Kishner reduction,[110] afforded a hydrocarbon whose spectroscopic properties confirm the obvious structure 1α,5-cyclo-5α-cholestane (158). The infra-red spectrum had no readily assignable peaks, although a shoulder at 3005 $cm.^{-1}$ is probably due to the C-H stretch of the lone cyclopropyl proton on C-1. The p.m.r. spectrum had a peak at τ 8.78, assigned to the C-19 methyl group. The compound gave a faint yellow colour with tetranitromethane, compatable with it being a cyclopropyl hydrocarbon.

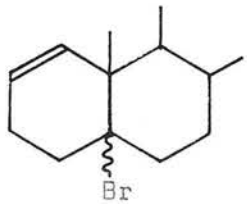
This cyclo-cholestane (158) was also isolated as the only product of catalytic reduction of the parent hydrocarbon, 1α,5-cyclo-cholest-2-ene (128), using the soluble catalyst, tris-(triphenylphosphine)-rhodium chloride.[156,157] Reduction of unhindered disubstituted steroid olefins, such as cholest-1-ene, cholest-2-ene and cholest-3-ene to 5α-cholestane is effected easily with this soluble catalyst in benzene solution, under one atmosphere of hydrogen. More highly substituted olefins are not reduced, for example cholest-4-ene and cholest-5-ene. Similarly disubstituted olefins in conjugation with carbonyl groups, cholest-1-en-3-one, are easily reduced, while the more substituted cholest-4-en-3-one is not. It is probable therefore that the

olefin group of the hydrocarbon 'H' will be reduced by this catalyst, while the cyclopropane ring remains intact. The hydrocarbon 'H' took up one mole of hydrogen over four hours; the product isolated was as expected 1α,5-cyclo-cholestane (158), identical to that obtained from the cyclopropyl ketone (137). Under the same conditions, 3α,5-cyclo-cholest-6-ene was reduced to 3α,5-cyclo-5α-cholestane. An authentic sample of 3α,5-cyclo-5α-cholestane was prepared by reduction of cholesteryl tosylate with lithium aluminium hydride.[38]

Addition of Hydrogen Halides to 1α,5-cyclo-5α-cholest-2-ene

The acid catalysed addition of hydrogen halides to the hydrocarbon 'H' failed to give readily isolable products. The hydrocarbon was added to a solution of dry hydrogen chloride in acetic acid, and after half an hour, isolation of the steroid gave a brown glass. The product was recovered quantitatively from grade I alumina, by elution with petrol, no separation being achieved. On g.l.c. the now clear glass was shown to contain at least five components, in roughly equal amounts. The experiment was repeated using hydrogen bromide, and only allowed to proceed for ten minutes. The product again contained several constituents by g.l.c.; these could not be separated by alumina chromatography. A p.m.r. spectrum of the mixture showed a complex series of absorptions in the region τ 3.5-5.0; the absorption at τ 8.75 of the C-19 methyl group on the cyclopropyl ring had diminished but was still present. The expected product of this reaction would be 1-4 addition of hydrogen bromide to

give (159); further addition of hydrogen bromide to this olefin



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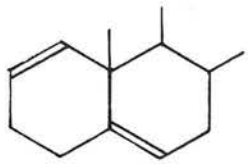
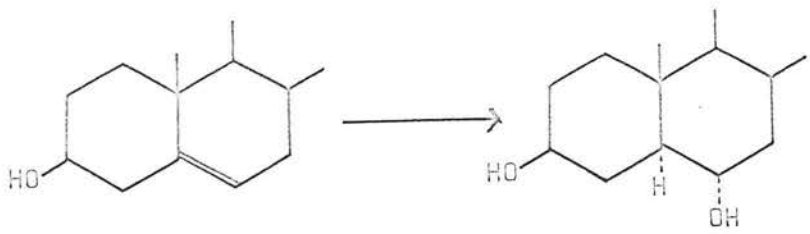


(160)

would be possible. In addition, 1,2-addition of hydrogen bromide to the double bond of the parent hydrocarbon (128) is feasible. It is also possible that rearrangement of the cyclopropyl olefin could take place in acidic conditions. The product of such a rearrangement may be the deconjugated diene (160). It was however not possible to separate the product mixture and identify the components.

An attempt to add water to the cyclopropyl olefin (128), the reaction being catalysed by acid, also failed. The expected product, by 1,4 addition, is 5α-cholest-1-en-3β-ol, however using 2% sulphuric acid (5N) in acetone, the hydrocarbon was recovered unreacted. Under stronger acid conditions, a mixture of products was obtained, which did not, from an infra red spectrum, appear to contain any alcohol. However, treatment of the hydrocarbon in glacial acetic acid with concentrated sulphuric acid gave a 60% yield of cholest-1-en-3β-ol acetate. It is possible that cholest-1-en-3β-ol, if formed during the reaction with water-sulphuric acid, may be rearranged in that system, and indeed treatment of cholest-1-en-3β-ol with sulphuric acid in acetone gave only 60% recovery of unchanged alcohol, with 15% unidentified hydrocarbon. The acetate is however stable under those conditions.



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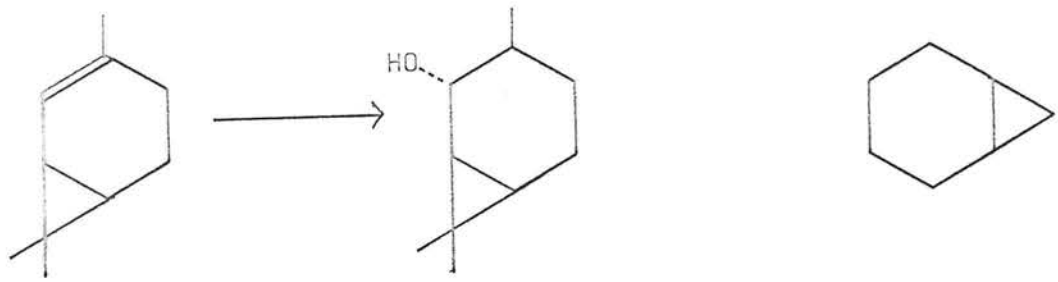

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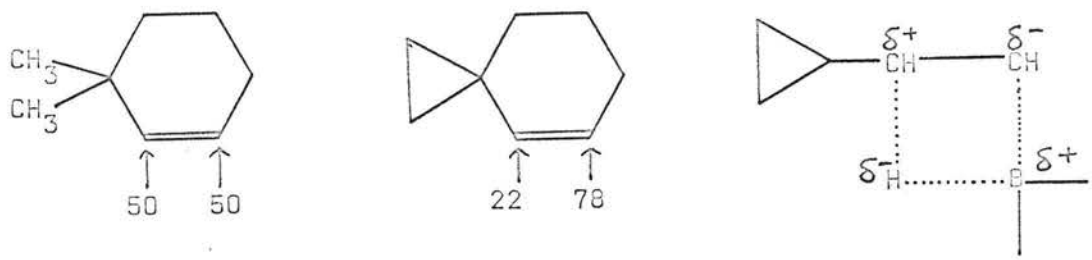

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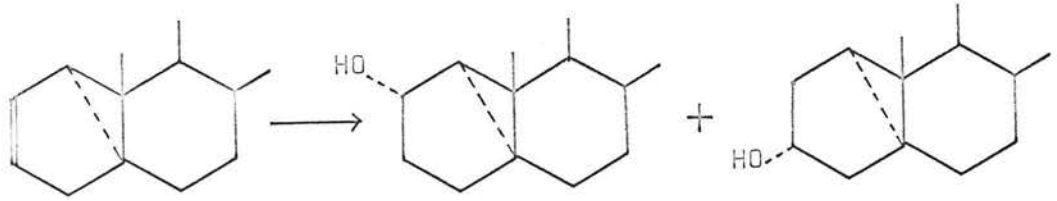


(128) (169) (170)

Hydroboration of 1α,5-cyclo-5α-cholest-2-ene

Hydration of olefins by hydroboration with diborane followed by oxidation, proceeds stereospecifically, giving anti-Markovnikov, cis addition of water to the double bond.[158] Thus hydroboration - oxidation of cholesterol (161) involves only cis hydration of the double bond to give a single diol (162).[159] Reaction of unsubstituted steroid olefins with diborane would be expected, in the absence of any directing influence, to give two products. From cholest-2-ene the two possible positionally isomeric alcohols have been obtained in almost equal amounts.[160] Attack occurs at the less hindered α side of the steroid nucleus giving rise to the α-alcohols.

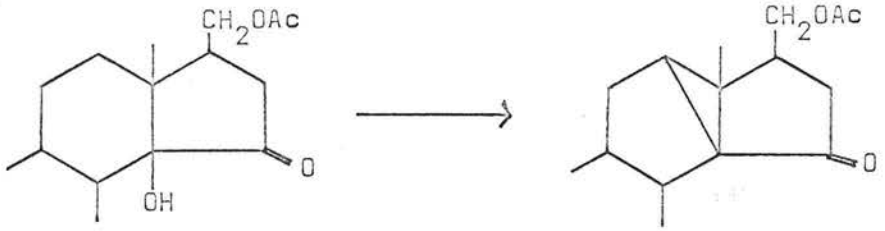
Hydroboration - oxidation of alkenylcyclopropane systems in terpenes has been reported by Acharya and Brown.[161] Hydration of 2-carene (163) gave the expected product 2-iso-caranol (164), in almost quantitative yield, establishing that there is no unusual difficulty in hydroboration of a double bond in conjugation with a cyclopropane ring, despite the reported cleavage of nor-carane (165) with diborane at 100°.[162]

In the case of 2-carene (163) the presence of the methyl group at C-3 will direct the attack of diborane to give only one alcohol by anti-Markovnikov addition. In the absence of substitution at the double bond, the cyclopropyl ring will exert a directive influence on the hydroboration.[163] Comparison of the product ratios in the hydroboration of 3,3-dimethylcyclohexene (166) and spiro[2,5]oct-4-ene (167) have been carried out.[163] While equal amounts of the two isomers were obtained in the

former case, with a cyclopropyl ring in conjugation, a greater proportion of attack (78%) took place at the position β to the cyclopropyl group. It is possible that this is caused by release of an electron by the cyclopropane ring during reaction, to give a transitional species such as (168). Such a polarised transition state was suggested by Brown[164] to be an important factor in controlling the anti-Markovnikov addition to an alkene, with electron release by the alkyl substituent being the directing factor.

Reaction of diborane, generated internally,[160] with 1α,5-cyclo-cholest-2-ene, followed by oxidation, gave an alcoholic product which was chromatographed on alumina to give unchanged hydrocarbon (44%) and a non-crystalline alcohol (51%). A p.m.r. spectrum of this alcohol had the following peaks; τ 6.64 (very broad, one proton), 8.78, 9.10, 9.19, 9.27 and 9.35. Gas chromatography showed this product to be a mixture of two components, with relative retention times 1.72 (22%) and 2.01 (78%). From the previous discussion, it would appear that these two compounds should be 1α,5-cyclo-cholestan-2α-ol (169) and 1α,5-cyclo-cholestan-3α-ol (170) respectively. The former of these has been prepared from the hydrocarbon oxide (132); the alcohols derived from the two sources had identical retention times. The 3α-alcohol was not prepared, but the 3β-isomer, whose preparation is described later, had a similar but not identical retention time. Minor peaks (less than 2%) in the gas chromatogram of the crude hydration product could be β-alcohols caused by attack

from the top side of the molecule to a very small extent. The mixture of alcohols was acetylated, but separation of the two main products by alumina chromatography was not possible.



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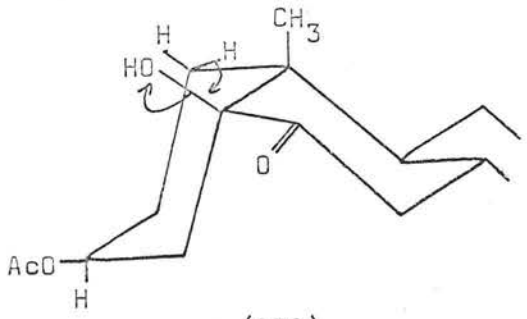

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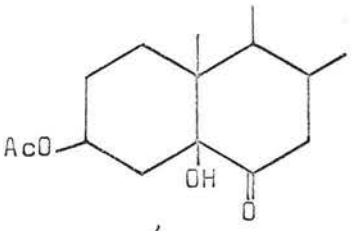

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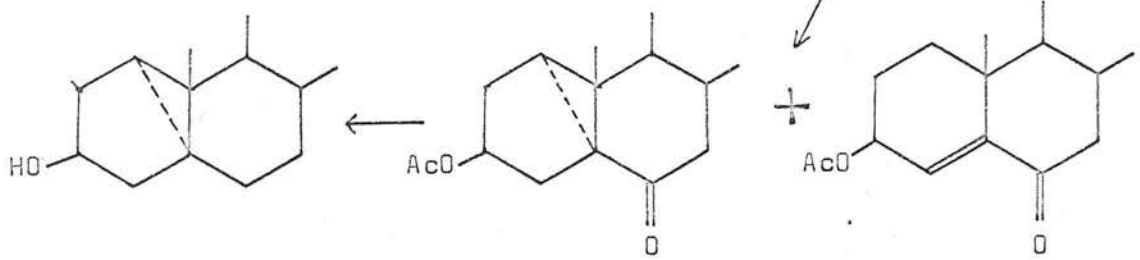

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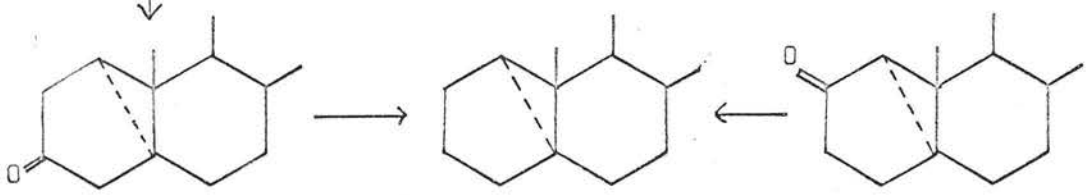


(177) (158) (137)

An Alternative Synthesis of 1α-5-Cyclo-steroids

The cyclo-steroid hydrocarbon obtained from cholest-1-en-3β-ol has been identified as 1α,5-cyclo-5α-cholest-2-ene, and the subsequent reactions already described are in agreement with this structure. However, conclusive proof that hydrocarbon 'H' is 1α,5-cyclo-5α-cholest-2-ene would involve an unequivocal synthesis.

Some syntheses of cyclopropyl steroids were summarised in the introduction, but most of these methods are not applicable for the introduction of a transannular bond in the A-ring. A reaction involving elimination of hydrogen halide from a 5β-halide-2-ketone would give the required 1α,5-cyclo-2-ketone, as previously described in the action of alkali on 5β-bromo-cholestan-2-one.

Application of the 12,14-cyclo-steroid synthesis of Reichstein (171)[57] to ring A should give the required 1α,5-cyclo-system. Dehydration of a 5β-alcohol with thionyl chloride will normally proceed to give trans elimination of water. By blocking the 4β and 6β positions, elimination will be forced to take place across a ring. By analogy with the C-ring reaction above, this should afford a 1α,5-cyclopropyl bridge by the mechanism indicated (172).

Synthesis of a suitably substituted 5β-alcohol could have proved time-consuming, therefore dehydration of 3β-acetoxy-cholestan-5β-ol-6-one was attempted. This 5β-alcohol was prepared by brominating 3β-acetoxy-cholestan-6-one in the 5α position,[165] then treating the product with potassium hydroxide

in ethanol followed by acetylation.[166] Crystallisation gave the 5β-alcohol (173) in 60% yield.

Treatment of this 5β-alcohol (173) with thionyl chloride in pyridine for one hour at 0° gave material which had three components by g.l.c., with relative retention times 1.98 (30%), 2.04 (20%) and 4.50 (50%). The more polar material was unchanged starting material. Chromatography on alumina separated the three products, starting material, 3β-acetoxy-cholest-4-ene-6-one (R_t 2.04) and a crystalline acetoxy ketone (R_t 1.98). The carbonyl frequency of this latter compound in the infra red was 1695 $cm.^{-1}$, characteristic of a conjugated cyclopropyl ketone. This compound gave no colouration with tetranitromethane, but the 3β-acetoxy-cholest-4-en-6-one produced a strong yellow colour. Generally, α,β-unsaturated ketones give a negative reaction with this reagent, but a colour is observed with this particular unsaturated ketone.[165] Blocking the 4β-position would have presumably prevented the formation of 3β-acetoxy-cholest-4-en-6-one (174). The unidentified acetoxy ketone, probably the cyclo-steroid (175), was reduced by the Huang-Minlong modification of the Wolff-Kishner reduction[110] to give an alcohol (176), which was readily acetylated. This alcohol must, from the reaction sequence, be a 3β-alcohol, yet its melting point and infra red spectrum show it is not cholestan-3β-ol. Oxidation with chromic acid gave a ketone (177) with carbonyl absorption at 1740 $cm.^{-1}$ in the infra red, characteristic of an unconjugated cyclopentanone. Removal of this ketone group

by Huang-Minlong reduction afforded a hydrocarbon (158), which gave a pale yellow colour with tetranitromethane, and proved to be identical with that derived from Huang-Minlong reduction of the cyclopropyl ketone (137). The alcohol (176) and the ketone (177) must therefore contain a cyclopropane ring, but the 3-carbonyl is not in conjugation. In the latter three compounds (176, 177, 158) the 19-methyl group has appeared in the p.m.r. spectra at or near τ 8.75.

An attempt to prepare the hydrocarbon 'H' itself from the cyclopropyl alcohol (176) was unsuccessful. Treatment of the alcohol with toluene-p-sulphonyl chloride in pyridine at 0^{o} for 24 hours, followed by adsorption of the product, in petrol, on alumina and allowing it to stand for 12 hours before eluting with petrol afforded a hydrocarbon mixture which by g.l.c. had four main components. None were identified, although one did have the same retention time as hydrocarbon 'H'.

The preparation of the cyclo-cholestane (158) from the cyclopropyl ketone (177) is however sufficient proof that the transannular bond is 1α-5α. The only other possibility for the product of dehydration of the 5β-hydroxy compound (173) is a 5α,9α-cyclopropane ring. The fact that ketone (177) is, from infra red evidence, a cyclopentanone precludes this course of reaction.

Attempts to form the tosylate of cholest-1-en-3β-ol by the normal procedure therefore afford a cyclo-steroid, 1α,5-cyclo-5β-cholest-2-ene, and a quaternary pyridinium salt. The structure of the hydrocarbon has been elucidated from spectra and chemical reactions; further reactions have synthesised several 1α,5-cyclo-steroids with oxygenated substituents, these reactions being in agreement with the assigned structure. Synthesis of some of these 1α,5-cyclo-steroids by a separate route has finally confirmed this structure.

Gas Chromatographic Behaviour of Allylic Steroid Alcohols

It has been reported that tosyl esters of sterols quantitatively decompose when they are subjected to gas chromatography.[167,168] The elimination reaction which occurs depends on the environment of the hydroxyl group especially the relative position of any unsaturation. Cholestan-3β-ol tosylate undergoes elimination to form cholest-2-ene only, but cholesteryl tosylate gives three elimination products; 3α,5-cyclo-cholest-6-ene, cholesta-3,5-diene and a second diene, probably the 2,5-diene. This reaction no doubt proceeds through a non-classical carbonium ion of the type mentioned previously (114, 115).[167] Cholest-5-en-3α-ol tosylate on g.l.c. gives only the 3,5-diene with no trace of a cyclo-steroid. This difference in behaviour of the two epimeric tosylates parallels the difference observed on solvolysis of the two compounds; the axial derivative does not react through a homoallylic cation, and gives mainly cholesta-3,5-diene by simple elimination. G.l.c. behaviour of various Δ^5-3β-hydroxy steroid tosylates has been examined by Vandenheuval.[168]

While the parent steroid Δ^5-3β-alcohols are stable on gas chromatography, allylic alcohols are not.[169,170] Chromatography of androst-4-en-3β-ol gives two peaks with retention times shorter than anticipated, one identified as a 3,5-diene and the other presumed to be a 2,4-diene.[169]

Gas chromatography of cholest-4-en-3β-ol gave two peaks identified by their retention times as 3α,5-cyclo-cholest-6-ene

and cholesta-3,5-diene. These retention times are quoted in table VIII. Ultra violet spectra of these two products, isolated by preparative g.l.c., confirm this assignment. The product with the significantly shorter retention time (R_t 0.79) can only be an alkenylcyclopropane; the u.v. spectrum shows no indication of the presence of a homoannular diene.

This elimination is not merely caused by the high temperature, 260° at the injector block, but is a surface reaction. Heating cholest-4-en-3β-ol at 260° sublimes the allylic alcohol, but absorption of the steroid on brick dust then heating it produces the same diene mixture as on g.l.c. This decomposition is temperature dependant and is also dependant on the amount of liquid phase on the column packing.[169] As the percentage of stationary phase on the support decreases, the decomposition increases.

Cholest-1-en-3β-ol also produces a cyclosteroid on gas chromatography. This has been identified as 1α,5-cyclo-cholest-2-ene, by comparison of its retention time and ultra violet spectrum with those of a sample prepared chemically. A second product of the elimination is cholesta-1,3-diene, which was similarly characterised.

While cholestan-3β-ol benzoates are stable on gas chromatography, the 7β-benzoates undergo elimination. Both cholest-5-en-3β,7β-diol dibenzoate and cholest-5-en-3β,7β-diol 3β-benzoate give a single peak at the same retention time, which is lower than expected. Table IX summarises these retention

times, and also gives retention values, relative to the solvent front, R_f, on thin layer chromatography. The product of elimination on g.l.c. was collected in each case and examined on thin layer chromatography. These products had the same R_f values and ultra violet spectra (λ_{max} 221 mu, ϵ 19,000) as cholesta-5.7-dien-3β-ol benzoate, prepared by refluxing cholest-5-en-3β,7β-diol dibenzoate in dimethyl aniline,[171] and also from 7-dehydrocholesterol.

Although 7-dehydrocholesterol benzoate was stable on g.l.c., the tosyl ester was not. Seven peaks were observed two of the major three (85%) being identified as cholesta-3,5,7-triene (60%) and 3,5-cyclo-cholest-6,8(14)-diene (18%). The third product R_t 0·67 (7%) and the minor products were not identified.

Gas Chromatography Data

The following tables summarise the retention times, relative to 5α-cholestane, of the various series of compounds mentioned throughout the previous discussion. Both analytical and preparative gas chromatography were carried out on a Perkin Elmer model 801 gas chromatograph, fitted with an all glass injector and column assembly, permitting on-column injections to be made. The injection area was normally heated to 20° above the temperature of the column, and the flame ionisation detector was at, or a few degrees above, column temperature. Relative peak areas were determined electronically by an integrator, model IE165, (Gas Chromatography Ltd.). The glass columns used for analytical g.l.c. had internal diameter 1.5 mm. and were 2 metres long. An inlet pressure of 30 p.s.i. carrier gas achieved a flow rate of 25 ml./min. The following column/temperature combinations are referred to in the tables and experimental section by their index letters.

A: 2.5% Silicon gum rubber E301 on A.W.-D.M.C.S. Chromosorb G (80-100 mesh); column temperature 220°; carrier gas nitrogen.

B: Column packed as A; column temperature 240°; carrier gas nitrogen.

C: SE30/Epon on A.W.-D.M.C.S. Chromosorb G (80-100 mesh); column temperature 180°; carrier gas helium.

Preparative g.l.c. was carried out by overloading the above glass columns, when the quantity of material required was small, for example for ultra violet spectra, but when larger amounts were required, steel columns 6 feet long and 3/16" internal diameter

were used. These were filled with the same 2.5% E301 on Chromosorb G packing. Although glass injection areas were used, these were not filled with column packing, so injections were not made directly onto the column. In the case of both glass and metal columns used preparatively, the column effluent was split, 20% to the detector and 80% collected. This fraction was passed out of the chromatograph oven through a glass tube. The steroid condensed on the cooler part of this tube, just outside the oven, and was readily recovered by washing with a solvent.

Since the main use of g.l.c. in this work has been the separation and identification of very non-polar material, the E301 (SE30) columns were chosen in preference to a more polar stationary phase. Here the selective retention effects due to functional groups are less significant than effects due to changes in molecular weight. Many of the compounds recorded in the tables were also chromatographed on a 1.5% FS-1265 (QF1) on H.M.D.S. Chromosorb W column. This proved completely unsatisfactory for hydrocarbon separations; very little difference in retention time compared with cholestane was found. Although this column should differentiate between oxygenated steroids better than E301, the particular column used was very prone to 'tailing,' and therefore giving reduced definition between compounds with similar retention times.

Table VIII

Relative Retention Times of Cholestane Hydrocarbons

	Column A	F60,224° [a]
5α-Cholestane	1.00[b]	1.00[c]
Cholest-5-ene	1.02	1.00
Cholest-2-ene	0.99	0.98
Cholestan-3β-ol tosylate*	0.99	0.98
1α,5-Cyclo-5α-cholestane	0.76	
3α,5-Cyclo-5α-cholestane	0.72	0.73
1α,5-Cyclo-cholest-2-ene	0.83	
3α,5-Cyclo-cholest-6-ene	0.79	0.80
Cholesta-1,3-diene	1.25	
Cholesta-3,5-diene	1.11	1.14
Cholesteryl tosylate*	0.79 1.02 1.11	0.80 1.02 1.10
Cholest-1-en-3β-ol*	0.83, 1.25	
Cholest-4-en-3β-ol*	0.80, 1.11	
3α,5-Cyclo-cholesta-6,8(14)-diene	0.92	
Cholesta-3,5,7-triene	1.20	
7-Dehydro-cholesteryl tosylate*	0.67, 0.90, 1.19	
Cholesta-2,5-diene	1.02	

[a] Values from Vandenheuvel et al.[167]

[b] Absolute retention time 22.9 min.

[c] Absolute retention time 10.3 min.

*Functional group eliminated on chromatography.

Table IX

Relative Retention Times of Cholestanyl Benzoates

	Column B	TLC R_f [a]
5α-Cholestane	1.00	
Cholestan-3β-ol	2.02	
Cholesterol	1.93	
Cholestan-3β-ol benzoate	1.84	
Cholest-5-en-3β,7β-diol dibenzoate*	1.06	0.742
Cholest-5-en-3β,7β-diol 3β-benzoate*	1.06	0.659
Cholesta-5,7-diene-3β-ol benzoate	1.06	0.825
Cholest-5-en-3β,6β-diol		0.124
Product from g.l.c. of compounds*		0.823

[a] Details of TLC procedure in experimental section

* C-7 functional group eliminated on gas chromatography.

Table X

Relative Retention Times of Androstane Hydrocarbons

(expressed relative to 5β-androstane)

	Column C
5α-Androstane	1.00 [a]
5α-Cholestane	16.2
1α,5-cyclo-androst-2-ene	0.89
Androsta-1,3-diene	1.13

[a] Absolute retention time 7.72 mins.

Table XI

Relative Retention Times of Substituted Cyclosteroids

	Column B
5α-Cholestane	1.00 [a]
1α,5-cyclo-cholest-2-ene	0.86
Cholestan-3-one	2.07
Cholestan-2-one	2.10
1α,5-cyclo-cholestan-2-one	1.79
Reduced 1α,5-cyclo-cholestan-2-one [b]	2.09
1α,5-cyclo-cholestan-3-one	1.81
Lumi-cholestenone	1.74
1(10→5α)-abeo-cholestan-2-one [c]	1.88
Cholestan-3β-ol	2.02
1α,5-cyclo-cholestan-2α-ol	1.72
1α,5-cyclo-cholestan-3α-ol	2.01
1α,5-cyclo-cholestan-3β-ol	1.97
Cholestan-2α-ol	2.06
Reduced 1α,5-cyclo-cholestan-2α-ol [b]	2.07

[a] Absolute retention time 10.7 mins.

[b] Products of high pressure reductions

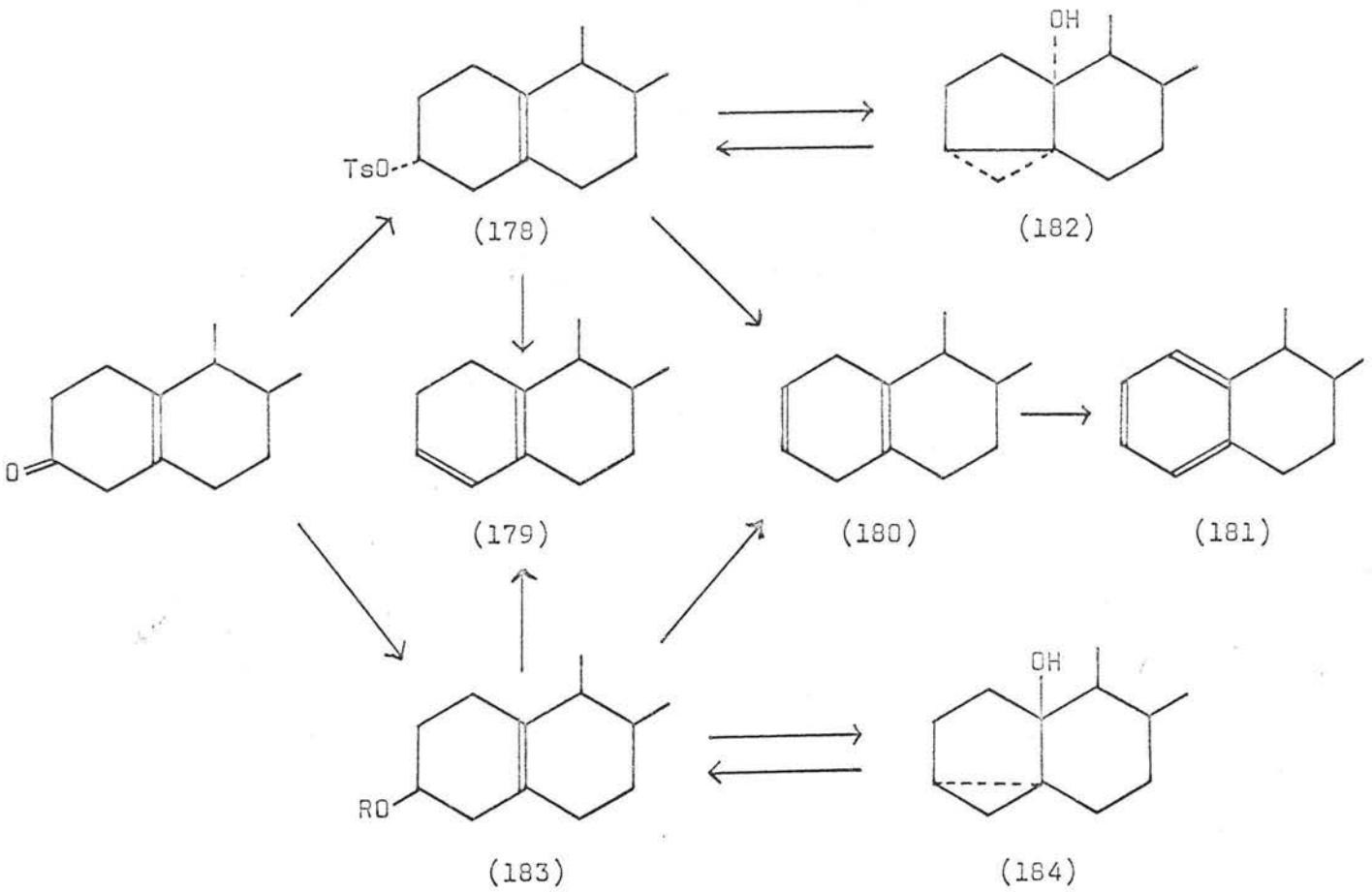
[c] Product of lithium in liquid ammonia reduction of 1α,5-cyclo-cholestan-2-one.

Solvolyses of 19-Nor Unsaturated Alcohols

The various unsaturated alcohol systems already considered have had an axial methyl group at C-10, imparting a certain rigidity to the steroid nucleus. An examination of similar reactions in 19-nor unsaturated alcohols was undertaken. Firstly a summary of previous work is presented.

The rearrangement of allylic alcohols having no C-10 substituent has been reported by Johns,[172] who studied the solvolysis of sulphonate esters of 3-hydroxy-ester-5(10)-en-17-one.

While Δ 5(10)-3-one systems are prepared without difficulty by metal ammonia reduction of compounds with an aromatic A-ring,[173] the system is very labile and isomerises to the conjugated ketone very readily. Reduction of the deconjugated ketone to the 3-hydroxy compound is possible without isomerisation, using lithium aluminium hydride or sodium borohydride, giving a mixture of the two isomeric 3-hydroxy compounds, in the ratio of 5:1. However, lithium tri-t-butoxy aluminium hydride reduction is more stereoselective, giving a 15:1 ratio of products. The major product in each case is the 3α-isomer.[174,175] This is the opposite configuration to that obtained by reduction of C-10 substituted steroids, and presumably results from the fact that in the Δ 5(10)-3-one system ring A will alternate between two half-chair conformations.[176] One of these conformations involves a strong non-bonded interaction between the equatorial 11α-proton and the 1β-proton. In this conformation, the 3β-substituent is


OH
TsO
(178)
(182)
O
(179)
(180)
(181)
OH
RO
(183)
(184)

equatorial; in the other, presumably preferred, conformation the 3α-substituent is equatorial. Therefore the preferred reduction product of a C-3 ketone will be the 3α-alcohol.

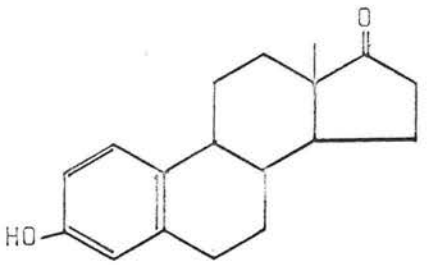
The tosylate of estra-5(10)-en-3α-ol (178) has been subjected[172] to the classical i-steroid transformation conditions; solvolysis in buffered aqueous acetone, and the products separated by chromatography. Elimination of the ester group resulted in a mixture of dienes, accounting for 60% of the product. The same mixture could be formed by passing the tosylate over activated alumina. By its ultra violet spectrum, this diene mixture was found to contain 20% of the conjugated diene (179) and a non-conjugated isomer (180). A third component was a triene (181), probably formed by autoxidation of the diene (180). The second product of this solvolysis was the 3β,5β-cyclo-steroid (182) in 30% yield, in a transformation exactly analogous to the i-steroid transformation. As expected, treatment of the cyclo-steroid (182) with acid regenerated the unsaturated alcohol (178). The third product, in 10% yield, was the inverted alcohol (183, R=H), the result of a typical S_N2 displacement of the sulphonate group without participation of the double bond. Solvolysis of the tosylate of the 3β-alcohol (183, R=Ts) gave similar products in the same proportions. The hydrocarbon mixture contained (179, 180 and 181) as before; the cyclosteroid isolated had the opposite configuration, the 3α,5α-cyclo-estran-10β-ol (184). S_N2 displacement gave the corresponding inverted 3α-alcohol.

In the i-steroid transformation of cholesteryl esters, the

3β-alcohol rearranges but the 3α-alcohol does not; an elimination reaction takes place. In this case however, both epimeric alcohols undergo rearrangement. In the case of cholesteryl esters the reason for the equatorial substituent to rearrange is because of the favourable overlap of the π electrons of the double bond with the carbonium ion formed at C-3, thus affording anchimeric assistance in the solvolysis. Since this overlap is not present in the axial substituent, the reaction takes a different course. In the case of the 19-nor alcohols, the ring system is much more flexible, therefore the isomeric 3-hydroxy substituents can both assume an equatorial conformation quite readily, both having a similar overlap with the π electrons of the 5-10 double bond.

It would therefore appear feasible that reactions of the tosylates of estr-1-en-3-ol, estr-4-en-3-ol and estr-5-en-3-ol might parallel the reactions reported earlier in the thesis for the corresponding 10β-methyl compounds,

Chart IV



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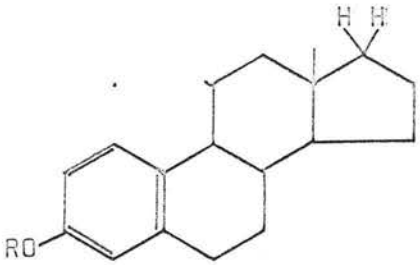
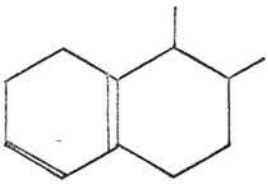


(186)

R = H, CH_3



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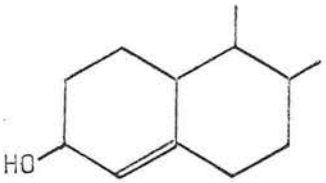

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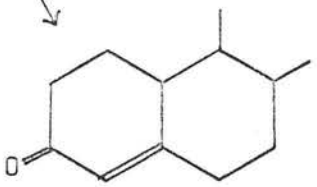

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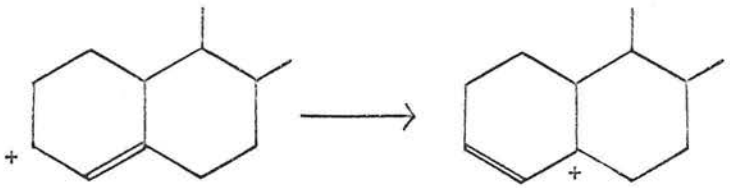
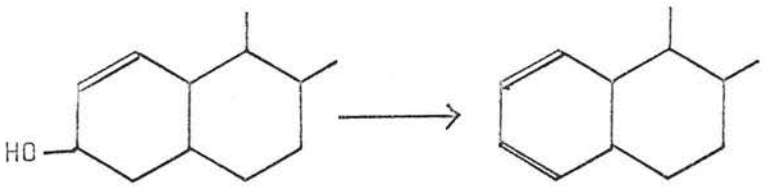


(190) (191)



(192) (193)

Preparation of the Tosylates of 19-Nor Unsaturated Steroid Alcohols

Estr-4-en-3-one was synthesised from estrone by standard reduction procedures.[177,178] The route is summarised in chart IV. Estrone (185) was reduced by the Huang-Minlong reaction to remove the 17-carbonyl.[110] The resulting phenol (186) was methylated and reduced with lithium in liquid ammonia. The initially formed estr-5(10)-en-3-one was not isolated, but prolonged acid washing during the work-up isomerised the double bond into conjugation.

Reduction of estr-4-en-3-one (187) was effected with lithium aluminium hydride in ether. After refluxing for one hour, the excess hydride was decomposed with ethyl acetate and the resulting alumina complex decomposed with hydrochloric acid (2N). The product had neither carbonyl nor hydroxyl absorption in the infra red; it was filtered through alumina in petrol and crystallised. The ultra violet spectrum was typical of a homoannular diene, λ_{max} 266 mμ (ε 5,800). P.m.r. spectrum showed two olefinic protons as a doublet at τ 4.42. The most likely structure satisfying these spectra is estra-3,5(10)-diene (188), although other dienes would be possible. This particular diene was obtained by Johns[172] as a solvolysis product, but not completely characterised.

A second reduction of estr-4-en-3-one with lithium aluminium hydride, decomposing the resulting complex with ammonium chloride instead of acid, resulted in an unsaturated alcohol being produced. This product appeared to be a mixture of the two C-3 epimers, but crystallisation enabled one to be obtained pure.

The major product of lithium aluminium hydride reduction of 19-nor-testosterone is the 3β-alcohol;[174] the crystalline product isolated here will therefore be estr-4-en-3β-ol (189).

As in the case of cholest-4-en-3β-ol, treatment of estr-4-en-3β-ol with toluene-p-sulphonyl chloride in pyridine at 0° for 18 hours did not produce any tosyl ester. After alumina chromatography, the main product isolated (62%) was estra-3,5(10)-diene, identical to that from the previous experiment. This rearrangement probably occurs via a carbonium ion, where the positive charge is located first at C-3 (190); a movement of π electrons occurs to give the more stable ion (191) with the positive charge at the tertiary centre. Loss of a proton from a second tertiary centre, C-10, gives the stable 3,5(10)-diene.

Stereospecific reduction of estr-4-en-3-one with lithium in liquid ammonia afforded 5α-estran-3-one;[179] catalytic reduction, as in the case of 10-β methylated analogue, gives a mixture of the 5α- and 5β-isomers. Bromination of the saturated ketone with bromine in acetic acid gave a non-crystallizible oil, a mixture of 2β-bromo- (70%) and 4β-bromo-estran-3-one (30%).[180,181] Dehydrobromination with lithium carbonate in dimethylformamide followed by alumina chromatography afforded estr-1-en-3-one in 45% overall yield from the saturated ketone.[182] Reduction of this unsaturated ketone with lithium aluminium hydride in ether, using ammonium chloride to decompose the alumina complex formed, afforded estr-1-en-3β-ol.(192). The configuration of the hydroxyl group is inferred by analogy with reduction of

androst-1-en-3-one under the same conditions, since the stereochemistry of the A-ring is identical.

Attempted preparation of the tosylate of estr-1-en-3β-ol resulted, as expected, in the formation of a hydrocarbon in 63% yield. G.l.c. indicated that this was homogeneous. Ultra violet and p.m.r. spectra pointed to this being a homoannular diene. The u.v. maximum was 262 mμ (ε 4,900), that calculated from Fieser's rules is 263 mμ for a 1,3-diene. The p.m.r. spectrum showed four olefinic protons in an unresolved multiplet at τ 4.25. Comparison with corresponding spectra of cholesta-1,3-diene substantiated the conclusion that this compound was estra-1,3-diene (193).

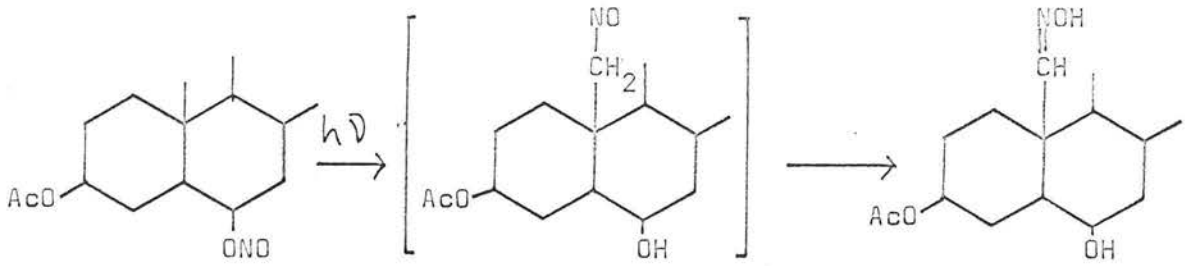
In view of the ease of elimination of the hydroxyl group from both these 19-nor unsaturated alcohols with dilute acid, it is probable that elimination would occur in pyridine also, with no formation of the corresponding tosylate. Even if these tosylates formed, the rate of elimination would be correspondingly much greater than in the cholestane series, and probably no participation of the π electrons of the double bond would occur. This would mean only diene formation occurring, with no possibility of cyclosteroid formation.

Syntheses of 19-Nor Steroids

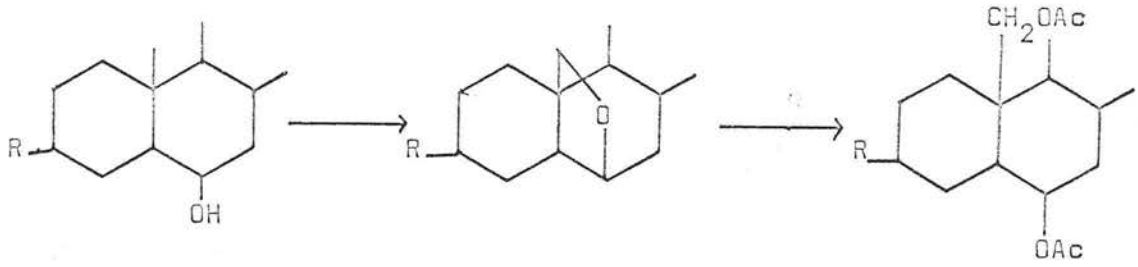
Before carrying out the rearrangement experiments on 19-nor unsaturated steroid alcohols reported in the previous section, attempts were made to find a convenient synthesis of 19-nor steroids from a less expensive starting material than estrone or its derivatives. Since the Birch reduction of estrone is a very convenient reaction, a synthesis of a ring-A aromatic steroid from starting material with a 19-methyl group would form a convenient route. Alternatively, a shorter reaction sequence might result from a reaction on the 19-methyl group itself, thereby removing this without the need of modifying the A-ring.

Non-aromatic 19-nor steroids have gained importance in the last decade as anabolic agents[196] and as progestogens for ovulation control.[197] Until 1961, the preparation of these compounds was based almost entirely on Birch reduction[173,198] of estrone, the only 19-nor steroid generally available. The recent increase in medical interest in 19-nor steroids has promoted intensive chemical research in this field. Efforts have been made to develop total syntheses of estrone from readily available starting materials, and also to eliminate, through chemical reactions, the usually unreactive 19-methyl group.

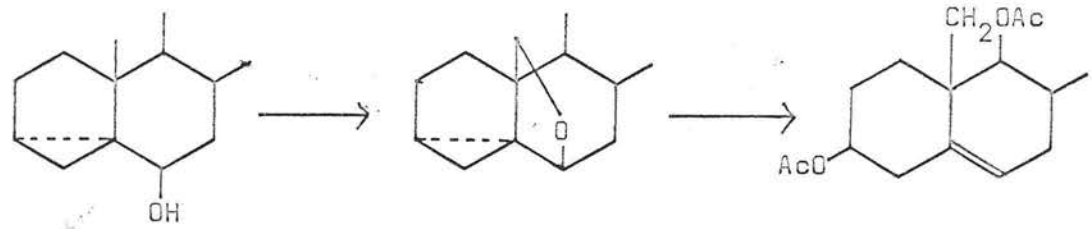
There are very few naturally occurring steroids with an oxygenated function at C-19 (e.g. strophanthidin) and those are not available in any quantity; a reactive group must therefore be introduced on C-19. Intramolecular attack on a non-activated methyl group is initiated by abstraction of one of its hydrogen



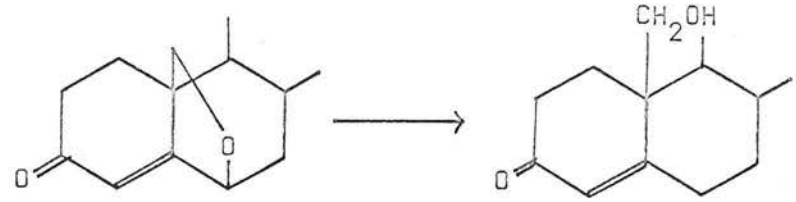
(194) Dimer (195)



(196) (197) (198)



(199) (200) (201)



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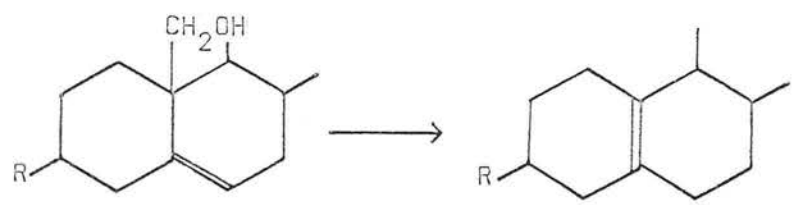


(204) (205)

atoms and this is brought about by oxygen radicals generated by homolysis of an -OR bond, (R being NO, halogen or $Pb(OAc)_3$), attached to a γ-carbon atom relative to the C-19 methyl group. Steroids containing an axial functional group on either carbon atoms 2, 4, or 6 meet these requirements for C-19 attack.

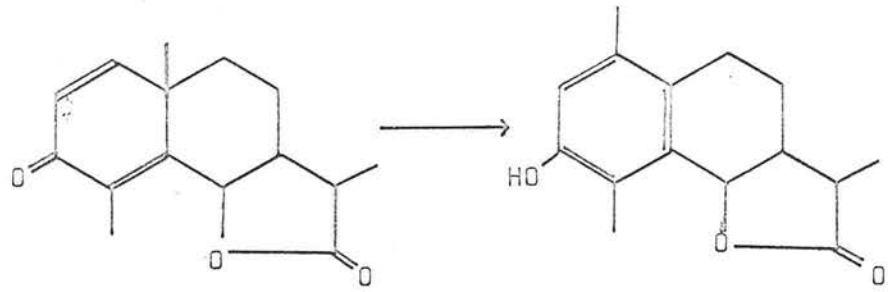
The first such hydrogen abstraction from a C-19 methyl group was reported by Barton in 1960.[199] This involved photolysis of the 6β-nitrite (194) to give the 19-oxime (195) through a dimeric nitroso derivative. 6β-19-Cyclic ethers have been formed from 6β-hydroxy compounds in several ways. The action of lead tetraacetate in refluxing benzene on the alcohol (196) produced the transannular cyclic ether (197) which could be cleaved with boron trifluoride in acetic anhydride to give the diacetate (198).[200] The yield for the preparation of (197) is improved by treating the alcohol (196) with lead tetraacetate and a trace of iodine in the presence of light.[201]

The application of the lead tetraacetate reaction to 3α,5-cyclo-steroids has recently been studied.[202,203] In one step, suitable starting material for this reaction can be prepared from readily available Δ^5-3-hydroxy steroids. Solvolysis of a Δ^5-3β-tosylate provides a 6β-alcohol and at the same time protects the 3β-position. Oxidation of the cyclo-steroid (199) with lead tetraacetate gave the cyclic ether (200), which on treatment with acetic acid gave the unsaturated 3,19-diacetate (201).

Various reactions have been utilized for removal of a substituted C-19 methyl group. Dehydration of a 19-oxime (195) leads to a 10β-cyano group, easily converted into a carboxylic

acid which can then be decarboxylated by pyrolysis[204,205] or use of Girard's reagent T.[206] Cleavage of cyclic ethers, e.g. (197), (202), with zinc in acetic acid or ethanol affords the 19-alcohol (203),[207] which can be oxidised to a 19-carboxyl derivative and then eliminated. Treatment of the alcohol (204) with lead tetraacetate or chromic acid leads directly to (205) by elimination of the hydroxyl group and carbon 19.[208,209] Using these reactions, pregnanolone acetate has been converted into 19-nor progesterone in 37% overall yield.[210]

Despite the progress that has been made in removing the 19-methyl group, the easiest route to 19-nor steroids is by Birch reduction[173,198] of aromatic A-ring steroids. The need for quantities of synthetic estrogens therefore increased and processes for their synthesis have been evolved that are more efficient than the pyrolysis in mineral oil of $\Delta^{1,4}$dien-3-ones to give a 1,3,5(10)-trien-3-ol, with expulsion of the 19-methyl group.[211,212] This process involves breaking the 19-methyl carbon-carbon bond, not ordinarily regarded as a reduction, but two hydrogen atoms have to be supplied for this pyrolysis to proceed; these must come from the mineral oil solvent and the solvent can therefore be considered as a reducing agent catalysed by heat. This reduction is not achieved by other purely chemical reducing agents. Estrone has however been synthesised in 75% yield by the reductive aromatization of androsta-1,4-dien-3,17-dione, 17-ethylene ketal with an excess of the radical anion derived from



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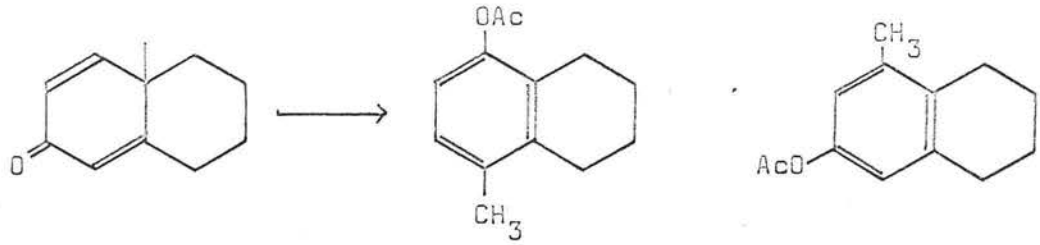
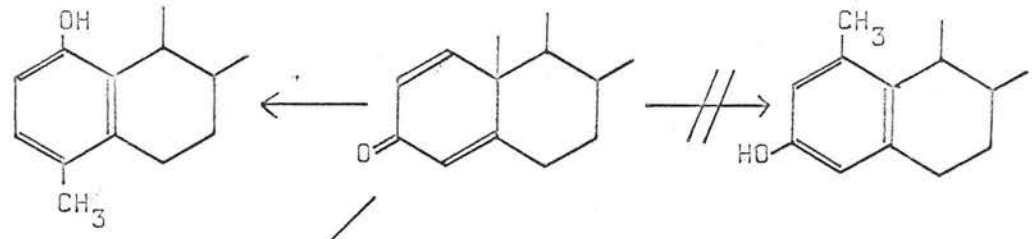
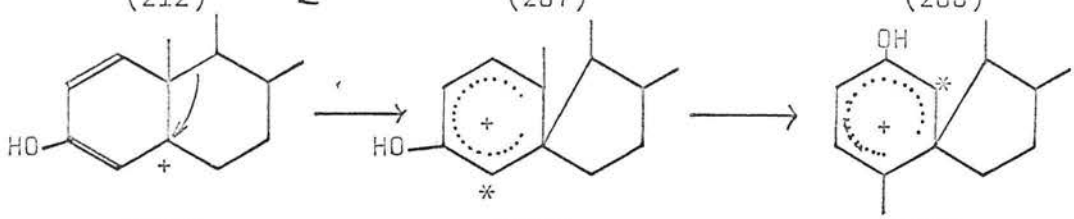


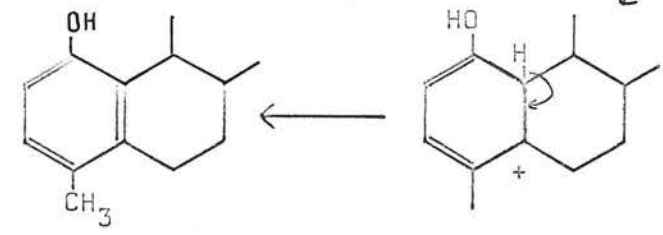
(209) (210) (211)



(212) (207) (208)



(214) (213) (213)

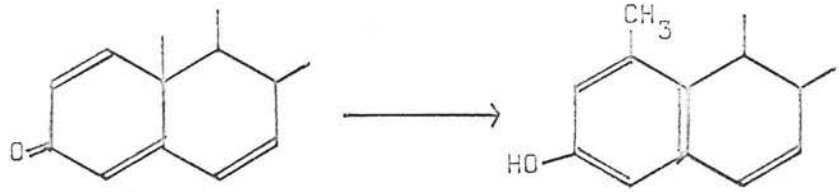


(212)

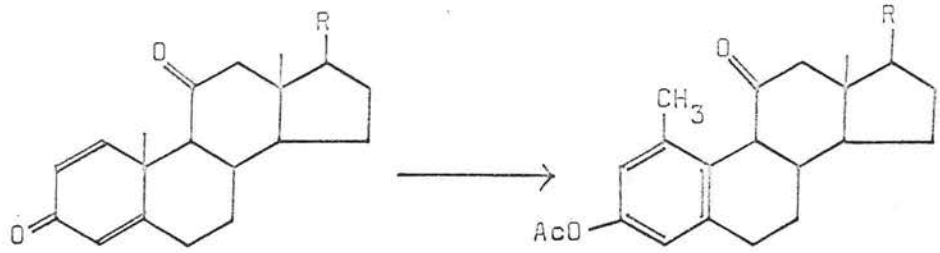
lithium metal and biphenyl.[213] This process has also been used in the synthesis of 19-nor-cholesta-1,3,5(10)-trien-3-ol.[214] Other rearrangements leading to aromatization of ring A, generally do not occur with elimination of the 19-methyl group, the product having a methyl substituent in the A-ring.[215]

The transformation of cross-conjugated dienones to phenols by acetic anhydride and sulphuric acid was first reported by Inhoffen.[216] By analogy with earlier work on the rearrangement of the terpenoid santonin (206),[217] the structure of the product of rearrangement of steroidal 1,4-dien-3-ones (207) was incorrectly formulated as (208) arising from methyl migration from C-10 to C-1.[194] Later work showed that this rearrangement in steroids directionally controlled by internal as well as external factors.[218] Rearrangement of the model system, 2-keto-10-methyl-$\Delta^{1(9),3}$-hexahydronaphthalene (209) with acetic anhydride and sulphuric acid was found to give the product of ring methylene migration (210) and not that of methyl migration (211).[219] It was therefore inferred that a similar rearrangement occurred in the case of the steroid (207), the product being the 1-hydroxy-4-methyl derivative (212).[218] This rearrangement occurs through an intermediate spiran structure (213) by the mechanism indicated.[218]

In cases where the migration terminus of this reaction sequence [carbon 4, C* in (213)] is substituted, angular methyl rearrangement takes place instead, as in the case of santonin (206).[217] Ring-bond movement is also restricted by the presence of additional unsaturation in conjugation with the dienone system. Treatment of the trienone (215) with acetic anhydride and sulphuric

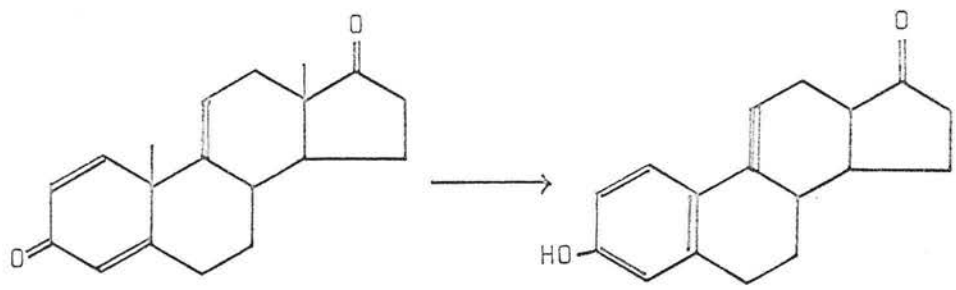


(215) (216)



(217) (218)

(219) (220)



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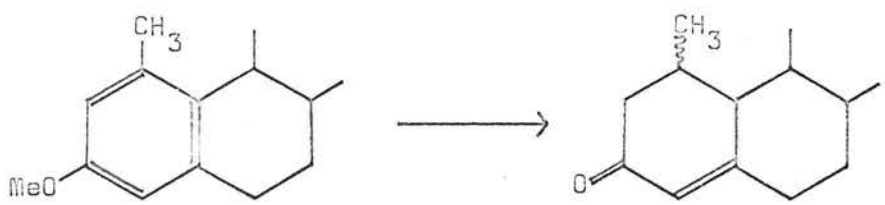


(223)

acid gives the 3-hydroxy-1-methyl compound (216).[218,220] This has been attributed to the 5-6 bond in the intermediate ion (214) having some double bond character which inhibits formation of the spirane intermediate. The presence of an 11-carbonyl group also has an effect on the rearrangement. Treatment of prednisone acetate (217) with acetic anhydride and sulphuric acid effects aromatisation but the 3-hydroxy-1-methyl compound (218) results.[221] In this case, movement of the 9-10 bond would require the presence of an unfavourable positive charge adjacent to the carbonyl dipole. As expected, a 1,4-dien-3-one with an 11β-hydroxyl substituent rearranges to a 1-hydroxy-4-methyl derivative.

A difference in the rearrangement products of dienones is seen with aqueous acid reagents.[222] Treatment of the dienone (219) with concentrated aqueous hydrochloric or hydrobromic acid leads to the 3-hydroxy-1-methyl triene (220) in 55% yield. The corresponding 1-hydroxy-4-methyl compound is also produced in 10% yield.

Cross-conjugated dienone systems have been aromatized with zinc in aqueous pyridine, dimethylformamide or ethylene glycol.[223] Androsta-1,4,9(11)-trien-3,17-dione (221) readily undergoes A-ring aromatization with elimination of the angular methyl group to form Δ^9-estrone (222) in 75% yield. Similar treatment of a 1,4-dien-3-one (207) results in the formation of a 1-hydroxy-4-methyl-triene (212) in 80% yield, together with only 4% estrone. Reaction of androsta-1,4,6-trien-3,17-dione affords Δ^6-estrone in 15% yield.

The synthesis of 19-nor steroids from A-ring phenolic compounds is readily achieved by Birch reduction.[173,198] However, in the majority of aromatizations discussed above, the 19-methyl group is not eliminated, but appears as a substituent on the A-ring. Birch reduction of these compounds with methyl substituents (e.g. 223), generally results in a mixture of isomers, not readily seperable.[215] It would therefore be advantageous if this substituent could be removed. Clearly, this must be achieved easily if the reaction sequence is to have any advantage over the direct removal of the methyl group while still a C-10 substituent. Reacting the methyl group as an aromatic substituent will be more facile than a corresponding alicyclic derivative.

Generally the reagents commonly used to oxidise the side chain of a benzene derivative are rather strong, and may well be unsuitable for use with a steroid molecule with other substituents present. In particular, a methylene group α to the aromatic A-ring (carbon 6) will be activated and may be oxidised also. Reaction of chromic acid with estradiol diacetate introduces a carbonyl group at C-6.[225] Wilds and Djerassi[194] failed to effect an oxidation of 1-methoxy-4-methyl-19-norcholesta-1,3,5(10)-triene with boiling alkaline permanganate.

Recently the use of cerium (IV) for the partial oxidation of aliphatic side chains on aromatic systems has been investigated.[226,227] Syper[227] reports the oxidation of various substituted toluenes to the corresponding benzaldehyde derivatives, in high yields, with a four-fold excess of ceric ammonium sulphate in aqueous acid solution. Solvents used were nitric acid

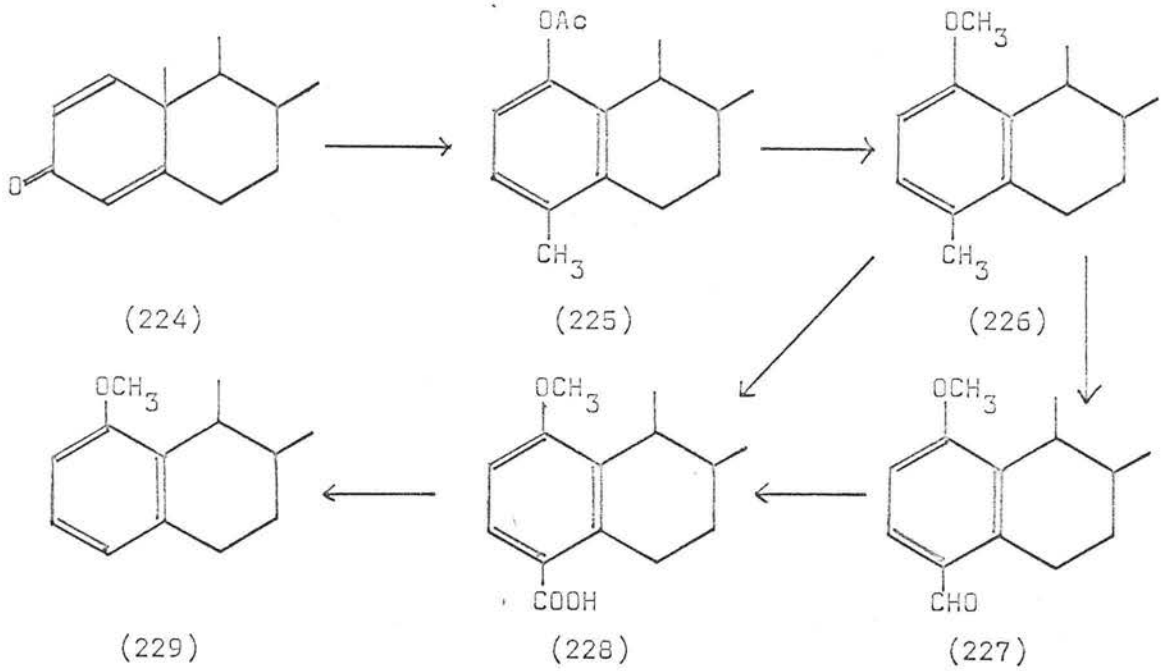
(3.5N), perchloric acid (6N) or acetic acid (50%). Under the conditions used, the aldehydes were not further oxidised to the corresponding acid, nor were second methyl groups attacked, p-xylene being oxidised to p-tolualdehyde.[226,227] Since both ortho- and para-methoxy-toluenes were oxidised to the corresponding methoxy-benzaldehyde, oxidation of a methyl group on a steroid phenol, e.g. 1-methyl-estrone methyl ether (223), should proceed to give a 1-aldehyde. It is reported however that cerium (IV) oxidises ethyl benzene to acetophenone;[227] the reagent might therefore attack C-6 on the steroid nucleus.

The oxidation of various methyl substituted steroid phenol ethers with cerium (IV) has been investigated, and the methyl group was found to be oxidised to an aldehyde or carboxylic acid. Treatment of estrone methyl ether with cerium (IV) under conditions found to oxidise an aromatic methyl group, produced no reaction. The reagent, four-fold excess of ceric ammonium nitrate in acetic acid (90%) at room temperature, must therefore be strong enough to oxidise an aromatic methyl group, but not to react with an activated methylene group (carbon 6) or with a ketone. For oxidation of alcohols and ketones a much stronger acid (e.g. perchloric or nitric) is invariably used as a solvent.[228]

The steroid aldehydes resulting from this oxidation have been further oxidised with potassium permanganate to their carboxylic acids, but attempts to decarboxylate these were not successful. The reaction time of the cerium oxidation was adjusted so that the only product was the aldehyde and these

compounds have been decarbonylated with tris-(triphenylphosphine)-rhodium chloride (TRC).[229]

Acyl aldehydes can be decarbonylated by metallic palladium at 200°.[230] However, TRC is converted easily into bis-(triphenylphosphine)-rhodium chlorocarbonyl by reaction with aldehydes at room temperature.[231] By this means aldehydes can be smoothly decarbonylated into the corresponding hydrocarbons. α-Hydroxy benzaldehyde phenol, in toluene, was refluxed with TRC for 20 min. and a 70% yield of phenol was produced.[229] It is probable therefore, that aldehyde substituents on the A-ring of steroid phenols would be readily decarbonylated by this reagent. The synthesis of A-ring aromatic precursors for 19-nor steroids, utilizing the reactions outlined, is described below.


OAc
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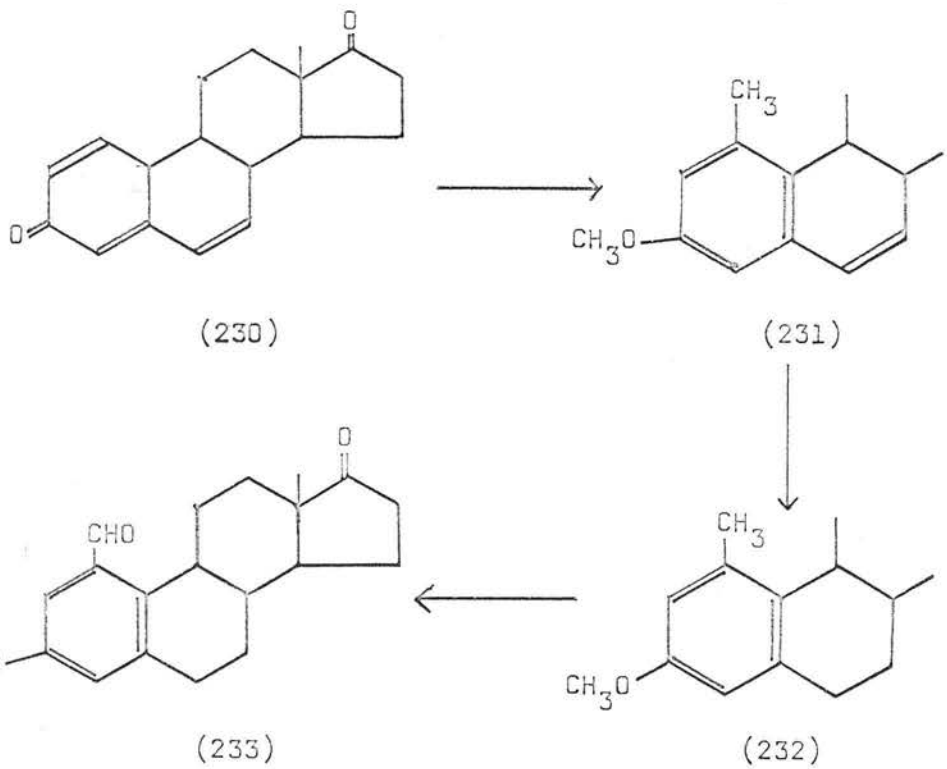
CH3
O
(224)
(225)
(226)
(227)
(228)
(229)
COOH
CHO


O
CH3
CH3O
(230)
(231)
(232)
(233)
CHO

Estrone Synthesis from 19-methyl Steroids.

1-Methoxy-4-methyl-19-nor-cholesta-1,3,5(10)-triene (226)[194,218] was synthesised from cholesta-1,4-diene-3-one (224) by reaction with acetic anhydride and sulphuric acid; the initial product, an acetate (225), being hydrolysed and methylated.[232] Although this particular phenol would not, on Birch reduction, afford an oxygenated substituent at C-3 on the resulting 19-nor steroid, its ease of preparation from readily available starting material made it useful as a model compound. The difference between a 1-methoxy-4-methyl compound prepared here and a 3-methoxy-1-methyl compound prepared below, is clear from the p.m.r. spectra. In the former, the two ortho aromatic protons (on C-2 and C-3) show as a quartet, τ 3.34, J = 12 c.p.s. The two meta aromatic protons (on C-2 and C-4) of the 3-methoxy-1-methyl compound appear in the p.m.r. spectrum as a singlet, τ 3.37.

Treatment of 1-methoxy-4-methyl-19-nor-cholesta-1,3,5(10)-triene (226) with four moles of ceric ammonium nitrate in acetic acid (90%) for 16 hours at room temperature gave a pale yellow glass, whose infra red spectrum had peaks (1700 and 2700 $cm.^{-1}$) characteristic of an aldehyde group, and also absorption at 1770 $cm.^{-1}$ and a broad peak at 2400-3600 $cm.^{-1}$, characteristic of a carboxylic acid. The p.m.r. spectrum of this material had absorption due to the aldehyde proton at τ -0.03. The signal at τ7.88 of the C-4 methyl group in the starting material was absent. The product would therefore appear to be a mixture of the aldehyde (227) and the acid (228). The material was not purified further, but was dissolved in acetone and oxidised with potassium

permanganate at room temperature. The product isolated was no longer an aldehyde and the intensities of the acidic peaks in the infra red had increased. The material was crystallised to give the acid (228).

Attempts were made to decarboxylate this compound with quinoline and with copper chromite and quinoline.[233] Refluxing the acid in quinoline for two hours gave steroidal material (20% recovery) which appeared to have no carboxyl group. Chromatography of this material was not successful in isolating the pure product (229). A second decarboxylation, with copper chromite in quinoline, was not successful either. The recovery of steroid was improved, although the product consisted of starting material and unidentified material, with no carboxyl group and no aromatic protons.

The product of a second cerium oxidation of 1-methoxy-4-methyl-19-nor-cholesta-1,3,5(10)-triene (226) was filtered through deactivated alumina, and the aldehyde (227) obtained free from the corresponding acid (228). This aldehyde, in benzene, was refluxed with an equimolar amount of tris-(triphenylphosphine)-rhodium chloride for one hour. The crude product was filtered through alumina in benzene to give a clear glass. Although this would not crystallise, it was clear from infra red and p.m.r. spectra that decarbonylation had occurred, to give 1-methoxy-19-nor-cholesta-1,3,5(10)-triene (229).

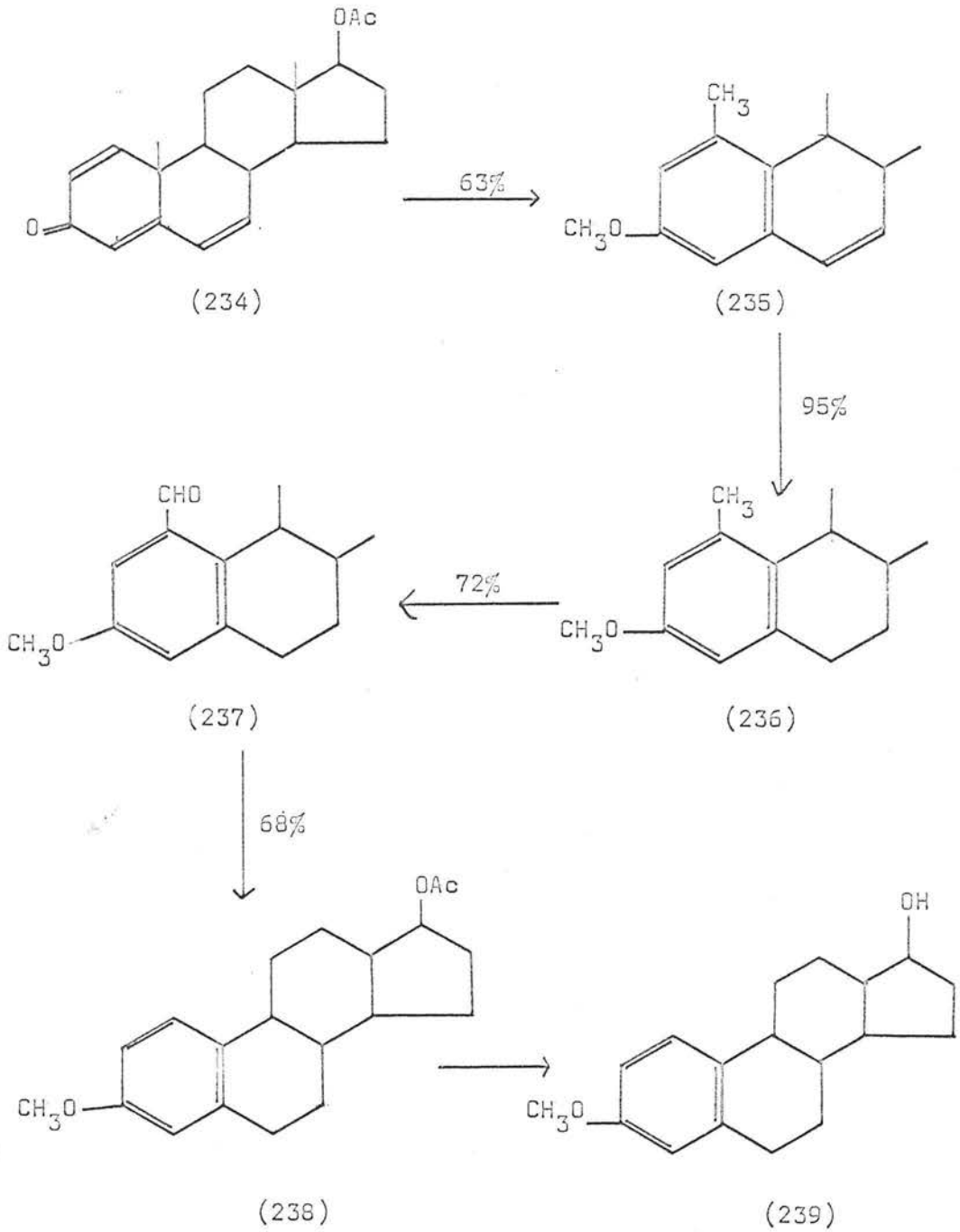
Since the reaction scheme for removal of aromatic methyl groups appears to have been successful in the cholestane series,

it was applied to the synthesis of an estrone derivative.

Rearrangement of a 1,4,6-trien-3-one system produces a phenol where the hydroxyl group is on C-3;[218,220] this system can be readily synthesised. Bromination, followed by dehydrobromination of testosterone acetate gave 17β-acetoxy-androsta-1,4,6-trien-3-one (234).[234] An easier synthesis of the corresponding 17β-alcohol, in slightly higher yield, was carried out by refluxing androst-5-en-3β,17β-diol with three moles of dichlorodicyanobenzoquinone.[235] Similarly, reaction of DDQ with 3β-hydroxy-androst-5-en-17-one gave androsta-1,4,6-trien-3,17-dione (231).

Aromatization of androsta-1,4,6-trien-3,17-dione (230) with acetic anhydride and sulphuric acid gave a mixture of the expected phenol acetate, about 25% from p.m.r. spectra, and unreacted trienone. The material was treated with the same reagents for a longer period, ten hours compared with three hours, and the reaction was found to have proceeded very little further. The mixture was hydrolysed and the phenol methylated. Alumina chromatography gave 3-methoxy-1-methyl-estra-1,3,5(10),6-tetraen-17-one (231) in 17% yield. It is probable that a reaction also occurs at the 17-carbonyl group and that this in some way affects the aromatization reaction. Catalytic reduction at atmospheric pressure, using 10% palladium on charcoal catalyst, removed the 6-7 double bond to give the corresponding triene (232).

3-Methoxy-1-methyl-estra-1,3,5(10)-trien-17-one (232) was treated with a four-fold excess of ceric ammonium nitrate in acetic acid, and stirred at room temperature. In order to determine the most favourable reaction time for preparation of the 1-aldehyde,



(234) (235) (236) (237) (238) (239)

with little or no carboxylic acid produced, the course of the reaction was followed. After (a) 1.5 hr., (b) 4.5hr. and (c) 10 hr., portions of the solution were removed, and the steroid isolated. The main bulk of the reaction was allowed to proceed for 22 hr. (d). P.m.r. spectra of the samples showed that (a) and (b) still contained a substantial amount, though decreased considerably in (b), of the starting material (peak at τ7.85 due to the aromatic methyl group). This had disappeared in (c) and (d). Sample (c) showed an aldehyde proton (τ -0.03), but this had diminished in (d). Infra red spectra of (c) and (d) showed that (c) contained aldehyde, but very little acid, while (d) represented a mixture of approximately equal amounts of aldehyde and acid.

It would appear therefore, that the optimum period for this oxidation will be 10 hours, for maximum yield of 1-formyl-3-methoxy-estra-1,3,5(10)-trien-17-one (233). In view of the low yield in the aromatization reaction with a 17-carbonyl in the molecule, the same reaction sequence was carried out on 17β-acetoxy-androsta-1,4,6-trien-3-one (234). In this case, the yield of aromatized product was increased. Aromatization was effected with sulphuric acid and acetic anhydride as before. The crude diacetate was hydrolysed, treated with methyl toluene-p-sulphonate in alkali and then acetylated with acetic anhydride in pyridine to give 3-methoxy-1-methyl-estra-1,3,5(10),6-tetraen-17β-ol acetate (235) in 63% yield from the trienone. Hydrogenation of this material proceeded rapidly with palladium on charcoal catalyst at room temperature to give 3-methoxy-1-methyl-

estra-1,3,5(10)-trien-17β-ol acetate (236). Oxidation of this compound with cerium (IV) in acetic acid gave 1-formyl-3-methoxy-estra-1,3,5(10)-trien-17β-ol acetate (237) after ten hours reaction. There was no evidence of any starting material present and only a trace of acid. The aldehyde, in benzene, was treated with an equimolar amount of tris-(triphenylphosphine)-rhodium chloride and the solution refluxed for one hour. The product, 3-methoxy-estra-1,3,5(10)-trien-17β-ol acetate (238) was obtained after alumina chromatography of the reaction mixture, and had melting point 101-103°, the literature[236] melting point being 101-102.5°. Hydrolysis of the acetate gave estradiol-3-methyl ether (239). The infra red and p.m.r. spectra of the last two compounds were identical to those of authentic samples.

The overall yield of this reaction sequence from the trienone (234) to the methyl ether (238) is 29%, the overall yield from 3β-hydroxy-androst-5-en-17-one (DHA) to estradiol methyl ether is 14%, the decrease due mainly to the poor yield in the preparation of the trienone (234). It is possible that this could be greatly increased by a more detailed study of the reactions involved. The reaction sequence does, however, provide a facile route for the removal of A-ring methyl substituents from phenolic steroids, prior to their reduction to 19-nor compounds.

E X P E R I M E N T A L

General Notes on Experimental Section

Melting points were determined on a Kofler block and are corrected. Optical rotations at 289 mμ were measured at room temperature on a Perkin Elmer 141 Polarimeter, for chloroform solutions in a 1 dm. cell. The concentration values quoted in brackets after each rotation are given in g./100 ml. Infra red spectra were recorded for carbon disulphide solutions (unless otherwise specified) on either a Unicam SP200 spectrophotometer or a Perkin Elmer 237 spectrophotometer. Ultra violet spectra were recorded for ethanol solutions on a Unicam SP800 spectrophotometer. The visible spectra of TNM complexes were recorded on a Perkin Elmer 137 UV spectrophotometer. Proton magnetic resonance spectra were recorded for deuterochloroform solutions using tetramethylsilane as an internal standard on a Perkin Elmer R 10 (60 mc/s) spectrometer. Rotatory dispersion measurements were recorded on a Bendix Ericsson Polarmatic 62 spectropolarimeter. The mass spectrum of hydrocarbon 'H' was determined on an LKB 9000 gas-chromatograph-mass spectrometer, by Dr. C. J. W. Brooks, Glasgow University.

Column chromatography was carried out by the method of Reichstein and Shoppee,[224] and the activity of the alumina used was defined by the method of Brockmann and Schodder.[81] In the following section, 'alumina' infers Peter Spence type 'H' alumina, activity II. 'Deactivated alumina' infers Spence type 'H' alumina treated with 5% (by volume) of 10% acetic acid, and was activity III. 'Alumina, activity I' infers Woelm neutral alumina, activity I. Thin layer chromatography was carried out

on Silica Gel 'G' (E. Merck & Co.), and the chromatograms were rendered visible with iodine vapour.

Solutions were dried over magnesium sulphate. The petrol used had b.p. 60-80°.

Literature constants quoted without a specific reference are from Fieser and Fieser 'Steroids'.

Cholestan-3β-ol[64]

Cholesterol (120 g.) in ethyl acetate, was reduced by catalytic hydrogenation over 10% palladium on charcoal catalyst, at 30° and 1 atmos. pressure. Perchloric acid (8 drops) was added as promoter.[64] The acid was neutralised with solid sodium hydroxide, and the catalyst filtered off. The volume of solvent was reduced and cooling afforded crystalline cholestan-3β-ol (93.3 g.; 77% yield), m.p. 141-142°, (lit. 142°), $[\alpha]_D$ +24° (c.0.17), (lit. +23°).

Cholestan-3-one[66]

Cholestan-3β-ol (30.0 g.) in acetone (2 l.) was oxidised with 8N chromic acid (Jones reagent[65]) (60 ml.). The reaction was allowed to proceed for 4 min. and excess reagent was decomposed with methanol (200 ml.). Water (200 ml.) was added, the acetone removed under reduced pressure and the steroid extracted into ether. The ethereal solution was washed with dilute hydrochloric acid, with water, until the washings were neutral, and with saturated sodium chloride solution. The solution was dried and the solvent removed under reduced pressure to give cholestan-3-one, which was recrystallised from acetone, to give needles (28.3 g.; 95%) with m.p. 128-129°, (lit. 129°), $[\alpha]_D$ +43° (c 0.16), (lit. +43.5°).

2α-Bromo-cholestan-3-one[66,67]

A solution of bromine (4.5 g.) and 48% hydrobromic acid (0.3 ml.) in glacial acetic acid (20 ml.) was added over 10 min.

to a vigorously stirred solution of cholestan-3-one (10.0 g.) in acetic acid (300 ml.). After stirring for 2 min., the resulting suspension was cooled to 10°, the bromoketone filtered off and washed free of bromine with acetic acid. Crystallisation from ethanol-acetone gave 2α-bromo-cholestan-3-one (7.2 g.; 60%), m.p. 166-169°, (lit. 168-169°). A second recrystallisation from ethanol, where heating was avoided to prevent decomposition, gave material with m.p. 168-169°.

Cholest-1-en-3-one[68,69,70]

2α-Bromo-cholestan-3-one (5.0 g.) was added to lithium carbonate (5.0 g.) in refluxing dimethylformamide (65 ml.), and the mixture refluxed for 30 min. The solution was allowed to cool and the inorganic material filtered off. Dilute hydrochloric acid (30 ml.) was added and the steroid extracted into ether. The ethereal solution was washed with water until it was neutral, washed with saturated sodium chloride solution and dried. Removal of the solvent afforded a white crystalline solid. Recrystallisation from ethanol gave cholest-1-en-3-one (3.20 g., 78%), m.p. 94-98° (lit.98°). An infra red spectrum showed the presence of a saturated ketone in the crystallised product as a shoulder at 1710 $cm.^{-1}$ on the carbonyl peak at 1680 $cm.^{-1}$ The experiment was repeated with bromoketone which had been carefully purified to exclude non-brominated material, but the product still showed the presence of the saturated impurity. Use of lithium bromide or chloride in place of the carbonate did not

improve the quality of the product.

Dehydrobromination with finely divided calcium carbonate ('Calofort U,' J. and E. Sturge, Ltd.) in dimethylacetamide also gave cholest-1-en-3-one containing saturated ketone.[69] The ultra violet spectra of these various products did not have an absorption at 241 mμ attributed to cholest-4-en-3-one,[69] indicating that this was not formed during the reaction.

Since the saturated ketone in the reaction product could not be removed by either crystallisation or alumina chromatography, it was separated chemically. The product of dehydrobromination (3.2 g.) was reduced by refluxing it with lithium aluminium hydride (2 g.) in ether (50 ml.) for one hour. The excess hydride was decomposed by the cautious addition of ethyl acetate, and the alumina complex formed was decomposed with dilute hydrochloric acid. The solution of steroid in ether was separated and washed again with dilute hydrochloric acid; it was then washed with water till neutral and washed with saturated sodium chloride solution and dried. Evaporation of the solvent gave crude cholest-1-en-3β-ol (3.1 g.). The allylic alcohol was selectively oxidised by shaking the crude product with manganese dioxide[71] (20 g.) and chloroform (250 ml.) for 5 hr. The manganese dioxide was filtered off and the solvent removed under reduced pressure. The product was crystallised from ethanol to give cholest-1-en-3-one (2.8 g.), m.p. 101-102° (lit. 98°), $[\alpha]_D$ +59° (c 0.14), (lit.+57°); $\lambda_{max.}$ 230 mμ (ϵ 10,200).

Cholest-1-en-3-one Semicarbazone[69]

A solution of 2α-bromo-cholestan-3-one (4.9 g.) in acetic acid (400 ml.) was heated at 60° with semicarbazide hydrochloride (1.3 g.) for 30 min. The mixture was diluted with water and the steroid extracted into chloroform. This solution was washed with water until neutral, washed with saturated sodium chloride solution, dried and the solvent removed. Crystallisation from ethanol-chloroform gave the semicarbazone (2.42 g.; 52%), m.p. 232-235°, (lit. 233-235°), λ_{max} 266 mμ (ε 25,700).

Cholest-1-en-3-one from its Semicarbazone[69]

Cholest-1-en-3-one semicarbazone (0.52 g.) was dissolved in acetic acid (2.5 ml.) and redistilled pyruvic acid (0.3 g.) added with water (0.5 ml.). The solution was refluxed for 10 min., water (2.5 ml.) added and the mixture cooled. The solid product was filtered off and refluxed for 5 min. with water (5 ml.). After cooling, the steroid was dissolved in ether and the ether solution washed with sodium carbonate solution, with water until it was neutral and then with saturated sodium chloride solution. After drying, the solvent was evaporated to give a pale brown solid. Crystallisation from ethanol gave cholest-1-en-3-one (0.34 g.,; 74%), m.p. 95-98°. The material was free from the saturated ketone present in other syntheses. This experiment was repeated using ten times the above quantities; the crude product in this case however was a black tar, from which no cholestenone could be obtained on chromatography.

A large scale semicarbazone cleavage was effected by Djerassi's method.[72] Cholest-1-en-3-one semicarbazone (5.0 g.) in dioxan (100 ml.) was treated with 43% sulphuric acid (50 ml.) and the solution warmed at 90° for 10 min., during which time its colour changed from pale yellow to deep red. Water was added, the steroid extracted into ether and washed free of acid with sodium carbonate solution, which also removed the red colour. The solution was washed with water and saturated sodium chloride solution, dried and the solvent evaporated. The resulting cholest-1-en-3-one was crystallised from ethanol to give a product free from saturated ketone (3.7 g.; 85%), m.p. 99-100°.

Reaction of Dichlorodicyanobenzoquinone with Cholestan-3-one.

Dry hydrogen chloride (2.72 g.) was dissolved in dioxan (30 g.) and cholestan-3-one (0.30 g.) added with dichlorodicyanobenzoquinone. After standing at room temperature for 12 hr. a pale brown precipitate appeared. The solution was warmed at 50° for 2 hr. during which time more hydrogen chloride was bubbled in. The solution was cooled and filtered. Ether was added to the filtrate and the acid removed by washing with sodium carbonate solution and water. The solvent was removed after drying, to give a white solid. An infra red spectrum showed that the product was largely unchanged cholestenone, with only small traces of unsaturated material.

Cholest-1-en-3β-ol[75]

Cholest-1-en-3-one (5.0 g.) in dry ether (30 ml.) was added

slowly to a solution of lithium aluminium hydride (3.0 g.) in dry ether (60 ml.) and the solution refluxed for 1 hr. After cooling, the excess lithium aluminium hydride was decomposed with ethyl acetate and the alumina complex decomposed with dilute hydrochloric acid. The solution of steroid in ether was separated, washed with water and dried; evaporation of the solvent gave a clear glass which solidified on cooling. Crystallisation from acetone gave cholest-1-en-3β-ol (4.82 g.; 9.6%), m.p. 129-131° (lit. 131°), $[\alpha]_D$ +53° (c 0.18), (lit. +54°).

Cholestan-3β-ol Tosylate[62]

Cholestan-3β-ol (1 g.) was dissolved in pyridine (10 ml.) and cooled to 0°. This solution was added to a solution, also at 0°, of toluene-p-sulphonyl chloride (1 g.) in pyridine (10 ml.), and the mixture was left at room temperature for 28 hr. The solution was poured into water and the steroid extracted into ether. The ethereal solution was washed free of pyridine with dilute hydrochloric acid and water, followed by a sodium carbonate wash to remove residual acid. The solution was washed with water until it was neutral and then dried. Evaporation of the solvent gave a white solid, which was recrystallised from ethanol to give cholestan-3β-ol tosylate (0.71 g.; 52%), m.p. 142-144° (lit.135°), $[\alpha]_D$ +7° (c 0.19), (lit. +6.5°), Maxima at 1189 and 1176 $cm.^{-1}$ were present in the infra red spectrum of the compound.[62] The p.m.r. spectrum had the following absorptions: τ 2.04, 2.10, 2.59, 2.71 (4 aromatic protons), 7.55 (aryl methyl group), 9.09, 9.18, 9.23 and 9.36. Despite the discrepancy in

melting point, the product is therefore the required tosylate.

Cholest-5-en-3β-ol Tosylate[19]

Cholesterol (10 g.) was treated with toluene-p-sulphonyl chloride (10 g.) in pyridine (100 ml.) as in the previous experiment. Crystallisation of the product from acetone gave cholest-5-en-3β-ol tosylate (10.3 g.; 74%), m.p. 130-131°, (lit. 131.5-132.5°), $[\alpha]_D$ -37° (c 0.14), (lit.-39.5°). The i.r. and p.m.r. spectra were very similar to those of cholestanol tosylate.

Attempted Preparation of Cholest-1-en-3β-ol Tosylate

Cholest-1-en-3β-ol (2 g.) was dissolved in pyridine (30 ml.) and cooled to 0°. To this was added a solution of toluene-p-sulphonyl chloride (2 g.) in pyridine (30 ml.) also at 0°. The mixture was allowed to warm to room temperature and left for 39 hr. Ice was then added and the steroid extracted with dichloromethane. This solution was well washed with sodium carbonate solution, then with water. It was then washed with dilute hydrochloric acid and water until the washings were neutral. The solution was dried and evaporated to give a pale brown gum. Despite there being no free pyridine or toluene-p-sulphonic acid in the product, both i.r. and p.m.r. spectra showed the presence of more aromatic unsaturation than in the two previously synthesised tosylates. The product could not be induced to crystallise.

An attempt was made to purify this material (2.9 g.) by chromatography on alumina, but not all the material would dissolve in petrol; a white amorphous solid was insoluble. This was

filtered off and crystallised from benzene to give white plates of N-(cholest-1-en-3α-yl)-pyridinium tosylate (0.62 g.) m.p. 220-222°, $[\alpha]_D$ +35° (c 0.099), (Found: C, 75.1; H, 9.1; N, 2 4; S, 5.0. $C_{39}H_{57}O_3NS$ requires C, 75.5; H, 9.2; N, 2.3; S, 5.1%); $\nu_{max.}$ 3450, 3050, 1205, 1130, 1045, 1025, 830, 795, 695 cm.$^{-1}$

The petrol soluble material was adsorbed on deactivated alumina, and elution with petrol afforded a clear glass (0.45 g.) which was crystallised with difficulty from acetone. Elution with petrol-benzene (98:2) gave cholest-1-en-3β-ol (0.62 g.) m.p. 129-131°. On continued elution with increasingly polar solvents, no material was eluted until a pale brown solid with chloroform-methanol (95:5). This was crystallised from benzene to give N-(cholest-1-en-3α-yl)-pyridinium tosylate (0.23 g.), m.p. 220-222°, undepressed on admixture with the material isolated before chromatography.

The steroid eluted with petrol was rechromatographed on neutral alumina, activity I, from which it was eluted with petrol. Crystallisation from acetone gave needles of 1α,5-cyclo-5α-cholest-2-ene (0.424 g.) m.p. 71-72°, $[\alpha]_D$ +40° (c 0.07), (Found: C, 88.0; H, 11.8. $C_{27}H_{44}$ requires C, 88.0; H, 12.0%). A mass spectrum indicated a molecular weight of 368: $C_{27}H_{44}$ = 368.6. $\nu_{max.}$ 3025, 1340, 1030, 965, 883, 775, 740, 703 cm.$^{-1}$; $\lambda_{max.}$ 212, 237, 246, 263 mμ.

This experiment was repeated several times; in each case the same ratio of products was obtained. Chromatography on Florisil instead of alumina also produced the same product ratio.

Dehydration of Cholest-1-en-3β-ol with Phosphorus Oxychloride

Phosphorus oxychloride (1 ml.) was added to a solution of cholest-1-en-3β-ol (0.5 g.) in pyridine (10 ml.) and the solution refluxed for 30 min., during which time it turned pale brown. Water was added and the steroid extracted into ether. The ether solution was washed with water and dried; evaporation of the solvent gave a pale brown oil. This was chromatographed on neutral alumina, activity I. Elution with petrol afforded a clear glass (0.27 g.) which was crystallised from acetone to give 1α,5-cyclo-cholest-2-ene (0.254 g.; 51%) m.p. 70-72°. Spectra were identical to those of the sample prepared by the previous method.

Attempted Benzoylation of Steroid 'S'

Steroid 'S' (0.13 g.) was refluxed with benzoyl chloride (1 ml.) in pyridine (5 ml.) for 2 hr.; during this time it darkened in colour. Water was added and after standing for 30 min. the steroid was extracted into dichloromethane. This solution was washed well with sodium carbonate and water before being dried. Evaporation of the solvent gave a brown oil (0.11 g.), which could not be crystallised. The strong hydroxyl peak at 3450 $cm.^{-1}$ in the i.r. spectrum of steroid 'S' had only diminished slightly in the spectrum of the product; this spectrum was not typical of a benzoate. Treatment of the product with lithium aluminium hydride (0.1 g.) in dry ether (20 ml.), the mixture being refluxed for 1 hr. before addition of ethyl acetate and dilute hydrochloric acid to decompose the excess

reagent and the alumina complex respectively, gave after isolation in the usual manner, a brown oil whose i.r. spectrum did not resemble that of steroid 'S'. It was not identified.

Attempted Methylation of Steroid 'S'

A solution of diazomethane in ether was prepared by placing nitrosomethylurea[183] (0.5 g.), water (20 ml.), ether (20 ml.) and potassium hydroxide (0.5 g.) in a stoppered separating funnel. The diazomethane generated in the aqueous layer was dissolved in the ether as it rose through it. The aqueous alkali was run off and the ethereal solution of diazomethane was dried over solid potassium hydroxide. Steroid 'S' (0.1 g.) in ether (10 ml.) was added to the diazomethane solution, and after standing at room temperature for 2 hr., the ether and excess diazomethane were removed under reduced pressure. Examination of an i.r. spectrum of the product showed that no reaction had occurred.

Treatment of Steroid 'S' with Sodium Iodide

Steroid 'S' (0.5 g.) was dissolved in hot ethanol (1 ml.) and this solution added to sodium iodide (0.5 g.) in hot ethanol (5 ml.). On cooling a pale yellow precipitate formed which was filtered off. Recrystallisation from ethanol gave N-(cholest-1-en-3α-yl)-pyridinium iodide (0.43 g.), m.p. 283-285°, $[\alpha]_D$ +57° (c 0.11).

Cholest-1-en-3β-ol Benzoate[75]

Cholest-1-en-3β-ol (5 g.) was dissolved in pyridine (40 ml.) and benzoic anhydride (5 g.) added. The solution was refluxed for 2 hr., water added and refluxed for a further 15 min. The

steroid was extracted into ether, washed with dilute acid, with water, with sodium carbonate solution and then with water until it was neutral. The solution was dried and the solvent evaporated. The product was crystallised from acetone to give cholest-1-en-3β-ol benzoate (5.2 g.; 82%) m.p. 138-141° (lit. 141°). $\nu_{max.}$ 1720, 1282, 760, 720 cm.$^{-1}$

Cholesta-1,3-diene.[59]

Cholest-1-en-3β-ol benzoate (0.95 g.) in petrol was adsorbed on neutral alumina, activity I, and allowed to stand for 22 hr. Elution with petrol gave a clear oil, which was crystallised from acetone to give cholest-1,3-diene (0.13 g.) m.p. 66-68° (lit. 67-68°). Elution with petrol-benzene (90:10) gave unchanged benzoate (0.19 g.). An alcohol eluted with ether was not identified.

Action of Toluene-p-sulphonyl Chloride in Pyridine on Cholesta-1,3-diene

Cholesta-1,3-diene (0.02 g.) was refluxed in pyridine (5 ml.) with toluene-p-sulphonyl chloride (0.02 g.) for 1 hr. Water was added and refluxing continued for 30 min. The steroid was extracted into ether, washed with acid, water, sodium carbonate solution and finally with water until it was neutral, before being dried. Evaporation of the solvent gave a clear glass which was crystallised from acetone to give unchanged cholesta-1,3-diene (0.017 g.).

Microhydrogenation of 1α,5-cyclo-cholest-2-ene

Quantitative microhydrogenation of the hydrocarbon 'H' was undertaken using the apparatus of Clauson-Kaus and Limborg,[84] which enables hydrogen uptake of up to 5 ml. to be accurately determined. Preliminary experiments with sorbic acid (approx. 0.008 g. samples were taken) gave results indicating that 2.00 and 2.03 double bonds were present. Hydrogenation of cholest-5-ene (0.02645 g.) using ethanol as solvent and 10% palladium on charcoal catalyst (0.020 g.) showed that there were 1.07 double bonds present in the molecule.

Hydrocarbon 'H' (0.01324 g.) was reduced under the same conditions as cholest-5-ene and from the volume of hydrogen taken up, it could be calculated that there were 1.93 double bonds present in the molecule. A repeat determination indicated 1.96 double bonds.

The products of both hydrogenations, after removal of catalyst, were combined and filtered in petrol through alumina (activity I). The product was crystallised from acetone to give 5α-cholestane (0.027 g.) m.p. 80-82° (lit. 80°), this was undepressed on admixture with an authentic sample derived from cholest-5-ene by a larger scale reduction.[190] The i.r. and p.m.r. spectra of the two products were identical. Methyl groups gave peaks in the p.m.r. spectrum at τ 9.09, 9.18, 9.22 and 9.35.

Cholest-1-ene[187,188]

Cholest-1-en-3β-ol benzoate (3.5 g.) was dissolved in anhydrous ethylamine (15 ml.) in a 250 ml. flask fitted with a stopcock in the stopper. Small pieces of lithium (0.1 g.) were added and

after effervescence ceased, the flask was shaken for 4 hr., during which time a blue colour appeared. The stopcock was opened periodically to prevent a build-up of pressure. The mixture was allowed to stand overnight and then water was added. The steroid was extracted into ether and the ether solution washed with dilute acid, water, sodium carbonate solution and finally with water until it was neutral. The solution was dried and the solvent evaporated, to give a clear glass, which was filtered through alumina (activity I) in petrol. The product was crystallised from acetone to give cholest-1-ene (1.95 g.; 75%) m.p. 69-70°, (lit. 70°). $\nu_{max.}$ 3015, 746, 720, 700 cm.$^{-1}$ A p.m.r. spectrum had the following peaks; τ 4.2 to 4.8 (2 olefic protons; complex multiplet; see fig.104), 9.10, 9.20, 9.24 and 9.37 (methyl groups).

Cholest-2-ene.[62]

Cholestan-3β-ol tosylate (5 g.) was dissolved in petrol (30 ml.) and adsorbed on alumina (200 g.). After 65 hr. the column was eluted with petrol (800 ml.). The product was crystallised from acetone to give cholest-2-ene (3.2 g.; 84%) m.p. 74-75°, (lit. 75°). $\nu_{max.}$ 3010, 1195, 980, 960, 790, 675 cm.$^{-1}$ A p.m.r. spectrum had the following peaks: τ 4.34, (shoulder 4.39), 9.09, 9.18, 9.23, 9.33.

Cleavage of Olefins with Periodate-Permanganate[87,88]

Initial attempts to cleave cholest-1-ene with this reagent, following the experimental details of J. T. Edward and his co-workers,[88] were only partially successful. This was attributed

to precipitation of the steroid during the reaction. The final solvent in this case was a mixture of equal parts water and t-butanol-water azeotrope. While this must have been satisfactory for large scale reactions, on scaling down there was too much water present for the steroid to remain in solution. In the following experiments, the volumes of water and t-butanol used are such that the final solution has the proportions of the azeotrope. Precipitation on inorganic material is preferred to steroid being insoluble.

1,2-Seco-Cholestane-1,2-dioic Acid

Cholest-1-ene (0.20 g.) was dissolved in t-butanol (15 ml.) and potassium carbonate (0.20 g.) in water (2 ml.) added. Sodium periodate (1.6 g.) and potassium permanganate (0.008 g.) were then added and the mixture stirred vigorously. After 15 min. the colour of the solution had changed from that of permanganate to a deep brick red. After 3 hr. potassium permanganate (0.02 g.) was added and stirring continued for a further 17 hr. The mixture was acidified with dilute hydrochloric acid and sodium bisulphite added till the solution became pale yellow. The steroid was extracted into ether, the solution washed with water, dried and the solvent removed under reduced pressure. The product was crystallised from petrol to give 1,2-seco-cholestane-1,2-dioic acid (0.187 g.; 80%) m.p. 177-180°, (lit. 179° [184]) $[\alpha]_D$ -15° (c 0.11), (lit. -14°). $\nu_{max.}$ 950, 980, 1130, 1310, 1710 (strong; carbonyl), and 2400-3550 $cm.^{-1}$ (strong; hydrogen-bonded hydroxyl).

The acid was methylated with diazomethane. A solution of diazomethane in ether, prepared from nitrosomethylurea (0.2 g.) as previously described, was added to the diacid (0.08 g.) in ether (10 ml.) and after standing at room temperature for 3 hr., the solution was evaporated to dryness under reduced pressure. The resulting ester was crystallised from acetone-petrol to give 1,2-seco-cholestane-1,2-dioic dimethyl ester (0.07 g.) m.p. 59-62°, (lit. 60°),[185] $[\alpha]_D$ -7° (c 0.10), (lit. -6°). A p.m.r. spectrum had the following peaks: τ 6.33 (doublet, 6 protons, ester methyl groups), 7.59, 7.65, 9.09, 9.20, 9.34. $\nu_{max.}$ 1740 $cm.^{-1}$

2,3-Seco-Cholestane-2,3-dioic Acid

A sample of this diacid was prepared from cholest-2-ene (0.40 g.) by exactly the same procedure as the preparation of the 1,2-diacid, the reagent quantities being doubled. The reaction product was crystallised from petrol to give 2,3-seco-cholestane-2,3-dioic acid (0.34 g.; 73%) m.p. 193-195° (lit. 196°),[186] $[\alpha]_D$ +29° (c 0.12), (lit. +31°). The i.r. spectrum was very similar to that of the 1,2-seco diacid.

This diacid was methylated with diazomethane as above. The product was crystallised from acetone-petrol to give 2,3-seco-cholestane-2,3-dioic dimethyl ester (0.10 g. from 0.12 g. diacid) m.p. 54-59° (lit. 60°), $[\alpha]_D$ +20° (c 0.14), (lit. +23°). $\nu_{max.}$ 1740 $cm.^{-1}$ The two methyl ester groups appeared as a single peak at τ 6.31. Other p.m.r. peaks were τ 7.58, 7.65, 9.09, 9.20, 9.34.

The g.l.c. retention times quoted for the methyl esters in

Table IV are recorded for column B.

Reaction of Periodate/Permanganate with 1α,5-cyclo-Cholest-2-ene

1α,5-Cyclo-cholest-2-ene (0.20 g.) was dissolved in t-butanol (15 ml.) and to this was added potassium carbonate (0.20 g.) in water (1.6 ml.), solid sodium periodate (3.2 g.) and potassium permanganate (0.015 g.). The mixture was stirred vigorously and developed a brick-red colour. After 3 hr. more potassium permanganate (0.08 g.) was added and stirring continued for 18 hr. The solution was acidified with dilute hydrochloric acid and sodium bisulphite added until the solution was almost colourless. The steroid was extracted into ether, and this solution washed with acid and then water until it was neutral. After drying, the solution was evaporated to dryness. Crystallisation from petrol gave 1α,5-cyclo-2,3-seco-5α-cholestane dioic acid (0.060 g.; 38%) m.p. 172-177°, $[\alpha]_D$ +8° (c 0.10); $\nu_{max.}$ 1710, 2400-3500 $cm.^{-1}$; (Found: C, 74.9; H, 10.6. $C_{27}H_{44}O_4$ requires C, 74.9; H, 10.3%).

This diacid was methylated with diazomethane. A solution of diazomethane in ether, made from nitrosomethylurea (0.10 g.) as previously described, was distilled before adding it to a solution of the acid (0.05 g.) in ether. After standing for 3 hr. at room temperature, the solvent and excess reagent were removed by distillation at reduced pressure. Crystallisation from acetone-petrol gave 1α,5-cyclo-2,3-seco-5α-cholestane-2,3-dioic dimethyl ester (0.04 g.) m.p. 73-75°, $[\alpha]_D$ +46° (c 0.11); $\nu_{max.}$ 1740 $cm.^{-1}$ A p.m.r. spectrum had the following peaks: τ 9.35, 9.20, 9.09, 8.74, 7.77, 7.69. A peak at τ 6.33 integrated for 6 protons (two methyl ester groups).

Catalytic Reduction of 1α,5-cyclo-2,3-seco-5α-cholestane dioic dimethyl ester

The dimethyl ester (0.01 g.) was dissolved in glacial acetic acid (20 ml.) and 10% palladium on charcoal catalyst added. The solution was kept under 1 atmosphere of hydrogen for 24 hr., but after initial uptake by the catalyst, there was no uptake of hydrogen by the steroid, and the diester was recovered unchanged.

The experiment was repeated using Adam's platinum oxide catalyst. After 4 hr. there was no uptake of hydrogen and the solution was heated to 100° and left for 20 hr. at 1 atmosphere. Again the diester was recovered unchanged.

The reduction was attempted using a 'High Active' platinum catalyst.[92] Potassium borohydride (4.2 g.) was dissolved in a solution made from sodium hydroxide (0.4 g.) dissolved in ethanol (95 ml.) and water (5 ml.). Ether (20 ml.) and ethanol (20 ml.) were placed in the reaction vessel and chloroplatinic acid (0.5 ml.) added. The flask was flushed out with nitrogen and 4 ml. of the borohydride solution added with vigorous stirring. After 1 min., hydrochloric acid (6M, 2 ml.) was added and the steroid diester (0.02 g.). The mixture was stirred for 2.5 hr. during which 40 ml. of the borohydride solution was added slowly. As the addition proceeded, effervescence occurred, and a very fine precipitate of platinum appeared. The solution was filtered through celite, washed with dilute hydrochloric acid and water, dried and the solvent removed. The diester was recovered unchanged.

Since these reduction methods were unsuccessful, it was

attempted at higher pressure. The diester (0.02 g.) was dissolved in ethanol (20 ml.) and Adam's catalyst (0.5 g.) added. This solution was treated with hydrogen at 100 atmospheres for 1 hr. at room temperature. It was heated to 240° and the pressure rose to 140 atmospheres. It was held at this temperature for 3 hr. and then allowed to cool to room temperature over 20 hr. By this time the pressure had dropped to 90 atmospheres and this was released. The solution was filtered and the solvent removed under reduced pressure. The product was filtered through alumina, in petrol-benzene. An infra red spectrum was very similar to that of the starting material. A p.m.r. spectrum however showed that the τ 8.75 peak assigned to the C-19 methyl group was absent. Examination of this material, which would not crystallise, on g.l.c. (column B) showed three constituents with the following relative retention times: 1.56 (20%), 2.05 (20%) and 2.72 (60%). This last constituent has the same retention time as 2,3-seco-cholestane-2,3-dioic dimethyl ester (see table IV).

The high pressure reduction was repeated on 0.10 g. of the cyclopropyl diester, to give a product which had the same three components as before, but g.l.c. showed 72% 2,3-seco-cholestane-2,3-dioic dimethyl ester was present. Chromatography of this material on alumina (activity I), afforded a crystalline diester. This was recrystallised from acetone-petrol to give 2,3-seco-cholestane-2,3-dioic dimethyl ester (0.03 g.), m.p. and mixed m.p. 59-60°, $[\alpha]_D$ +22° (c 0.13). The i.r. and p.m.r. spectra of the material were identical to those of authentic samples.

The Tetranitromethane Test

The olefin under examination (one thirtieth of the molecular weight in milligrams; for a cholestene this is about 0.01 g.) was weighed accurately into a 1 cm. u.v. spectrometer cell, with a ground glass stopper. A standard solution (0.95 M) of tetranitromethane in carbontetrachloride was prepared, and 3.00 ml. was added to the compound. The same TNM solution was put in the back cell. As soon as the compound had dissolved, the visible spectrum was recorded at room temperature. A Perkin Elmer 137UV spectrophotometer was used for this work. From the optical density measurements [$D(\lambda)$], log $E^*(\lambda)$ was calculated. The yellow solutions decolourised on standing; the more intense quite quickly, the less intense over about 24 hr.

3α,5-Cyclo-Cholest-6-ene[19]

Potassium acetate (6 g.) was refluxed in acetic anhydride (90 ml.) for 15 min. and cooled. To this finely divided suspension of potassium acetate, cholesteryl tosylate (2 g.) was added and the mixture heated to 80°. Stirring at 80° was continued for 48 hr., when the mixture was allowed to cool and water (100 ml.) was added. The steroid was extracted into ether and the solution washed with potassium carbonate solution, water and saturated sodium chloride solution. After drying the solution, the solvent was removed under reduced pressure, to give a white crystalline solid, which was chromatographed on alumina (activity I). Elution with petrol gave a clear glass which was crystallised from acetone to give 3α,5-cyclo-5α-cholest-6-ene (0.39 g.) m.p. 71-73°, (lit. 73°), $[\alpha]_D$ -45°, (lit. -47°);

$\lambda_{max.}$ 210 mμ (ε 11,000); $\nu_{max.}$ 3040, 1640, 1035, 950, 930, and 750 cm.$^{-1}$ P.m.r. absorptions at τ 4.4-4.9 (2 olefinic protons), 9.03, 9.09, 9.18, 9.28, 9.47-9.68 (2 cyclopropyl protons). Further elution with petrol-benzene gave cholesteryl acetate (5.48 g.) m.p. 115-116° (lit. 116°). Any 3α,5-cyclo-cholestan-6β-ol or acetate formed must have been isomerised on the alumina.

Cholesta-3,5-diene[102]

Cholesterol (15 g.) and anhydrous copper sulphate (20 g.) were heated on an oil bath at 200° for 30 min. The mixture was cooled and extracted several times with hot petrol, the solid residue being filtered off. The volume of petrol was reduced and the solution filtered through alumina. Evaporation of the solvent gave a pale yellow solid which was recrystallised from acetone to give cholesta-3,5-diene (8.5 g.; 59%) m.p. 79.5-80° (lit. 80°); $[\alpha]_D$ +124° (c 0.16), (lit. +123°). P.m.r. absorptions at τ3.95, 4.12, 4.58 (3 protons), 9.04, 9.09, 9.17, 9.29. $\lambda_{max.}$ 234 mμ (ε 20,000).

3α,5-Cyclo-cholesta-6,8(14)-diene

7-Dehydrocholesterol (0.5 g.) and toluene-p-sulphonyl chloride (0.5 g.) in pyridine (25 ml.) were refluxed for 30 min.; water was added and the steroid extracted into ether. The ether solution was washed with dilute acid, water, sodium carbonate solution and then with water until it was neutral. It was dried and the solvent removed, to give a pale brown oil. This was filtered through alumina (activity I) in petrol. Crystallisation of the clear glass from acetone gave 3α,5-cyclo-cholesta-6,8(14)-

diene (0.23 g.; 41%) m.p. 73-76°, $[\alpha]_D$ -76° (c 0.13); $\lambda_{max.}$ 260 mμ. (ε 17,000). P.m.r. absorptions at τ 3.74, 3.90, 4.70, 4.87 (2 olefinic protons), 9.10, 9.18, 9.21 (methyl groups), 9.41, 9.54, 9.63 (2 cyclopropyl protons).

Cholesta-5,7-dien-3β-ol Benzoate

7-Dehydrocholesterol (1.0 g.) was dissolved in pyridine (25 ml.) and benzoyl chloride (1 ml.) added. After standing overnight at room temperature, the solution was poured into water and the steroid extracted into ether. The ether solution was washed with dilute acid, water, sodium carbonate solution and with water until it was neutral; evaporation of the solvent gave a white solid which was recrystallised from acetone to give cholesta-5.7-dien-3β-ol benzoate (0.94 g.; 79%) m.p. 139-140° (lit. 140°).[191]

Cholesta-3,5,7-triene

Cholesta-5,7-diene-3β-ol benzoate (0.5 g.) was adsorbed on alumina (40 g.) in petrol and was allowed to stand for 20 hr. before being eluted with petrol to give a clear glass. This was crystallised from acetone to give cholesta-3,5,7-triene (0.13 g.; 48%) m.p. 67-69° (lit. 69°);[103] $[\alpha]_D$ -124°, (lit. -122°). $\lambda_{max.}$ 315 mμ. (ε 19,600).

17β-Hydroxy-5α-androstan-3-one[106,107]

a. Testosterone (10 g.) was dissolved in ethyl acetate (100 ml.), 10% palladium on charcoal catalyst (1 g.) added and the mixture was shaken under hydrogen at 30° for 30 min. The solution was

filtered and evaporated to give a white solid, which was crystallised from acetone to give 17β-hydroxy-5α-androstan-3-one (0.5 g.; 45%), m.p. 177-180° (lit. 184°). A second crop melted at 110-142° and gave two equal peaks on g.l.c. (column A); a mixture of C-5 isomers. This second crop (5.1 g.) was dissolved in hot ethanol and this solution added to a hot solution of semicarbazide hydrochloride (6 g.) and sodium acetate (6 g.) in ethanol-water. The mixture was warmed at 70° for 20 min. and allowed to cool. The crystalline product was filtered off, and was washed with water, ethanol and ether before being dried. Recrystallisation from ethanol-chloroform gave 17β-hydroxy-5α-androstan-3-one semicarbazone (2.2 g.) m.p. 254-256° (lit. 253-254.5°). From the melting point it would appear that this is only one C-5 isomer, the 5β-isomer having been separated during crystallisation. This semicarbazone was hydrolysed by refluxing it for 3 hr. in ethanol (400 ml.) with concentrated hydrochloric acid (50 ml.) and water (50 ml.). The ethanol was distilled off and the steroid extracted into ether; the solution was washed with water and dried. Evaporation of the solvent gave a white solid, which still gave two peaks on g.l.c. although the proportion of 5β isomer had decreased.

b. Testosterone (10 g.) in dioxan (100 ml.) and ether (100 ml.) was added over 10 min. to a solution of lithium (1.2 g.) in liquid ammonia (1200 ml.). The solution was stirred for 30 min. during which the initial blue colour disappeared. Ammonium chloride (8 g.) was added, and then water (200 ml.). When the ammonia remaining had evaporated, the steroid was extracted into chloroform;

this solution was washed with dilute acid and water before being dried and the solvent removed. The crude product, which gave only one peak on g.l.c., was recrystallised from ethyl acetate - petrol to give 17β-hydroxy-5α-androstan-3-one (9.3 g.; 93%) m.p. 197-199°, (lit. 184-185°). $[\alpha]_D$ +92° (c 0.17) (lit.+90°); $\nu_{max.}$ 1700 cm.$^{-1}$

2α-Bromo-17β-hydroxy-androstan-3-one[108]

17β-Hydroxy-androstan-3-one (4 g.) in glacial acetic acid (200 ml.) were treated with a solution of bromine (2.209 g.) and hydrobromic acid (0.3 ml.) in acetic acid (25 ml.) over 20 min. with vigorous stirring. The solution was poured into water and the steroid extracted into chloroform; the solution was washed with water, dried and the solvent removed. The crude product was crystallised from acetone to give 2α-bromo-androstan-3-one (4.9 g.; 96%) m.p. 168-169°(lit. 164°); $\nu_{max.}$ 1720 cm.$^{-1}$

17β-Hydroxy-5α-androst-1-en-3-one[109,192]

2α-Bromo-17β-hydroxy-androstan-3-one (4.8 g.) was added to lithium carbonate (5 g.) in refluxing dimethylformamide (100 ml.), and refluxed for 30 min. After cooling, the solution was filtered and the filtrate poured into dilute hydrochloric acid. The steroid was extracted into chloroform, and the solution washed with water and dried. Evaporation of the solvent gave a white solid, whose infra red spectrum had $\nu_{max.}$ 1670 with a shoulder at 1700 cm.$^{-1}$ Chromatography of this material on deactivated alumina gave, on elution with petrol-benzene (50:50), 17β-hydroxy-androstan-3-one (0.74 g.; 20%). Continued elution with the same solvent

gave mixed fractions and then pure 17β-hydroxy-androst-1-en-3-one (2.1 g.; 55%) m.p. 154.5-156°, (lit. 157-159°); $[\alpha]_D$ +55° (c 0.14) (lit. +57°); $\nu_{max.}$ 1670 cm^{-1}; $\lambda_{max.}$ 230 mμ. (ε 9,500); p.m.r. absorptions: τ2.72, 2.88, 4.02, 4.19 (2 protons) 6.3 (17α proton), 8.97 (19-methyl), 9.21 (18-methyl).

Androst-1-en-3β,17β-diol.[192]

17β-Hydroxy-androst-1-en-3-one (1.7 g.) in dry ether (30 ml.) was added to lithium aluminium hydride (1.0 g.) in dry ether (300 ml.), and the mixture refluxed for 1 hr. Ethyl acetate was added and then dilute hydrochloric acid; the ether layer was separated and washed with water, dried and the solvent removed. The crude product was crystallised from acetone to give 5α-androst-1-en-3β,17β-diol, (1.4 g.; 82%) m.p. 162-163° (lit. 163°); $[\alpha]_D$ +37° (c 0.15) (lit. +38°).

Androst-5-en-3β-ol[110,111]

a. 3β-Hydroxy-androst-5-en-17-one (DHA) (5 g.) was dissolved in ethanol (3 ml.) and added to a solution of hydrazine hydrate (2 ml.) and hydrochloric acid (0.2 ml.) in ethanol (2 ml.). DHA hydrazone (5.1 g.) precipitated and was filtered off and washed. DHA hydrazone (4 g.) was added in small portions over 6 hr. to a rapidly stirred solution of potassium t-butoxide (5 g.) in dry dimethyl sulphoxide (10 ml.). Water and chloroform were added and the chloroform solution was washed with water and dried. Evaporation of the product gave DHA hydrazone.

b. DHA (10 g.) was dissolved in ethanol (5 ml.) and this solution added to potassium hydroxide (5 g.) and 99% hydrazine hydrate (7

ml.) in digol (80 ml.). The clear pale yellow solution was refluxed for 1 hr. at 90°, then water and ethanol were allowed to evaporate and the temperature of the solution increased to 205°; refluxing at this temperature was continued for 2.5 hr. The solution became water-clear and a white solid formed in the condenser. The solution was cooled, water added and the steroid extracted into chloroform. This solution was washed with water, dried and the solvent removed to give a white solid. This was crystallised from acetone to give androst-5-en-3β-ol (9.23 g.; 97%) m.p. 134-135° (lit. 133°), $[\alpha]_D$ -46° (c 0.17), (lit. -47°).

5α-Androstan-3β-ol[112]

Androst-5-en-3β-ol (9 g.) in ethanol (100 ml.) was hydrogenated at atmospheric pressure over a palladium on charcoal catalyst (10%), perchloric acid (0.3 ml.) being added as promotor. After 10 hr. the uptake of hydrogen had ceased and the catalyst was filtered off, the solution neutralised with solid potassium hydroxide. Water was added and the ethanol removed under reduced pressure. The steroid was extracted into ether, the solution washed with water and dried evaporation of the solvent gave a clear glass, which had, by g.l.c., three components. The product was chromatographed on alumina; elution with petrol gave an oil (0.75 g.) which could not be crystallised. A p.m.r. spectrum of this oil had three peaks; τ 9.07, 9.21 and 9.30 in the ratio of 1:1:2. G.l.c. showed this to be a mixture of two compounds in equal amounts, and from the p.m.r. spectrum, it was

deduced that these were 5α- and 5β-androstane. Elution with benzene-ether gave a white solid, which was crystallised from acetone to give 5α-androstan-3β-ol (7.93 g.; 88%) m.p. 153-154° (lit. 151-152°); $[\alpha]_D$ +2° (c 0.15), (lit. +2°).

5α-Androstan-3-one[112]

Androstan-3β-ol (6.0 g.) was dissolved in acetone (400 ml.) at 30° and 8N chromic acid[65] (12 ml.) added over 3 min. with vigorous stirring. After standing for 1 min., methanol (40 ml.) was added. After the addition of water, the organic solvents were distilled off and the steroid extracted into ether. The ether solution was washed with water, dried and the solvent removed, to give a white crystalline solid, which was recrystallised from acetone to give 5α-androstan-3-one (4.76 g.; 79%) m.p. 104-105° (lit. 105°), $[\alpha]_D$ +17°,(c 0.19), (lit. +18°).

2α-Bromo-androstan-3-one

Androstan-3-one (2.6 g.) in acetic acid (50 ml.) was treated with a solution of bromine (0.76 g.) and hydrobromic acid (0.3 ml.) in acetic acid (10 ml.) over 20 min. A precipitate gradually appeared and was filtered off after the reaction mixture had been cooled. After being washed with hot water and dried, the bromo-ketone was crystallised from acetone to give 2α-bromo-androstan-3-one (2.75 g.; 82%), m.p. 201-205° (decomp.); $\nu_{max.}$ 1715 $cm.^{-1}$; p.m.r. absorptions at: τ 5.08, 5.19, 5.30, and 5.41 (1 proton); 8.91 (19-methyl group); 9.29 (18-methyl group).

5α-Androst-1-en-3-one[109]

2α-Bromo-androstan-3-one (1.3 g.) was added to lithium carbonate (1.5 g.) in refluxing dimethylformamide (50 ml.) and the mixture refluxed for 35 min. After cooling, the inorganic material was filtered off, water added and the steroid extracted into dichloromethane. The solution was washed with dilute acid and with water before being dried and the solvent removed to give a white solid, which, from i.r., contained both saturated and unsaturated ketone. The mixture was separated on an alumina column. Elution with petrol-benzene (90:10) gave androstan-3-one (0.306, 30%) and material containing both ketones. Elution with petrol-benzene (50:50) gave androst-1-en-3-one, recrystallised from acetone, (0.42 g.; 41%), m.p. 99.5-101° (lit. 102°); $[\alpha]_D$ +44° (c 0.15), (lit. +43.5°); $\nu_{max.}$ 1675 cm.$^{-1}$

5α-Androst-1-en-3β-ol

Androst-1-en-3-one (0.35 g.) in dry ether (10 ml.) was added to a solution of lithium aluminium hydride (0.3 g.) in dry ether (30 ml.) and the solution refluxed for 1 hr. After decomposition of excess reagent with ethyl acetate, dilute acid was added. The solution of steroid in ether was washed with water and dried; evaporation of the solvent gave a white solid which was crystallised from acetone to give androst-1-en-3β-ol (2.6 g.; 73%) m.p. 102.5-104.5°; $[\alpha]_D$ +36° (c 0.17). $\nu_{max.}$ 755, 1030, 1070, 3550 cm.$^{-1}$; p.m.r. absorptions at τ3.98, 4.14, 4.42, 8.43, 8.70, 9.09 (19-methyl group) and 9.29 (18-methyl group).

Protection of the 17α-hydroxyl group

a. Attempted formation of trityl ether of testosterone[114]

Triphenyl methyl chloride (0.5 g.) and testosterone (0.5 g.) were dissolved in pyridine (10 ml.) and refluxed for 2 hr. under nitrogen. On cooling, the solution was poured into water and the steroid extracted with dichloromethane. This solution was washed with water and dried before the solvent was removed under reduced pressure to give unchanged testosterone (0.48 g.).

b. Attempted formation of the tetrahydropyranyl ether of testosterone[115]

Testosterone (0.3 g.) was added to a solution of redistilled dihydropyran (2 ml.) and phosphorus oxychloride (0.05 ml.) in dichloromethane (2 ml.), and the solution was allowed to stand at room temperature for 24 hr. Chloroform and water were added, the organic layer separated, washed thoroughly with water, dried and the solvent evaporated. Examination of an i.r. spectrum of the product showed a slight diminution of the hydroxyl peak - reaction had occurred to a small extent. The experiment was repeated as above, but the solution was left at room temperature for 90 hr. An i.r. spectrum showed that partial reaction with the hydroxyl group had occurred, but also the intensity of the carbonyl peak had decreased by more than half.

c. Attempted methylation of testosterone

1. Testosterone (0.1 g.) was dissolved in dichloromethane and a solution of fluoboric acid (0.01 ml.) in ether (2 ml.) added. A solution of diazomethane in dichloromethane was added slowly

over 20 min., the solution being kept at 0°. The solution became pale blue, but on adding water and dichloromethane, this blue colour disappeared. The organic layer was separated and washed with potassium bicarbonate solution, then water, dried and the solvent removed. An i.r. spectrum showed no change.

2. Testosterone (0.1 g.) was dissolved in methanol (15 ml.) and refluxed while dimethyl sulphate (10 ml.) and a solution of potassium hydroxide (5 g.) in methanol water (50:50, 10 ml.) were simultaneously dropped slowly in, over 1 hr. Water was added and the steroid extracted with dichloromethane. The solution was well washed with water, dried and the solvent removed under reduced pressure, to give unchanged testosterone.

3. Testosterone (0.1 g.) in tetrahydrofuran (distilled from lithium aluminium hydride; 10 ml.) was treated with methyl iodide (1 ml.) and sodium hydride (50% dispersion in oil; 0.05 g.). The mixture was stirred under nitrogen for 22 hr. Water was added and the steroid extracted into chloroform; the solution was washed with water, dried and the solvent evaporated. The hydrocarbon oil was removed on alumina. No reaction had occurred, testosterone being recovered quantitatively.

4. Testosterone (0.1 g.) was dissolved in methyl iodide (10 ml.) and silver oxide (0.1 g.) added. The mixture was stirred and refluxed for 17 hr. The insoluble material was filtered off and the methyl iodide distilled off under reduced pressure. A white solid remained which was crystallised from petrol to give 17β-methoxy-androst-4-en-3-one (0.09 g.) m.p. 125-26.5°, (lit. 127°).

17β-Methoxy-androstan-3-one

17β-Hydroxy-androstan-3-one (6.6 g.), silver oxide (12 g.) and methyl iodide (50 ml.) were stirred vigorously and the mixture refluxed for 18 hr. After cooling, the solution was filtered and evaporated to dryness. Crystallisation from petrol gave 17β-methoxy-androstan-3-one (6.4 g.; 92%) m.p. 125-126°, $[\alpha]_D$ +14°, (c 0.14); $\nu_{max.}$ 1720, 1130 cm.$^{-1}$; p.m.r. absorptions; τ 6.66 (ether methyl group), 8.99 (19-methyl group), 9.25 (18-methyl group).

17β-Methoxy-androst-1-en-3-one

17β-Methoxy-androstan-3-one (4.2 g.) was dissolved in dimethylformamide (100 ml.) and treated over 15 min. with a solution of bromine (1.1 g.) and hydrobromic acid (0.4 ml.) in dimethyl formamide (25 ml.). The solution was then stirred for 3 hr. Lithium carbonate (5 g.) was added and the solution was refluxed for 1 hr. After cooling solid material was filtered off, water added and the steroid extracted into ether. The ethereal solution was washed with dilute acid and with water, then dried and the solvent removed. The product was again a mixture of saturated and unsaturated ketones, which were separated on deactivated alumina. Elution with petrol-benzene (50:50) gave starting material (2.21 g.) and several fractions containing both ketones. Further elution with the same solvent gave 17β-methoxy-androst-1-en-3-one, recrystallised from acetone-petrol, (1.38 g.; 33%) m.p. 117-118°; $[\alpha]_D$ +21° (c 0.17). $\nu_{max.}$ 1680, 1120 and 785 cm.$^{-1}$; p.m.r. absorptions at τ2.73, 2.93, 4.06, 4.23, 6.65 (ether methyl group), 8.98 (19-methyl group) and 9.20

(18-methyl group).

17β-Methoxy-androst-1-en-3β-ol

17β-Methoxy-androst-1-en-3-one (0.9 g.), dissolved in dry ether (10 ml.) was added to lithium aluminium hydride (0.8 g.) in dry ether (30 ml.) and the solution refluxed for 1 hr. The excess reagent was decomposed with ethyl acetate, dilute acid was added and the ether layer separated. This was washed with water, dried and the solvent removed. Crystallisation from methanol gave 17β-methoxy-androst-1-en-3β-ol (0.84 g.; 93%) m.p. 164.5-166°. $[\alpha]_D$ +9° (c 0.16).

Attempted Tosylation of Androst-1-en-3β,17β-diol

Androst-1-en-3β,17β-diol (0.35 g.) was dissolved in pyridine (5 ml.) and cooled to 0°. To this was added a solution of toluene-p-sulphonyl chloride (0.35 g.) in pyridine (5 ml.) also cooled to 0°. The mixture was allowed to warm to room temperature and left for 22 hr. The solution was poured into ice-water and the steroid extracted with dichloromethane. This solution was washed with sodium carbonate solution, water, dilute acid, then with water until it was neutral. The solution was dried and evaporated to give a pale brown gum. Infra red and p.m.r. spectra of this compound were similar to the crude product from the corresponding cholestenol experiment. Chromatography of the mixture on deactivated alumina was attempted. In this case all the material was soluble in petrol-benzene (50:50) and it was applied to the column in this solution. Elution with the same solvent produced no material, but elution with benzene afforded a clear gum, which could not be crystallised. A p.m.r.

spectrum had peaks τ 7.56 (aryl methyl group of the tosyl ester), 8.74 and 9.20 (angular methyl groups). There were no distinct peaks in the region of olefinic absorption. No further material was eluted until chloroform-methanol (95:5) eluted a second non-crystalline gum. The p.m.r. spectrum of this material had a complex region between τ o.6 and 3.0, indicating a pyridinium tosylate group.

Androstan-17β-ol[112]

17β-Hydroxy-androstan-3-one (1 g.) in ethanol (7 ml.) was added to hydrazine hydrate (5 ml.) and potassium hydroxide (3 g.) in digol (50 ml.), and the solution refluxed for 30 min. at 90°. The temperature was raised to 210°, by allowing the water and ethanol to evaporate; refluxing was continued at 210° for 2 hr. The solution was cooled and water was added. The steroid was extracted into ether, the solution washed with water, dried and the solvent evaporated. Crystallisation from methanol gave androstan-17β-ol (0.82 g.) m.p. 164-166° (lit. 166°); $[\alpha]_D$ +11° (c 0.19), (lit. +10°).

Preparation and Hydrolysis of Androsan-17β-ol Tosylate

Androstan-17β-ol (0.5 g.) in pyridine (5 ml.) at 0° was added to toluene-p-sulphonyl chloride (0.5 g.) in pyridine (5 ml.) and left at room temperature for 24 hr. The solution was poured into water, the steroid extracted into ether washed with water, dried and the solvent evaporated, to give a crystalline solid. This solid (0.35 g.) was dissolved in petrol and adsorbed on alumina, where it was left for 50 hr. Elution with petrol gave

a hydrocarbon (0.29 g.), which could not be crystallised. A p.m.r. spectrum indicated that two olefinic protons were present, and this material was assumed to be androst-16-ene, although g.l.c. showed that other compounds were present, representing 10% of the material.

Attempted Tosylation of Androst-1-en-3β-ol

Androst-1-en-3β-ol (0.3 g.) was dissolved in pyridine (5 ml.) and cooled to 0°. This was added to a solution of toluene-p-sulphonyl chloride (0.3 g.) in pyridine (5 ml.), and the combined solution was allowed to stand at room temperature for 16 hr. It was poured into water and the steroid extracted into dichloromethane, and this solution was washed with sodium carbonate solution, water, dilute acid and finally water until it was neutral. After drying, the solvent was evaporated, to give a pale brown gum. This was dissolved in petrol and adsorbed on alumina. Elution with petrol gave a clear glass (0.10 g.) which would not crystallise. A p.m.r. spectrum showed absorptions at τ 8.75, 9.23 and 9.30. Several small peaks were in the 3.5-4τ region. $\lambda_{max.}$ 212 mμ (ε 6,300), 261 mμ (ε 4,100). Analytical g.l.c. showed this to be a mixture of two components, R_t 0.89 (60%) and 1.14 (40%) (column C). It was not possible to separate these two compounds on alumina (activity I). Preparative g.l.c. with 6' x 3/16" E301 columns enabled the two hydrocarbons to be obtained pure. Injections of 0.003 g. were made, and collection of the effluent from several injections gave ca.0.003 g. of each component. The less polar material

had $\lambda_{max.}$ 212 mμ (ϵ 7,100); its p.m.r. spectrum showed two equal peaks at τ 8.74 and 9.30. The spectrum, obtained with a computer for averaging transients, was not sufficiently intense for any olefinic protons to be observed. The second component had $\lambda_{max.}$ 262 mμ. P.m.r. absorptions were at τ 4.28 (4 protons), 9.23, 9.30 (angular methyl groups). Elution with chloroform-methanol (95:5) gave a brown gum (0.25 g.) which gave crystals from benzene but these melted over a wide range. The material appeared from spectra to be a pyridinium tosylate, but was not investigated further.

Reduction of the hydrocarbon mixture

Crude hydrocarbon mixture (0.01 g.) from the previous experiment was dissolved in acetic acid and hydrogenated over a 10% palladium on charcoal catalyst for 3 hr. The catalyst was filtered off, water added and the steroid extracted into ether. The solution was washed with water, dried and evaporated to dryness. Examination of the product (0.01 g.) on g.l.c. showed that there was now only one component. This had the same retention time as 5α-androstane.

Androst-1-en-3β-ol Benzoate

Androst-1-en-3β-ol (0.04 g.) was dissolved in pyridine (2 ml.) at 0° and added to a solution of benzoyl chloride (0.05 ml.) in pyridine (2 ml.). After standing at room temperature for 16 hr., the solution was poured into water and the steroid extracted into ether. The ethereal solution was washed thoroughly with sodium carbonate solution, water, dilute acid, and finally with water

until the washings were neutral. The ether solution was dried and the solvent removed under reduced pressure. The crude product (0.03 g.) was dissolved in petrol, absorbed on alumina and left on the column for 48 hr. Elution with petrol gave a clear oil (0.015 g.), which gave one peak on g.l.c. R_t 1.13 (column C). The material could not be crystallised, but had $\lambda_{max.}$ 262 mμ (ϵ 5,100).

Attempted Tosylation of 17β-Methoxy-androst-1-en-3β-ol

17α-Methoxy-androst-1-en-3β-ol (0.4 g.) dissolved in pyridine (5 ml.) was added to a solution of toluene-p-sulphonyl chloride (0.4 g.) in pyridine at 0°. After standing for 18 hr. at room temperature, the mixture was poured into water and the steroid extracted into dichloromethane. This solution was washed with sodium carbonate solution, water, dilute acid and then water until it was neutral. The solution was dried and the solvent evaporated, to give a brown solid, whose spectra were similar to those from previous attempted tosylations. Thin layer chromatography (solvent: petrol-ether, 1:1) showed that at least eight components were present in the product. The least polar spot (R_f 0.68), estimated to be 75% of the material, probably represented a methoxyandrostene. Other spots on the t.l.c. were at R_f 0.59, 0.47, 0.38, 0.28 and 0.18; the spot R_f 0.28 has the same retention time as 17β-methoxy-androst-1-en-3β-ol and the spot R_f 0.18 could be androst-1-en-3β,17β-diol, present in the starting material. The product was chromatographed on alumina and elution with petrol gave a clear glass (0.32 g.; 80%)

$\nu_{max.}$ 1120 cm.$^{-1}$ On g.l.c. this fraction was found to have two components. Further elution of the alumina column gave very small amounts of polar material which was not identified.

Rechromatography of the glass on alumina (activity I) was carried out, and elution with several small volumes of petrol gave two hydrocarbons. The first to be eluted (0.25 g.) would not crystallise. It gave a yellow colour with tetranitromethane, from which it could be deduced that it was an alkenylcyclopropane, 17β-methoxy-1α,5-cyclo-androst-2-ene. This was supported by the u.v. spectrum; $\lambda_{max.}$ 212 mμ (ϵ 7,000). P.m.r. absorptions at τ 4.36 (2 olefinic protons), 6.70 (methoxyl group), 8.75 (19-methyl group), 9.25 (18-methyl group). The second hydrocarbon to be eluted, 17β-methoxy-androsta-1,3-diene (0.068 g.); $\lambda_{max.}$ 264 mμ, could not be crystallised either. P.m.r. absorptions τ 4.27 (4 protons), 9.22 and 9.26.

Catalytic Reduction of 17β-Methoxy-1α,5-cyclo-androst-2-ene

17β-Methoxy-1α,5-cyclo-androst-2-ene (0.04 g.) was dissolved in ethanol and hydrogenated over a 10% palladium on charcoal catalyst. After 4 hr., the catalyst was filtered off and the solvent evaporated. A p.m.r. spectrum of the resulting 17β-methoxy-5α-androstane had the following peaks: τ 6.70 (methoxyl), 9.20 (19-methyl group) and 9.26 (18-methyl group).

Cholest-4-en-3-one[124]

Cholesterol (150 g.) was converted into cholest-4-en-3-one (98 g.; 65%) m.p. 80-81° (lit. 81-82°) by the four stage

synthesis of Fieser.[124] $[\alpha]_D$ +89° (c 0.08), (lit. +88°); $\lambda_{max.}$ 244 mμ (ε 16,400).

1β,5-Cyclo-5β,10α-cholestan-2-one[11]

Cholest-4-en-3-one (2.5 g.) in t-butanol (1000 ml.) was irradiated with a 125 watt medium pressure mercury vapour lamp (Hanovia) housed in a water cooled immersion type thimble for 110 hr. The radiant energy was filtered through pyrex glass, and sufficient heat was supplied to the solution to prevent the solvent solidifying. The volume of solution was reduced to 100 ml. and a solid (photodimer; 0.04 g.) filtered off. The solution was evaporated to dryness and crystallised from ethanol to give cholest-4-en-3-one (1.87 g.). The mother liquors were chromatographed on deactivated alumina. Elution with petrol-benzene (50:50) gave a white solid which was recrystallised from acetone to give lumicholestenone (0.177 g.; 7.1%). m.p. 164.5-166° (lit. 165-166°); $[\alpha]_D$ +68° (c 0.13), (lit. +70°); $\lambda_{max.}$ 212, 284 mμ, (ε 8,000 and 60); $\nu_{max.}$ 898, 1183, 1680, 1715, 3050 $cm.^{-1}$; p.m.r. absorptions at: τ 7.91, 8.33, 8.83, 9.09, 9.18 and 9.35. No resonance higher than 9.35τ. A small sample of lumicholestenone (0.01 g.) was dissolved in deuteromethanol (CH_3OD) (2 ml.) and sodium (0.005 g.) added. The solution was refluxed for 1 hr., poured into D_2O and the steroid extracted into ether. The ether solution was dried and evaporated. A p.m.r. spectrum of this deuterated material was similar to that of the undeuterated, but the multiplet centred on τ 7.91 had almost disappeared.

Cholest-4-en-3β-ol[125]

Cholest-4-en-3-one (10 g.) was dissolved in dry ether (30 ml.) and added slowly to a solution of lithium aluminium hydride (5 g.) in dry ether (50 ml.). The solution was refluxed for 1 hr., ethyl acetate added and the mixture poured into dilute hydrochloric acid. The ether solution was washed with water, dried and the solvent evaporated, to give a white solid which was recrystallised from methanol-acetone to give cholest-4-en-3β-ol (7.2 g.; 72%) m.p. 130-132° (lit. 132°); $[\alpha]_D$ +46° (c 0.15), (lit. +46°).

Attempted Tosylation of Cholest-4-en-3β-ol

Cholest-4-en-3β-ol (3 g.) in pyridine (10 ml.) was added to a solution of toluene-p-sulphonyl chloride acid (3 g.) in pyridine (10 ml.) at 0°. After standing at room temperature for 24 hr., the solution was poured into water and the steroid extracted with dichloromethane. The solution was well washed with sodium carbonate solution, water, dilute acid and finally with water until the washings were neutral. The solution was dried and the solvent evaporated to give a brown gum. Chromatography of this material on alumina was attempted, but a rather large volume of petrol (60 ml.) was required to dissolve all the material. Elution of the column with petrol gave a clear glass, which was crystallised from acetone to give cholesta-3,5-diene (0.63 g.; 27%) m.p. and mixed m.p. 79-80° (lit. 80°); $[\alpha]_D$ +122° (c 0.18), (lit. +123°). While this material gave only one peak

on g.l.c. (R_t 1.11; column A), examination of the mother liquors from the crystallisation showed that they had two components, the 3,5-diene and a component with R_t 0.79, representing 12% of the original hydrocarbon mixture. This retention time of 0.79 is identical to that of 3α,5-cyclo-cholest-6-ene. A sample of this minor product was obtained by preparative g.l.c. (by overloading column A) and an i.r. spectrum, obtained using a beam condenser, was identical to a spectrum of an authentic sample recorded under the same conditions.

Elution with petrol-benzene afforded unchanged cholest-4-en-3β-ol. No material was eluted until a pale brown solid was eluted with chloroform-methanol (95:5). This was crystallised from benzene to give N-(cholest-4-en-3α-yl)-pyridinium tosylate, (0.68 g.; 28%) m.p. 170-172°; $[\alpha]_D$ +32° (c 0.09). $\nu_{max.}$ 3450, 3040, 1190, 1120, 1035, 1010, 820 and 680 $cm.^{-1}$

Attempted tosylation of Cholest-1-en-3β-ol in o-Toluidine

Cholest-1-en-3β-ol (0.05 g.) was added to a solution of toluene-p-sulphonyl chloride (0.05 g.) in o-toluidine (5 ml.), and the solution left at room temperature for 17 hr. The solution was poured into dilute acid and the steroid extracted into ether. This solution was thoroughly washed with dilute acid, water, sodium carbonate solution, and finally with water until the washings were neutral. After drying, the solvent was evaporated to give a brown oil. Chromatography on alumina gave the same products as the reaction in pyridine. Elution with petrol gave 1α,5-cyclo-cholest-2-ene (0.013 g.) m.p. 70-72°, elution with petrol-benzene gave

unchanged cholest-1-en-3β-ol (0.021 g.) and elution with chloroform-methanol (95:5) gave N-(cholest-1-en-3α-yl)-pyridinium tosylate (0.022 g.) m.p. 220-221°.

Attempted Tosylation of Cholest-1-en-3β-ol in Acetone

Cholest-1-en-3β-ol (0.05 g.) was dissolved in acetone (5 ml.) and water (0.1 ml.), sodium bicarbonate (0.05 g.) and toluene-p-sulphonyl chloride (0.05 g.) were added. This mixture was stirred at room temperature overnight. It was poured into water, the acetone evaporated and the steroid extracted into ether. The ether solution was washed with water, dried and the solvent removed to give unchanged Cholest-1-en-3β-ol (0.047 g.)

Deuterated Acetic Acid (CH_3COOD)[130]

Deuterium oxide (99.7%; 19.8 g.) was refluxed for 1.5 hr. with redistilled acetic anhydride (110 g.), care being taken to exclude water. The resulting solution was distilled, the fraction boiling between 115 and 120° being collected. This was redistilled through a vigreux column to give deuterated acetic acid (90 ml.). The purity of this was found to be 94%, i.e. 6% CH_3COOH present, from the p.m.r. spectrum of the product.

Deuteration of Cholesteryl Acetate[131]

Cholesteryl acetate (10.5 g.) was dissolved in deuterated acetic acid (90 ml.) and Adam's catalyst (1.42 g.) added. The solution was kept in an atmosphere of deuterium for 1.5 hr., during which time 850 ml. of deuterium reacted. Most of the

acetic acid was distilled off, and the steroid crystallised from this solution. It was filtered off, washed with methanol and dried to give 5α-cholestan-3β-ol acetate labelled at C-5α and C-6 with deuterium, (10.06 g.; 96%); m.p. 114-115° (lit. 115°). There was no noticeable difference between the p.m.r. spectra of deuterated and non-deuterated cholestan-3β-ol. The i.r. spectrum of this material (in CCl_4) showed peaks at 2130, 2165 and 2205 $cm.^{-1}$

Deutero-cholestan-3-one[131]

5,6-Deutero-cholestan-3β-ol acetate (9 g.) was dissolved in methanol (200 ml.) and benzene (20 ml.) and potassium hydroxide (7.5 g.) in methanol (150 ml.) added. The solution was refluxed for 1 hr., then most of the solvent was removed. Water was added and the steroid extracted into ether, the ether solution washed with water and dried and the ether evaporated. Crystallisation of the product from acetone gave 5,6-deutero-cholestan-3β-ol (7.78 g.; 96%) m.p. 142-143° (lit. 141-142°).

Deutero-cholestan-3β-ol (7.5 g.) was dissolved in acetone (500 ml.) at 30° and chromic acid[65] (15 ml.) added. The solution was stirred for 4 min. and then methanol (50 ml.) added, followed by water. The organic solvents were distilled off and the steroid extracted into ether. The ethereal solution was washed with dilute acid then with water and dried. Evaporation of the solvent gave a white solid which was recrystallised from acetone to give 5,6-deutero-cholestan-3-one (6.8 g.; 91%) m.p. 129-130°, (lit.129°); $\nu_{max.}(CCl_4)$ 2130, 2170, 2210 $cm.^{-1}$

5,6-Deutero-cholest-1-en-3-one

Deutero-cholestan-3-one (2.07 g.) was dissolved in acetic acid (60 ml.) and a solution of bromine (0.83 g.) and hydrobromic acid (0.02 ml.) in acetic acid (4 ml.) added over 10 min. with vigorous stirring. The solution was cooled slightly and the precipitated steroid filtered off. This was washed with water and dried over phosphorus pentoxide, to give deuterated 2α-bromo-cholestan-3-one (1.63 g.) m.p. 168-170° (lit. 168-169°).

This bromo-ketone (1.6 g.) was added to lithium carbonate (2 g.) in dimethylformamide (50 ml.) and the mixture refluxed for 1 hr. The solution was cooled and filtered. Dilute acid was added to the filtrate and the steroid extracted into ether. The ether solution was washed with water, dried and the solvent evaporated. Recrystallisation of the product from acetone gave 5,6-deutero-cholest-1-en-3-one (1.02 g.; 38% from the saturated ketone) m.p. 99-101°, (lit.98°); $[\alpha]_D$ +58° (c 0.16), (lit. +57°); $\lambda_{max.}$ 230 mμ (ϵ 10,300); $\nu_{max.}$ 1675 $cm.^{-1}$ There was no saturated ketone present in the reaction product, i.e. no shoulder at 1710 $cm.^{-1}$ in the i.r. spectrum.

5,6-Deutero-cholest-1-en-3β-ol

Deutero-cholest-1-en-3-one (0.6 g.) in dry ether (8 ml.) was added to a solution of lithium aluminium hydride (0.5 g.) in dry ether (30 ml.) and the solution refluxed for 1 hr. Ethyl acetate was added and then dilute acid. The ether solution was washed with water, dried and the ether evaporated, to give a white solid, which was crystallised from acetone to give 5,6-

deutero-cholest-1-en-3β-ol (0.58 g.; 96%) m.p. 127-129° (lit. 131°). $[\alpha]_D$ +52° (c 0.16), (lit +54°); $\nu_{max.}$ 3400, 1040 and 760 cm.$^{-1}$ (CS_2). In CCl_4, $\nu_{max.}$ 2200, 2170 and 2135 cm.$^{-1}$

5α-Fluoro-cholestan-3β-ol[133]

Cholesteryl acetate (10 g.) was dissolved in dichloromethane (100 ml.) and this solution was added to a stirred solution of anhydrous hydrogen fluoride (ca.30 ml.) in dichloromethane (100 ml.) cooled in dry ice. The solution was allowed to stand for 30 min. and poured into an ice - sodium carbonate solution mixture. The steroid in dichloromethane solution was washed thoroughly with water, the solution dried and the solvent evaporated, to give a pale yellow oil. This was chromatographed on 'Florisil;' elution with petrol-benzene (9:1) gave unreacted cholesteryl acetate (3.45 g.). Elution with benzene gave 5α-fluoro-cholestan-3β-ol acetate, crystallised from methanol, (4.15 g.; 39%) m.p. 112-115° (lit.116°); $[\alpha]_D$ +15° (c 0.10), (lit. +20°). 5α-Fluoro-cholestan-3β-ol acetate (4.0 g.) was dissolved in dry ether (10 ml.) and added to a solution of lithium aluminium hydride (0.8 g.) in dry ether (30 ml.) and allowed to stand at room temperature for 20 min. Ethyl acetate was added and the ammonium chloride solution. The ethereal solution was washed with water and then dried. Evaporation of the solvent gave a clear glass, which was crystallised from methanol to give 5β-fluoro-cholestan-3β-ol (3.1 g.; 77%) m.p. 129-133° (lit.133-134°). Both the 3β-acetate and the alcohol gave positive tests for fluorine.

Qualitative test for Fluorine[136]

a. Alizarin fluorine blue (0.0385 g.) was dissolved in sodium hydroxide solution (0.5N; 20 ml.) and the solution diluted to 150 ml. with water. Sodium acetate (0.02 g.) was added and the solution brought to pH 5 with dilute hydrochloric acid. A solution of sodium acetate (2.5 g.) and acetic acid (2.5 ml.) in water (30 ml.) was then added and the whole solution made up to 200 ml.

b. Cerous nitrate (0.0217 g.) was dissolved in water (200 ml.) and dilute nitric acid (1 drop) and hydroxlamine hydrochloride (0.1 g.) added.

c. Tribenzylamine (0.1 g.) was dissolved in warm n-pentanol - sec-butanol mixture (3:1 ; 35 ml.), the solution cooled and diluted to 100 ml. with the same mixture.

A small amount (ca.0.0004 g.) of steroid was combusted in a flask filled with oxygen, and the residue dissolved in water (1 ml.). Five drops of this solution were placed in a small tube and the alizarine fluorine blue solution (a) (2 drops) added and the contents mixed. Cerous nitrate solution (b) (2 drops) was then added and mixed. A change to blue within 1 min. indicated fluorine present. The extraction solution (c) (10 drops) was added and the blue colour was absorbed into the organic layer.

5α-Fluoro-cholestan-3-one

Chromium trioxide (1 g.) in water (10 ml.) and acetic acid (200 ml.) were added to 5α-fluoro-cholestan-3β-ol (2.0 g.) and the mixture stirred until the steroid had dissolved (45 min.).

Stirring was continued for a further 45 min. when water was added and the steroid extracted into ether. The ether solution was washed with water, dried and the solvent evaporated. The semicrystalline product was recrystallised from methanol to give 5α-fluoro-cholestan-3-one (1.36 g.; 66%), m.p. 143-148° (lit. 150°). $\nu_{max.}$ 1710 cm.$^{-1}$

5α-Fluoro-cholest-1-en-3-one

5α-Fluoro-cholestan-3-one (0.5 g.) was enol-acetylated[135] by dissolving it in a solution of 72% perchloric acid (0.05 ml.) and acetic anhydride (4.8 ml.) made up to 50 ml. with ethyl acetate, allowing it to stand at room temperature for 5 min., then pouring the solution into ice-water. The ethyl acetate was evaporated and the steroid extracted with ether, the solution washed with water, dried and evaporated. The crude enol acetate was dissolved in acetic acid (30 ml.) to which sodium acetate (0.11 g.) had been added. A solution of bromine (0.20 g.) in acetic acid (10 ml.) was added and the solution allowed to stand at room temperature for 5 hr. Water was added and the steroid extracted into ether. The solution was washed with sodium carbonate solution and water, then dried and the ether evaporated at room temperature in a rotary evaporator.[134]

This crude 2α-bromo-5α-fluoro-cholestan-3-one (0.38 g.) was dissolved in acetic acid (30 ml.) containing semicarbazide hydrochloride (0.15 g.). The solution was allowed to stand at room temperature for 3 hr. then warmed to 50° for 1 hr. The mixture was diluted with water and the steroid extracted into

dichloromethane. The solution was washed with sodium carbonate solution and water, then dried and the solvent removed at room temperature. This semicarbazone was dissolved in dioxan (20 ml.) and sulphuric acid (40%; 15 ml.) added. The solution was warmed at 50° for 10 min. and poured into ice-water. The steroid was extracted into ether, the solution washed with water, dried and evaporated to dryness. Crystallisation of the brown product from acetone gave 5α-fluoro-cholest-1-en-3-one (0.18 g.; 36%), m.p. 89-93°; $[\alpha]_D$ +72° (c 0.14); $\lambda_{max.}$ 230 mμ (ε 9,900).

5α-Fluoro-cholest-1-en-3β-ol

5α-Fluoro-cholest-1-en-3β-one (0.15 g.) dissolved in dry ether (5 ml.) was added to a solution of lithium aluminium hydride (0.1 g.) in dry ether, and the resulting solution refluxed for 1 hr. Ethyl acetate was added and then a solution of ammonium chloride. The ethereal solution was washed with water, dried and the solvent evaporated. The resulting clear glass (0.14 g.) could not be crystallised. A test for fluorine was positive, giving approximately the same intensity as the tests of the saturated and unsaturated fluoro-ketones. $\nu_{max.}$ 3440, 1143 (C-F stretch), 1035, 760 $cm.^{-1}$

Attempts to purify this material by chromatography on Florisil were not successful; crystallisation could not be achieved, although the material gave only one spot on t.l.c.; R_f 0.54 (solvent: petrol-ether 2:1).

Attempted Tosylation of 5,6-Deutero-cholest-1-en-3β-ol

Deutero-cholest-1-en-3β-ol (0.20 g.) was dissolved in pyridine (5 ml.) and added to a solution of toluene-p-sulphonyl ~~acid~~ chloride (0.2 g.) in pyridine at 0°, and allowed to stand at room temperature for 18 hr. The solution was then poured into water and the steroid extracted into ether. The ethereal solution was washed with sodium carbonate solution, water, dilute acid and finally with water until it was neutral. The solution was dried and the ether evaporated, to give a pale brown glass. This was chromatographed on alumina (activity I); elution with petrol gave a clear glass which was crystallised from acetone to give 3,6-deutero-1α,5-cyclo-5α-cholest-2-ene (0.045 g.; 22%) m.p. 70-72°, $[\alpha]_D$ +39° (c 0.08). $\lambda_{max.}$ 212 mμ (ε 7,200); $\nu_{max.}(CS_2)$ 3020, 1030, 960, 780, 740 703 $cm.^{-1}$ $\nu_{max.}(CCl_4)$ 2240, 2170, 2135 $cm.^{-1}$ P.m.r. absorptions at τ 4.42 (1 proton), 8.45, 8.75, 9.10, 9.19, 9.25, 9.35.

Attempted Tosylation of 5α-Fluoro-cholest-1-en-3β-ol

5α-Fluoro-cholest-1-en-3β-ol (0.10 g.) in pyridine (5 ml.) was added to a solution of toluene-p-sulphonyl chloride (0.10 g.) in pyridine (5 ml.) at 0°. After standing at room temperature for 28 hr. the solution was poured into water and the steroid extracted into ether. The ether solution was washed with sodium carbonate solution, water, dilute acid, and finally with water until it was neutral. The solution was dried and the solvent evaporated to give a brown oil. Chromatography on deactivated neutral alumina (activity II-III) afforded on elution

with petrol, a clear glass (0.03 g.). This could not be crystallised, but gave a single spot on t.l.c., R_f 0.61 (solvent: petrol-ether 2:1). $\lambda_{max.}$ 263 mμ (ϵ 5,300); p.m.r. absorptions at τ 4.25 (4 protons, unresolved multiplet), 9.10, 9.19, 9.39. Testing for fluorine in the compound semi-quantitatively, proved that there was still one fluorine atom in the molecule.

Reaction of 1α,5-Cyclo-5α-cholest-2-ene

p-Nitro-perbenzoic Acid[141]

A slurry of p-nitro-benzoic acid (40 g.) in methane sulphonic acid (116 g.) was stirred at 0° while hydrogen peroxide (87%; 35 ml.) was added, over 10 min. The temperature rose to 34° during the addition, and the slurry was stirred at this temperature for 3 hr. Ice was then added, and water before the mixture was filtered and the solid material washed with water. The peracid was dried over phosphorus pentoxide to constant weight. Yield 40.8 g.; 98%.

The purity of this peracid was determined by reacting a known weight (0.25 g.) of acid with sodium iodide in acetic acid-chloroform (3:2). Water was added and the liberated iodine titrated with standard sodium thiosulphate solution (0.1 N). The product was found to contain 97% peracid.

1α,5-Cyclo-5α-cholestane-2α,3α-epoxide

1α,5-Cyclo-cholest-2-ene (0.5 g.) was dissolved in dry ether (40 ml.) and p-nitro-perbenzoic acid (0.25 g.) added; the solution was allowed to stand at room temperature for 28 hr. The ether solution was washed with sodium sulphite solution until there was no further reaction with acidified starch-iodide paper. The ether solution was washed with water and sodium carbonate solution, and finally with water until it was neutral. The solution was dried and the solvent evaporated to give a white solid which was crystallised from acetone to give

1α,5-cyclo-5α-cholestane-2α,3α-epoxide (0.48 g.; 93%) m.p. 97-98°, $[\alpha]_D$ +34° (c 0.12); (Found: C,83.8; H, 11.2. $C_{27}H_{44}O$ requires C, 84.3; H, 11.5%); analysis for oxirane oxygen gave 3.99%; $C_{27}H_{44}O$ requires 4.16%. $\nu_{max.}$ 820 cm.$^{-1}$; p.m.r. absorptions at τ6.85, 8.17, 8.77 (19-methyl group), 9.12, 9.21, 9.27 and 9.38 (18-methyl group).

This epoxidation was repeated using a threefold excess of peracid (0.75 g.) over hydrocarbon (0.5 g.) in dry ether (50 ml.). The product was isolated as before and crystallised from acetone to give the same epoxide (0.47 g.) m.p. 97.5-98°.

1α,5-Cyclo-cholestan-2α-ol

The epoxide (0.4 g.) in dry ether (10 ml.) was added to lithium aluminium hydride (0.3 g.) in dry ether (30 ml.) and the solution refluxed for 1 hr. Ethyl acetate was added, followed by dilute hydrochloric acid. The ethereal solution of the steroid was washed with water, dried and the solvent evaporated to give a white solid. This was crystallised from acetone to give 1α,5-cyclo-cholestan-2α-ol (0.38 g.; 95%) m.p. 161-162°; $[\alpha]_D$ +24° (c 0.078); (Found: C, 83.6; H, 11.9. $C_{27}H_{46}O$ requires C, 83.8; H, 11.9%); $\nu_{max.}$ 3600, 1040, 1010 cm.$^{-1}$; p.m.r. absorptions at τ5.95, 8.75 (19-methyl group), 9.10, 9.19, 9.22, 9.34 (18-methyl group). After shaking the sample with D_2O, the peak at τ5.95 disappeared.

This alcohol (0.05 g.) was treated with acetic anhydride (0.3 ml.) in pyridine (5 ml.) and allowed to stand at room temperature for 16 hr. The solution was poured into water and

the steroid extracted into ether. The ether solution was washed with dilute acid, water, sodium carbonate solution and finally with water until it was neutral. The solution was dried and the solvent evaporated to give a white solid, recrystallised from acetone to give 1α,5-cyclo-cholestan-2α-ol acetate (0.05 g.; 89%) m.p. 101-102°; $[\alpha]_D$ +31° (c 0.13); (Found: C, 81.4; H, 11.3. $C_{29}H_{48}O_2$ requires C, 81.3; H, 11.3%). $\nu_{max.}$ 3020, 1735, 1250, 1040 cm.$^{-1}$; p.m.r. absorptions at τ7.96, 8.75, 9.09, 9.18, 9.34.

Reduction of 1α,5-cyclo-cholestan-2α-ol acetate

a. The acetate (0.02 g.) in acetic acid (5 ml.) with Adam's catalyst (0.01 g.) was heated to 80° in an atmosphere of hydrogen for 18 hr. The catalyst was filtered off and the solvent evaporated, the final traces being removed by azeotroping with acetone. Crystallisation of the product from acetone gave unchanged acetate. The reduction was attempted in methanol-benzene solution, with Adam's catalyst at 100 atmospheres and room temperature. After 3 hr., the catalyst was filtered off and the solvent evaporated. Crystallisation of the product from acetone gave cholestan-2α-ol acetate (0.013 g.; 65%) m.p. 87-89° (lit. 90°); $[\alpha]_D$ -2° (c 0.15), (lit. -1°).

The product of this reduction (0.01 g.) was dissolved in ethanol (5 ml.) and potassium hydroxide (0.01 g.) added. The solution was refluxed for 40 min., poured into water and the ethanol evaporated. The steroid was extracted into ether, the solution washed with water, dried and evaporated. The crude

material was dissolved in acetone (25 ml.) and 8N chromic acid (1 ml.) added. The solution was stirred for 3 min. and methanol (5 ml.) added. Water was added and the organic solvents evaporated. The steroid was extracted into ether, the solution washed with dilute acid and water before being dried and the ether evaporated. The product was crystallised from acetone to give cholestan-2-one (0.007 g.) m.p. 129-131° (lit. 131°). A mixed melting point with an authentic sample gave no depression. $[\alpha]_D$ +48° (c 0.08), (lit. +50°).

1α,5-Cyclo-5α-cholestan-2-one

1α,5-Cyclo-cholestan-2α-ol (0.7 g.) was dissolved in acetone (100 ml.) and 8N chromic acid (2 ml.) added with vigorous stirring. Stirring was continued for 4 min. and methanol (20 ml.) added, followed by water. The organic solvents were distilled off and the steroid extracted into ether. The ether solution was washed with dilute acid and water, before being dried and the solvent evaporated. Crystallisation from acetone gave 1α,5-cyclo-5α-cholestan-2-one (0.65 g.; 93%). m.p. 141-143°, $[\alpha]_D$ +37° (c 0.102); (Found: C, 84.4: H, 11.3. $C_{27}H_{44}O$ requires C, 84.3; H, 11.5%). $\lambda_{max.}$ 215 mμ (ε 7,800); 327 mμ (ε 630). $\nu_{max.}$ 3018, 1710, 760, 720 cm.$^{-1}$; p.m.r. absorptions at τ7.58, 7.78, 7.90, 8.79 (19-methyl group), 9.01, 9.20, 9.32 (18-methyl group). A small sample treated with sodium in deuteromethanol (CH_3OD) under reflux for 30 min., poured into deuterium oxide, extracted with ether and recovered from this solution, showed no absorptions at τ7.58, 7.69, 7.78, and 7.90.

The rotatory dispersion curve of this ketone (figure III) was recorded for a solution in methanol (c 0.094): $[\Phi]_{500}$ +38°, $[\Phi]_{308}$ +1110°, $[\Phi]_{273}$ -690°, $[\Phi]_{260}$ -510°.

Reduction of 1α,5-Cyclo-cholestan-2-one

a. The cyclopropylketone (0.05 g.) was dissolved in acetic acid and hydrogenated over Adam's catalyst at room temperature and atmospheric pressure for 6 hr. The catalyst was filtered off and the solvent removed to give, on crystallisation from acetone, unreacted 1α,5-cyclo-cholestan-2-one.

b. The reduction was attempted on the cyclopropyl ketone (0.05 g.) in acetic acid using Adam's catalyst (0.03 g.) at 120 atmospheres pressure of hydrogen at room temperature. After 6 hr., the catalyst was filtered off and the solvent removed. An i.r. spectrum showed the presence of a ketone (1725 $cm.^{-1}$) and an acetate (1250, 1740 $cm.^{-1}$). The product was chromatographed on deactivated alumina. Elution with petrol-benzene (90:10) gave a ketone (0.13 g.) which was crystallised from acetone. Further elution with petrol-benzene, increasing the proportion of benzene, afforded an acetate, crystallised from acetone to give cholestan-2α-ol acetate (0.022 g.) m.p. 89-90° (lit. 90°), $[\alpha]_D$ -1.5° (c 0.096) (lit. -1°).

Hydrolysis of this acetate (0.02 g.) was effected by refluxing it for 30 min. in ethanol (10 ml.) with potassium hydroxide (0.02 g.). Water was added and the ethanol evaporated. The steroid was extracted into ether, the solution washed with water, dried and the solvent evaporated. The crude alcohol,

dissolved in acetone (30 ml.) was treated with 8N chromic acid (2 ml.) with vigorous stirring. After 4 min. methanol (10 ml.) was added, followed by water. The organic solvents were removed and the steroid extracted into ether. The ether solution was washed with dilute acid then water, dried and the solvent evaporated. Crystallisation from acetone gave a ketone (0.017 g.) m.p. 129-130°, $[\alpha]_D$ +47°, (c 0.12). This ketone had identical i.r. and p.m.r. spectra to that isolated directly from the chromatogram; a mixed m.p. showed no depression. From melting point, mixed melting point with an authentic sample, optical rotation, and comparison of i.r. spectra, this ketone was identified as cholestan-2-one. Rotatory dispersion curves of this product and of cholestan-2-one and cholestan-3-one were recorded in methanol, with and without the addition of hydrochloric acid (0.05 ml.) to catalyse hemi-ketal formation. The amplitudes are recorded in table VII, and show the product to be cholestan-2-one.

Reduction of 1α,5-Cyclo-cholestan-2-one with Lithium in Liquid Ammonia

1α,5-Cyclo-cholestan-2-one (0.06 g.) was dissolved in t-butanol (30 ml.) and liquid ammonia (200 ml.) added. The mixture was stirred for 3 hr. while lithium (2 g.) was added in small portions, to maintain a blue colour in the reaction mixture. Water was added and the mixture left overnight to allow any remaining ammonia to evaporate. The steroid was extracted into ether; the ether solution was washed with dilute acid, then

water, dried and the solvent evaporated. An i.r. spectrum of this product had $\nu_{max.}$ 1715, 1745 cm.$^{-1}$ and g.l.c. showed two components to be present, R_t 1.88 (80%) and 2.09 (20%) (column B). From retention time (2.09) and i.r. spectrum (1715 cm.$^{-1}$) this ketone could be identified as cholestan-2-one. The mixture was chromatographed on alumina (activity I), and elution with benzene-ether (99:1 to 95:5) gave fractions containing both ketones. The earlier fractions were richer in the cyclohexanone, but it could not be obtained free from the second product. Later fractions contained the cyclopentanone (1745 cm.$^{-1}$) free from the cyclohexanone. Although this was homogeneous on g.l.c., it could not be crystallised. A p.m.r. spectrum of this compound had the following absorptions: τ8.98, 9.03, 9.09, 9.20, 9.32.

Addition of Hydrogen Bromide to 1α,5-Cyclo-cholestan-2-one

To a solution of the ketone (0.05 g.) in acetic acid (5 ml.) was added hydrogen bromide in acetic acid (45%; 1 ml.). The solution was refluxed for 30 min., water added and the steroid extracted into ether. The ether solution was washed with sodium carbonate solution, with water until it was neutral and then dried. Evaporation of the solvent gave a pale yellow oil, which was crystallised from ethanol to give 5β-bromo-cholestan-2-one (0.036 g.; 59%) m.p. 107-111°, $[\alpha]_D$ +17° (c 0.093); (Found: C, 70.6; H, 9.8. $C_{27}H_{45}OBr$ requires C, 70.0; H, 9.4%). $\nu_{max.}$ 1720 cm.$^{-1}$; p.m.r. absorptions at τ8.90 (19-methyl group), 9.10, 9.18, 9.32.

Reduction of 5β-Bromo-cholestan-2-one

The bromoketone (0.01 g.) was dissolved in ether (2 ml.) and added to liquid ammonia (30 ml.). Sodium (0.005 g.) was added and the mixture stirred for 20 min. Water was added and the ammonia allowed to evaporate. The steroid was extracted with ether, the ether solution washed with dilute acid and water, dried and the solvent evaporated. Crystallisation of the product from acetone gave 5β-cholestan-2-one (0.008 g.) m.p. 86-88° (lit. 87.5-88°); $[\alpha]_D$ +21° (c 0.07), (lit. +20.5°).[153] P.m.r. absorptions at τ8.97, 9.08, 9.17, 9.30.

Regeneration of 1α,5-Cyclo-cholestan-2-one

5β-Bromo-cholestan-2-one (0.01 g.) was dissolved in ethanol (10 ml.) and a solution of sodium hydroxide in water (5%; 0.5 ml.) added. The solution was refluxed for 20 min., diluted with water, the ethanol evaporated and the steroid extracted into ether. The solution was washed, dried and the solvent evaporated. Crystallisation of the product from acetone gave 1α,5-cyclo-cholestan-2-one (0.006 g.) m.p. and mixed m.p. 141-143°, $[\alpha]_D$ +35° (c 0.02).

1α,5-Cyclo-5α-cholestane from 1α,5-Cyclo-cholestan-2-one

1α,5-Cyclo-cholestan-2-one (0.02 g.) was dissolved in digol (3 ml.) and ethanol (0.3 ml.). Hydrazine hydrate (99%; 0.5 ml.) and potassium hydroxide (0.05 g.) were added and the solution refluxed at ca.100° for 30 min. The temperature of the solution was raised to 210° by allowing the water and ethanol to evaporate,

and refluxing continued for 2 hr. The solution was cooled, water added and the steroid extracted into ether. The solution was washed with water, dried and evaporated to dryness. The product, a clear glass, was crystallised from acetone to give 1α,5-cyclo-5β-cholestane (0.017 g.; 85%) m.p. 73-74.5, $[\alpha]_D$ +20° (c 0.06); (Found: C, 87.3; H, 12.4. $C_{27}H_{46}$ requires C, 87.5; H, 12.5%); $\nu_{max.}$ 3005, 1030, 965, 945, 745. P.m.r. absorptions at τ 8.78 (19-methyl group), 9.09, 9.19, 9.27, 9.34 (18-methyl group). The compound gave a pale yellow colour with TNM.

1α,5-Cyclo-5α-cholestane from 1α,5-Cyclo-cholest-2-ene

The catalyst was prepared by refluxing rhodium trichloride trihydrate (1.0 g.) and triphenylphosphine (6 g.) in ethanol (120 ml.) for 30 min. The solution was cooled and the tris-(triphenylphosphine)-rhodium chloride (3.5 g.) filtered off. The crystals were washed with ethanol and then with ether.[156]

1α,5-Cyclo-cholest-2-ene (0.05 g.) was dissolved in benzene (5 ml.) and tris-(triphenylphosphine)-rhodium chloride (0.03 g.) added. The mixture was hydrogenated at room temperature and atmospheric pressure for 4 hr. The catalyst quickly dissolved as the solution became saturated with hydrogen. The solution was adsorbed on an alumina column and eluted with benzene, to give a clear glass, which was crystallised from acetone to give 1α,5-cyclo-5α-cholestane (0.04 g.; 80%) m.p. 73.5-75°, $[\alpha]_D$ +21° (c 0.08). A mixed melting point with the material from the previous experiment gave no depression. The i.r. and p.m.r. spectra were identical.

Reduction of 3α,5-Cyclo-cholest-6-ene

The cyclosteroid (0.05 g.) was dissolved in benzene (5 ml.) and tris-(triphenylphosphine)-rhodium chloride (0.03 g.) added. The mixture was hydrogenated at room temperature and atmospheric pressure for 4 hr. The solution was adsorbed on alumina and elution with benzene gave a clear glass. This was crystallised from acetone to give 3α,5-cyclo-cholestane (0.045 g.) m.p. 77.5-79°, (lit.79°); $[\alpha]_D$ +79° (c 0.086), (lit. +80°); p.m.r. absorptions at: τ 8.99, 9.09, 9.18, 9.30; 9.60, 9.66, 9.72 (2 cyclopropyl protons). The p.m.r. and i.r. spectra were identical to those of an authentic sample.

3α,5-Cyclo-5α-cholestane[38]

Cholesteryl tosylate (0.5 g.) in dry ether (5 ml.) was added to a solution of lithium aluminium hydride (0.3 g.) in dry ether (30 ml.) and the solution refluxed for 1 hr. Ethyl acetate was added followed by dilute acid. The ether solution was washed with water and dried before evaporation of the solvent. G.l.c. of the product showed two constituents. The material was chromatographed on alumina (activity I). Elution with small quantities of petrol gave two hydrocarbon fractions. The first eluted, 3α,5-cyclo-5α-cholestane (0.42 g.; 84%) m.p. 78-79°, (lit. 79°); $[\alpha]_D$ +81° (c 0.13), (lit. +80°), was crystallised from acetone. The second product eluted was cholest-5-ene (0.06 g.).

The Reaction of 1α,5-Cyclo-cholest-2-ene with Hydrogen chloride

The hydrocarbon (0.05 g.) was dissolved in acetic acid (20

ml.) and dry hydrogen chloride bubbled through for 30 min. Water was added and the steroid extracted into ether, washed with sodium carbonate solution and water, then dried and the solvent evaporated to give a brown oil (0.043 g.). The product was chromatographed on alumina and elution with petrol gave a clear glass (0.042 g.), which had, by g.l.c., five non-polar constituents in equal amounts. These could not be separated on alumina (activity I) and were not identified.

The Reaction of 1α,5-Cyclo-cholest-2-ene with Hydrogen Bromide

The hydrocarbon (0.03 g.) was dissolved in acetic acid (10 ml.) and a solution of hydrogen bromide in acetic acid (45%; 10 ml.) added. After 10 min. water was added and the steroid extracted into ether. The ether solution was washed with sodium carbonate solution and water and then dried. The solvent was evaporated to give a brown glass. On g.l.c. this was shown to have at least four constituents. Chromatography on alumina (activity I) failed to effect a separation.

Addition of Water to 1α,5-Cyclo-cholest-2-ene

a. The hydrocarbon (0.05 g.) in acetone (25 ml.) was treated with 5N sulphuric acid (0.5 ml.), and the solution refluxed for 3 hr. Water was added, the acetone removed and the steroid extracted into ether. This solution was washed with water, dried and the solvent evaporated. An infra red spectrum of the product was almost identical to that of the hydrocarbon; no alcohol was present.

b. The hydrocarbon (0.03 g.) was added to concentrated sulphuric acid, warmed for 5 min. and the solution poured into water. The steroid was extracted into ether, washed with sodium carbonate solution and water, dried and the solvent evaporated. The brown oil (0.019 g.) produced was not, from i.r. spectra, an alcohol. It was not identified.

c. The hydrocarbon (0.05 g.) was dissolved in acetic acid (10 ml.) and concentrated sulphuric acid (0.5 ml.) added. After 15 min. this was poured into water, the steroid extracted into ether, the solution washed with sodium carbonate solution then water and dried. Evaporation of the solvent gave a brown glass (0.041 g.). This was chromatographed on alumina and elution with petrol-benzene gave cholest-1-en-3β-ol acetate (0.03 g.; 65%), m.p. 85-88° (lit. 87°), $[\alpha]_D$ +58° (c 0.073), (lit. +60°).[75]

Reaction of Cholest-1-en-3β-ol with Sulphuric Acid

Cholest-1-en-3β-ol (0.04 g.) in acetone (10 ml.) was treated with 5N sulphuric acid (0.5 ml.) and refluxed for 3 hr. The solution was poured into water, the acetone evaporated and the steroid extracted into ether. The ether solution was washed with water, dried and evaporated to give a pale brown glass. Chromatography on alumina gave cholest-1-en-3β-ol (0.024 g.; 60%). The above experiment was carried out on the corresponding acetate and gave crystalline starting material in 90% yield.

Hydroboration-Oxidation of 1α,5-Cyclo-cholest-2-ene

Redistilled boron trifluoride etherate (2 ml.) in dry ether

(100 ml.) was added to the hydrocarbon (0.3 g.) in dry ether (3 ml.). A solution of lithium aluminium hydride (0.1 g.) in dry ether (6 ml.) was added dropwise over 1 hr. while the solution, under nitrogen, was stirred at 0°. The solution was allowed to warm up to room temperature and stirred for a further hour. Water was added, the organic layer was washed with sodium carbonate solution then water, dried and the solvent evaporated.

The resulting organoborane compound was dissolved in ether (10 ml.) and treated with sodium hydroxide solution (10%, 5 ml.) and cooled to 0°. The solution was stirred while 30% hydrogen peroxide (5 ml.) was added. Stirring at 0° was continued for 1 hr., then the solution was diluted with water and ether, the organic layer was washed with sodium bisulphite solution and water, dried and the solvent evaporated. The product was chromatographed on alumina; elution with petrol gave unchanged hydrocarbon (0.13 g.; 44%). Elution with petrol-benzene (90:10) gave an alcohol (0.16 g.; 51%), which could not be crystallised. A p.m.r. absorption: τ 8.78, 9.10, 9.19, 9.27, 9.35. G.l.c. showed that there were two compounds present in the reaction product; R_t 1.72 (22%) and R_t 2.01 (78%). These could not be separated on alumina.

3β-Acetoxy-5α-bromo-cholestan-6-one[165]

A solution of bromine in acetic acid (5%, 64 ml.) was added over 1 hr. to a vigorously stirred solution of 3β-acetoxy-cholestan-6-one[193] (8 g.) in acetic acid (20 ml.) and ether (80 ml.) at 0°. The ether was evaporated and the precipitated product filtered off. Recrystallisation from petrol gave 3β-acetoxy-5α-bromo-cholestan-6-one (5.99 g.; 64%), m.p. 161.5-162° (lit. 162°) $[\alpha]_D$ -130° (c 0.12) (lit. -133°), p.m.r. absorptions at τ7.98, 9.02, 9.09, 9.18, 9.35.

3β-Acetoxy-5β-hydroxy-cholestan-6-one[166]

3β-Acetoxy-5α-bromo-cholestan-6-one (2.9 g.) was dissolved in ethanol (40 ml.) and potassium hydroxide (2 g.) added. The mixture was stirred at room temperature for 5 hr.; after 1 hr ethanol (15 ml.) was added. The mixture was diluted with ether (100 ml.) washed with saturated sodium chloride solution and dried. Evaporation of the solvent gave an oil which was dissolved in pyridine (15 ml.) and acetic anhydride (20 ml.) added. After standing for 26 hr. at room temperature, the solution was poured into dilute hydrochloric acid-ice and the resulting precipitate filtered off. The product was crystallised from acetone - methanol to give 3β-acetoxy-5β-hydroxy-cholestan-6-one (1.52 g.; 60%), m.p. 143-144°, (lit. 144.5°) $[\alpha]_D$ -21° (c 0.097°), (lit. -22°); $\nu_{max.}$ 3400, 1730, 1710, 1260, 1175, 1060, 1025 cm.$^{-1}$, p.m.r. absorptions at τ7.96, 9.10, 9.19, 9.25, 9.34.

Dehydration of 3β-Acetoxy-5β-hydroxy-cholestan-6-one

The steroid (0.1 g.) dissolved in pyridine (5 ml.) at 0° was treated with thionyl chloride (0.1 ml.) and allowed to stand at 0° for 2 hr. The mixture was poured into ice, and the steroid extracted into ether. The solution was washed with dilute hydrochloric acid, water before being dried and the solvent evaporated. G.l.c. showed that the reaction product had three components, which were separated on alumina. Elution with benzene gave 1α,5-cyclo-cholestan-3β-ol-6-one acetate which was recrystallised from acetone to give white plates (0.03 g.; 30%) R_t 1.98; m.p. 107-108°, $[\alpha]_D$ -34° (c 0.096), (Found: C, 78.7; H, 10.3. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.5%); $\nu_{max.}$ 3030, 1735, 1695, 1245, 1040 cm.$^{-1}$; p.m.r. absorptions at τ 7.96 (acetate), 8.76, 8.99, 9.10, 9.19, 9.37. Elution with benzene-ether (99:1) gave, after crystallisation from acetone, 3β-acetoxy-cholest-4-en-6-one (0.02 g.; 20%), R_t 2.04, m.p. 108-110° (lit. 110°), $[\alpha]_D$ -52° (c 0.13) (lit. -50°)[165] $\nu_{max.}$ 1735, 1720, 1250, 1045 cm.$^{-1}$; this compound gave a yellow colour with TNM. Elution with benzene-ether (95:5) afforded unchanged starting material (0.05 g.; 50%).

1α,5-Cyclo-cholestan-3β-ol

3β-Acetoxy-1α,5-cyclo-cholestan-6-one (0.025 g.) was dissolved in ethanol (5 ml.) and added to a solution of potassium hydroxide (0.5 g.) and hydrazine hydrate (3 ml.) in digol (30 ml.). The solution was refluxed at 90° for 30 min. and then the temperature raised to 210° and refluxing continued for 2.5 hr. The solution

was cooled and poured into water, the steroid extracted into ether, washed with water, dried and the solvent evaporated. The product was crystallised from acetone to give 1α,5-cyclo-cholestan-3β-ol (0.022 g.; 88%) m.p. 131-133°, $[\alpha]_D$ +31° (c 0.074), (Found: C, 83.8; H, 11.6. $C_{27}H_{46}O$ requires C, 83.9; H, 11.9%). P.m.r. absorptions at τ5.83, 8.76, 9.10, 9.19, 9.22, 9.34.

This alcohol (0.01 g.) was treated with acetic anhydride (0.1 ml.) in pyridine (5 ml.), and the solution allowed to stand at room temperature for 18 hr. The solution was poured into water and the steroid extracted into ether; the ether solution was washed with sodium carbonate solution, water, dilute acid and finally with water until it was neutral. The solution was dried and the solvent evaporated to give a white solid which was crystallised from acetone to give 1α,5-cyclo-cholestan-3β-ol acetate (0.009 g.) m.p. 114-116° $[\alpha]_D$ +49° (c 0.063).

1α,5-Cyclo-cholestan-3-one

1α,5-Cyclo-cholestan-3β-ol (0.12 g.) was dissolved in acetone (30 ml.) at 30° and 8N chromic acid (0.5 ml.) added. The solution was stirred for 2 min. and methanol (5 ml.) added, followed by water. The organic solvents were evaporated and the steroid extracted into ether. The ether solution was washed with dilute acid then water, dried and the solvent evaporated. The resulting white solid was recrystallised from acetone to give 1α,5-cyclo-cholestan-3-one (0.11 g.; 94%) m.p. 128-130°, $[\alpha]_D$ +12° (c 0.12), (Found: C, 84.5; H, 11.3. $C_{27}H_{44}O$ requires C, 84.3; H, 11.5%). $\lambda_{max.}$ 208 mμ (ε 6,400), 287 mμ (ε 570); $\nu_{max.}$ 1740 cm.$^{-1}$

P.m.r. absorptions at τ8.78, 9.08, 9.19, 9.22, 9.32.

1α,5-Cyclo-cholestane

1α,5-Cyclo-cholestan-3-one (0.08 g.) was dissolved in digol (5 ml.) and ethanol (0.5 ml.). Hydrazine hydrate (0.8 ml.) and potassium hydroxide (0.1 g.) were added and the solution refluxed at 100° for 30 min. The temperature was raised to 210°, and refluxing continued for 2 hr. The solution was cooled and poured into water, the steroid was extracted into ether and the ether solution washed with water, dried and evaporated to dryness. The product was filtered in petrol through an alumina column, and crystallised from acetone to give 1α,5-cyclo-5α-cholestane (0.064 g.; 79%) m.p. 74-75°, $[\alpha]_D$ +20° (c 0.09). A mixed melting point with material prepared previously from Hydrocarbon 'H' showed no depression. The i.r. and p.m.r. of samples from the two sources were identical. This material also gave a pale yellow colour with TNM.

Attempted preparation of 1α,5-Cyclo-5α-cholest-2-ene

1α,5-Cyclo-cholestan-3β-ol (0.05 g.) was dissolved in pyridine (3 ml.) and added to a solution of toluene-p-sulphonic acid (0.05 g.) in pyridine (5 ml.) at 0°. After standing at room temperature for 24 hr., the solution was poured into water and the steroid extracted into ether. The ether solution was washed with dilute acid, water, sodium carbonate solution and finally with water. Evaporation of the solvent gave a pale brown glass, which from an i.r. spectrum appeared to contain a tosyl

ester group. The material was not investigated further, but adsorbed on alumina in petrol solution. After 18 hr., the column was eluted with petrol to give a clear glass (0.037 g.) which was examined on g.l.c. and found to have four main components, R_t 0.84, 0.99, 1.11 and 1.20 (column A). These hydrocarbons could not be separated on alumina, and were therefore not identified. The material R_t 0.84 did however have the same retention time as 1α,5-cyclo-cholest-2-ene.

Sublimation of Cholest-4-en-3β-ol

Cholest-4-en-3β-ol (0.05 g.) was placed in a glass tube, sealed at the lower end. The lower end, containing the steroid, was heated to 260° in an electrically heated metal block. A crystalline deposit appeared on the cooler upper part of the tube, and was removed. Examination of i.r. and p.m.r. spectra showed this to be unchanged cholest-4-en-3β-ol.

Cholest-4-en-3β-ol (0.1 g.) in ether (2 ml.) was added to brick dust (g.l.c. column support material) (0.5 g.) and the ether was allowed to evaporate. The steroid coated brick dust was heated at 260° as above, and a clear glass appeared on the upper walls of the tube. This was collected and filtered through alumina in petrol. A p.m.r. spectrum of this material showed a complex multiplet between τ 4.1 and 5.0. There were also cyclopropyl protons present at τ 9.45 to 9.70. Examination of this hydrocarbon on g.l.c. showed that it was a mixture of cholesta-3,5-diene and 3α,5-cyclo-cholest-6-ene, in the same proportions as produced by gas chromatography of the alcohol.

Cholest-5-en-3β,7β-diol 3β-benzoate

3β-Acetoxy-cholest-5-en-7-one (0.5 g.) in dry ether (10 ml.) was added to a solution of lithium aluminium hydride (0.3 g.) in dry ether (30 ml.) and the solution refluxed for 1 hr. Ethyl acetate was added, followed by dilute acid. The ether solution was washed with water, dried and the solvent evaporated. The crude diol was dissolved in pyridine (5 ml.) and added to a solution of benzoyl chloride (0.4 ml.) in pyridine (5 ml.).

After standing overnight at room temperature, the solution was poured into water and the steroid extracted into ether. The ethereal solution was washed with sodium carbonate solution, water, dilute acid and finally with water. The solution was dried and the solvent evaporated. The resulting product was crystallised from acetone to give cholest-5-en-3β,7β-diol 3β-benzoate (0.43 g.) m.p. 190-192° (lit. 192°). P.m.r. absorptions at τ4.64, 8.89, 9.09, 9.18, 9.30.

Cholesta-5,7-dien-3β-ol benzoate[171]

Cholest-5-en-3β,7β-diol dibenzoate (0.03 g.) in dimethylaniline (5 ml.) was refluxed for 6 hr. The solution was poured into dilute hydrochloric acid and the steroid extracted into ether. The ether solution was washed with water, dried and the solvent evaporated. Crystallisation from acetone gave cholesta-5,7-dien-3β-ol benzoate (0.014 g.) m.p. 136-139° (lit. 140°), $\lambda_{max.}$ 221 mμ (ϵ 19,000). Thin layer chromatography of these compounds, and the products from g.l.c. was carried out using petrol-ether 1:2 as solvent. The R_f values are quoted in table IX.

Estra-1,3,5(10)-trien-3-ol[110,178]

Estrone (3.6 g.) was dissolved in digol (40 ml.) and potassium hydroxide (2.5 g.) and hydrazine hydrate (3 ml.) added. The solution was refluxed at 90° for 30 min. and then the temperature raised to 210° and refluxing continued for 2 hr. The solution was cooled and poured into water. The steroid was extracted into ether, the solution washed thoroughly with water and dried; the solvent was removed by distillation under reduced pressure. The product was crystallised from methanol to give estra-1,3,5(10)-trien-3-ol (3.2 g.; 84%) m.p. 140-141° (lit. 134°)[11]

3-Methoxy-estra-1,3,5(10)-triene[178,232]

Estra-1,3,5(10)-trien-3-ol (2.2 g.) was added to a solution of methyl toluene-p-sulphonate (4 g.) in 10% aqueous potassium hydroxide solution (10 ml.) and the resulting suspension stirred at 90° for 3 hr. After 1 hr., a further 10 ml. of 10% alkali solution was added. The mixture was cooled and the product filtered off, washed with water and dried over phosphorus pentoxide. The crude product was crystallised from methanol to give 3-methoxy-estra-1,3,5(10)-triene (2.05 g.; 89%). m.p. 79.5-80° (lit. 78-79°); p.m.r. absorptions at τ6.20, 9.25.

Estr-4-en-3-one[178]

3-Methoxy-estra-1,3,5(10)-triene (1.4 g.) was dissolved in tetrahydrofuran (125 ml.) and t-butanol (125 ml.) and this solution added to liquid ammonia (250 ml.). This solution was stirred vigorously while lithium (5 g.) was added over 6 hr. The lithium

was added at such a rate that the solution remained deep blue throughout the reaction period. The liquid ammonia was allowed to evaporate, and water was added. The organic solvents were evaporated, and the steroid extracted into ether. The ether solution was washed several times with dilute acid and then with water, dried and the solvent evaporated. The product was crystallised from acetone to give estr-4-en-3-one (0.71 g.; 51%) m.p. 65-66° (lit. 66.7°), $[\alpha]_D$ +42° (c 0.13) (lit. +44°). $\lambda_{max.}$ 240 mμ (ϵ 16,000); $\nu_{max.}$ 1685, 970, 890 $cm.^{-1}$; p.m.r. absorption at τ 4.11, 9.22.

Reduction of Estr-4-en-3-one

Estr-4-en-3-one (0.5 g.) was dissolved in dry ether (10 ml.) and the solution added to lithium aluminium hydride (0.5 g.) in dry ether (20 ml.). The resulting solution was refluxed for 1 hr., cooled and ethyl acetate added, followed by dilute acid. The ether solution was washed with water, dried and the solvent evaporated. A clear glass was produced, whose i.r. spectrum showed no characteristic absorption of either a carbonyl or hydroxyl group. The product was filtered through alumina in petrol and crystallised from acetone to give estra-3,5(10)-diene (0.41 g.; 84%) m.p. 66-69°, $\lambda_{max.}$ 266 mμ (ϵ 5,800), p.m.r. absorptions at τ 4.42 (2 protons), 9.24.

Estr-4-en-3β-ol

Estr-4-en-3-one (0.5 g.) was dissolved in dry ether (10 ml.) and the solution added to lithium aluminium hydride (0.4 g.) in dry ether (20 ml.). The resulting solution was refluxed for 1

hr., cooled and ethyl acetate added. The alumina complex was dissolved by adding ammonium chloride solution. The ether solution was washed with water, dried and the solvent evaporated. Crystallisation of the product from acetone gave estr-4-en-3β-ol (0.12 g.; 24%) m.p. 71-74°. A second crop could not be obtained, presumably this was due to two isomers being present. P.m.r. abs. τ9.23.

Attempted Tosylation of Estr-4-en-3β-ol

Estr-4-en-3β-ol (0.05 g.) in pyridine (3 ml.) was added to a solution of toluene-p-sulphonyl chloride (0.05 g.) in pyridine (3 ml.) at 0°, and the solution allowed to stand at 0° for 18 hr. The solution was poured into water, the solvents removed by distillation at ca.40° on a rotary evaporator and the residue taken up in ether and sodium carbonate solution. The ether solution was washed with water, briefly with very dilute acid and finally with water, dried and the solvent evaporated. The product, a clear brown glass, was chromatographed on alumina, and elution with petrol gave a clear glass, crystallised from acetone to give estra-3,5(10)-diene (0.03 g.; 62%). m.p. 66-71°. The spectra of this material were identical to those from the previous preparation.

5α-Estran-3-one

Estr-4-en-3-one (0.75 g.) was dissolved ether (25 ml.) and dioxan (25 ml.), and this solution added to liquid ammonia (125 ml.). Lithium (0.030 g.) was added in small pieces and the

solution stirred for 30 min. then solid ammonium chloride (5 g.) added. The ammonia was allowed to evaporate and water was added. The ether solution of steroid was washed with water, dried and the solvent evaporated. The crude product was dissolved in acetone (60 ml.) and 8N chromic acid (1 ml.) added. The solution was stirred for 3 min. and methanol (15 ml.) added, followed by water. The organic solvents were evaporated and the steroid extracted into ether. The ether solution was washed with dilute acid and water, then dried and the solvent evaporated. The product was crystallised from acetone to give 5α-estran-3-one (0.41 g.; 55%) m.p. 72-75°, $\nu_{max.}$ 1705 cm.$^{-1}$; p.m.r. abs.: τ9.26.

Estr-1-en-3-one[180,181,182]

Estran-3-one (0.15 g.) in acetic acid (10 ml.) was treated with bromine (0.05 g.) in acetic acid (2 ml.) and the mixture stirred for 20 min. The solution was poured into water, and the steroid extracted into ether. The ether solution was well washed with water, dried and evaporated to dryness. The oily product was dissolved in dimethylformamide (10 ml.), lithium carbonate (0.1 g.) added and the mixture refluxed for 1 hr. After cooling, the solution was poured into dilute hydrochloric acid and ether added. The solution of steroid in ether was washed with water, dried and the solvent evaporated. This reaction product appeared from i.r. to be a mixture of Δ^4 and Δ^1-3-ketones; $\nu_{max.}$ 1680, 880, 760 cm.$^{-1}$ The product was chromatographed on alumina; elution with benzene gave estr-1-en-3-one,

(0.079 g.) which could not be crystallised. $\nu_{max.}$ 1680, 760 cm.$^{-1}$ Further elution with benzene and with benzene-ether (99:1) gave estr-4-en-3-one (0.045 g.), $\nu_{max.}$ 1680, 880 cm.$^{-1}$

Estr-1-en-3β-ol

Estr-1-en-3-one (0.05 g.), dissolved in ether (5 ml.) was added to lithium aluminium hydride (0.05 g.) in dry ether (10 ml.) and the solution refluxed for 1 hr. Ethyl acetate was added, followed by dilute acid. The ether solution was washed with water, dried and the solvent evaporated. The resulting oil (0.046 g.), estr-1-en-3β-ol, could not be crystallised.

Attempted tosylation of Estr-1-en-3β-ol

Estr-1-en-3β-ol (0.03 g.) in pyridine (3 ml.) was added to toluene-p-sulphonyl chloride (0.03 g.) in pyridine (5 ml.) at 0°. The solution was allowed to stand at room temperature for 18 hr. then it was poured into dilute acid. The steroid was extracted into ether and the ether solution washed with sodium carbonate solution, water, dilute acid, and finally water. The solution was dried and the solvent evaporated to give a brown oil. This oil was chromatographed on alumina and elution with petrol gave a clear glass (0.019 g.) which could not be crystallised. The glass had the following spectra: $\lambda_{max.}$ 262 mμ (ϵ 4,900), p.m.r. absorption at τ 4.25 (complex multiplet, 4 protons), 9.9.31.

Action of Cerium (IV) on Estrone Methyl Ether

Estrone methyl ether (0.1 g.) in acetic acid (10 ml.) was stirred at room temperature while ceric ammonium nitrate (0.5 g.) in acetic acid (90%, 10 ml.) was added over 30 min. Stirring was continued for 12 hr. during which time no difference in the pale yellow colour of the solution was noticed. The solution was poured into water, the steroid extracted into dichloromethane, washed with sodium carbonate solution and water, dried and the solvent evaporated. The solid product was recrystallised from methanol to give unchanged estrone methyl ether (0.08 g.) m.p. 168.5-170° (lit. 169°). Examination of the mother liquors from this crystallisation on t.l.c. showed that no other more polar material was present.

Cholesta-1,4-dien-3-one[194,195]

Cholest-4-en-3-one (12 g.) was dissolved in dry benzene (200 ml.). The last traces of water were removed by distilling off benzene (100 ml.), the first fraction being benzene-water azeotrope. Dichlorodicyanobenzoquinone (7.8 g.) was added and the solution was refluxed for 17 hr. After cooling, the precipitated quinol was filtered off, the solution reduced to 25 ml. and petrol (25 ml.) added. This solution was adsorbed on alumina, and eluted with petrol-benzene (1:1). The pale yellow glass was crystallised from acetone to give cholesta-1,4-dien-3-one (11.6 g.; 97%) m.p. 112-113.5° (lit.112°), $[\alpha]_D$ +29° (c 0.15) (lit. +28°); λ max. 242 mμ (ϵ 14,900).

1-Methoxy-4-methyl-19-nor-cholesta-1,3,5(10)-triene[194,218]

Cholest-1,4-dien-3-one (9.02 g.) in acetic anhydride (100 ml.) was treated with concentrated sulphuric acid (1.5 ml.) in acetic anhydride (20 ml.). The solution was allowed to stand at room temperature for 3 hr., during which time it turned from pale yellow to dark green. More concentrated sulphuric acid (0.75 ml.) in acetic anhydride (10 ml.) was added, and after standing for a further hour, the solution was poured slowly into an ice cold solution of potassium hydroxide (90 g.) in water (200 ml.). After the resulting solution had cooled, the steroid was extracted into ether, the ether solution washed with sodium carbonate solution, then water and dried. Evaporation of the solvent gave a clear glass. This crude product was refluxed for 1 hr. in methanol (100 ml.) with potassium hydroxide solution (45%; 20 ml.). This solution was poured into water and the steroid extracted into dichloromethane. The organic solution was washed with water, dried and the solvent removed. Crystallisation of the product from petrol (b.p. 40-60°) gave 4-methyl-19-nor-cholesta-1,3,5(10)-trien-1-ol (7.74 g.; 86%) m.p. 147-148° (lit. 145-146°). $\nu_{max.}$ 3650, 1320, 1285, 1275, 1170, 815 cm.$^{-1}$ P.m.r. absorptions at τ 3.34 (quartet, J = 12 c.p.s.; 2 protons), 5.44 (hydroxyl), 7.86 (aryl methyl group), 9.09, 9.18 and 9.27.

This phenol (6.5 g.) was stirred at 90° with methyl toluene-p-sulphonate (6.5 g.) in aqueous potassium hydroxide solution (10%, 150 ml.) for 3 hr. After 1 hr., a further 50 ml. of this solution was added. The suspension was poured into water, and

the steroid extracted into ether. The ethereal solution was washed with water, dried and the solvent evaporated. Crystallisation of the product from methanol gave 1-methoxy-4-methyl-19-nor-cholesta-1,3,5(10)-triene (6.1 g.; 94%) m.p. 103-105°, (lit. 104.5-105°), $\nu_{max.}$ 1260, 1245, 1085, 805 cm.$^{-1}$ P.m.r. abs. at τ 3.27 (quartet, J = 12 c.p.s.; 2 protons), 6.30 (methoxyl group), 7.88, 9.10, 9.19, 9.30.

Cerium Oxidation of 1-methoxy-4-methyl-19-nor-cholesta-1,3,5(10)-triene

The ether (0.5 g.) was dissolved in acetic acid (50 ml.) and a solution of ceric ammonium nitrate (2.7 g.) in acetic acid (10 ml.) and water (10 ml.) added slowly over 2 hr. The solution was stirred at room temperature for 16 hr., poured into water, and the steroid extracted into ether. The ethereal solution was washed with sodium carbonate solution, then water and dried. Evaporation of the solvent gave a pale yellow glass, which would not crystallise. $\nu_{max.}$ 3600-2400, 1740, 1700, 1280, 1090, 860. P.m.r. abs. at τ -0.03, 2.32 - 3.30, 6.17, 9.09, 9.18, 9.28.

1-Methoxy-19-nor-cholesta-1,3,5(10)-triene-4-carboxylic acid

The crude product from the previous reaction (0.46 g.) was dissolved in acetone (40 ml.) containing 5% water, and treated with potassium permanganate (0.1 g.). The resulting solution was stirred at room temperature for 5 hr., then poured into water. Sodium bisulphite was added and the steroid extracted into ether. The ethereal solution was washed with dilute acid, then water,

dried and the solvent evaporated. Crystallisation of the brown glass from acetone gave 1-methoxy-19-nor-cholesta-1,3,5(10)-triene-4-carboxylic acid (0.28 g.; 61%) m.p. 143-147°; $\nu_{max.}$ 3400 (broad band), 1770, 1700, 1260, 830 cm.$^{-1}$ P.m.r. absorptions at τ 2.43 - 3.30 (complex multiplet; 2 protons), 6.18, 9.10, 9.20, 9.31.

Attempted Decarboxylation of 1-methoxy-19-nor-cholesta-1,3,5(10)-triene-4-carboxylic acid

a. The acid (0.1 g.) was refluxed in quinoline (20 ml.) for 2 hr., the solution was cooled and poured into dilute hydrochloric acid. The steroid was extracted into ether, the solution washed with dilute acid then water and dried. Evaporation of the solvent gave a brown oil (0.02 g.; 20% recovery). The infra red spectrum of this material was very similar to that of the starting material, although the intensities of the peaks at 1770 and 1700 cm.$^{-1}$ had diminished considerably and the hydroxyl absorption (3400 cm.$^{-1}$) had almost disappeared. Chromatography of this material on deactivated alumina, afforded no fractions containing significant quantities of material.

b. The acid (0.1 g.) and copper chromite[237] were refluxed in quinoline (20 ml.) for 1 hr. The solution was filtered and the filtrate poured into dilute hydrochloric acid. The steroid was extracted into ether, the ether solution was washed with dilute acid then water, dried and the solvent evaporated, to give a brown oil (0.08 g.). It was apparent from an infra red spectrum, that this material was ca.50% acid. It was chromatographed on

deactivated alumina. Elution with petrol gave a clear glass (0.02 g.), $\nu_{max.}$ 1280, 820 cm.$^{-1}$; p.m.r. abs. at τ 6.18, 9.09, 9.18, 9.34. Elution with benzene - ether (95:5) gave unchanged starting material (0.06 g.).

4-Formyl-1-methoxy-19-nor-cholesta-1,3,5(10)-triene

1-Methoxy-4-methyl-19-nor-cholesta-1,3,5(10)-triene (1.0 g.) was oxidised with cerium (IV) as above, to give a pale yellow solid (0.93 g.). This material had identical spectra to the previous oxidation product and was filtered through deactivated alumina in benzene to give a white solid (0.11 g.), $\nu_{max.}$ 2,700, 1695, 1630, 1230, 1080, 805 cm.$^{-1}$; p.m.r. abs. at τ -0.03, 2.32, 2.48, 3.19, 3.34, 6.17, 9.09, 9.18, 9.28.

1-Methoxy-19-nor-cholesta-1,3,5(10)-triene

4-Formyl-1-methoxy-19-nor-cholesta-1,3,5(10)-triene (0.05 g.) in benzene (10 ml.) was treated with tris-(triphenylphosphine)-rhodium chloride (0.15 g.) and the solution refluxed for 1 hr. The solution was cooled and filtered through alumina, elution with benzene being continued. Evaporation of the solvent gave a clear glass (0.012 g.) which could not be crystallised; $\nu_{max.}$ 1630, 1260, 830, 740 cm.$^{-1}$; p.m.r. abs. at τ 6.20, 9.09, 9.18, 9.27.

17β-Acetoxy-androsta-1,4,6-trien-3-one[234]

Testosterone acetate (10 g.) was dissolved in dry ether (320 ml.), cooled in ice and hydrogen bromide in acetic acid (45%; 0.1 ml.) added. A solution of bromine (9.5 g.) in acetic acid (100 ml.) was added over 10 min. with vigorous stirring. The ether,

and some acid, were evaporated under reduced pressure and the precipitated product filtered off, washed with ethanol and dried. This crude bromo-ketone (11.0 g.) was added to lithium carbonate (20 g.) in refluxing dimethylformamide (150 ml.) and the mixture refluxed for 1 hr. After cooling, the solid material was filtered off, and the filtrate poured into water. The steroid was extracted into ether, the solution washed with dilute acid then water, dried and the solvent evaporated. Crystallisation of the oily product from acetone - petrol gave 17β-acetoxy-androsta-1,4,6-trien-3-one (4.6 g.; 46%), m.p. 153-155° (lit. 151-153°); ν_{max} 1720, 1650, 1240, 1040, 900 cm.$^{-1}$; p.m.r. abs. at τ7.95, 8.80, 9.09.

Androst-5-en-3β,17β-diol

3β-Acetoxy-androst-5-en-17-one (12 g.) in dry ether (250 ml.) and tetrahydrofuran (250 ml.) was added to lithium aluminium hydride (7 g.) in dry ether (50 ml.) and the solution refluxed for 1.5 hr. The excess reagent was decomposed with ethyl acetate, and dilute acid was added to decompose the complexes formed. The organic solvents were evaporated and the steroid extracted with dichloromethane. This solution was washed with dilute acid and water, dried and the solvent evaporated. Crystallisation of the product from methanol - acetone gave androst-5-en-3β,17β-diol (10.7 g.; 91%) m.p. 182-184° (lit.184°).

17β-Hydroxy-androsta-1,4,6-trien-3-one[234,235]

Androst-5-en-3β,17β-diol (7.3 g.) was dissolved in dioxan (300 ml.) and dichlorodicyanobenzoquinone (16.8 g.) added.

The solution was refluxed for 20 hr., cooled, the precipitated quinol filtered off, and the solvent evaporated. The dark material was filtered through deactivated alumina in benzene - ether (50:50), and the material crystallised from petrol - acetone to give 17β-hydroxy-androst-1,4,6-trien-3-one (5.1 g.; 69%) m.p. 155-157° (lit. 156-157.5°); $\lambda_{max.}$ 222 mμ (ε 14,100), 257 mμ (ε 13,500), 297 mμ (ε 15,000).

Androsta-1,4,6-trien-3,17-dione[234,235]

3β-Hydroxy-androst-5-en-17-one (6.5 g.) and dichlorodicyano-benzoquinone (14.9 g.) were dissolved in dioxan (220 ml.) and the solution refluxed for 21 hr. After cooling, the quinol was filtered off and the filtrate evaporated to dryness. The dark red material was chromatographed on alumina; elution with benzene - ether (50:50) gave a yellow solid which was crystallised from petrol-acetone to give androsta-1,4,6-trien-3,17-dione (4.16 g.; 63%) m.p. 164-166° (lit. 165-166°) $\nu_{max.}$ 1735, 1650 cm.$^{-1}$ λ_{max} 220 mμ (ε 14,200), 257 mμ (ε 13,000), 293 mμ (ε 17,100).

3-Methoxy-1-methyl-estra-1,3,5(10),6-tetraen-17-one[218]

Androsta-1,4,6-trien-3,17-dione (3.1 g.) in acetic anhydride (100 ml.) was treated with sulphuric acid (0.1 ml.) in acetic anhydride (10 ml.), and set aside for 3 hr. at room temperature. The solution was poured into potassium hydroxide solution (40%; 100 ml.) and ice. The steroid was extracted into dichloro-methane, the solution washed with water, dried and the solvent evaporated. The product appeared, from an i.r. spectrum, to

contain ca.75% unreacted trienone. The crude product was treated with acetic anhydride and sulphuric acid as above, but allowed to stand at room temperature for 10 hr. Isolation of the product in the same manner, afforded a brown glass, which still had more than 60% starting material present. The crude product (2.7 g.) was dissolved in methanol (100 ml.) and potassium hydroxide (4.5 g.) added. The solution was refluxed for 1 hr., poured into water and the methanol distilled off. The steroid was extracted into dichloromethane, the solution washed with water, dried and the solvent evaporated. This crude product in methanol (20 ml.) was stirred at 90° with potassium hydroxide solution (15%; 30 ml.) and methyl toluene-p-sulphonate (4 g.) for 4 hr. Water was added, the steroid extracted into dichloromethane and this solution washed with dilute acid, then water, dried and the solvent evaporated. The crude product was chromatographed on deactivated alumina. Elution with benzene gave 3-methoxy-1-methyl-estra-1,3,5(10),6-tetraen-17-one, crystallised from methanol (0.54 g.; 17%) m.p. 151.5-152°; $\nu_{max.}$ 1735, 1320, 1080, 890, 870, 700 cm.$^{-1}$; p.m.r. abs. at τ 3.48 (2 protons), 3.75 (quartet, J = 9 c.p.s.), 6.23, 7.47, 9.07. Elution with benzene - ether (98:2) gave starting material (1.22 g.).

1-Methyl-estrone methyl ether[215]

3-Methoxy-1-methyl-estra-1,3,5(10),6-tetraen-17-one (0.3 g.) was dissolved in ethanol (20 ml.) and hydrogenated at 1 atmosphere over 10% palladium on charcoal catalyst (0.05 g.), 1 mole of

hydrogen being taken up within 10 min. The catalyst was filtered off and the solvent removed. Crystallisation from methanol gave 1-methyl-estrone methyl ether (0.28 g.; 94%) m.p. 128-130° (lit. 130°), $[\alpha]_D$ +227° (c 0.13), (lit. +238°).

Cerium Oxidation of 1-Methyl-Estrone Methyl ether

1-Methyl-estrone methyl ether (0.21 g.) in acetic acid (10 ml.) was treated with ceric ammonium nitrate (1.58 g.) in 90% acetic acid (10 ml.) over 30 min., the solution being stirred at room temperature. Stirring was continued for a further hour, when a sample of the reaction mixture (4 ml.) was removed, poured into water and the steroid extracted into ether. The ethereal solution was washed with sodium carbonate solution, then water, dried and the solvent removed. I.r. and p.m.r. spectra showed this to be largely unchanged starting material, although the signal at τ7.85 (aromatic methyl group) had slightly diminished. After 4.5 hr. a second sample (4.0 ml.) was removed and the steroid isolated as above. Spectra showed some starting material still present (ca. 40%), but an aldehyde was also present in high yield (60%). P.m.r. abs. at τ -0.03, 6.18, 7.85, 9.08. A third sample was removed after 10 hr. and the steroid isolated. No starting material was present; the material appeared to be an aldehyde. $\nu_{max.}$ 2,700, 1700, 1630, 1245, 820 cm.$^{-1}$; p.m.r. abs. at τ -0.04 (1 proton), 6.18, 9.08. After a total of 22 hr. the remainder of the reaction was worked up as above; spectra of this material showed that it contained an aldehyde and acidic material in equal amounts. $\nu_{max.}$ 3400 (broad band), 2700, 1765, 1735, 1700, 1630, 1250, 815 cm.$^{-1}$; p.m.r. abs. at τ -0.03, 6.18, 9.10. The p.m.r. spectra of all these fractions showed complex multiplets between 2.3 and 3.4 τ .

3-Methoxy-1-methyl-estra-1,3,5(10),6-tetraen-17β-ol acetate

17β-Acetoxy-androsta-1,4,6-trien-3-one (5.7 g.) in acetic anhydride (150 ml.) was treated with sulphuric acid (1.5 ml.) in acetic anhydride (20 ml.) and set aside for 3 hr. More sulphuric acid (1.0 ml.) in acetic anhydride (10 ml.) was added and after a further hour, the solution was poured into potassium hydroxide solution (40%; 200 ml.) and ice. The steroid was extracted into dichloromethane, the solution washed with water, dried and the solvent evaporated. The crude product, in methanol (100 ml.) was refluxed for 1 hr. with potassium hydroxide solution (45%; 25 ml.), water was added and the steroid extracted into dichloromethane. This solution was well washed with water, dried and the solvent evaporated. The crude phenol (5.2 g.) was dissolved in methanol (30 ml.) and this added to methyl toluene-p-sulphonate (6 g.) in aqueous potassium hydroxide solution (15%, 150 ml.) and the suspension stirred at 90° for 3 hr. The steroid was extracted into dichloromethane, washed with dilute acid and water, dried and the solvent evaporated. The crude product was dissolved in pyridine (30 ml.) and acetic anhydride (30 ml.). After standing at room temperature for 26 hr., this solution was poured onto ice, the steroid extracted into ether, the solution washed with water, dried and the solvent evaporated. The product was crystallised from acetone - petrol to give 3-methoxy-1-methyl-estra-1,3,5(10),6-tetraen-17β-ol acetate (3.6 g.; 63%) m.p. 128-133°. $\nu_{max.}$ 1740, 1630, 1265, 1245, 1085, 830 cm.$^{-1}$; p.m.r. abs. at τ 3.47 (2 protons), 3.78 (quartet, J = 8 c.p.s., 2 protons) 6.28, 7.51, 7.98, 9.16.

3-Methoxy-1-methyl-estra-1,3,5(10)-trien-17β-ol Acetate

The product from the above reaction (3.0 g.) in ethanol (40 ml.) was hydrogenated at room temperature over 10% palladium on charcoal catalyst. The catalyst was filtered off and the solvent evaporated. Crystallisation from methanol gave 3-methoxy-1-methyl-estra-1,3,5(10)-trien-17β-ol acetate (2.8 g.; 95%) m.p. 159-161°; $\nu_{max.}$ 1735, 1245, 1140, 1050, 1030, 820 cm.$^{-1}$; p.m.r. abs. at τ 3.32 (2 protons), 6.34, 7.48, 7.96, 9.16.

1-Formyl-3-methoxy-estra-1,3,5(10)-trien-17β-ol Acetate

3-Methoxy-1-methyl-estra-1,3,5(10)-trien-17β-ol acetate (2.6 g.) in acetic acid (100 ml.) was treated over 1 hr. with ceric ammonium nitrate (20 g.) in acetic acid (10 ml.) and water (15 ml.), with vigorous stirring. The stirring was continued, at room temperature, for 10 hr., and the solution was poured into water and the steroid extracted into ether. The ether solution was washed with sodium carbonate solution and water, dried and the solvent evaporated at room temperature. This material could not be crystallised and, since heat appeared to decompose the aldehyde, prolonged attempts to crystallise the material were not made. The material had only one spot on thin layer chromatography. $\nu_{max.}$ 2700, 1740, 1700, 1635, 1240, 1135, 820 cm.$^{-1}$; p.m.r. abs. at τ -0.04 (1 proton), 3.16 (2 protons), 6.29, 7.96, 9.18.
Yield 1.8 g.; 72%.

3-Methoxy-estra-1,3,5(10)-trien-17β-ol Acetate

The crude aldehyde from the previous reaction (0.7 g.), dissolved in dry benzene (100 ml.), was treated with tris-

(triphenylphosphine)-rhodium chloride (2.1 g.) and the solution refluxed for 1 hr. The volume of benzene was reduced to 20 ml. and the solution absorbed on alumina. Elution with benzene gave a pale yellow oil, which was crystallised from ethanol to give 3-methoxy-estra-1,3,5(10)-trien-17β-ol acetate (0.47 g.; 68%) m.p. 101-103°, (lit. 101-102.5°);[236] $\nu_{max.}$ 1740, 1625, 1245 (broad peak), 1135, 1050, 1040, 880, 730 cm.$^{-1}$; p.m.r. abs. at τ 2.7-3.4 (complex multiplet, 3 protons), 6.23, 7.86, 9.18.

Estradiol methyl ether.

The ester from the above experiment (0.2 g.) was dissolved in ethanol (20 ml.) and potassium hydroxide (0.3 g.) added. The solution was refluxed for 2 hr., cooled and poured into water. The ethanol was distilled off, and the steroid extracted into chloroform. This solution was washed with water, dried and the solvent evaporated. Crystallisation of the product from benzene - petrol gave estradiol methyl ether (0.18 g.) m.p. 120-121° (lit. 121°)[236] $\nu_{max.}$ 3650, 1620, 1260, 1240, 1135, 1060, 1050, 880, 825, 785 cm.$^{-1}$; p.m.r. absorptions at τ 2.7-3.4 (complex multiplet, 3 protons), 6.23, 9.22.

B I B L I O G R A P H Y

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ABSTRACT

The action of toluene-p-sulphonyl chloride in pyridine on cholest-4-en-3β-ol and on 5α-cholest-1-en-3β-ol has been investigated; in neither case was a tosyl ester isolated. The former reaction produced 3α,5-cyclo-5α-cholest-6-ene, cholesta-3,5-diene and N-(cholest-4-en-3α-yl)-pyridinium tosylate. In the latter case, the products isolated were 1α,5-cyclo-5α-cholest-2-ene and N-(cholest-1-en-3α-yl)-pyridinium tosylate.

1α,5-Cyclo-5α-cholest-2-ene has been characterised by both its spectral properties and chemical reactions. The ultra violet and infra red spectra are consistent with the assigned alkyl-cyclopropane structure; indeed the proton magnetic resonance spectrum also supports the structure, but assignment of peaks was uncertain. The ambiguity was resolved by consideration of the p.m.r. spectrum of 17β-methoxy-1α,5-cyclo-5α-androst-2-ene, synthesised from 17β-methoxy-5α-androst-1-en-3β-ol by the same method. This reaction also produced 17β-methoxy-5α-androsta-1,3-diene in addition to the cyclosteroid.

The major reactions of 1α,5-cyclo-5α-cholest-2-ene are summarised below. Catalytic reduction using palladium on charcoal catalyst afforded 5α-cholestane, but use of the soluble catalyst, tris(triphenylphosphine)rhodium chloride, gave 1α,5-cyclo-5α-cholestane. Oxidative fission of the olefinic bond followed by reductive cleavage of the cyclopropane ring afforded the known diacid, 2,3-seco-cholestan-2,3-dioic. Reaction of peracid with the hydrocarbon gave a mono-oxide, cleaved with

lithium aluminium hydride to give 1α,5-cyclo-5α-cholestan-2α-ol. The cyclopropyl-ketone obtained from the oxidation of this alcohol was reduced catalytically to give 5α-cholestan-2-one. 1α,5-Cyclo-5α-cholestane was obtained from this ketone by Wolff-Kishner reduction.

1α,5-Cyclo-5α-cholestane has also been synthesised by a separate reaction sequence, the cyclopropane ring being introduced by dehydration of a suitably substituted 5β-hydroxy compound, and proved to be identical with the material obtained in the above reactions.

A possible mechanism for the formation 1α,5-cyclo-5α-cholest-2-ene from 5α-cholest-1-en-3β-ol has been proposed, involving transannular migration of the 5α-hydrogen atom. Results from tosylation experiments on 5α-deutero and 5α-fluoro-cholest-1-en-3β-ol have substantiated this mechanism.

Attempted tosylation of 19-nor-androst-4-en-3β-ol and 19-nor-5α-androst-1-en-3β-ol gave estra-3,5(10)-diene and estra-1,3-diene respectively; no cyclosteroids were isolated.

A synthesis of estradiol from 17β-acetoxy-androst-1,4,6-trien-3-one has been carried out. Aromatisation of this trienone with sulphuric acid under anhydrous conditions gave 17β-acetoxy-1-methyl-estra-1,3,5(10)-trien-3-one. Removal of the 1-methyl group was effected in two stages. Oxidation with cerium (IV) gave the 1-formyl derivative, which was decarbonylated with tris-(triphenylphosphine) rhodium chloride.