Title of Thesis

The Synthesis of 10α-Cholesterol

or

The Synthesis of 10-alpha-Cholesterol

by

Nelson Ernest Lawson

THE SYNTHESIS OF 10α-CHOLESTEROL

by

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ABSTRACT

The Synthesis of 10α-Cholesterol

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The first epimer of natural cholesterol, 10α-cholesterol, was produced by an eleven-step synthesis. Ring A of cholesterol was removed by a sequence of five reactions to give the tricyclic BCD intermediate, des-A-cholest-9-en-5-one. Conformational and steroelectronic principles were applied to this compound to predict the position and direction of the subsequent methyl vinyl ketone alkylation. By this means a 37% yield of the product, 10α-cholest-9(11)-en-5α-ol-3-one could be obtained. This was easily separable from the 10β-by-product which eliminated water under the conditions of the reaction to give cholesta-4,9(11)-dien-3-one. The 9,11- double bond of the 10α-intermediate was hydrogenated, and the resulting saturated ketol converted to a mixture of 10α-cholest-4-en-3-one and 10α-cholest-5-en-3-one. From this mixture the enol acetate could be formed, and reduction of this by sodium borohydride in aqueous methanol resulted in 10α-cholesterol. The overall yield was 6.4%.

In an appendix to this thesis the Bamford-Stevens reaction of equilenin methyl ether tosylhydrazone is described.

ACKNOWLEDGEMENTS

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To Sharon

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F O R W O R D

In this thesis, ordinary Arabic numerals will refer to formulae, and the numerals in superscript will indicate the appropriate references. The detailed synthetic scheme is incorporated in a one-page fold-out section at the end of this volume. This scheme includes formulae 1-21 and should be consulted when reading this thesis.

The Synthesis of 10α-Cholesterol

Introduction

Since the time when man first appeared on the face of the earth, his life has been shortened and made miserable by disease. In highly developed societies today, the chief cause of death is atherosclerosis[1], a disease in which the large and medium arteries gradually become narrowed by an accumulation of fatty, fibrous deposits, leading to lesions, thrombii, and high blood pressure with its attendant strain on the heart. Since 1847 it has been known[2,8,9], that cholesterol, its esters, and transformation products form a large part of these deposits (called plaques), and that high levels of cholesterol in the body usually lead to atherosclerosis[3,10].

Although all nucleated mammalian cells can synthesize cholesterol, the chief site of endogenous production is the liver[14]. The sterol (both free and esterified) is transported throughout the body in the blood plasma in the form of lipoprotein complexes[14,15], and may be filtered out of the blood through the intima (lining) of the artery, thus accumulating into atherosclerotic plaques[16]. Although a fat-free, cholesterol-free diet will reduce the plasma cholesterol level and hence the possibility of atherogenesis[4,9], lowering only the exogenous cholesterol intake will result in increased body production, possibly from acetic acid from the degradation of fat[5,9,11].

A high plasma cholesterol level is not the sole cause of atherosclerosis, however, since it has been found that this level was not

appreciably higher for atherosclerotic patients than for normal persons[17]. Other factors such as hormones[12], and enzymes[18] have a profound effect upon the development of this disease.

Only recently has it been recognized that atherosclerosis is not an inevitable product of aging[6] and that the disease can actually be reversed[7,13]. Current research on chemotherapeutic agents, based on the above premises, has been most often directed at interfering with endogenous syntnesis of the sterol[19,20]. Few encouraging results have been obtained, however, since inhibition of one step of the biosynthesis[21] usually results in accumulation of some atherosclerotic precursor[20]. As atherosclerosis is at least partly due to an alteration of cholesterol metabolism[2] it would be desirable to know more about the degradation of this sterol in the body.

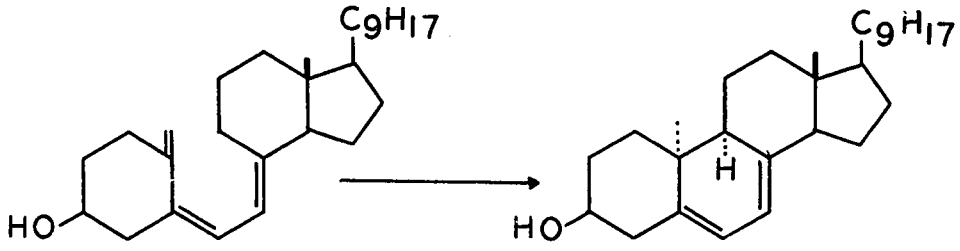
One general method of accomplishing this is to vary the structure of the parent compound, and then to investigate what the organism will do with it. For the special case of steroids, one can either change the positions and types of the functional groups attached to the perhydrocyclopentenophenanthrene skeleton, or one can change the shape of the skeleton itself. In some cases the physiological results upon varying the latter are remarkable; therefore, it was decided that the epimer at C-10 would be synthesized, as the first variation on the cholesterol skeleton.

Previous Synthesis of 10α-Steroids

Prior to the commencement of this work only two research

groups had concerned themselves with the synthesis of 10α-steroids. The discovery of the dramatic clinical effects of 9β, 10α-("retro") steroids[22,23] put greater emphasis upon the synthesis of the 10α-epimers, and newly developed synthetic methods enabled several workers to fashion elegant schemes for their production.

Pyrocalciferol (10α-ergosterol) (23)



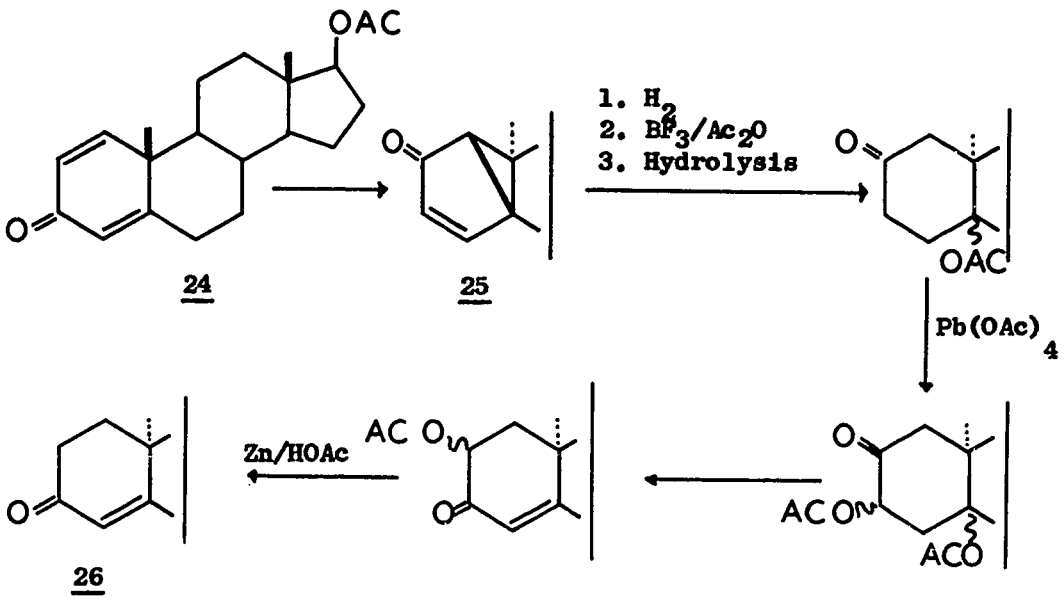
22 23

This isomer of ergosterol was known as far back as 1932, when it was produced by the pyrolysis of vitamin D_2[24] (22). Recent work by Jones et al[25] established that the stereochemistry originally assigned 9β, 10α, was actually 9α, 10α.

10α-Testosterone (26)

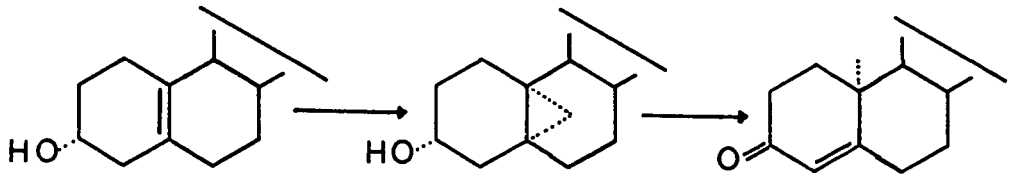
This compound was first synthesized[26] by photochemical transformation of the conjugated dienone 24 into the 2-oxo-10α-1,5-cyclosteroid 25. This was converted to 10α-testosterone by rupture of the cyclopropane ring, and subsequent transferral of the oxygen function to

C-3 by a novel reaction sequence involving lead tetra-acetate.



24 25 26

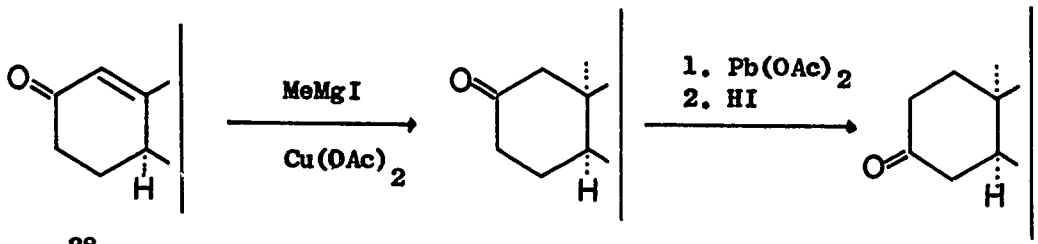
In a simpler synthesis, Ginsig and Cross[27] treated 27 (which is easily available from Birch reduction of estrone) with the Simmons-Smith reagent to form the 5α, 10α-methylene bridge. Oxidation and bridge rupture by base led to 10α-testosterone (26).



27 26

Dihydro-10α-testosterone (29) has also been produced by 1,4-Grignard addition to the conjugated ketone 28 and subsequent ketone

isomerization to C-3 via the lead tetra-acetate method[28].



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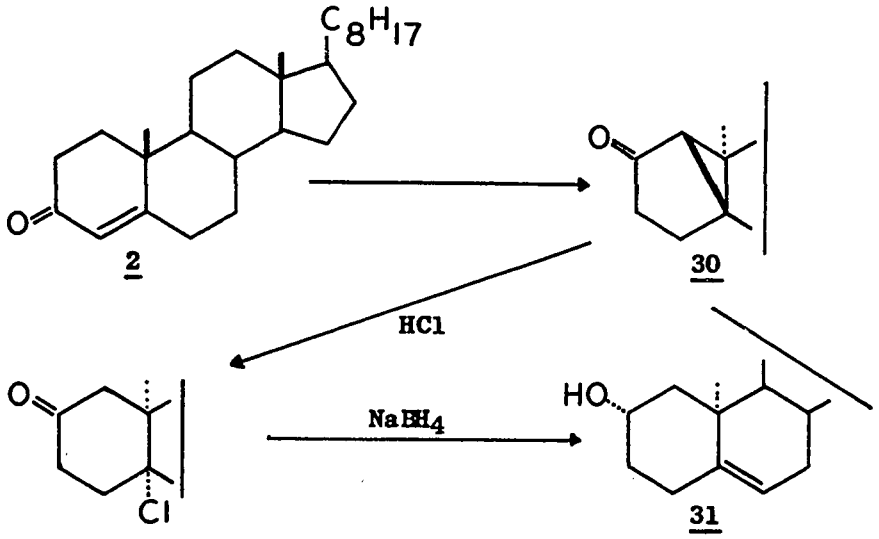
10α-Cholest-5-en-2-ol (31)

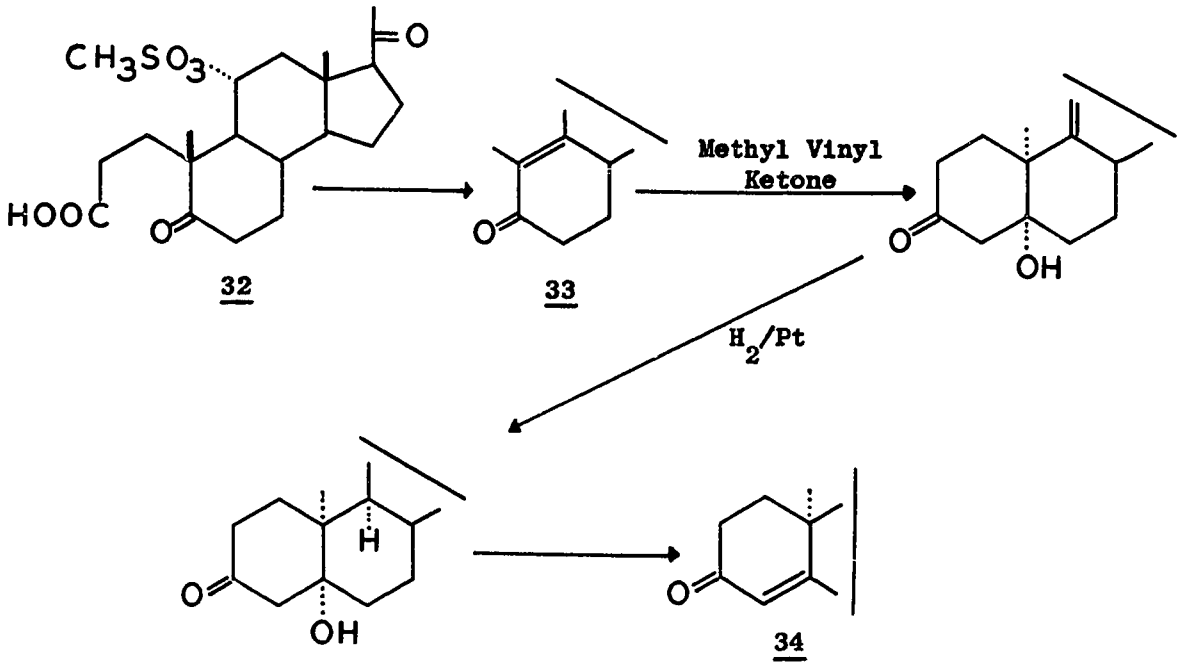
This positional isomer of 10α-cholesterol was also produced by photolysis[29], this time of cholestenone (2). Acid cleavage of the cyclosteroid 30 and reduction gave the product (31). Potentially this represents the easiest route to 10α-cholesterol, since the only further requirement is a transposition of the oxygen function to C-3.



2 30 31

10α-Progesterone (34)

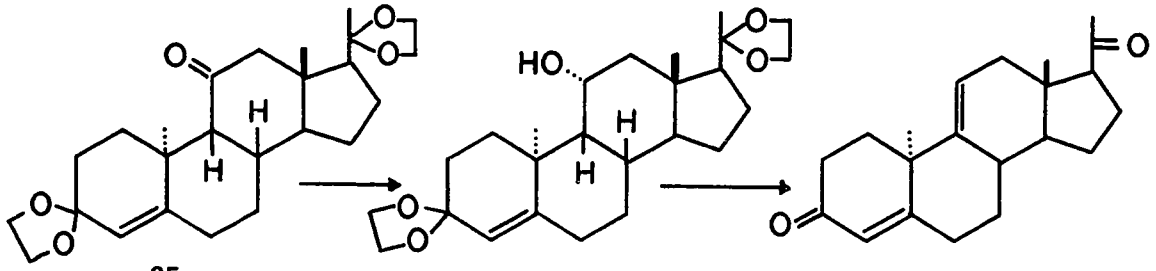
Coincidently with the work described in this thesis, this compound was synthesized[30] by a route similar to the one chosen by us to produce 10α-cholesterol. However, details are lacking in the preliminary note in which this work was reported.



Pyrolysis of 32 in sodium phenyl acetate combined a retro-Michael reaction, an elimination, and a shift of the resultant double bond into conjugation with the C-5 ketone group to give the tricyclic intermediate 33 in one step. This was treated with methyl vinyl ketone, and the product hydrogenated. Elimination of water then yielded the product (34).

10α-Pregna-4,9(11)-diene-3,20-dione (36)

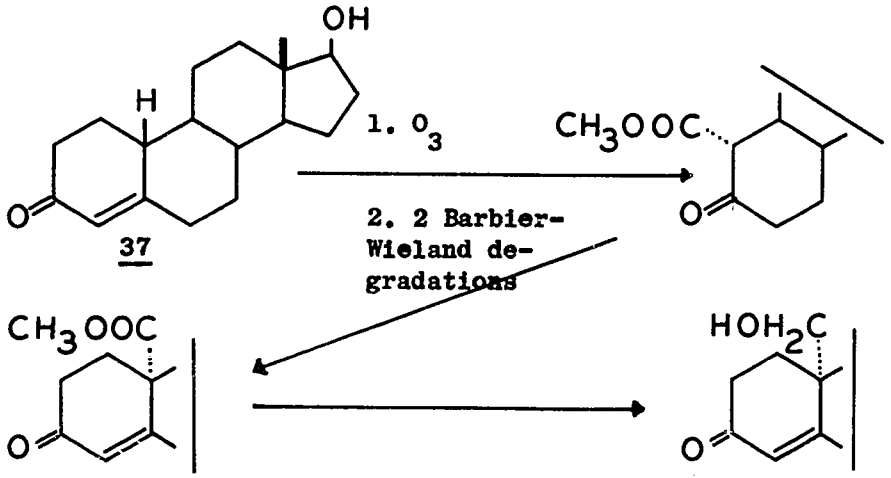
This scheme used a 9β, 10α-("retro")-steroid (35) as starting material, and thus suffers from the lack of a readily available precursor[31].



35 36

19-Hydroxyl-10α-testosterone (38)

Although this 19-substituted steroid falls somewhat outside the scope of this thesis, it is included here since it was the first 10α-steroid epimer to be intentionally synthesized[32]. The scheme started from 19-nortestosterone (37), and involved partial degradation of the A-ring and its re-formation via methyl vinyl ketone alkylation.



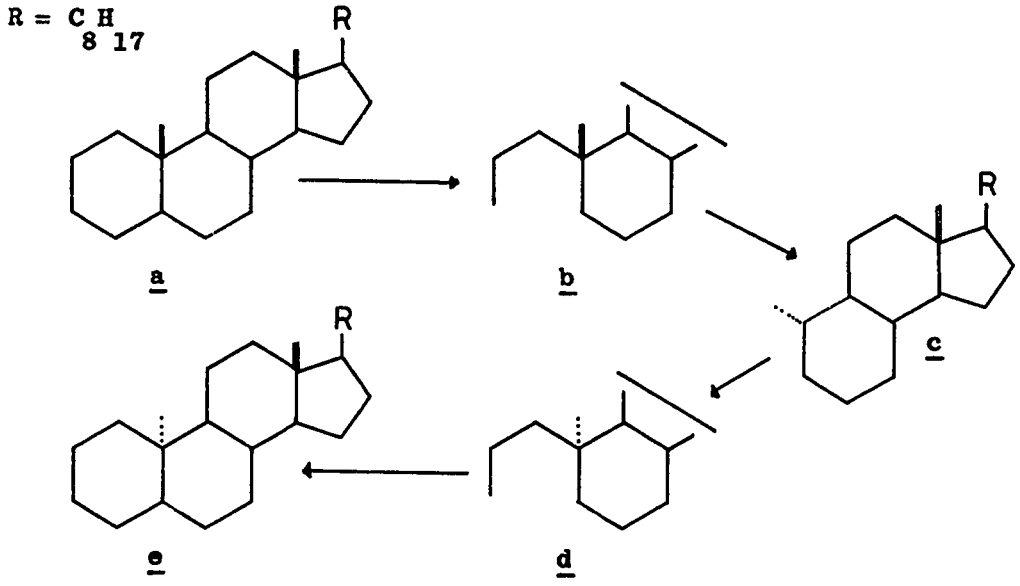
37 38

General Description of the Reaction Scheme

From the point of view of convenience and cost, natural cholesterol ((a) in figure 1) was the obvious choice for the starting material. The general reaction scheme shown in figure 1 involved removal of ring A by first splitting it to give b, and then removing the side chain thus formed to give the tricyclic compound c. At this point a side chain was to be re-attached to c but in the opposite direction so as to leave the C-10 methyl group in the α-position (pointing downwards), and the side chain then cyclized to give the 10α-steroid skeleton e. Although some experimentation was required to find suitable reagents for this scheme, it was followed through to the conclusion.

FIGURE 1

General Reaction Scheme for the Synthesis of 10α-Cholesterol



(Functional groups have been omitted for simplicity).

Degradation of Ring A

The first objective was to build up a supply of the tricyclic intermediate, des-A-cholestan-5-one (4), commonly called Inhoffen's ketone. This compound has been produced in different ways by Inhoffen[33], Robinson[34], Julia[35], and Hartshorn and Jones[36], but it was the latter method which was finally used.

Cholesterol (1) was converted to cholest-4-en-3-one (2) in 85% yield by means of an Oppenauer oxidation[37], during which the 5,6-double bond shifted into the conjugated 4,5-position. Ring A was then cleaved by permanganate-periodate reagent as described by Edward et. al.[38] to give Windaus keto-acid (3) in 74% yield.

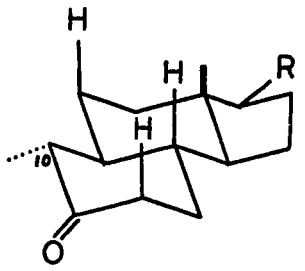
The remainder of ring A was next removed in a retro-Michael reaction by pyrolyzing the sodium salt of keto-acid 3 in a mixture of sodium phenylacetate and asbestos fibres under a pressure of 0.05 mm. of mercury. The oil which distilled over was purified to give white crystals of 4, m.p. 61°-62°, in 69% yield. Although Hartshorn and Jones also obtained Inhoffen's ketone by this reaction[36], they gave few experimental details, and it proved impossible to reach their quoted yield of 79%.

Molecular models of Inhoffen's ketone clearly indicate that the C-10 methyl is more stable in an equatorial α-configuration (see figure2), and should therefore epimerize to this configuration under the strongly basic conditions of the pyrolysis. That this is so, was demonstrated by Hartshorn and Jones[36].

FIGURE 2

Molecular Model of Inhoffen's Ketone (4)

($R = C_8H_{17}$)



Conformational Analysis of Polycyclic Systems

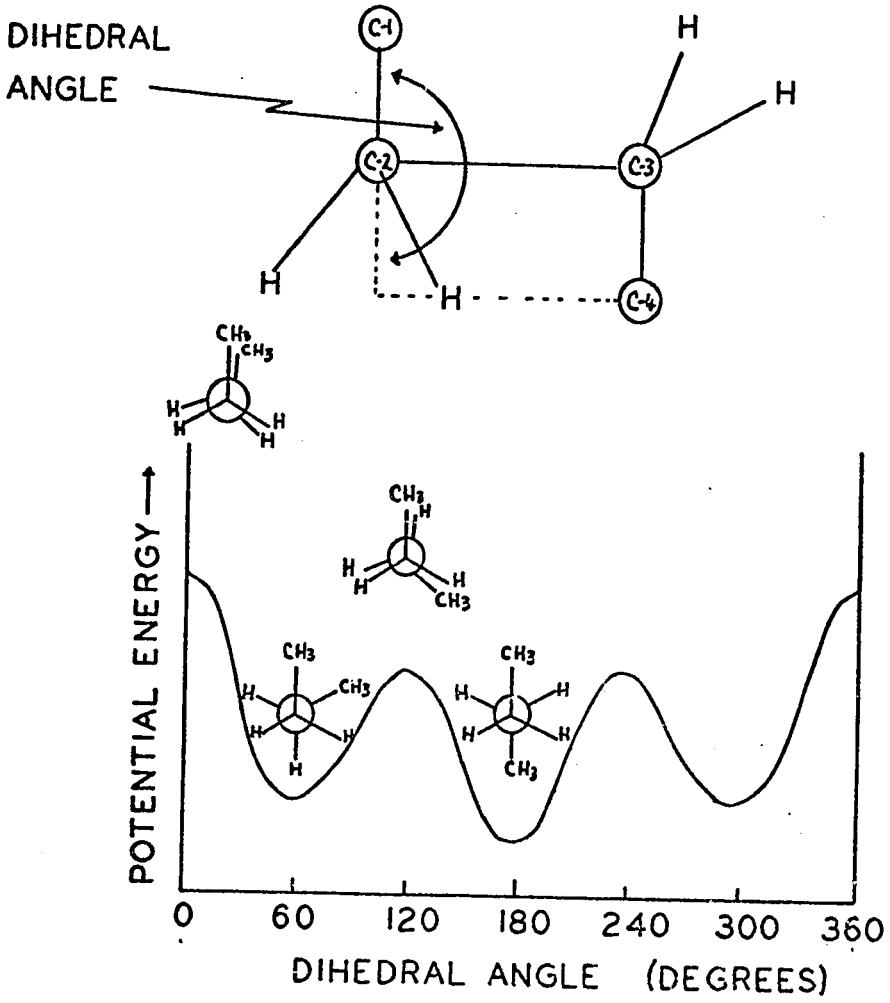
It would be outside the scope of this thesis to attempt a detailed coverage of the conformational analysis of polycyclic ring systems; yet it is necessary to discuss some of the basic principles if one is to understand their application to the steroid intermediates discussed in this text. Several papers have appeared on this topic[39-41] especially by Bucourt[42-44] to which the reader is referred for a more detailed exposition of the ideas involved.

It is well known that a chain of four carbon atoms can exist in various conformations which can be described by the dihedral angle. (This is the angle which the plane composed of C-1, C-2, and C-3 makes with the plane composed of C-2, C-3, and C-4). The potential energy or strain of such a system is a function of this dihedral angle (see figure 3).

Dihedral angles of 180° ("anti"-conformation) and 60° ("gauche"-conformation) represent energy minima.

FIGURE 3

Dihedral Angle and Strain of the Various Conformations of Butane.[45]

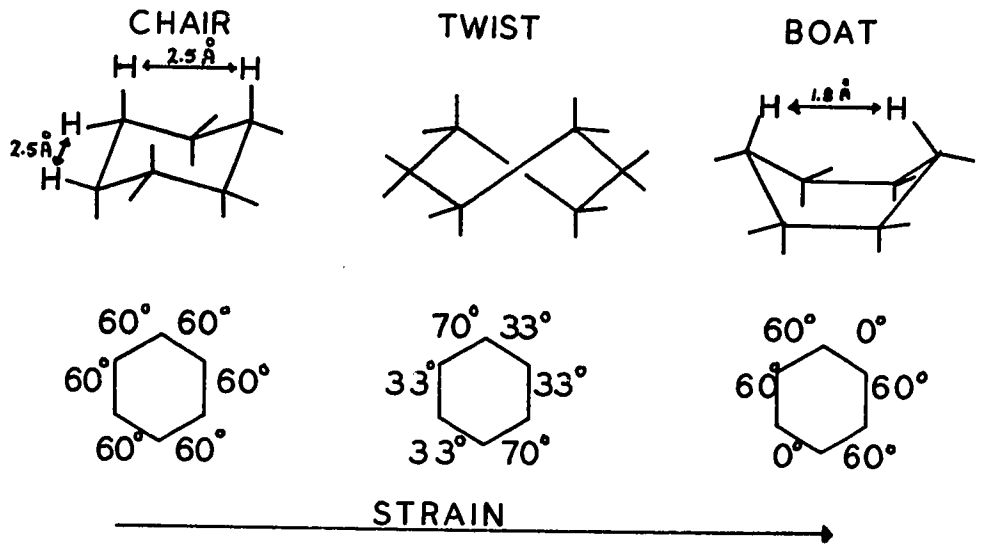


The cyclohexane ring can be thought of as a cyclic combination of these butane units, each contributing its own conformational problem.

The most stable conformation of the cyclohexane ring is the chair form. This is because all dihedral angles are 60° (onergy minimum) and non-bonded interactions are minimized. It can be seen in figure 4 that the other conformations, the twist and the boat, are handicapped in this regard.

FIGURE 4

Conformations of Cyclohexane

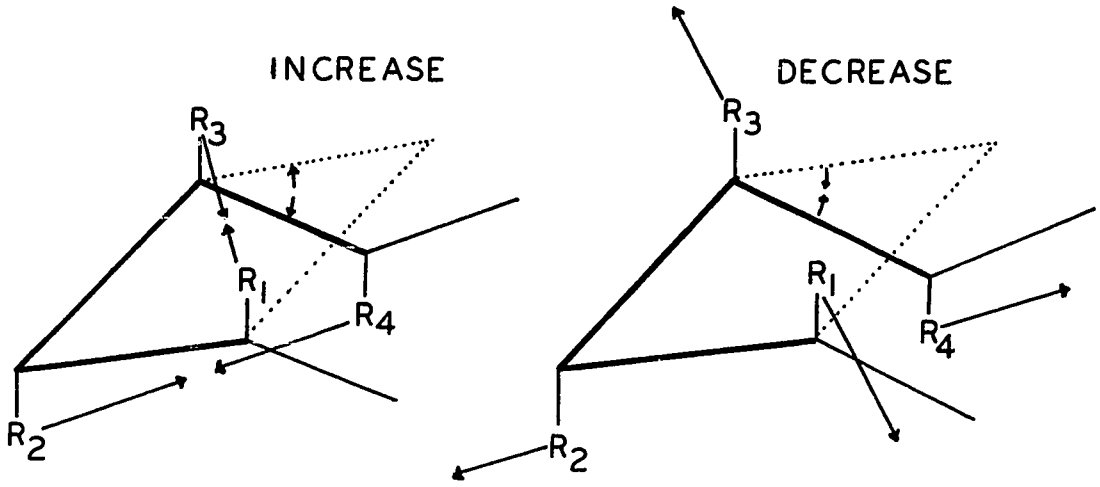


In the chair form the most significant non-bonded interactions are the 1,3-diaxial and the 1,2-diequatorial interactions. Slight changes in the distance between 1,3-diaxial hydrogens have little effect on the stability of the system, but this is not so for larger groups such as methyl. For example, the strain caused by 1,3-diaxial methyl-methyl interaction is estimated to be 5.5 k. cal./mole greater than if the methyls were diequatorial[40]. Naturally the cyclohexane ring will tend to minimize repulsions such as these, and if it is part of a rigid system, it will do so by moving the interacting groups somewhat apart, thus distorting the dihedral angle from its normal 60°. This leads to changes in the other dihedral angles of the ring. Conversely, changes in the dihedral angles which cause increased 1,3-repulsions

will not be favoured, whereas those which relieve strain will be more likely to occur. It has been found[42-44] that <u>increasing the dihedral angle in the cyclohexane ring from its normal value of 60° leads to increased 1,3-diaxial interactions, whereas the opposite is the case for a decrease in the dihedral angle</u> (see figure 5)+.

FIGURE 5

Effects of Changing the Dihedral Angle in a Cyclohexane Ring



R_1 approaches R_3 — R_1 moves away from R_3

R_2 approaches R_4 — R_2 moves away from R_4

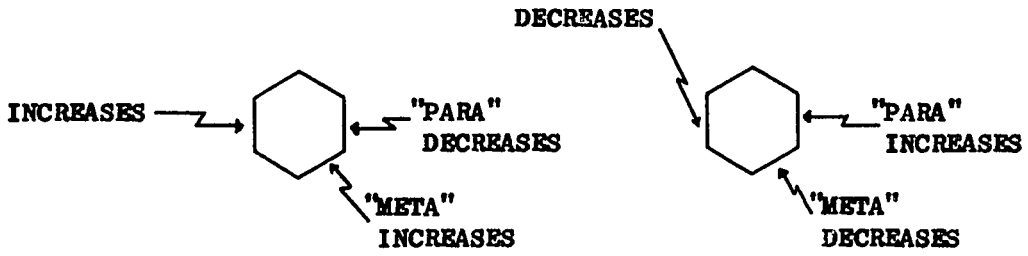
The effect is greatest if a bulky substituent is located on one of the carbon atoms which form the "vertex" of the dihedral angle (ie. substituents R_2 and R_3 in figure 5).

+ This can be seen most easily from molecular models.

From a study of molecular models and from theoretical considerations[42-44], it can be shown that changes in one dihedral angle in a cyclohexane ring affect the other dihedral angles in a definite way. Specifically, increasing a dihedral angle causes the dihedral angle directly across the ring ("para") to decrease, and the dihedral angle indirectly across the ring ("meta") to increase. Decreasing the dihedral angle caused the opposite effects (see figure 6).

FIGURE 6

Effects of Changing a Dihedral Angle



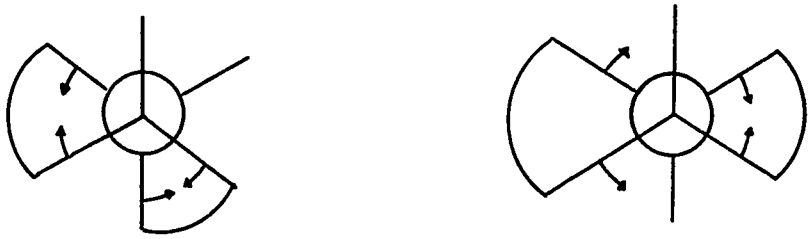
It has been estimated that introducing a double bond into the cyclohexane ring (which is, in effect, a reduction of the dihedral angle to nearly 0°), causes changes of $+10^{\circ}$ in the "para" dihedral angle and -10° in the "meta" dihedral angle [42-44].

If more than one ring is fused together (as in a steroid), distortions of the dihedral angles in one ring will cause distortions in the other rings; this is the phenomenon of conformational transmission[41]. Examining the Newman projection at the ring junction of

two cis-fused rings clearly shows that an increase of the dihedral angle at the ring junction in one ring causes a similar increase in the dihedral angle at the junction in the other ring, and a decrease in one causes a decrease in the other (see figure 7).

FIGURE 7

The Effects of Dihedral Angle Changes at Ring Junctions



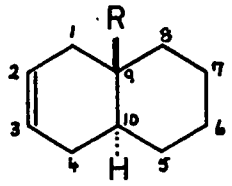
CIS-FUSED RINGS TRANS-FUSED RINGS

For the case of two trans-fused rings, a change in the dihedral angle at the ring junction causes an equal but opposite change in the corresponding dihedral angle of the second ring (see figure 7).

If the underlined postulates are kept in mind, then conformational analysis of the relative stabilities of polycyclic systems can be made with a remarkable degree of success. A few applications relative to the work in this thesis will now be given.

Δ^2- and Δ^3-trans-Octalin

Δ^2-trans-Octalin (39; R = H) and Δ^3-trans-octalin (40; R = H) with no substituents at C-9 are almost equally stable, but a C-9 substituent such as R = methyl changes this markedly.



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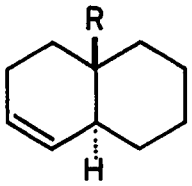
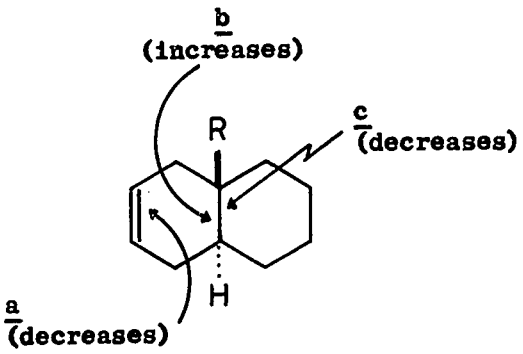


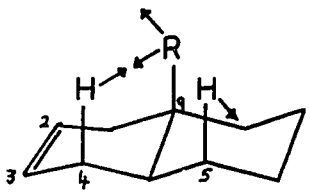
40

Introduction of a 2,3-double bond involves the decreasing of the dihedral angle between carbon atoms 1, 2, 3, and 4 (angle a, figure 8) from the normal 60°, and this causes "para"-dihedral angle b to increase. Therefore, the axial substituents on dihedral angle b will approach each other; specifically there will be increased interaction between the

FIGURE 8

Dihedral Angle Changes in Δ^2-trans-Octalin



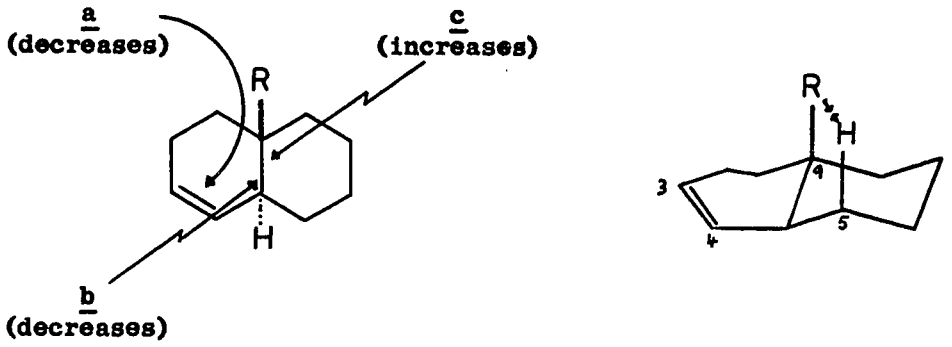


R-group and the 4β-hydrogen (see figure 8). However, the rings are trans-fused, so increasing dihedral angle b will cause dihedral angle c to decrease, thus decreasing the 1,3-diaxial interaction between R and the 5β-hydrogen. There will therefore be no gain or loss of non-bonded repulsions.

In Δ^3-trans-octalin, decreasing the dihedral angle a by introduction of a 3,4-double bond causes "meta"-angle b to decrease also (see figure 9).

FIGURE 9

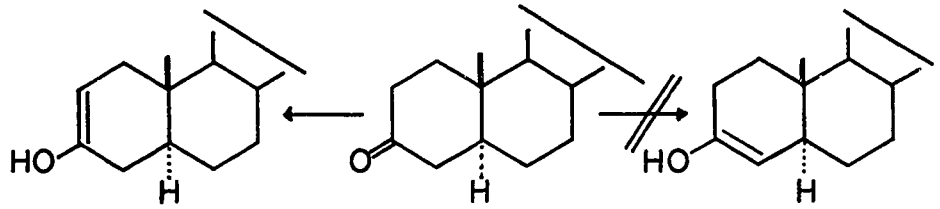
Dihedral Angle Changes in Δ^3-trans-Octalin



This introduces no favourable decrease in non-bonded interaction, since there is no 4β-hydrogen to move away from R.[+] However, dihedral

+ This removal of a 1,3-diaxial interaction by loss of the 4β-hydrogen due to the 3,4-double bond has its counterpart in Δ^2-trans-octalin in the loss of the 2β-hydrogen which also removes a non-bonded R-H interaction; therefore, there is no difference between the Δ^2- and Δ^3-octalins on this account.

angle c is increased since the rings are trans-fused; hence the R group and the 5β-hydrogen approach each other. Thus a net increase in strain results relative to Δ^2-trans-octalin. This is enough to make the Δ^2-isomer more stable than the Δ^3-, and explains why 3-oxo-A/B-trans-steroids (42) form Δ^2-enols (41) and not Δ^3-enols (43)[46].



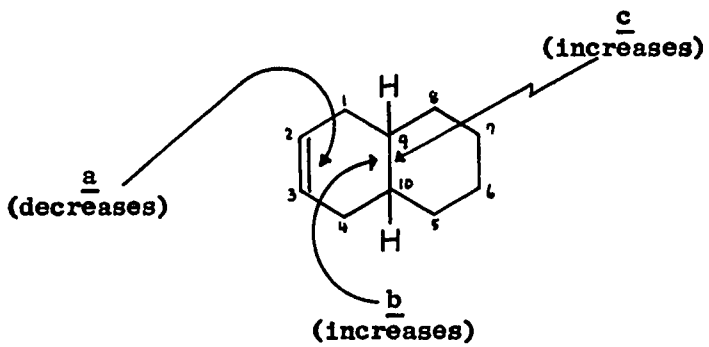
41 42 43

Δ^1-, Δ^2-, and Δ^3-cis-Octalins+

By an analysis similar to the above, it is easy to show that Δ^2-cis-octalin is the least stable of the three isomers. Dihedral angles b and c are both increased by the decrease in a, and hence 1,3-diaxial repulsions will be increased in both rings (see figure 10).

FIGURE 10

Dihedral Angle Changes for Δ^2-cis-Octalin

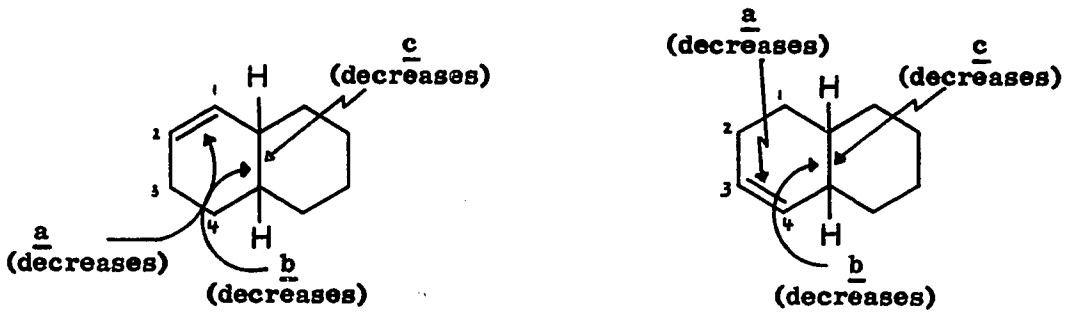


+ It is assumed that the octalins are fixed in the steroid conformation, so that the Δ^1- and Δ^3-isomers cannot become equivalent.

In Δ^1-cis-octalin the 1,2-double bond causes "meta"-dihedral angle b to decrease and hence c will also decrease (cis-fused rings) (see figure 11). This leads to a decrease of 1,3-diaxial interactions.

FIGURE 11

Dihedral Angle Changes for Δ^1- and Δ^3-cis-Octalin



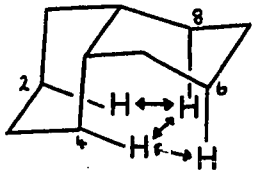
Δ^3-cis-Octalin has the same steric relief (see figure 11), and in addition, introduction of a 3,4-double bond eliminates two non-bonded interactions between the 4α- and 6α- and 8α-hydrogens (see figure 12). A 1,2-double bond relieves only the 2α-8α-hydrogen-hydrogen repulsion. The relative order of stabilities of the cis-octalins is therefore

$$\Delta^3 > \Delta^1 > \Delta^2$$

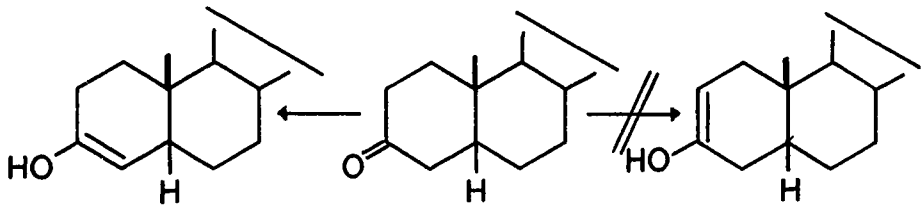
It should be noted from figure 12 that a carbon-carbon bond of one ring is always an axial group on the "vertex" of the junction dihedral angle of the other ring. For example, the 4,10-bond is axial to ring B. This makes relief of 1,3-diaxial strain important; adding a substituent at C-9 increases this effect, and thus a ready explanation

FIGURE 12

Hydrogen-Hydrogen Interactions in Two cis-Fused Rings



of the preferred enolization of 3-oxo-A/B-cis-steroids (45) in the 3,4-position (44) (and not the 2,3-position (46)) is evident[47].



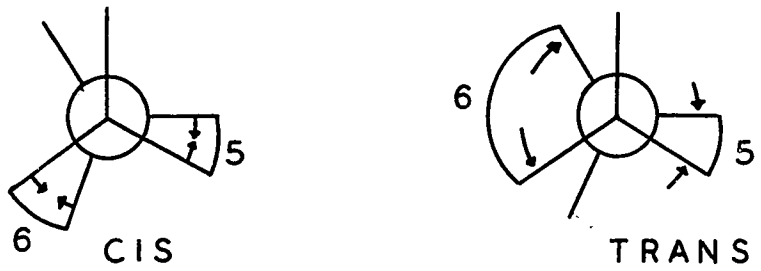
44 45 46

cis- and trans-Tetrahydroindanes

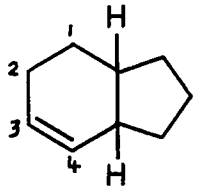
The dihedral angles of a cyclopentane ring are considerably less than in cyclohexane (in the order of 20^{o}-30^{o} [48]); consequently a five-membered ring cis-fused to a six-membered ring will cause the junction dihedral angle in the latter ring to decrease also, whereas if they are trans-fused, it will increase (see figure 13). It thus becomes obvious that cis-tetrahydroindane (47) will prefer to have the double bond in the 3,4-position since the dihedral angle at this point is "meta" to the ring junction and decreases when the junction dihedral

FIGURE 13

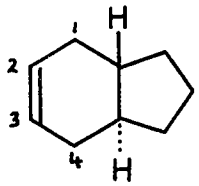
Dihedral Angle Changes in cis- and trans-Tetrahydroindanes



angle decreases (see figure 13).



47



48

trans-Tetrahydroindane (48) will prefer the 2,3-double bond since its "para"-position will complement the opening of the junction dihedral angle in the six-membered ring. This example should be kept in mind since it will become important later in this thesis.

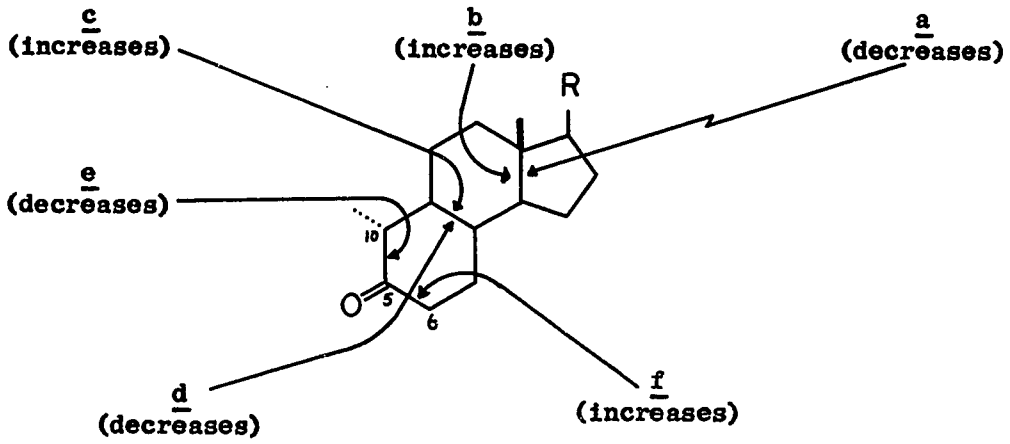
Inhoffen's Ketone (4)

In principle Inhoffen's ketone (4) presents only a slightly more difficult case. It can be thought of as a combination of the trans-tetrahydroindane and trans-octalin examples (see figure 14).

FIGURE 14

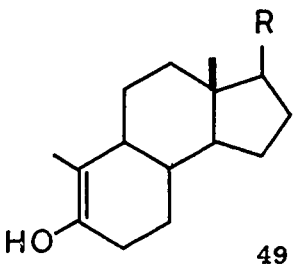
Conformational Transmission Through Inhoffen's Ketone

R = C_8H_{17}



The five-membered D-ring decreases junction dihedral angle a, causing b to increase and hence c also, since it is "meta" to b. This causes d to decrease, since the B and C-rings are trans, and e to decrease since it is "meta" to d. Hence f should increase since it is "para" to d. Of course, the easiest way to decrease dihedral angle e is by introducing a 5,10-double bond; thus it was predicted that the most stable enol of Inhoffen's ketone would be the 5,10-enol (49).

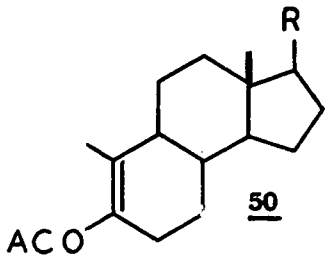
R = C_8H_{17}



49

Some justification of this prediction was indicated by the results of Hartshorn and Jones[36], who found that under thermodynamically controlled conditions, Inhoffen's ketone afforded a 70% yield of the 5,10-enol acetate (50).

$R = C_8H_{17}$



50

Stereoelectronic Effects During Enolization and Alkylation+

The intermediate state* of the reaction between an enolate anion and an attacking reagent has been postulated[40,49,50] to resemble partly the ketonic product (see figure 15). In an intermediate state of this type, the attacking reagent (X) will make a dihedral angle of 90° with the two carbon atoms (C-1 and C-2) and ketonic oxygen# if it is to preserve the maximum overlap with the negatively charged sp^2 orbital on C-2 (see figure 15). This is called the principle of perpendicular attack[40].

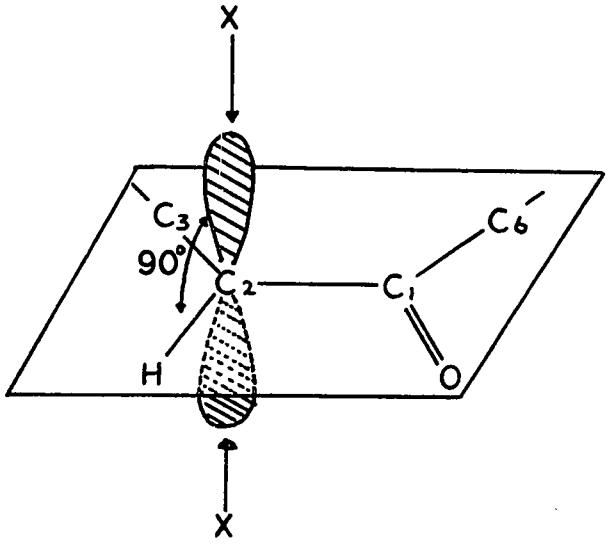
+ A more complete discussion is available in ref. 40.

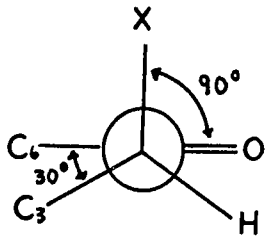
* The course of C-alkylation of cyclohexanones is still a matter of controversy; therefore, the intermediate state referred to may not necessarily represent the true transition state, although it is probably close to it.

i.e. dihedral angle composed of X, C-2, C-1, and O.

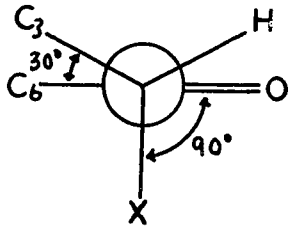
FIGURE 15

Intermediate State for Attack on a Cyclohexanone Enolate





ATTACK OF X FROM THE TOP



ATTACK OF X FROM THE BOTTOM

The same principle also applies to the departure of a substituent during enolization. Examination of the dihedral angles of cyclohexanone[+] viewed along the C-2, C-1 bond (figure 16) shows that far less distortion[*] is involved in moving the axial hydrogen from its normal

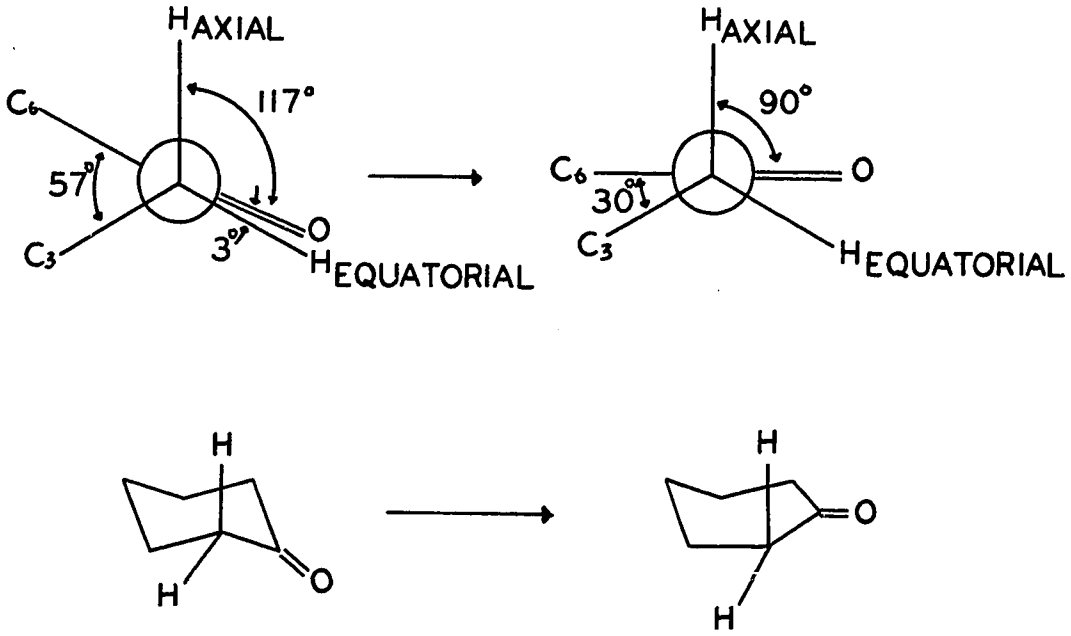
+ The geometry of cyclohexanone is similar to that of cyclohexane.[51]

* i.e. of the dihedral angle of the cyclohexanone ring, composed of C-3, C-2, C-1, and C-6.

dihedral angle[+] of 117° to the 90° required for easy departure, than to move the equatorial hydrogen to a similar angle. By the principle of microreversibility, the same processes are involved in re-addition of the hydrogen to the enol or enolate; to generalize, substituents ∝ to the carbonyl group of cyclohexanone in the chair form will be gained or lost at the axial position.

FIGURE 16

Dihedral Angle Changes Prior to the Enolization of Cyclohexanone



This axial attachment which is encountered in many reactions of enols and enolates (protonation, halogenation, and alkylation) has been interpreted by Corey in his rule of axial attack[39], but it is

+ composed of H-axial, C-2, C-3, and O.

important to note that this is really a consequence of the more general principle of perpendicular attack. A cyclohexanone ring does not necessarily have to exist only in the chair conformation, although this is the most stable form. If the approach to the enolate function from the axial direction is hindered, then the ring may assume a twist form in order to preserve the required perpendicularity of the attacking reagent with the ketonic oxygen; this will result in equatorial substitution of the chair-shaped ring. However, unless this hinderance is severe, the initial enolization and subsequent attack will be from an axial direction.

Application of Conformational and Stereoelectronic Principles to Base-Catalyzed Alkylation

The general method for the attachment of a side chain α to a carbonyl group is a base-catalyzed alkylation reaction.+ The first step of this reaction is the abstraction of a proton from an α-carbon to give an enolate anion, which can then be attacked by an electrophile to give the adduct. If the mechanism of alkylation is similar to that of the base-catalyzed halogenation or deuteration of ketones (which is very probable[40]), then the rate-determining step is the initial formation of the enolate[92]. Thus, for an unsymmetrical ketone which can form two different enolates, the ratio of the two products will be the same as the ratio of the rates of formation of their enolate precursors.

+ Or its formal equivalent, alkylation of the corresponding enamine.

There are two main factors which affect the relative rates of formation of enolates in a polycyclic system. The first is the activation energies of the respective transition states.

It was shown earlier in the section on "direction of attack", that the transition state during the enolization of a cyclohexanone requires that the leaving proton be perpendicular to the C=O bond, and that, hence, the dihedral angle in the cyclohexanone ring must decrease to 30° (see figure 16). It seems reasonable to expect that of two axial protons α to the carbonyl group, the one which will be lost will be that which causes the least strain in the polycyclic system when its associated dihedral angle decreases to 30°. In other words, since the transition state requires a dihedral angle decrease, then the overall activation energy will be less when this decrease is introduced at a point in the ring system where the conformational effects tend to "desire" a decrease.

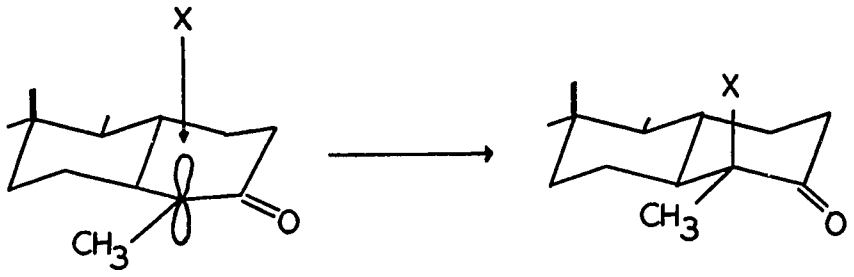
The conclusion to be reached from this is <u>that in the abscence of other effects, the more stable enolate will also be formed faster</u>. This is because the same conformational factors are responsible for both the relative stability of the respective enolates and the relative strain in the transition state. Two examples which support this statement are the 3-oxo-A/B-<u>trans</u>-steroid system which brominates at C-2[47] (the most stable enol is the 2,3-enol) and the 3-oxo-A/B-<u>cis</u>-steroid system which brominates at C-4 (the most stable enol is the 3,4-enol).

The second major factor affecting the relative rates of enol-

ization is the accessibility of the protons to be abstracted. This scarcely needs elaboration except to state that the least hindered axial proton will be preferentially lost to the attacking base, in the abscence of over-ruling conformational effects in the transition state.

Application to Inhoffen's Ketone (4)

It was predicted earlier that the 5,10-enolate (51) would be the more stable one to be formed from Inhoffen's ketone. From all the preceding arguments, it can be concluded that alkylation should take place in the 10β-position (52). This is a necessary requirement for our elaboration of a 10α-steroid.



51 52

Alkylation of Inhoffen's Ketone

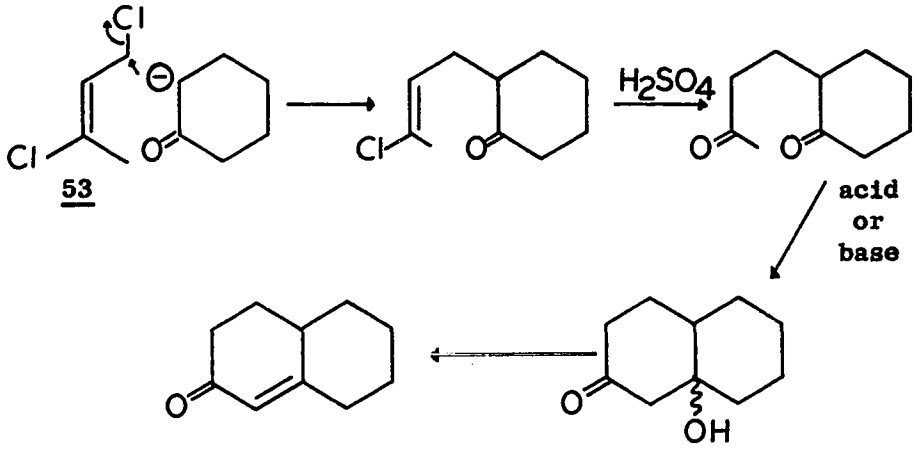
Now that the position and direction of attack had been predicted to be favourable, a practical method had to be found to attack a side-chain to Inhoffen's ketone (4) in the 10β-position. Alkylation with

methyl vinyl ketone (Robinson annellation) had been shown not to work with Inhoffen's ketone[34] and the Woodward approach using acrylonitrile seemed to be useless for producing a 10α-steroid[52,53].

The method first chosen was to employ 1,3-dichlorobut-2-ene (53) in a base-catalyzed reaction. This reagent had been used by the Roussel-Uclaf group to form six-membered rings in high yield[54-56]. The utility of this reagent is due to the fact that one of the chlorine atoms is allylically activated and is easily displaced by an enolate. (See figure 17). The resulting vinyl chloride group can be converted to a ketone by cold concentrated sulphuric acid,+ and the resultant

FIGURE 17

1,3-Dichlorobut-2-ene Method for Forming Rings

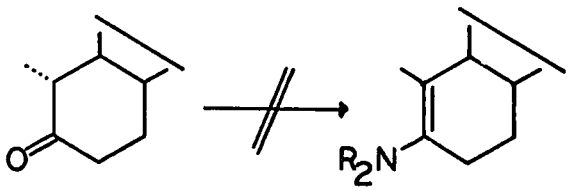


diketone cyclized by acid or base.

The Roussel-Uclaf group generally used enamine alkylation, instead of base catalysis. Although Gurowitz and Joseph[58] showed that it is sometimes possible to produce enamines with the double bond ex-

+ Commonly called the Wichterle Reaction[57].

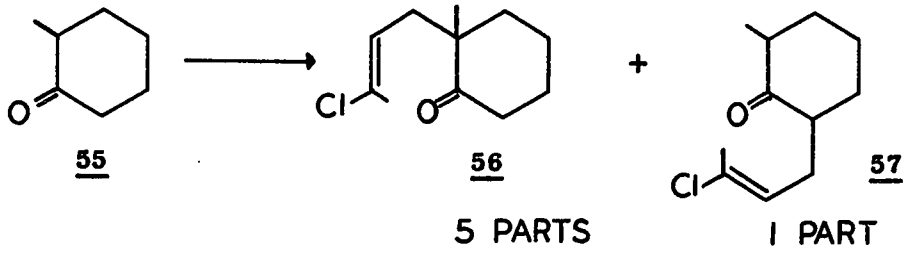
tending towards the more substituted carbon (e.g. 54), under no conditions could any enamine (54) of Inhoffen's ketone (4) be made, although several methods were tried[54,58-61].



4 54

The basic catalyst finally chosen for the alkylation was sodium tertiary-amylate[62]; this base was bulky enough so that it would preferentially abstract a proton from Inhoffen's ketone instead of attacking the 1,3-dichlorobut-2-ene (53) itself, and was also soluble in benzene, the reaction solvent.

The reaction was first applied to the model system 2-methylcyclohexanone (55) and the crude product was separated by preparative vapour-phase chromatography into unreacted starting material and two products which were formed in the ratio of 5:1. Both had infrared spectra which corresponded to structures 56 and 57 viz. 3040 $cm.^{-1}$ and 1665 $cm.^{-1}$ (double bond) and 1720 $cm.^{-1}$ (ketone). The major product gave an NMR spectrum having a singlet at 1.05 p.p.m. due to the quaternary methyl group, a singlet at 2.05 p.p.m. due to the vinyl methyl group, and a small triplet at 5.35 p.p.m. due to the vinyl hydrogen, and must therefore be 56. The minor product is accordingly 57. Julia[62] had previously reported that this reaction gave only 56.



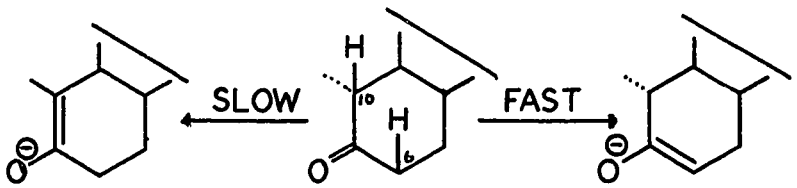
55 56 57

5 PARTS 1 PART

Thus encouraged, we applied the reaction to Inhoffen's ketone (4). The product, which was separated in 43% yield, had the typical infrared spectrum of the alkylation products of 2-methylcyclohexanone. However, the NMR spectrum left no doubt that alkylation had taken place at C-6 and not C-10, since the C-10 methyl group was still a doublet at 0.80 p.p.m. (J = 4.5 c.p.s.). Thus the product must be formulated as 5.+

The failure of Inhoffen's ketone to alkylate at C-10 as predicted was probably due to the fact that the conformational effects predicted earlier, had to operate through three rings, and consequently were too weak to make the activation energy of the 5,10-enolate (58) significantly less than that of the 5,6-enolate (59). If this were the case, then the accessibility factor would have become the directing influence; since the 6β-proton is less hindered, it was attacked in preference to the 10β-proton.

+ Under the basic reaction conditions the originally 6β-axial product will epimerize to the more stable 6α-equatorial.



58 4 59

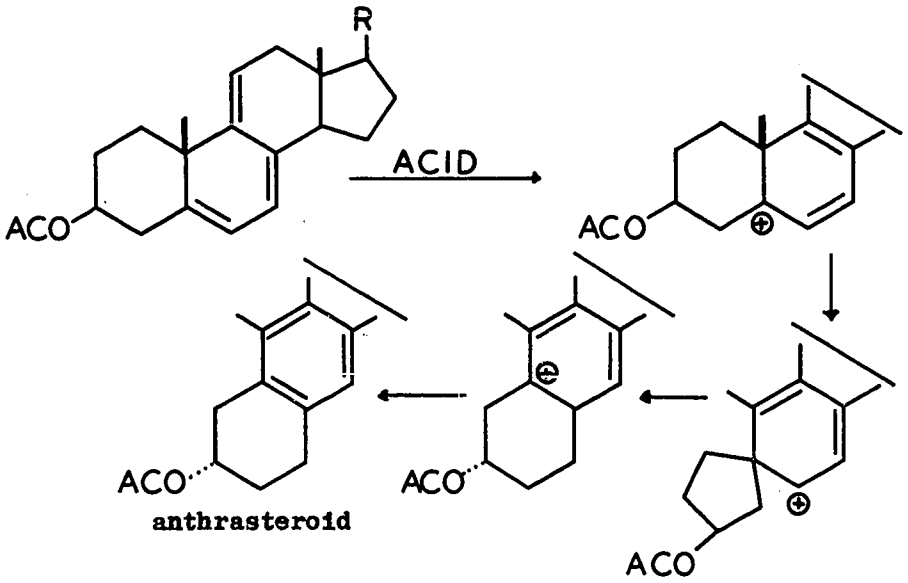
The product of undesired alkylation at C-6 (5) was used to test the subsequent steps of the proposed synthesis. Treatment with cold, concentrated sulphuric acid gave a more polar product, which was assigned structure 6 on the basis of its infrared spectrum, which showed that the only functional group present was ketone (1720 cm.$^{-1}$). When 6 was left overnight in a solution of acetic acid with a small amount of hydrochloric acid, it was cyclized to the anthrasteroid 7, m.p. 122.5°-123.5°. This compound had the typical α,β -unsaturated ketone absorptions in the infrared (1680 cm.$^{-1}$) and ultraviolet spectra ($\lambda_{max.}$ 244 mμ)+. The NMR spectrum showed that the 19-methyl hydrogens absorbed as a doublet at 0.85 p.p.m. (J = 3.5 c.p.s.).

Anthrasteroids are compounds with the skeletal structure shown in figure 18; usually ring B is aromatic. Until now they have been formed solely by acid-catalyzed rearrangements of multiply unsaturated steroids[73,74], such as the one illustrated in figure 18[74].

+ Woodward's rules[63] predict 244 mμ.

FIGURE 18

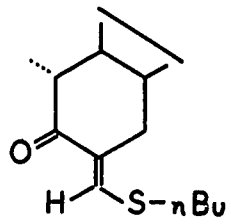
The Anthrasteroid Rearrangement



Alkylation of Dehydro-Inhoffen's Ketone (9) with 1,3-Dichlorobut-2-ene

It was now obvious that conformational effects in Inhoffen's ketone were insufficient to direct alkylation to the C-10 position. Blocking the 6-position with some group which could be readily removed after alkylation (such as the n-butylthiomethylene group (60)[64]) was considered, but rejected as introducing unnecessary complications.

The solution to the problem was found in the introduction of a 9,10-double bond to give dehydro-Inhoffen's ketone (des-A-cholest-9-en-5-one) (9). Pinder and Robinson[34] had attempted to produce it by treatment of Inhoffen's ketone with bromine, but they could not isolate

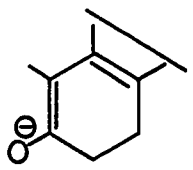


60

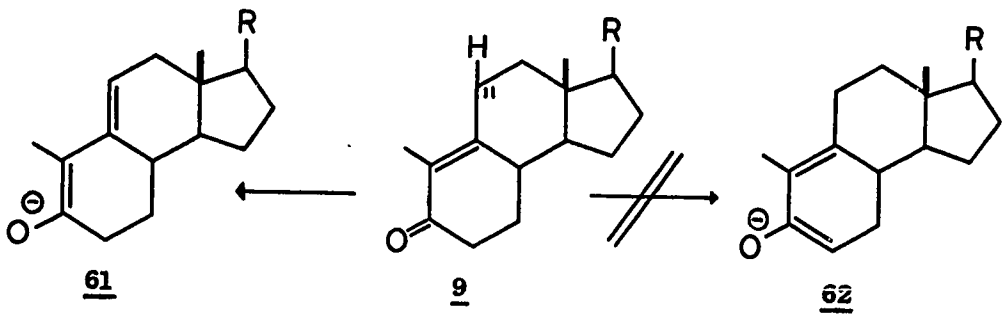
any product. This compound had been previously produced by Hartshorn and Jones[36], who separated the mixture of products resulting from the enol acetylation of Inhoffen's ketone, isolated the 5,10-enolate and brominated it, to give 10β-bromo-des-A-cholestan-5-one. This was dehydrobrominated to give 9. The method used here is simpler and less time-consuming.

Bromination by N-bromo-succinimide permitted a selective attack at C-10 to give 8, which could be dehydrohalogenated by treatment with lithium chloride in dimethyl formamide, giving 9 in an overall yield of 55%.

Conformational analysis of the two[+] enolates derived from dehydro-Inhoffen's ketone (9) shows why alkylation could be expected to occur at C-10.

+ A third enolate  is not considered likely but in any case would still lead to alkylation at C-10.

R = C_8H_{17}

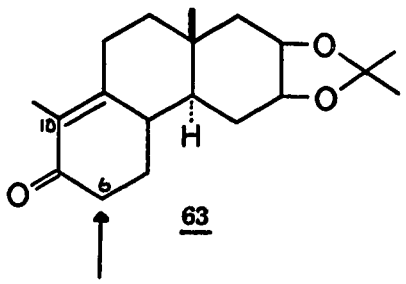


61 9 62

Rings C and D can be considered equivalent to the trans-tetrahydroindane system which was studied earlier. There, it was shown that a double bond "para" to the ring junction gives the most stability to the system. Now, enolate 61 provides exactly this, whereas enolate 62 does not. Furthermore, abstraction of an 11β-hydrogen removes a 1,3-diaxial interaction with the 18-methyl group; this should also favour 61.

In practice the conformational effect of the five-membered D-ring in the "C-D tetrahydroindane" system is decisive. For example, in the Woodward steroid total synthesis[52], intermediate 63 with a six-membered D-ring required blocking at C-6 to force alkylation to occur at C-10.+ In a later modification, Barkely et. al.[53] found that the five-membered D-ring intermediate 64 required no blocking group; alkylation spontaneously occurred at C-10.

+ Otherwise the predominate attack was at C-6[52].



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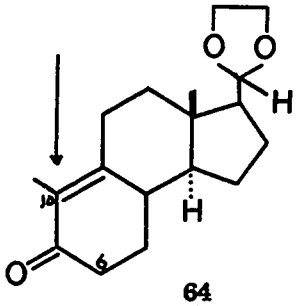


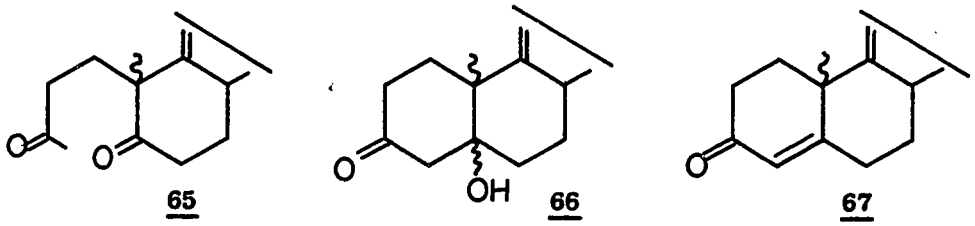
64

The prediction of alkylation at C-10 was correct, as it turned out. Alkylation of dehydro-Inhoffen's ketone (9) with 1,3-dichlorobut-2-ene in the presence of sodium tertiary-amylate gave, after purification, a yellow oil in 78% yield. This had the infrared spectrum typical of 1,3-dichlorobut-2-ene alkylation products, and the NMR spectrum showed a singlet at 0.78 p.p.m. for the quaternary 19-methyl. However, neither the 2,4-dinitrophenylhydrazone nor the semi-carbazone derivatives could be obtained crystalline, probably because the product consisted of a mixture of 10α- and 10β-epimers 10 and 11.

Even more discouraging was the difficulty in converting the vinyl chloride group to the ketone by sulphuric acid. Numerous attempts to utilize other strong acids and to vary the reaction conditions finally led to the isolation of a more polar product in 45% yield which had an infrared spectrum compatible with 65.

Treatment of this product with sodium tertiary-amylate, which would be expected to cause the intramolecular aldol condensation of 65, had no effect. Sodium ethoxide in ethanol/dioxane gave a product

oil in 7% yield. However, the infrared spectrum contained numerous bonds which could not possibly be due to 66 or 67; obviously some deep-rooted change had taken place in the molecule, but this was not further pursued. Although futile in the end, this part of the work established that dehydro-Inhoffen's ketone (9) could be alkylated easily at C-10.



65 66 67

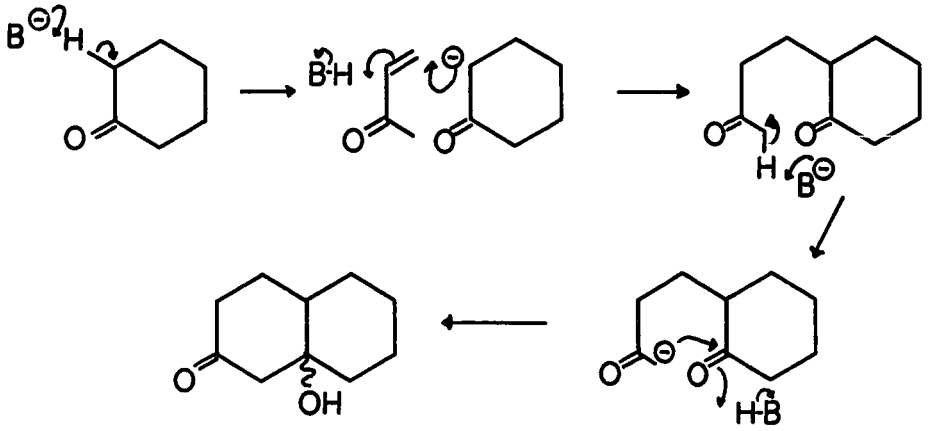
Alkylation of Dehydro-Inhoffen's Ketone with Methyl Vinyl Ketone

At this point it was decided to attempt to alkylate dehydro-Inhoffen's ketone (9) with methyl vinyl ketone (the Robinson annellation reaction). Generally this method gives poor yields with large or complicated molecules , but it does have the advantage of forming a ring all in one step. The mechanism, shown in figure 19 in simplified form, amounts,in effect, to a base-catalyzed Michael addition, followed immediately by a base-catalyzed intramolecular condensation to give a ketol.

FIGURE 19

The Mechanism of Methyl Vinyl Ketone Alkylation

(The Robinson Annellation)



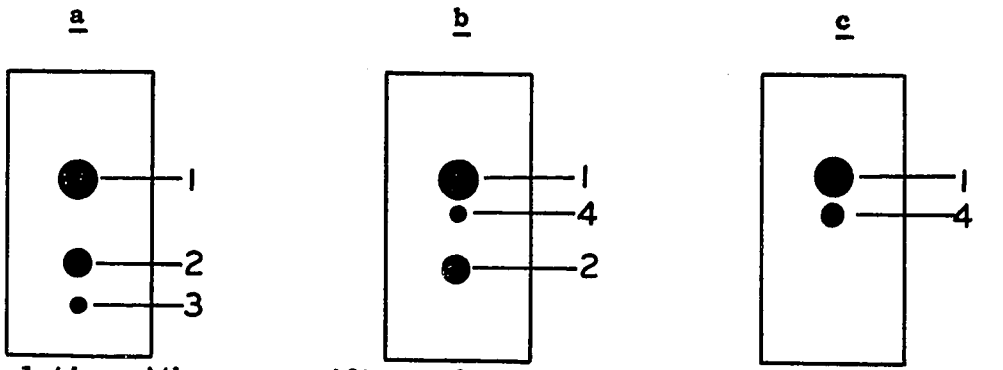
Occasionally this reaction gives bicyclic derivatives similar to 68[65], but these can readily be identified by their nuclear magnetic resonance spectra. No bicyclic derivatives of this type were found during this research.

When methyl vinyl ketone was added to a cold solution of dehydro-Inhoffen's ketone (9) in the prescence of sodium ethoxide, two new polar products were formed in reasonable yield (see (a), figure 20). It was also found that refluxing the crude product with sodium ethoxide caused the most polar product (spot 3) to be converted into a new, less

polar product, spot 4 (see b, figure 20), and that subsequent reflux in benzene in the presence of para-toluenesulphonic acid caused spot 2 to disappear and spot 4 to increase in size (see c, figure 20).

FIGURE 20

Thin-Layer Chromatography* of Reaction Products



Alkylation with methyl vinyl ketone

After reflux with ethoxide

After benzene/para-toluenesulphonic acid reflux

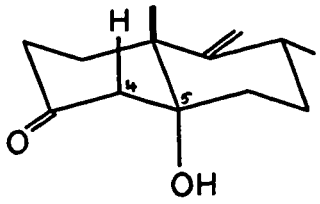
* Silica gel slides, eluant-benzene-20% ether. Spot 1 is unreacted dehydro-Inhoffen's ketone.

These preliminary results suggested that spot 3 was the product of alkylation at C-10 which gave the natural 10β-epimer (13) and that spot 2 was the desired 10α-epimer (12). The reason for these assignments can be seen from an examination of molecular models of 12 and 13. (See figure 21).

The model of 10β-ketol 13 shows that the 4β-hydrogen and the 5α-hydroxyl are trans-diaxial, and hence well disposed to E_2 elimination by ethoxide ion to give the α,β-unsaturated ketone 14[66].

FIGURE 21

Molecular Models of Ketols 12 and 13



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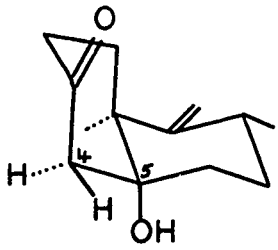
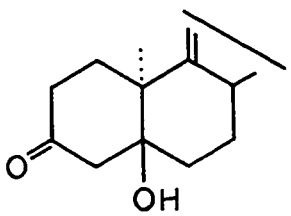


12

However, the model of 10α-ketol 12 demonstrates that neither C-4 hydrogen is trans-diaxial to the 5α-hydroxyl; hence, base will not effect the elimination of water from this 10α-ketol.+ However, reflux in benzene with a catalytic amount of para-toluenesulphonic acid would be expected to eliminate water from ketol 12 because it operates through a different mechanism (E_1). This will be discussed later.

In principle, this base elimination presented an efficient

+ The two other isomers at C-5 and C-10, 69 and 70 are not likely because of steric hinderance.



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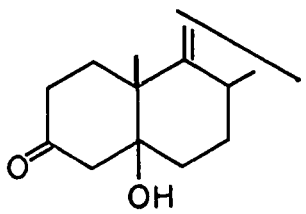
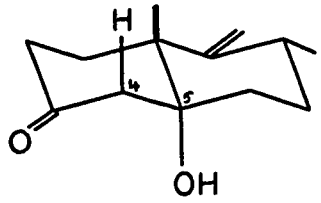


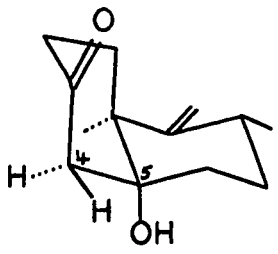
70

FIGURE 21

Molecular Models of Ketols 12 and 13



13

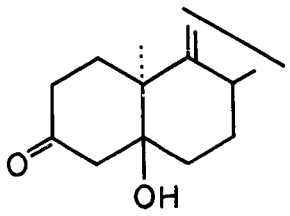


12

However, the model of 10α-ketol 12 demonstrates that neither C-4 hydrogen is trans-diaxial to the 5α-hydroxyl; hence, base will not effect the elimination of water from this 10α-ketol.+ However, reflux in benzene with a catalytic amount of para-toluenesulphonic acid would be expected to eliminate water from ketol 12 because it operates through a different mechanism (E_1). This will be discussed later.

In principle, this base elimination presented an efficient

+ The two other isomers at C-5 and C-10, 69 and 70 are not likely because of steric hinderance.



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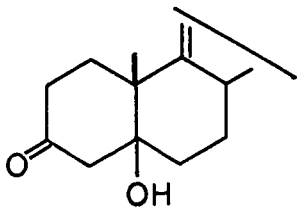


70

method for separation of the 10α- and 10β-epimers, 12 and 13. In practice, it was simply sufficient to leave the reaction mixture under nitrogen in the refrigerator for several days, during which time the sodium ethoxide alkylation catalyst effectively eliminated water from the 10β-epimer (13). The 10α-epimer (12) could then be easily separated by column chromatography, and purified by crystallization.

The reaction yields were considerably improved by using very pure reagents, and by very slow addition of the methyl vinyl ketone in dilute solution, so that the tendency for polymerization was reduced. In this manner 10α-ketol 12 could be obtained in 37% yield from dehydro-Inhoffen's ketone (9) with an 18% recovery of starting material. The ketol 12 had m.p. 148.5°-149.5° and $[\alpha]_D$ +61°.

Assignment of structure 12 was based on:

(a) the aforementioned elimination reactions,

(b) the fact that the hydroxyl could not be oxidized,

(c) the infrared spectrum, which showed OH absorption at 3600 cm.$^{-1}$, double bond at 3050 and 1675 cm.$^{-1}$ and ketone at 1720 cm.$^{-1}$, and

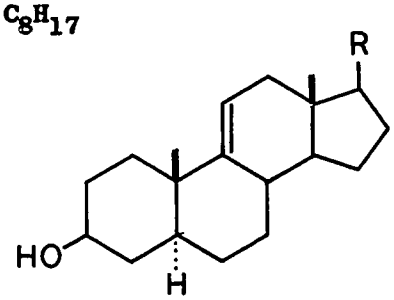
(d) the NMR spectrum, which indicated a singlet at 0.82 p.p.m. for the 19-methyl, a singlet at 1.98 p.p.m. (which disappeared on D_2O exchange) due to the hydroxyl proton, a broad peak at 5.63 p.p.m. due to the C-11 vinyl proton, and no peaks for protons α to an hydroxyl (3.4-4.5 p.p.m.).

From the less polar eluants of the chromatographic purification of ketol 12, crystals of m.p. 116°-116.5°, $[\alpha]_D$ +81° could be obtained in 5% yield. This by-product corresponded to spot 4 on TLC b (figure 20)

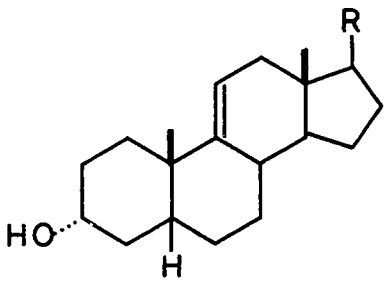
and was therefore assigned structure 14, cholesta-4,9(11)-dien-3-one. This was supported by infrared peaks at 3040 and 1610 cm.$^{-1}$ (double bond) and 1680 cm.$^{-1}$ (α,β-unsaturated ketone). The ultraviolet spectrum ($\lambda_{max.}$ 241 mμ; cholest-4-en-3-one has $\lambda_{max.}$ 241 mμ[63]), and the NMR spectrum, which is closely analogous to that of cholest-4-en-3-one with the addition of a broad peak at 5.45 p.p.m. due to the vinyl proton on C-11, also support this structure.

An attempt was made to transform 14 into the known 5α-cholest-9(11)-en-3-β-ol (71)[67] (m.p. 123°, $[\alpha]_D$ +27°; acetate m.p. 105°, $[\alpha]_D$ +22.5°), by hydrogenation of the 4,5-double bond in acid medium and reduction of the ketone group. Two products were isolated which were unsaturated alcohols (by infrared), but only the major one could be crystallized; m.p. 134°-135°, $[\alpha]_D$ +25°; acetate m.p. 98°-99°, $[\alpha]_D$ +14°. Probably hydrogenation of the 4,5-double bond gave a mixture of the two epimers at C-5, and it was the 5β-cholestane compound (72) which was isolated. This is a common phenomenon[68]. Therefore, in view of the fact that the by-product could not be converted to a

R = C_8H_{17}



71



72

known derivative, the assignment of 14 must still be regarded as tentative.

Hydrogenation of the 9,11-Double Bond

This step provided no significant difficulties as the unsaturated ketol 12 was easily hydrogenated by platinum in acetic acid to the saturated diol 15. The principal byproduct (about 20% yield) was 10α-cholestan-5α-ol (16) m.p. 79.5°-80°, $[\alpha]_D$ +53°, which was formed by hydrogenolysis of the C-3 hydroxy group. This structure was assigned on the basis of its infrared spectrum, which showed hydroxyl peaks at 3640 and 3350 $cm.^{-1}$, and of the fact that it was not oxidized by Jones reagent.

Such extensive hydrogenolysis is not a usual characteristic of ordinary secondary alcohols. A possible explanation of this anomalous behaviour is that the catalyst must approach the C-3 ketone group from the back side (α-direction), since β-attack would be too hindered (see figure 22). This would yield the axial 3β-alcohol, which would be in a very hindered position, and thus rather susceptible to loss by hydrogenolysis.

Several other catalysts and hydrogenation conditions were tried in an attempt to minimize this hydrogenolysis, but only platinum in acetic acid was effective for reduction of the 9,11-double bond. This is probably because of its rather hindered position.

Diol 15 was not purified but was immediately oxidized by chromic acid in acetone (Jones reagent[69]) to the saturated ketol 17

(10α-cholestan-5α-ol-3-one) in 74% overall yield from 12. The compound had a m.p. of 170.5°-171°, $[\alpha]_D$ +49°, and an infrared spectrum essentially the same as that of unsaturated ketol 12, except for bands at 3050 and 1675 $cm.^{-1}$, due to unsaturation, in the latter.

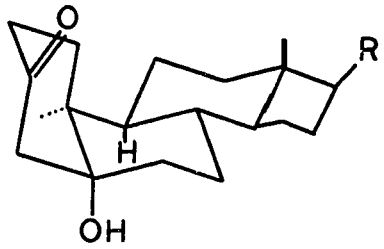
Proof of the Configuration of 10α-Cholestan-5α-ol-3-one (17)

Molecular models of the unsaturated ketol 12 had indicated the hydrogenation of the 9, 11-double bond would take place from the α or bottom side, because steric hinderance to the approach of the catalyst would then be at a minimum. This should lead to a 9α-hydrogen. At this point it was thought desirable to check the conclusions concerning the stereochemistry of both the methyl vinyl ketone addition and the hydrogenation, and to prove with certainty that the structure of 17 was that shown in figure 22.

FIGURE 22

Molecular Model of 10α-Cholestan-5α-ol-3-one (17)

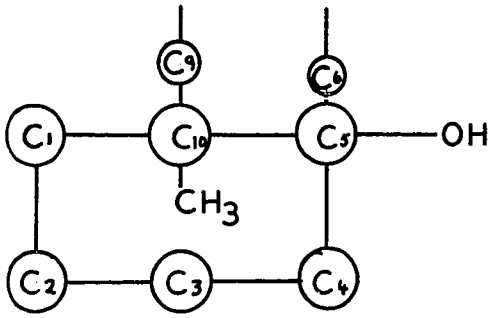
R = C_8H_{17}

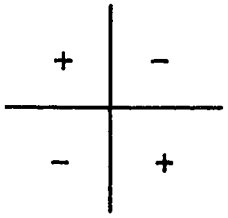


Models indicated that the most stable conformation for 17 would be the all-chair form, pictured in figure 22. If this is projected along the O=C-3 axis, figure 23 results. Applying the octant rule[70] to this projection, one can easily see that most of the substituents on the A-ring fall into the upper-right-rear octant, thus leading to the prediction of a negative Cotton effect.

FIGURE 23

Projection of 10α-Cholestan-5α-ol-3-one Along the O=C-3 Axis





Cotton Effect of Substituents in Rear Octants

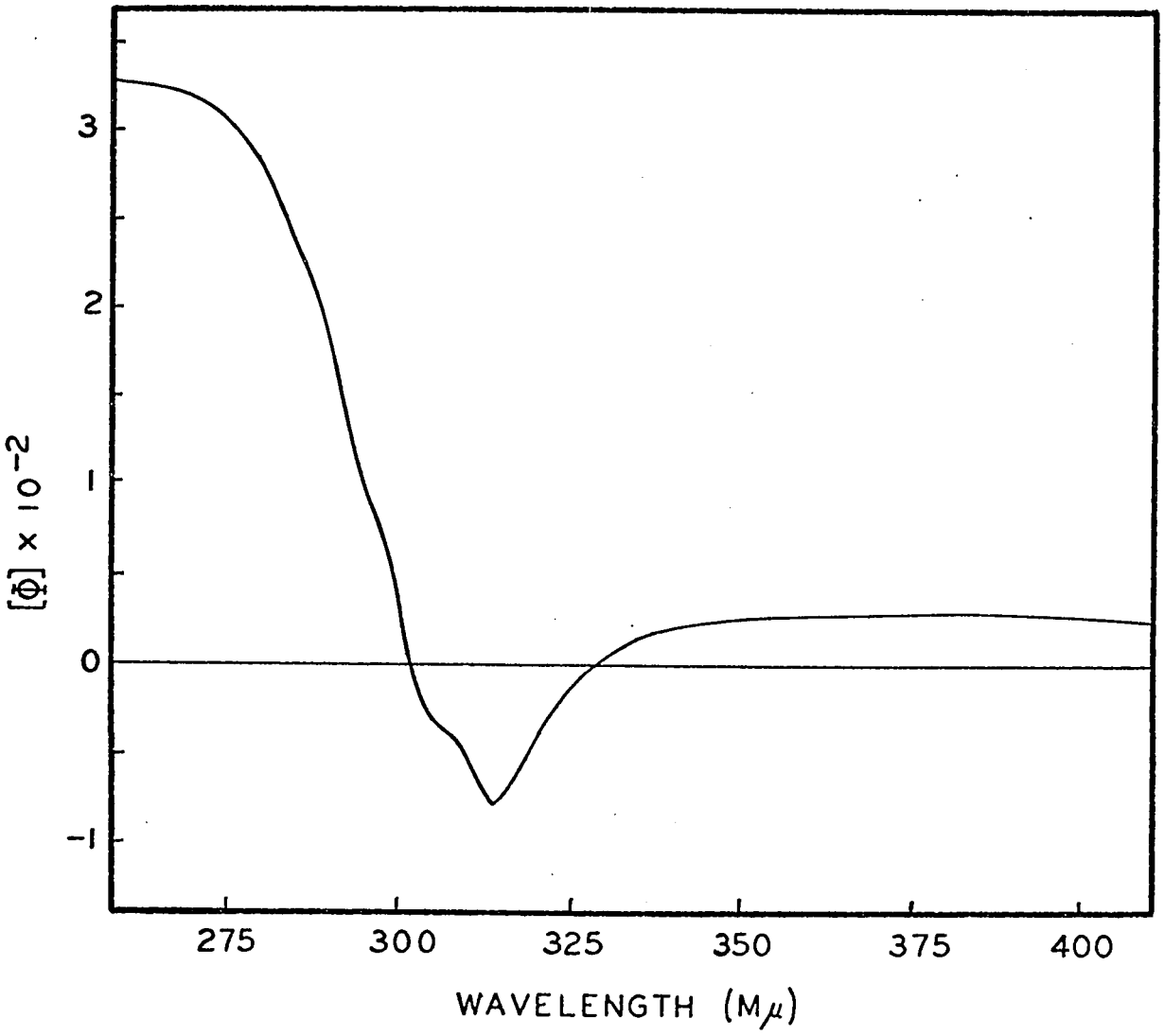
The optical rotatory dispersion curve shown in figure 24+ clearly justifies this prediction; therefore, the structure and configuration of 10α-cholestan-5α-ol-3-one (17) are confirmed.

+ This curve provides another interesting instance of a compound with a positive $[\alpha]_D$ and a negative Cotton effect.

FIGURE 24

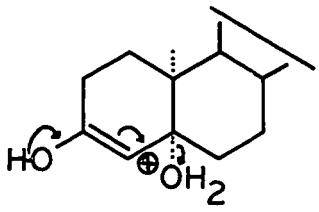
Optical Rotatory Dispersion Curve

of 10α-Cholestan-5α-ol-3-one



Formation of 10α-Cholest-4-en-3-one (18)

Elimination of water from ketol 17 was easily accomplished by refluxing it in benzene with a trace of para-toluenesulphonic acid. This reaction probably proceeds by protonation of the 5α-hydroxyl group, which can easily leave from enolic intermediate (73). Thus it is more in the nature of an E_1 elimination.



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Two unsaturated ketones were separated from the product mixture by chromatography. The major product (10α-cholest-4-en-3-one (18)) was a clear oil which did not crystallize but gave a crystalline red 2,4-dinitrophenylhydrazone melting at 185°-185.5°. The infrared spectrum of the oil showed that it was an α,β-unsaturated ketone (1680 $cm.^{-1}$) and the ultraviolet spectrum confirmed this ($\lambda_{max.}$ 243 mμ, natural cholest-4-en-3-one has $\lambda_{max.}$ 242 mμ [71]). Furthermore, the ultraviolet spectrum of the crystalline 2,4-dinitrophenylhydrazone is closely analogous to that of natural cholest-4-en-3-one[72]. (See table 1).

TABLE 1

Ultraviolet Absorbances of

10α- and 10β-Cholest-4-en-3-one-2,4-dinitrophenylhydrazone

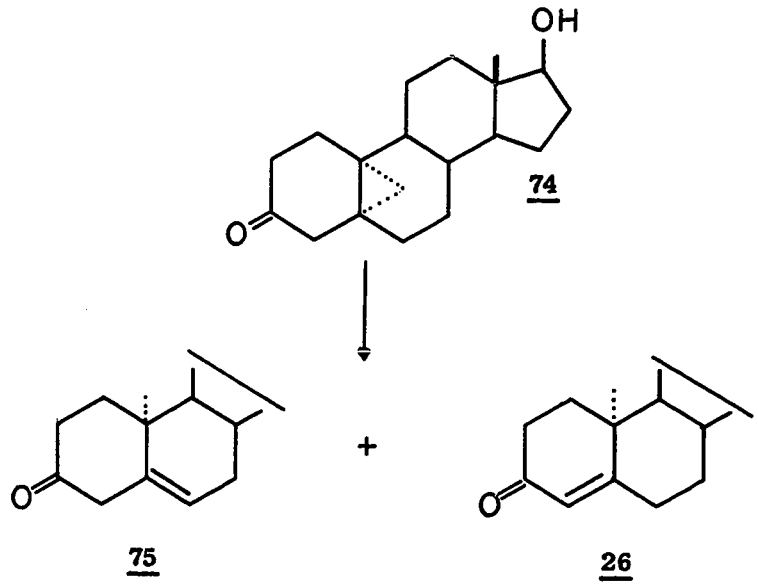
10α-		10β-	
$\lambda_{max.}$ (cm.$^{-1}$)	log $\epsilon_{max.}$	$\lambda_{max.}$ (cm.$^{-1}$)	log $\epsilon_{max.}$
260	4.23	256	4.33
		281	4.20
293	4.02	292	4.06
392	4.44	393	4.47

The minor product was a crystalline solid of m.p. 112°-112.5° and $[\alpha]_D$ -104°, and was assigned the structure 10α-cholest-5-en-3-one (19) from its infrared spectrum which showed double bond absorption at 3030 and 1680 cm.$^{-1}$ and unconjugated ketone at 1720 cm.$^{-1}$. Furthermore, both 18 and 19 could be converted into the same enol acetate (20) (described in the following section), and basic hydrolysis of 20 led to a mixture of 18 and 19 in the proportion two parts conjugated ketone (18) to one part unconjugated ketone (19).+

Ginsig and Cross[27] found similar behaviour when they treated 74 with potassium tertiary-butoxide in dimethylsulphoxide. The two ketonic products were isolated by chromatography in the ratio of two parts of unconjugated ketone (75) to one part conjugated ketone (26).*

+ Determined by the relative intensities of their carbonyl absorptions in the infrared spectrum.

* Determined from the yields of the products separated by chromatography.



74

75 + 26

Obviously there must be a strong steric reason for the preference of the double bond in ring B, since β,γ-unsaturated ketones usually reconjugate themselves almost completely in the presence of acid or base. Molecular models of conjugated (18) and unconjugated (19) ketones suggest one major explanation (see figure 25).

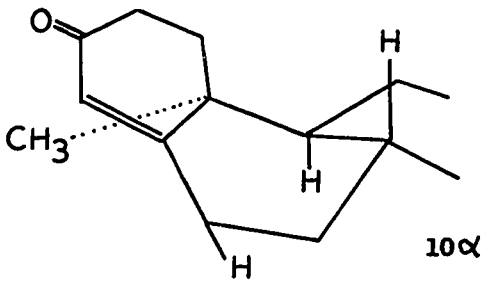
In 10-α-cholest-4-en-3-one (18) ring B is forced to exist in a skew-boat conformation, whereas in the unconjugated 10α-cholest-5-en-3-one (19), it can take up the more stable half-chair conformation.+ This would be expected to destabilize the conjugated ketone relative to the unconjugated one.

+ The half-chair form of cyclohexene is probably only a little more strained then the chair form of cyclohexane, but the skew-boat form is considerably more strained than the chair form[75].

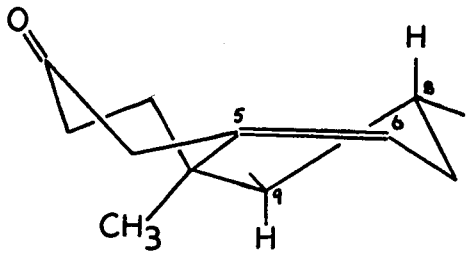
FIGURE 25

Molecular Models of 10α-Cholest-4-en-3-one (18)

and 10α-Cholest-5-en-3-one (19)



10α-Cholest-4-en-3-one



10α-Cholest-5-en-3-one

Formation of 10α-Cholesterol

As mentioned earlier, both unsaturated ketones 18 and 19 could be converted to the same enol acetate 20. In later experiments the crude mixture of 18 and 19 was not separated, but immediately treated with the perchloric acid-acetic anhydride reagent of Edwards and Rao[76] to give 20 in an overall yield (from 17) of 84%. This 10α-enol acetate (m.p. 93°-94°, $[\alpha]_D$ +32°) was identified by its infrared spectrum (double bond: 3030 and 1660 $cm.^{-1}$; acetate: 1755 $cm.^{-1}$), and its

ultraviolet spectrum ($\lambda_{max.}$ 236 mμ; the corresponding 10β-enol acetate has $\lambda_{max.}$ 236 mμ).

Final production of 10α-cholesterol (21) (m.p. 118.5°-119°, $[\alpha]_D$ -46°) was achieved by a modification of the method of Belleau and Gallagher[77]. The 10α-enol acetate (20) was reduced by sodium borohydride in aqueous methanol in 84% yield. Normally sodium borohydride is too weak to reduce esters, but in the solution made basic by the borohydride, the enol acetate was apparently hydrolyzed to the unconjugated ketone 19, which was reduced to the alcohol by the excess borohydride before any equilibration of the double bond could occur[78]. To prove that the double bond was in fact in the 5,6-position, the 10α-cholesterol was oxidized under mild conditions which could not have caused reconjugation of the double bond[69]; the product isolated was identical with 19.

Stereochemistry of the C-3 Hydroxyl Group

The configuration of the C-3 hydroxyl group was deduced from the nuclear magnetic resonance spectrum of 10α-cholesterol shown in figure 27. The broad peak at 3.45 p.p.m. is due to both the hydroxyl and C-3 protons; upon deuterium oxide exchange the former is removed and the peak becomes much sharper. (Width at half-height: 2.5 c.p.s.). It has been pointed out that the equatorial protons α to hydroxyl groups absorb at a lower field and give considerably sharper peaks than their axial counterparts[79,80]. Comparison of the sharp peak at 3.45 p.p.m. in the NMR spectrum of 10α-cholesterol with the very

broad peak of natural 10β-cholesterol at about 3.4 p.p.m. illustrates this difference. Thus, the hydroxyl group in the 10α-epimer must be axial, and hence, in the β-configuration (see figure 26).

In the reduction of unhindered ketones by metal hydrides, the most important factor in controlling the stereochemistry is the relative stability of the product alcohols. This is especially so for a small reducing species such as $^{\ominus}AlH_4$ (from lithium aluminum hydride); which is little affected by small differences in steric hinderance. Hence, the more stable equatorial alcohol will be the main product. This is called "product development control" [82,91].

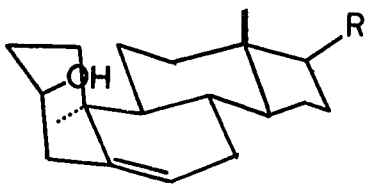
However, for the case of hindered ketones, the accessibility of the carbonyl function becomes the controlling factor, forcing the reducing species to approach from the least hindered direction, thus giving the more hindered and less stable alcohol as the principal product. This is called "steric approach control" [82,91]; it is especially important for bulky reducing agents such as sodium borohydride in aqueous methanol, where the borohydride ion is probably quite solvated[82].

As a molecular model of 10α-cholest-5-en-3-one (figure 25) shows, the β-direction at C-3 is fairly hindered by the rest of the steroid, whereas the "convex" α-face is quite open to attack, thus leading to the 3β-axial alcohol. Since only the axial alcohol could be isolated (in 84% yield) the reduction is an example of steric approach control.

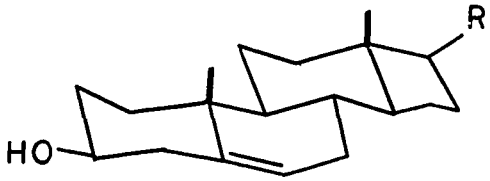
FIGURE 26

Molecular Models of 10α- and 10β-Cholesterol

$R = C_8H_{17}$



10α-Cholesterol



10β-Cholesterol

Comparison of the Spectral Properties of 10α- and 10β-Cholesterol

Figures 27 and 28 show that the NMR spectra for 10α- and 10β-cholesterol are very similar. Surprisingly, the peak due to the 19-methyl group has exactly the same chemical shift for both compounds (0.817 p.p.m.); it might be expected to be at lower field for the 10α-epimer, since it does not seem to be able to "see" as much of the steroid ring system[81].

Infrared spectra of the two epimers (see figure 29) are almost identical. The only difference is in the hydroxyl bending vibration which occurs at 1050 $cm.^{-1}$ for the 10α- and 1060 $cm.^{-1}$ for the 10β-compound.

10α-Cholesterol Acetate

This was made by acetylation of 10α-cholesterol by the perchloric acid-acetic anhydride method of Edwards and Rao[76]. It had a m.p. of 124^{o}-124.5^{o} and $[\alpha]_D$ -47^{o}. Its infrared spectrum and that of 10β-cholesterol acetate is found in figure 30.

Comparison of Melting Points and Optical Rotations of C-10 Epimers

It is evident from table 2 that there is no regularity in either melting point or optical rotation differences between the six 10α- and 10β-epimers listed. The only conclusion which can be drawn is that the melting points are generally lower and optical rotations generally more negative for the 10α-compounds; there are however, glaring exceptions.

TABLE 2

Comparison of Melting Points and Optical Rotations of C-10 Epimers

Compound	Melting Point			$[\alpha]_D$		
	X = α	X = β	Ref.	X = α	X = β	Ref.
10X-cholesterol*	119	150	83+	- 46	- 40	88+
10X-cholesterol acetate*	124	114	84+	- 47	- 43	88+
10X-cholest-5-en-3-one	112	126	85+	-104	- 8	85+
10X-cholesta-3,5-dien-3-ol acetate	94	84		+ 32	-100	89+
10X-cholestan-5α-ol	80	110	86+	+ 53	+ 11.2	86+
10X-cholestan-5α-ol-3-one	171	226	87+	+ 49	+ 41	87+

* It is worth noting that in these compounds the C-3 functional group is equatorial in the 10β-isomers and axial in the 10α-isomers. This might contribute differently to the overall optical rotation of the C-10 epimers.

\+ Refers to references to X = β only.

FIGURE 27

Nuclear Magnetic Resonance Spectrum of 10α-Cholesterol in Deutero-chloroform Solution

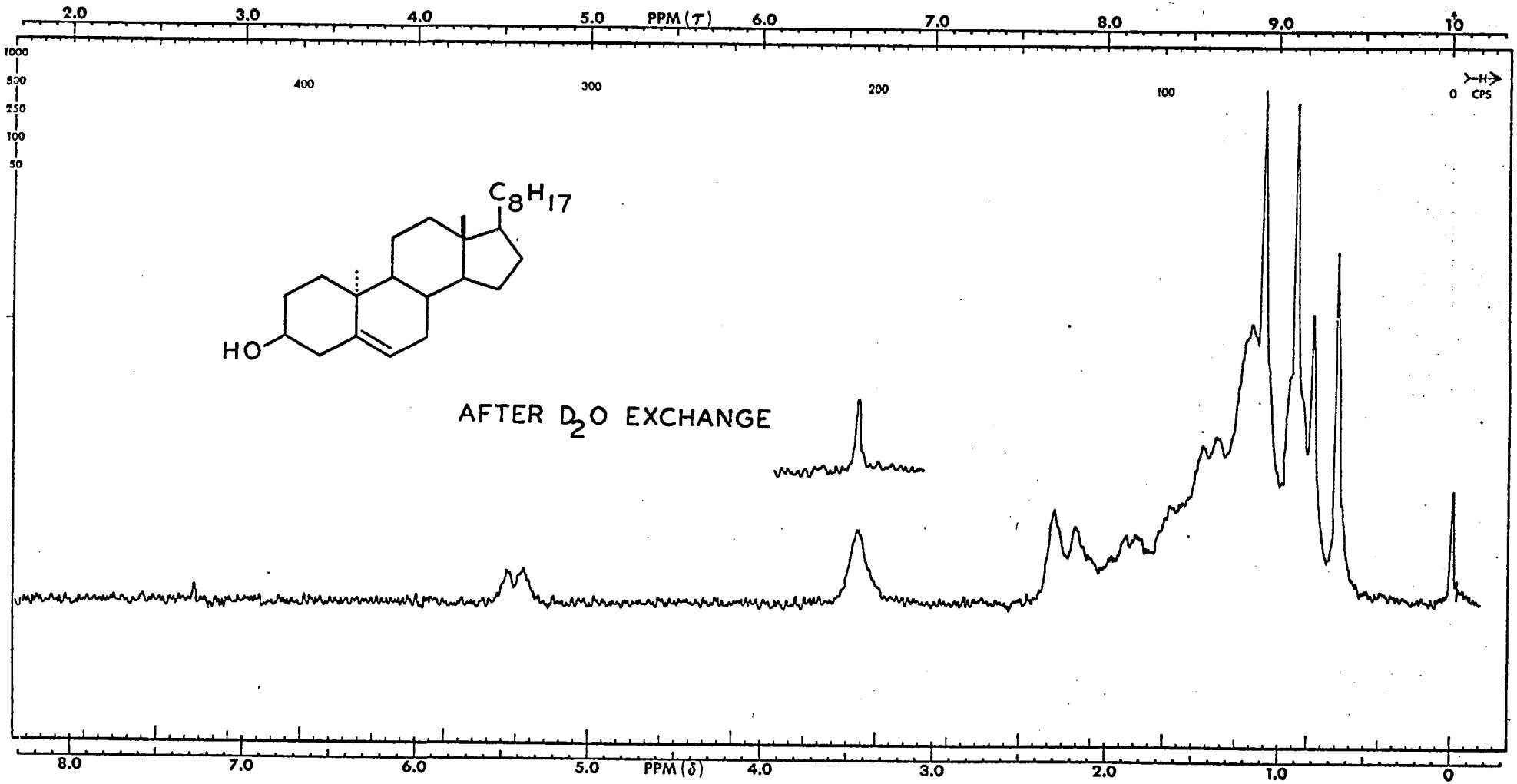

2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
9.0
10
1000
500
250
100
50
400
300
200
100
0
CPS
C8H17
HO
AFTER D2O EXCHANGE
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0

FIGURE 28

Nuclear Magnetic Resonance Spectrum of 10β-Cholesterol in Deutero-chloroform Solution

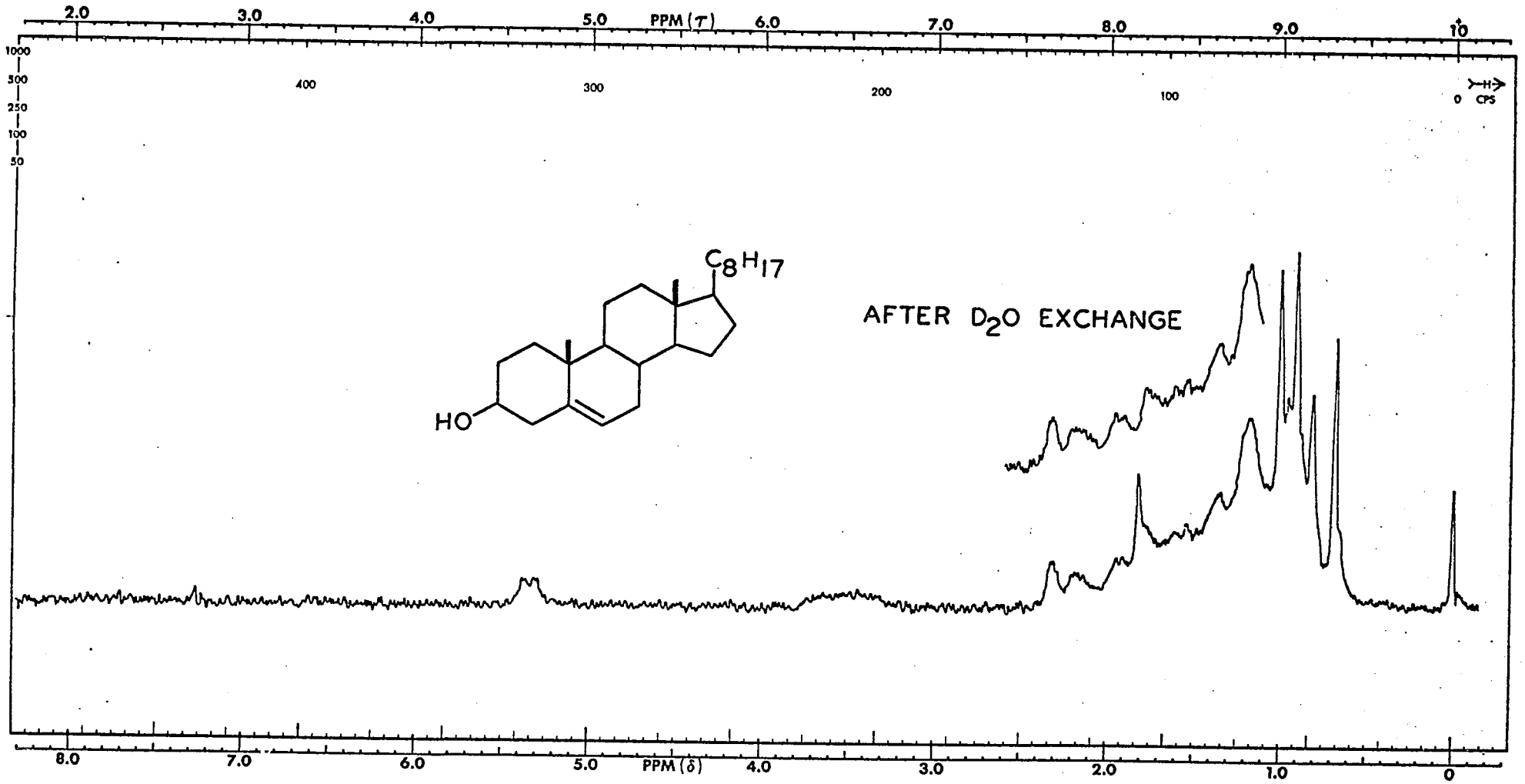
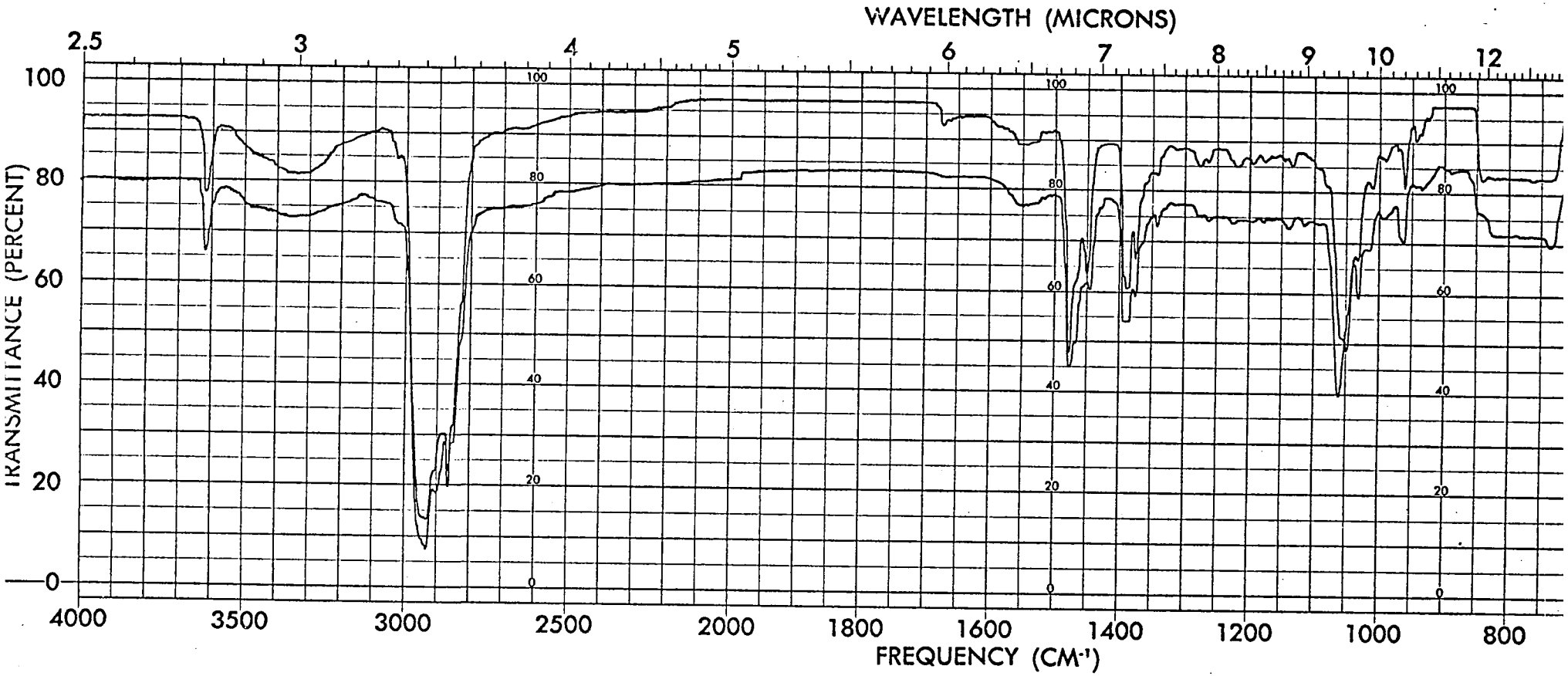

2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
9.0
10
1000
500
250
100
50
400
300
200
100
0
CPS
C8H17
HO
AFTER D2O EXCHANGE
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0

FIGURE 29

Infrared Spectra of

10α- and 10β-Cholesterol

in Carbon Tetrachloride Solution



Upper curve: 10α-cholesterol

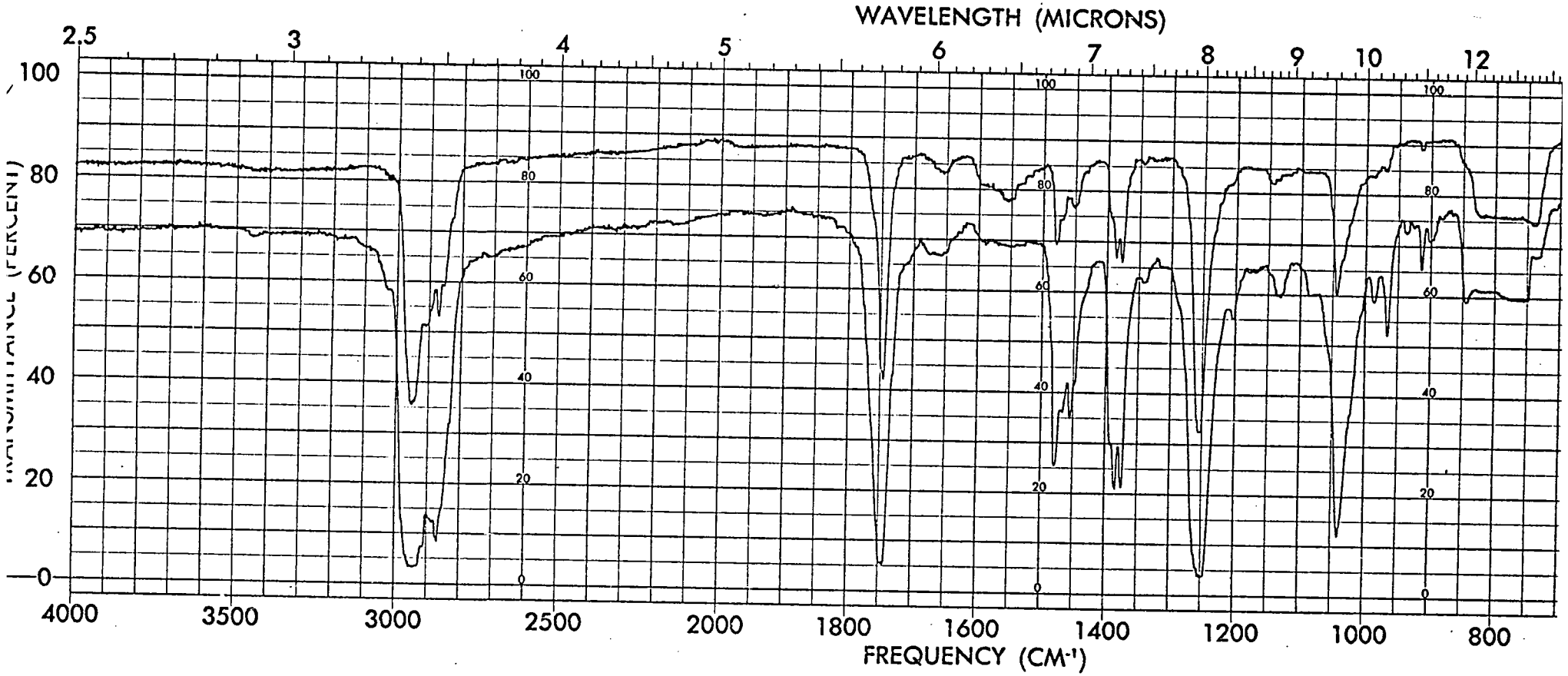
Lower curve: 10β-cholesterol

FIGURE 30

Infrared Spectra of

10α- and 10β-Cholesterol Acetate

in Carbon Tetrachloride Solution



Upper curve: 10β-cholesterol acetate

Lower curve: 10α-cholesterol acetate

Summary

The general results of this work indicate that the theoretical predictions made as to the position and direction of alkylation of dehydro-Inhoffen's ketone (9) were justified. Attack at C-10 from the predicted β-direction gave 88% of the product whereas α-attack resulted in only 12%.

On surveying the overall synthetic scheme the advantages of methyl vinyl ketone become obvious. Firstly, the product 12 was formed directly. Secondly, the mode of formation ensured that any product of α-attack would be converted into the less polar ketone 14, thus making the separation of the 10α- and 10β-epimers easy. Thirdly, the immediate product was a polar ketol with a relatively high melting point which facilitated purification by crystallization. Finally, the ketol structure provided an excellent mask for the 4,5-double bond, (later 5,6-double bond), while the 9,11-double bond was being hydrogenated.

With this method as the key step, cholesterol has been converted to 10α-cholesterol in an eleven-step synthesis in an overall yield of 6.4%. However, should 10α-cholesterol have biological or medical significance, one can be certain that improved methods and techniques will improve this, since necessity is truly the mother of invention.

EXPERIMENTAL

1. All chemicals used were reagent grade, unless otherwise specified.
2. Melting points were taken on a Gallenkamp melting point apparatus and are corrected.
3. Infrared spectra were made on Perkin-Elmer 337 and 521 grating spectrophotometers in 0.1 mm. sodium chloride cells using "spectral" grade carbon tetrachloride as solvent. Infrared absorption band intensities are listed as: (w) weak, (m) medium, (s) strong, and (sh) shoulder. Band positions are quoted in reciprocal centimeters ($cm.^{-1}$).
4. Ultraviolet spectra were taken on a Unicam SP-800 recording spectrophotometer in 1 cm. quartz cells. The solvent was 95% ethanol, unless otherwise noted.
5. Nuclear magnetic resonance spectra were made on a Varian A-60 spectrometer equipped with a time averaging computer. The solvents used were "spectral" grade carbon tetrachloride or deuterochloroform. Peak positions are given in parts per million (p.p.m.) relative to a tetramethylsilane internal standard.
6. Optical rotations were taken on a Carl Zeiss $.005^{o}$ photoelectric polarimeter, using chloroform solutions in a 1 decimeter cell.
7. The optical rotatory dispersion curve was made on a JASCO spectropolarimeter at the University of Ottawa through the kindness of Professor Peter Morand.
8. Elemental analyses were performed by Dr. C. Daessle, Montreal, and by Alfred Bernhardt, Mulheim (Ruhr).

9. Vapour phase chromatography was done on an Aerograph 705 Autoprep using nitrogen as carrier gas.
10. All column chromatography was done on Woelm neutral alumina, grade III.
11. All solutions were dried over anhydrous magnesium sulphate prior to evaporation.
12. The courses of all reactions and the purity of all compounds were checked by thin-layer chromatography on microscope slides coated with silica gel.

Cholesterol (1)

Cholesterol was used as received from Canada Packers Limited (m.p. 144°-145°).

Cholest-4-en-3-one (2)

Cholest-4-en-3-one (m.p. 80°-81°) was prepared by the method of Eastham and Teranishi[37] in 85% yield.

Windaus' Keto-acid (3,5-Secocholestan-5-one-3-oic acid) (3)

Windaus' keto-acid (m.p. 153°-154° was prepared by the method of Edward et al[38] in 74% yield.

Inhoffen's Ketone (10 α-des-A-cholestan-5-one) (4)

Essentially the procedure of Hartshorn and Jones[36] was followed; however these authors gave few experimental details.

In 300 ml. of methanol 23.5 gm. of Windaus keto-acid was dissolved with stirring, and enough sodium methoxide (6.7 gm.) was added nearly to neutralize the solution. A few drops of phenolphtalein in methanol were added, and the solution titrated with 0.25 N sodium methoxide in methanol until a violet colour just persisted. One drop of concentrated hydrochloric acid was then added, and the solution evaporated to dryness under vacuum. The resulting sodium salt was dried at 100°C. for two hours. Sodium phenylacetate was prepared from phenylacetic acid in the same manner.

The sodium salt of Windaus' keto-acid (25 gm.) and 100 gm. of

sodium phenylacetate were powdered together with 3 gm. of asbestos powder in a mortar, and the dry mixture poured into a 250 ml. round-bottom flask. This was connected by a short 90° elbow to a 100 ml. receiving flask equipped with a side-arm. The elbow was wrapped with heating tape and insulation, and the side-arm connected to a mercury-diffusion pump capable of maintaining a pressure of 0.05 mm. of mercury in the system.+

The flask containing the powdered solid was submerged in a bath of molten solder at 250°, the vacuum was cautiously connected, and the heating tape regulated so as to keep the elbow at about 250°. The solder bath was allowed to heat up to 310°, and was held there for two hours while the pale yellow oil distilled into the receiving flask which was cooled by a stream of cold water. After two hours the apparatus was removed and disassembled immediately to prevent joint seizure. The oil (which had a distinct odour of roses) weighed 19.3 gm. and contained a considerable amount of some non-polar impurity which could not be removed by steam distillation or chromic acid oxidation.

Chromatography of the crude product in two lots on columns of 300 gm. of alumina, using hexane as eluant, followed by crystallization from petroleum ether (30°-60°) gave 6.7 gm. of crystals. Further chromatography of the mother liquors yielded 6.5 gm. more. Total yield was 69%.

Melting Point: 61°-62° (literature m.p. 62°-63° [36]).

+ A liquid nitrogen trap is essential.

Infrared Spectrum: 2940 (s), 2860 (s), 1720 (s), 1465 (m), 1455 (m), 1375 (m), 1310 (w), 1280 (w), 1220 (w), 1190 (w), 1160 (w), 1120 (w), 1080 (w), 975 (w), 965 (w), 935 (w), 915 (w).

Nuclear Magnetic Resonance Spectrum: 0.71 (singlet), 18-methyl; 0.82 (doublet, J = 3.5 c.p.s.), 19-methyl.

The 2,4-dinitrophenylhydrazone derivative had a m.p. 173°-174° (literature m.p. 177°-178°).[35]

2-(3'-Chlorobut-2'-enyl)-2-methylcyclohexanone (56) and 6-(3'-Chlorobut-2'-enyl)-2-methylcyclohexanone (57)

The reaction was essentially described by Julia.[62] However, several improvements have been made, and the crude product analyzed by VPC, a tool not available to Julia.

Sodium tertiary-amylate solution in benzene (2N) was prepared by dissolving 4 ml. of redistilled tertiary-amyl alcohol in 20 ml. of sodium-dried benzene. Freshly cut sodium metal (1.2 gm.) was added, and the mixture stirred and refluxed under dry nitrogen for eight hours until no more sodium would react. The clear solution was cooled and a 1 ml. aliquot was withdrawn and added to 10 ml. of 0.2N standardized hydrochloric acid. This was titrated with 0.2N standard sodium hydroxide solution to the phenolphthalein end point. The solution could be kept for about two days under nitrogen, but after that time it formed a brown gummy mass.

In an oven-dried 25 ml. three-necked flask, 1 ml. of 2-methylcyclohexanone (55) and 1 ml. of freshly distilled 1,3-dichlorobut-2-ene were dissolved in 5 ml. of sodium-dried benzene. The flask was cooled

to 5°, dry nitrogen passed through it, and 5 ml. of 2N sodium tertiary-amylate solution added over a period of five minutes. The solution was stirred at 5° for one hour, and refluxed for one hour. It was then diluted with ether, washed with water and with saturated sodium chloride solution, dried, and evaporated under reduced pressure.

The crude residue was fractionated by preparative vapour-phase chromatography on a column 20' x 3/8" packed with 20% SE-30 on Chromosorb W (60-80 mesh). The column temperature was 250° and the carrier gas flow was 200 ml./min. Retention times were 11.0 min. for the 2-isomer (56) and 12.5 min. for the 6-isomer (57) and the ratio of peak areas was 5:1:1.5 (2-isomer:6-isomer:unreacted starting material).

Infrared Spectrum of 56 (pure liquid): 3400 (w), 3040 (w), 2970 (sh), 2940 (s), 1720 (s), 1665 (s), 1450 (s), 1430 (s), 1380 (s), 1345 (m), 1320 (m), 1260 (w), 1235 (m), 1210 (w), 1165 (w), 1130 (s), 1090 (m), 1070 (s), 1045 (w), 1025 (w), 990 (m), 965 (w), 925 (w), 900 (w), 855 (w), 800 (w), 700 (w), 630 (s).

Infrared Spectrum of 57 (pure liquid): 3400 (w), 3040 (w), 2975 (sh), 2950 (s), 2875 (s), 1720 (s), 1665 (m), 1465 (m), 1450 (m), 1440 (m), 1385 (m), 1340 (w), 1320 (w), 1260 (w), 1235 (w), 1130 (s), 1080 (s), 1020 (w), 985 (w), 960 (w), 930 (w), 900 (w), 855 (w), 800 (w), 680 (w).

Nuclear Magnetic Resonance Spectrum of the 2-Isomer (56): 1.05 (singlet), quaternary methyl; 2.05 (singlet), vinyl methyl; 5.35 (triplet),

vinyl proton.

6α-(3'-Chlorobut-2'-enyl)-10α-des-A-cholestan-5-one (5)

Sodium tertiary-amylate was prepared as in the previous example, except that only 10 ml. of benzene was used. Titration showed the concentration to be 3.8N.

Inhoffen's ketone (4) (1.50 gm.) and freshly distilled 1,3-dichlorobut-2-ene (0.71 gm.) were dissolved in 10 ml. of sodium-dried benzene under nitrogen in an oven-dried three-necked flask cooled to 5°. After adding 1.25 ml. of 3.8N sodium tertiary-amylate solution, stirring was continued for one hour while the flask warmed up to room temperature. Reflux for two hours followed. Dilution with ether, washing with water and with saturated sodium chloride solution, drying, and evaporation yielded a clear yellow oil. This was separated on 80 gm. of alumina to give 0.82 gm. (43%) of the pure chloroketone (5) which was a clear oil (eluted with hexane-5% ether). The preceding and ensuing fractions contained considerable amounts of product, however, they were not pure.

Infrared Spectrum: 3040 (sh), 2970 (s), 2885 (s), 1725 (s), 1675 (m), 1480 (m), 1460 (m), 1370 (m), 1350 (m), 1310 (w), 1290 (w), 1125 (w), 970 (m), 625 (m).

Nuclear Magnetic Resonance Spectrum: 0.71 (singlet), 18-methyl; 0.80 (doublet, J = 4.5 c.p.s.), 19-methyl; 2.2 (singlet), vinyl methyl; 5.47 (multiplet), vinyl proton.

In earlier attempts to improve the yield of alkylation, the following solvents were used in conjunction with sodium tertiary-amylate in benzene as the basic catalyst: dimethylformamide, dimethylsulphoxide-benzene (75-25), isopropyl ether, tertiary-amyl alcohol-benzene (7-3), toluene, and diglyme. Reaction times and temperatures were also varied, but in no case could the yield be increased. Analysis of the product mixtures was by vapour-phase chromatography on a glass column 10' x 4 mm. packed with 3% SE-30 on Chromosorb W. At 250° with a carrier gas flow of 90 ml./min., Inhoffen's ketone had a retention time of 4.5 min. and the alkylation product, 13.5 min.

6α-(3'-Oxobutyl)-10α-des-A-cholestan-5-one (6)

The product from the previous alkylation reaction (0.80 gm.) was dissolved in 5 ml. of glacial acetic acid and poured into 12 ml. of ice-cold concentrated sulphuric acid under nitrogen. The dark solution was stirred at 0° for 5 minutes, and then poured over 50 gm. of crushed ice. The resultant mixture was extracted twice with ether, and this was washed with water, saturated sodium bicarbonate solution, dried, and evaporated. The crude product (0.677 gm.) was a light yellow oil and was chromatographed on 20 gm. of alumina. Benzene eluted 0.283 gm. (37%) of a clear oil.

Infrared Spectrum: 2960 (s), 2940 (s), 2880 (s), 1720 (s), 1475 (m), 1455 (m), 1410 (w), 1380 (m), 1160 (w), 1150 (m), 940 (w).

10α,6β-Anthracholest-4-en-3-one+ (7)

The product of the above sulphuric acid treatment (6) was dissolved in 5 ml. of glacial acetic acid, 0.5 ml. of concentrated hydrochloric acid was added, and the solution was left overnight at room temperature. Dilution with water was followed by two ether extractions which were combined, washed with water and saturated sodium bicarbonate solution, dried, and evaporated at reduced pressure to give a yellow oil.

This was chromatographed on 20 gm. of alumina. Hexane-50% benzene eluted 0.122 gm. of the unsaturated ketone 7 which was crystallized twice from methanol. (Yield: 43%).

Melting Point: 122.5°-123.5°.

Infrared Spectrum: 3030 (sh), 2955 (s), 2865 (s), 1680 (s), 1625 (m), 1475 (m), 1460 (m), 1380 (m), 1340 (m), 1255 (m), 1205 (w), 1175 (w), 1150 (w), 1055 (w), 1020 (w), 975 (w), 910 (w), 900 (w), 880 (w), 720 (w), 690 (w).

Ultraviolet Spectrum: $\lambda_{max.}$ 244 mμ, $\log \epsilon_{max.}$ 4.10 .

Nuclear Magnetic Resonance Spectrum: 0.70 (singlet), 18-methyl; 0.85 (doublet, J = 3.5 c.p.s.), 19-methyl; 5.65 (singlet), C-4 vinyl proton.

Analysis calc. for $C_{27}H_{44}O$: C, 84.31; H, 11.53%

Found: C, 84.47; H, 11.33%

+ Numbering system suggested in ref. 74.

The unsaturated ketone formed a red 2,4-dinitrophenylhydrazone, m.p. 166.5°-167.5°.

Analysis calc. for $C_{33}H_{48}O_4N_4$: C, 70.18; H, 8.57%

Found: C, 70.21; H, 8.56%

Dehydro-Inhoffen's Ketone (Des-A-Cholest-9-en-5-one) (9)

Inhoffen's ketone (4) (6.94 gm.) and N-bromosuccinimide (3.92 gm.) were placed in a 1 liter round-bottom flask equipped with a stirring magnet and reflux condenser, and 100 ml. of pentane and 350 ml. of carbon tetrachloride were added. The solution was illuminated for 45 minutes by a 500 watt photoflood bulb while being stirred. The flask and lamp were encased in a jacket of aluminum foil, and a blower was necessary to cool the flask so that the solvent would not reflux too violently. After cooling and filtration, the solvent was removed at room temperature under reduced pressure to yield a brown oil, which was the crude bromide 8. This was immediately used in the next reaction.

The crude oil was dissolved in 35 ml. of dimethylformamide and 3.4 gm. of anhydrous lithium chloride added. This mixture was heated on the steam bath with occasional swirling, for four hours. After dilution with ether, water was added, the ether layer separated, washed with water, with 2N-hydrochloric acid, with water, and with saturated sodium chloride solution. It was then dried and concentrated at reduced pressure to give 6.85 gm. of a dark brown oil. This was chromatographed on 210 gm. of alumina. Hexane and hexane-benzene mixtures eluted 2.95 gm. of starting material, 0.88 gm. of mixture, and 2.46 gm. of dehydro-

Inhoffen's ketone (9). The recovered starting material could be recycled to raise the overall yield to 55%.

The product was a pale yellow oil but formed a dark red 2,4-dinitrophenylhydrazone m.p. 179.0°-179.5° (literature m.p. 179°-181° [36]).

Infrared Spectrum: 3050 (sh), 2960 (s), 2875 (s), 1675 (s), 1610 (m), 1475 (m), 1450 (sh), 1430 (m), 1380 (m), 1330 (m), 1320 (m), 1310 (m), 1250 (w), 1170 (m), 1135 (w), 1085 (w), 1010 (w), 935 (w).

Ultraviolet Spectrum: $\lambda_{max.}$ 249 mμ, log $\epsilon_{max.}$ 4.16 (literature $\lambda_{max.}$ 248.5 mμ, log $\epsilon_{max.}$ 4.21[36]).

Nuclear Magnetic Resonance Spectrum: 0.80 (singlet), 18-methyl; 1.69 (singlet), vinyl 19-methyl; no peaks due to olefinic protons.

10-(3'-Chlorobut-2'-enyl)-des-A-cholest-9(11)-en-5-one (10 and 11)

All equipment was oven-dried before use. Sodium tertiary-amylate solution was made up by the usual procedure and titrated at 2.42N.

Dehydro-Inhoffen's ketone (7.6 gm.) and 1,3-dichlorobut-2-ene (3.3 gm.) were dissolved in 400 ml. of dry benzene under nitrogen. The flask was cooled in an ice bath and 11.4 ml. of the sodium tertiary-amylate solution added through a dropping funnel at a rate of 1 drop/second while the solution was stirred. After addition, the solution was allowed to come to room temperature for one hour, and then refluxed for two hours. It was diluted with ether, washed twice with water and then with saturated sodium chloride solution, dried, concentrated, and chromatographed on 270 gm. of alumina. The product, which was eluted

with hexane and hexane-benzene mixtures, was not well separated, but 1.5 gm. was obtained pure and 6.0 gm. as a slightly impure mixture. (Yield: 78%). The pure fraction was a pale yellow oil. Attempts were made to prepare the 2,4-dinitrophenylhydrazone and semicarbazone derivatives for analysis, but only oils could be obtained.

Infrared Spectrum: 3040 (sh), 2970 (s), 2940 (sh), 2880 (s), 1720 (s), 1670 (m), 1475 (m), 1460 (m), 1435 (w), 1380 (m), 1160 (w), 1075 (w), 630 (w).

Nuclear Magnetic Resonance Spectrum: 0.63 (singlet), 18-methyl; 0.78 (singlet), 19-methyl; 2.02 (singlet), vinyl methyl; 5.27 (broad singlet), vinyl protons.

Analysis calc. for $C_{27}H_{43}OCl$: C, 77.40; H, 10.32%

Found: C, 77.32; H, 10.28%

Attempted Formation of Ring A Compounds from 10-(3'-Chlorobut-2'-enyl)-des-A-cholest-9(11)-en-5-one (10 and 11)

The product of the previous alkylation (0.388 gm.) was dissolved in 4 ml. of trifluoroacetic acid and the flask cooled in a dry ice-acetone bath. Concentrated sulphuric acid (80 ml.), precooled to -10^{o}, was poured in and the mixture was then frozen. This was allowed to warm up to 0^{o} in an ice bath while stirring under nitrogen. After 30 minutes it was poured over 200 gm. of ice and extracted with ether. This extract was washed with water and with saturated sodium bicarbonate solution, dried, and concentrated. The resulting dark brown oil residue was chromatographed on 80 gm. of alumina. Benzene

eluted 0.166 gm. (45%) of an oil. Experimental conditions were varied, but no increase in the yield was obtained.

Infrared Spectrum: 3030 (sh), 2940 (s), 2860 (s), 1720 (sh), 1710 (s), 1680 (sh), 1455 (m), 1430 (sh), 1380 (m), 1360 (m), 1290 (w), 1165 (m), 1100 (w), 970 (w).

The oil was dissolved in 10 ml. of anhydrous dioxane at 0° and a solution of 100 mg. of sodium metal in 25 ml. of absolute ethanol was added. The solution was left at 0° under nitrogen for five days, after which time 5 ml. of glacial acetic acid was added, and the solvent removed under reduced pressure at room temperature. Water and ether were added to the residue, and the ether layer washed with water and saturated sodium bicarbonate solution, dried, and evaporated.

Chromatography on 25 gm. of alumina yielded 11.5 mg. (7%) of a polar product (eluted by benzene-10% ether), which could not be crystallized.

Infrared Spectrum: 3500 (sh), 3450 (broad peak), 3100 (w), 3080 (w), 3045 (m), 2960 (s), 2870 (s), 1960 (w), 1735 (s), 1605 (w), 1595 (w), 1475 (m), 1395 (m), 1380 (s), 1125 (s), 1075 (m), 1040 (m), 1000 (w), 940 (w), 740 (w).

10α-Cholest-9(11)-en-5α-ol-3-one (12)

Dehydro-Inhoffen's ketone (9) (8.30 gm.) was dissolved in 60 ml. of anhydrous dioxane and a solution of 0.58 gm. of sodium dissolved in 190 ml. of absolute ethanol was added. The flask was cooled to -15° in an ice-salt bath and dry nitrogen was passed through it. A solution of 6.5 ml. of freshly distilled methyl vinyl ketone in 50 ml.

of anhydrous dioxane was dripped in over 9.5 hours, while stirring, and the reaction was left to proceed in the refrigerator (0^{o}) under nitrogen for two days. At this point 2 ml. of methyl vinyl ketone in 2 ml. of dioxane was added, the flask was re-flushed with nitrogen, and left at 0^{o} for two more days. Glacial acetic acid (10 ml.) was then added, and the yellow solution evaporated to dryness under reduced pressure at room temperature. Water was added, and the mixture extracted twice with ether. The ethereal layers were combined, washed with water and with saturated sodium bicarbonate solution, dried, and evaporated. Chromatography on 285 gm. of alumina gave 3.73 gm. (37%) of the unsaturated ketol 12, which was eluted by benzene-10% ether. This was recrystallized from petroleum ether (30^{o}-60^{o}) to give white crystals.

Melting Point: 148.5^{o}-149.5^{o}.

Optical Rotation: $[\alpha]_{D}$ + 61^{o} (C, 0.55).

Infrared Spectrum: 3600 (m), 3050 (w), 2955 (s), 2930 (s), 2865 (s), 1720 (s), 1675 (m), 1470 (m), 1425 (w), 1375 (m), 1320 (w), 1000 (w).

Nuclear Magnetic Resonance Spectrum: 0.59 (singlet), 18-methyl; 0.82 (singlet), 19-methyl; 1.98 (singlet), hydroxyl proton (disappeared on D_2O exchange); 1.5-3.0 (multiplet), protons α to the carbonyl group; 5.63 (broad singlet), vinyl proton at C-11.

Analysis calc. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07%

Found: C, 81.26; H, 10.96%

Cholesta-4,9(11)-dien-3-one (14)

In the chromatographic purification of the previous product (12), a brown oil was obtained from the earlier fractions. This was shown by thin-layer chromatography to be unreacted starting material (9) (about 18% yield) plus a small proportion of a slightly more polar compound. After several weeks, crystals of this by-product formed in the oil and were removed by decantation and filtration. Crystallization from petroleum ether (30°-60°) and methanol yielded 0.465 gm. (5% yield from 9) of cholesta-4,9(11)-dien-3-one (14).

Melting Point: 116°-116.5°.

Optical Rotation: $[\alpha]_D$ + 81° (C, 1.84).

Infrared Spectrum: 3040 (w), 2955 (s), 2930 (s), 2865 (s), 1680 (s), 1610 (m), 1465 (m), 1375 (m), 1340 (m), 1270 (m), 1230 (m), 1185 (w), 990 (w), 965 (w), 930 (w), 910 (w), 865 (m), 720 (w), 690 (w).

Ultraviolet Spectrum: $\lambda_{max.}$ 241 mμ, log $\epsilon_{max.}$ 4.18.

Nuclear Magnetic Resonance Spectrum: 0.65 (singlet), 18-methyl; 0.82 (singlet), 19-methyl; 5.45 (broad singlet), vinyl proton at C-11; 5.73 (sharp singlet), vinyl proton at C-4.

Analysis calc. for $C_{27}H_{42}O$: C, 84.75; H, 11.07%

Found: C, 84.55; H, 11.05%

5β-Cholest-9(11)-en-3α-ol (72) (?)

Cholesta-4,9(11)-dien-3-one (84 mg.) was hydrogenated over 61 mg. of pre-reduced Adam's catalyst (PtO_2) in 15 ml. of acetic acid

containing one drop of concentrated hydrochloric acid, until rapid uptake of hydrogen ceased (about 30 minutes). The solution was filtered, diluted with ether, washed twice with water and once with saturated sodium bicarbonate solution, dried, and concentrated under reduced pressure. The crude residue was dissolved in 50 ml. of acetone, and chromic acid (Jones reagent [69]) was added dropwise with stirring until a reddish colour persisted for three minutes. Excess oxidant was destroyed by the addition of 5 ml. of methanol, and the solution was diluted with ether. Washing with water and with saturated sodium bicarbonate solution was followed by drying and concentrating under reduced pressure.

The solid residue was dissolved in 10 ml. of anhydrous diglyme, and 200 mg. of lithium tri-tertiary-butoxyaluminum hydride was added with stirring. The solution was left overnight at room temperature; water was added to destroy the excess hydride, followed by 10 ml. of glacial acetic acid. The mixture was extracted with ether, and the ether solution was washed four times with water and once with saturated sodium bicarbonate solution. Drying and concentration of the solution yielded a clear oil which was chromatographed on 10 gm. of alumina. Hexane-50% benzene eluted 15 mg. of an oil which did not crystallize, and benzene eluted 20 mg. of a solid which was crystallized from methanol.

Melting Point: 134°-135°.

Optical Rotation: $[\alpha]_D + 25^{\circ}$ (C, 0.13).

Infrared Spectrum: 3625 (w), 3350 (broad peak), 3040 (w), 2955 (sh), 2930 (s), 2870 (s), 1465 (m), 1375 (m), 1100 (s), 1020 (s), 915 (w).

This solid was acetylated by the method of Edwards and Rao[76]. To 20 ml. of absolute ethyl acetate, 0.025 ml. of 72% perchloric acid and 2.4 ml. of acetic anhydride were added. The solution was made up to 25 ml. with ethyl acetate, and immediately poured on to the solid. After 15 minutes at room temperature, saturated sodium bicarbonate solution and ether were added. The ethereal layer was separated, washed well with bicarbonate solution, dried, and concentrated under reduced pressure. To the residue, a few milliliters of methanol containing a trace of pyridine was added, and the solvent again removed. The acetate was obtained as a white solid, and was recrystallized from methanol.

Melting Point: 98°-99°.

Optical Rotation: $[\alpha]_D$ + 14° (C, 0.17)

Analysis calc. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29%

Found: C, 81.21; H, 11.20%

10α-Cholestan-5α-ol-3-one (17)

10α-Cholest-9(11)-en-5α-ol-3-one (12) (266 mg.) in 20 ml. of glacial acetic acid was hydrogenated at atmospheric pressure over 196 mg. of pre-reduced Adams' catalyst (platinum oxide). After uptake of hydrogen ceased (about two equivalents were absorbed in about 4 hours), ether was added, and the catalyst filtered off. The filtrate was washed twice with water, and once with saturated sodium bicarbonate solution, dried, and concentrated under pressure to give a white solid which was a mixture of the saturated diol 15 and alcohol 16.

Infrared Spectrum: 3620 (m), 3400 (broad peak), 2950 (s), 2865 (s), 1465 (m), 1445 (sh), 1375 (m), 1265 (s), 1160 (w), 1100 (s), 1020 (s), 935 (w), 870 (w), 685 (w).

The crude mixture was dissolved in 50 ml. of acetone and Jones reagent[69] was added dropwise until a reddish tinge persisted for three minutes. Excess oxidant was destroyed by the addition of 5 ml. of methanol, and the solution was diluted with ether. This was washed with water and with saturated sodium bicarbonate solution, dried, and concentrated under reduced pressure. The resulting solid was crystallized from petroleum ether (30°-60°) to give 197 mg. of the ketol 17 (74% from 12) in the form of white needles. This crystallization was fairly critical, since, if the solution was too concentrated, or was cooled in the refrigerator, only a sludge resulted. Seed crystals were usually necessary for best results.

Melting Point: 170.5°-171°.

Optical Rotation: $[\alpha]_D$ + 49° (C, 0.24).

Infrared Spectrum: 3610 (m), 3400 (broad peak), 2950 (s), 2870 (s), 1710 (s), 1470 (m), 1430 (sh), 1375 (m), 1280 (w), 1240 (w), 1115 (w), 1060 (w), 1030 (w), 1000 (w), 945 (m).

Optical Rotatory Dispersion Curve: See figure 23.

Analysis calc. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52%

Found: C, 80.69; H, 11.22%

Attempts were made to improve the yield by the use of the following catalysts and solvents: platinum oxide in ethanol or ethyl acetate with a trace of perchloric acid, palladium in ethanol or acetic

acid, and tris-(triphenylphosphine)-rhodium chloride[90] in benzene. Hydrogenation pressures varied from atmospheric to 4 atmospheres. In none of these cases could the 9,11-double bond be completely hydrogenated.

10α-Cholestan-5α-ol (16)

The combined mother liquors from the crystallization of 10α-cholestan-5α-ol-3-one (17) were chromatographed on alumina. The main byproduct of the hydrogenation (10α-cholestan-5α-ol (16)) was eluted with hexane-10% benzene and represented approximately a 20% yield from 12. Crystallization from methanol gave white crystals.

Melting Point: 79.5°-80°.

Optical Rotation: $[\alpha]_D$ + 53° (C, 0.18).

Infrared Spectrum: 3640 (m), 3350 (broad peak), 2950 (s), 2870 (s), 1475 (m), 1450 (sh), 1375 (m), 1250 (w), 1175 (w), 1120 (w), 1030 (m), 980 (w), 940 (w), 930 (w).

Analysis calc. for $C_{27}H_{48}O$: C, 83.43; H, 12.45%.

Found: C, 83.72; H, 12.25%.

10α-Cholestan-5α-ol (16) (50 mg.) in acetone was treated with Jones reagent in the same manner as described in the section on 10α-cholestan-5α-ol-3-one (17), but only starting material was isolated.

10α-Cholest-4-en-3-one (18) and 10α-Cholest-5-en-3-one (19)

10α-Cholestan-5α-ol-3-one (17) was dissolved in 10 ml. of dry benzene, 3-4 small crystals of para-toluenesulphonic acid were added,

and the solution was refluxed under nitrogen for two hours. Dilution with ether, washing with saturated sodium bicarbonate solution, drying, and concentration under reduced pressure gave a clear oil which was chromatographed on 12 gm. of alumina. Hexane-25% benzene eluted 27 mg. (22% yield) of a white solid which was crystallized from methanol-water to give pure 10α-cholest-5-en-3-one (19).

Melting Point: 112°-112.5°.

Optical Rotation: $[\alpha]_D$ -104° (C, 0.68).

Infrared Spectrum: 3030 (w), 2950 (s), 2860 (s), 1720 (s), 1680 (sh), 1460 (m), 1375 (m), 1330 (w), 1310 (w), 1230 (m), 1170 (w), 1125 (w), 1025 (w), 1005 (w), 960 (w), 925 (w).

Analysis calc. for $C_{27}H_{44}O$: C, 84.31; H, 11.53%.

Found: C, 84.14; H, 11.56%.

Hexane-50% benzene eluted a clear oil (90 mg., 73% yield) which could not be crystallized, but did form a red, crystalline 2,4-dinitrophenylhydrazone. The oil was assigned the structure 10α-cholest-4-en-3-one (18).

Infrared Spectrum: 3030 (sh), 2960 (s), 2875 (s), 1680 (s), 1630 (m), 1475 (s), 1425 (w), 1380 (m), 1250 (m), 1120 (w), 1005 (w), 930 (w).

Ultraviolet Spectrum: $\lambda_{max.}$ 243 mμ, log $\epsilon_{max.}$ 4.15

The 2,4-dinitrophenylhydrazone melted at 185°-185.5°. Its ultraviolet spectrum is found in table 1.

Analysis calc. for $C_{33}H_{48}O_4N_4$: C, 70.18; H, 8.57%.

Found: C, 70.08; H, 8.73%.

10α-Cholesta-3,5-dien-3-ol Acetate (20)

Both conjugated and unconjugated ketones 18 and 19 were convertible into the enol acetate 20 by the method described below. In actual practice the crude mixture was not separated, but immediately transformed into 20 by the method of Edwards and Rao[76].

Perchloric acid (0.05 ml. of 72% $HClO_4$) was added to 50 ml. of absolute ethyl acetate, and 10 ml. of this solution poured into a 50 ml. volumetric flask with 30 ml. of absolute ethyl acetate and 4.8 ml. of acetic anhydride (in that order). After making up to 50 ml. with ethyl acetate, it was poured on to the crude mixture of 10α-cholest-4-en-3-one (18) and 10α-cholest-5-en-3-one (19) derived from 825 mg. of 17, and left at room temperature for ten minutes. Saturated sodium bicarbonate solution and ether were added, and the ether layer was well washed with bicarbonate solution. After drying the ether solution and concentrating it under reduced pressure, 10 ml. of methanol containing a trace of pyridine was added, and the solvent was removed again. The residual white solid was crystallized from methanol to give 733 mg. of the enol acetate (20) in the form of white plates. (84% yield from 17).

Melting Point: 93°-94°.

Optical Rotation: $[\alpha]_D$ +32° (C, 0.36).

Infrared Spectrum: 3030 (sh), 2950 (s), 2925 (sh), 2860 (s), 1755 (s), 1660 (w), 1460 (m), 1430 (m), 1375 (m), 1360 (s), 1220 (s), 1200 (s), 1125 (m), 1115 (m), 1110 (m), 1045 (w), 1020 (w), 920 (w), 825 (w).

Ultraviolet Spectrum: $\lambda_{max.}$ 236 mμ, log $\epsilon_{max.}$ 4.18.

Analysis calc. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87%.

Found: C, 81.75; H, 10.93%.

Alkaline Hydrolysis of 10α-Cholesta-3,5-dien-3-ol Acetate (20)

The title compound (20) (5 mg.) was dissolved in 2 ml. of methanol and 0.25 ml. of 5% aqueous sodium hydroxide solution was added. The solution was heated to boiling for several minutes, cooled, diluted with ether, washed with water and with saturated sodium chloride solution, dried, and concentrated at reduced pressure. The infrared spectrum of the product showed two strong peaks in the carbonyl region: at 1720 cm.$^{-1}$ due to the unconjugated ketone 19 and at 1680 cm.$^{-1}$ due to the conjugated ketone 18. Their absorption intensities were in the ratio 1:2.

10β-Cholesta-3,5-dien-3-ol Acetate

This was made for comparison purposes from 10β-cholest-4-en-3-one (2) by the same method as the 10α-analogue.

Melting Point: 83°-84°, clears at 110° (literature m.p. 80°, clears at 105°-110° [89]).

Ultraviolet Spectrum: $\lambda_{max.}$ 236 mμ, log $\epsilon_{max.}$ 4.26.

10α-Cholesterol (10α-Cholest-5-en-3β-ol) (21)

A solution of 10α-cholesta-3,5-dien-3-ol acetate (20) (615 mg.) in 400 ml. of methanol was cooled to about 5°. Sodium borohydride (3 gm.) dissolved in 30 ml. of 85% aqueous methanol was added with stir-

ring, and the solution allowed to warm up to room temperature. After stirring for 2½ hours, 0.5 gm. of sodium borohydride was added, and the solution stirred overnight. The methanol was removed under reduced pressure and ether was added. Enough 2N hydrochloric acid was added to make the aqueous layer slightly acidic, and this layer was extracted well with ether. The combined ether extracts were washed with water and with saturated sodium bicarbonate solution, dried, and concentrated at reduced pressure. A small amount of a less polar impurity was removed by chromatography on 30 gm. of alumina. The 10α-cholesterol was eluted by hexane-50% benzene, and was crystallized from methanol to give 468 mg. (84%) of white needles.

Melting Point: 118.5°-119°.

Optical Rotation: $[\alpha]_D$ -46° (C, 1.14).

Infrared Spectrum: See figure 29.

Nuclear Magnetic Resonance Spectrum: See figure 27.

Analysis calc. for $C_{27}H_{46}O$: C, 83.87; H, 11.99%.

Found: C, 83.76; H, 11.79%.

Oxidation of 10α-Cholesterol (21)

To 20.5 mg. of 10α-cholesterol in 5 ml. of acetone, Jones reagent[69] was added dropwise with stirring until a reddish tinge remained for three minutes. Methanol (2 ml.) was added, followed by ether and saturated sodium bicarbonate solution. The ether layer was dried and concentrated under reduced pressure to give a white solid

which was crystallized from methanol yielding white crystals of 10α-cholest-5-en-3-one (19). Their identity was proven by melting point and mixed melting point determinations.

10β-Cholesterol

A pure sample for comparison purposes was prepared by crystallizing commercial cholesterol from methanol and petroleum ether (30°-60°).

Melting Point: 149.5°-150° (literature m.p. 150°)[83]. The melting point was taken under nitrogen in a sealed tube.

10α-Cholesterol Acetate

This was prepared by acetylation of 10α-cholesterol by the method of Edwards and Rao[76] as discussed on page 77.

Melting Point: 124°-124.5°.

Optical Rotation: $[\alpha]_D$ -47° (C, 1.48).

Infrared Spectrum: See figure 30.

Analysis calc. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29%.

Found: C, 81.14; H, 11.40%.

10β-Cholesterol Acetate

This was made for comparison purposes by the above method.

Melting Point: 114.5°-115.5° (literature m.p. 114°)[84].

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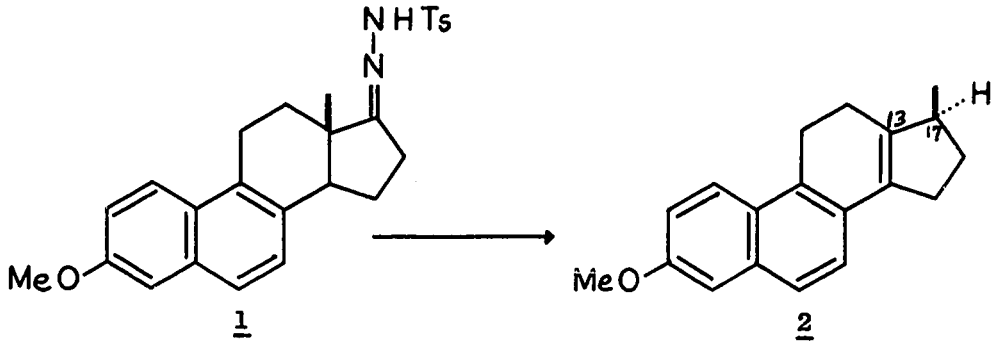
A P P E N D I X

The Protic Bamford-Stevens Reaction of Equilenin Methyl Ether Tosylhydrazone

Structure of the Reaction Product

As part of a study of the mechanism and scope of the Bamford-Stevens reaction begun by Edward and D' Anglais[1], the tosylhydrazone of equilenin methyl ether (1) was decomposed thermally in ethylene glycol in the presence of the alkoxide.



1 2

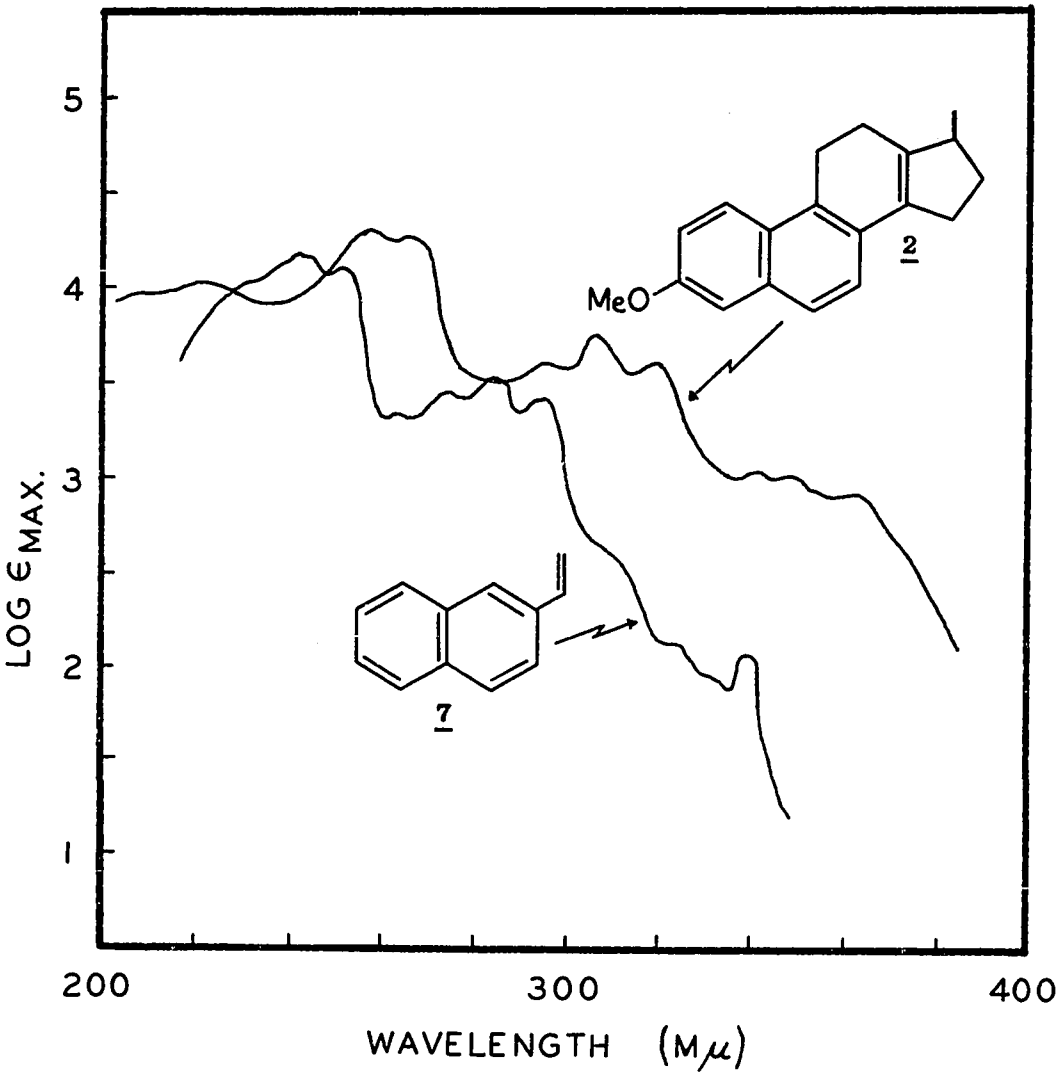
The brown product (m.p. 121°-122°; $[\alpha]_D$ +24.3°) which was obtained in 89% yield was assigned structure 2 on the basis of its nuclear magnetic resonance spectrum. This showed a doublet at 1.1 p.p.m. due to the methyl group at C-17, which was presumed to have shifted from C-13 during the reaction, and no peaks due to olefinic protons.

Further support for the assignment of 2 came from the ultraviolet spectra of 2 and 2-vinylnaphthalene (7), which show a striking similarity. (See figure 1).

However, while this work was in progress, Johns[2] reported that he had obtained a compound (6) of m.p. 84°-85° and $[\alpha]_D$ -1° by heating 17α-chloro-3-methoxyestra-1,3,5(10)-triene (4) in an ethanol

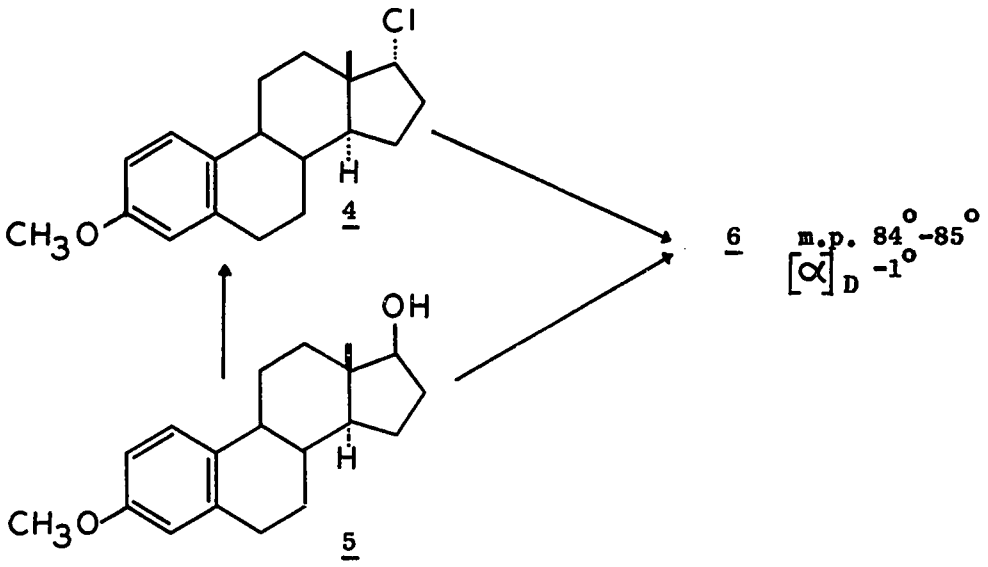
FIGURE 1

Ultraviolet Spectra of Decomposition Product of Equilenin Methyl Ether Tosylhydrazone (2) and of 2-Vinylnaphthalene (7)+



+ The spectrum of 2-vinylnaphthalene (7) was obtained from ref. 37.

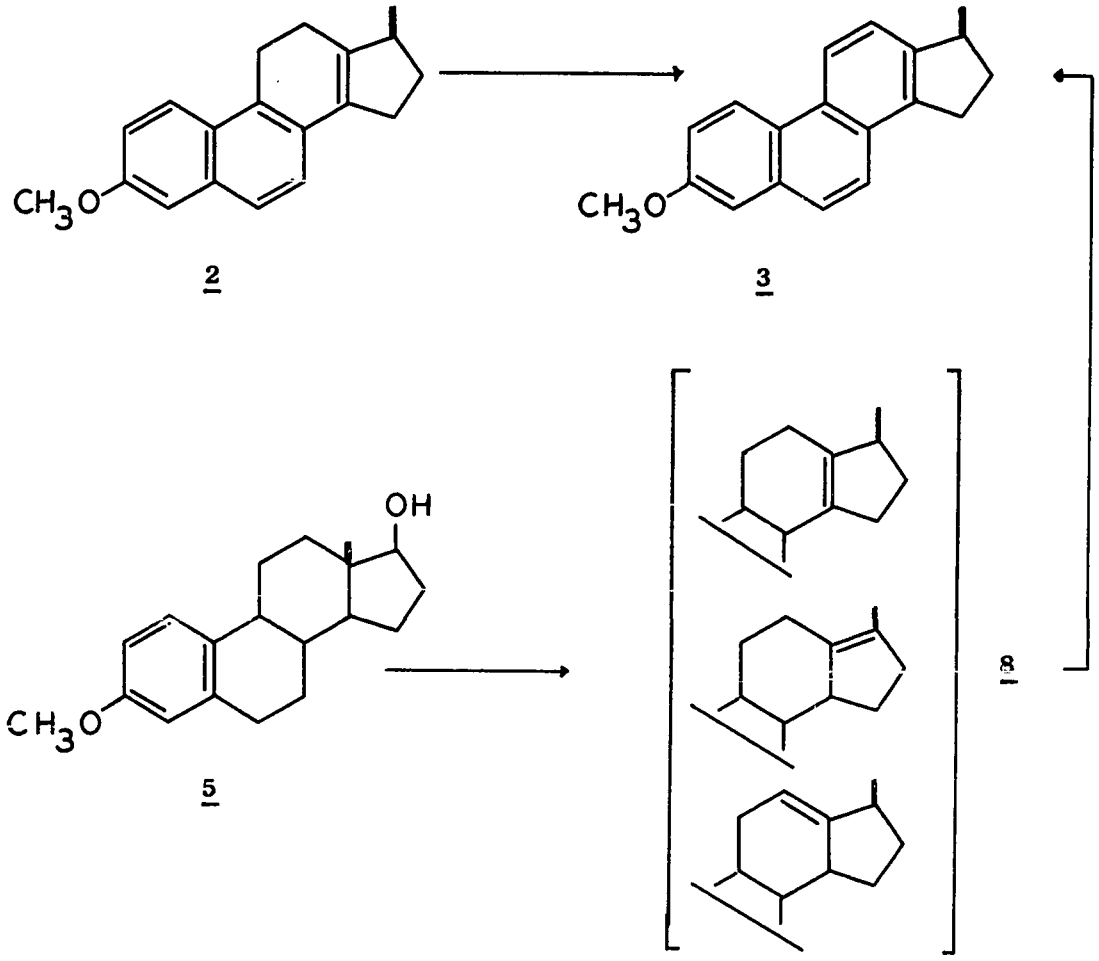
solution of potassium iodide and sodium acetate at 125° in a Paar bomb. The same product could be obtained in small yield by heating estradiol-3-methyl ether (5) in boric acid at 280° for one hour, or by simple chromatography of a crude preparation of 4 from treatment of 5 with phosphorus pentachloride.



Johns assigned structure 2 to his compound 6 on the basis of its nuclear magnetic resonance spectrum (3.818 p.p.m. and a doublet with peaks at 1.358 p.p.m. and 1.250 p.p.m.), and ultraviolet spectrum ($\lambda_{max.}$ 280 mμ; log $\epsilon_{max.}$ 3.41). However, it is difficult to imagine how such a compound could have arisen from Johns' reactions, but since some doubt had been cast on the structural assignment of the Bamford-Stevens reaction product, it was decided to provide definite chemical proof.

When 2 was refluxed in xylene with 5% palladium-on-charcoal,

it was quantitatively converted to the phenanthrene derivative 3. The properties of the product 3 agreed with those reported by Cohen, Cook, and Hewett[3], who had obtained it by heating estradiol-3-methyl ether (5) with zinc chloride, and dehydrogenating the mixture of olefins (8) thus obtained with selenium. This facile dehydrogenation of 2 to 3 established 2 as the structure for the Bamford-Stevens reaction product.



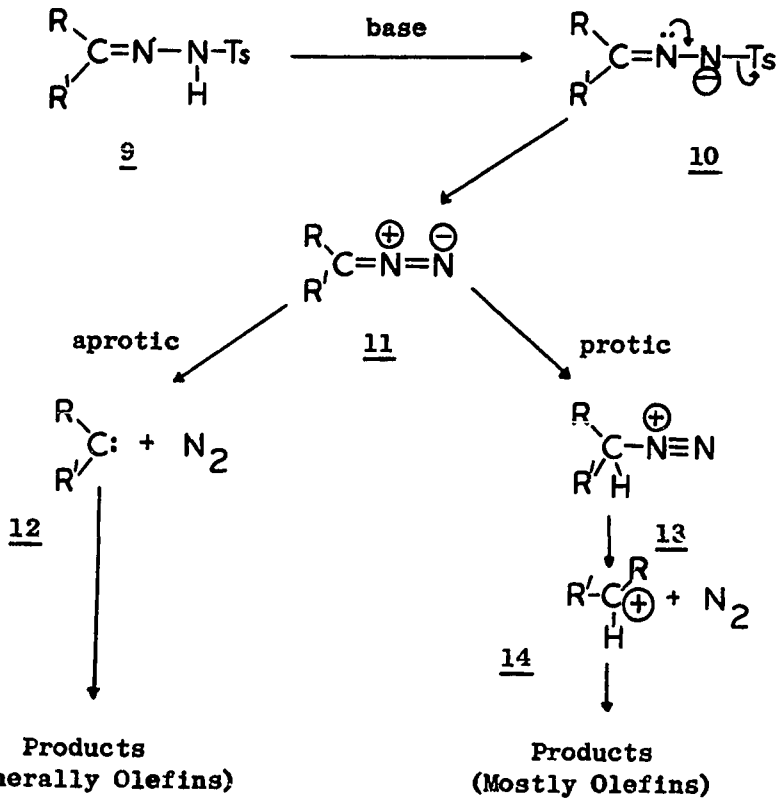
2 3 5 8

Mechanism of the Reaction

The usual course of the Bamford-Stevens reaction is to yield olefin(s), nitrogen, and p-toluenesulphinic acid when a solution of an aldehyde or ketone tosylhydrazone is heated in the presence of fairly strong base, such as an alkali alkoxide[4]. The accepted mechanism[5,6,13] involves the following steps+ (see figure 2):

FIGURE 2

The Mechanism of the Bamford-Stevens Reaction

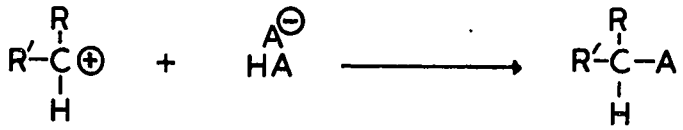


Ts = para-toluenesulphonyl

\+ A different mechanism results if sodium hydride or amide is used as the base[7].

First, base abstracts a proton from the nitrogen atom of the tosylhydrazone (9) to give anion 10, which immediately loses p-toluene-sulphinate to give the diazo compound 11. In aprotic solvents such as decalin or diglyme, nitrogen is lost directly to give a carbene (12), which reacts by intramolecular insertion into a carbon-hydrogen bond (commonly called an hydride shift[8]). Generally this results in an olefin.+ In protic solvents such as ethanol or ethylene glycol, the diazo form (11) is protonated to a diazonium ion (13). This loses nitrogen to form a carbonium ion (14) which can react in three ways:*

(a) It can react with an anion or the solvent.



(b) It can eliminate a proton to form an olefin.

+ Aprotic Bamford-Stevens reactions will not be considered further. For a review see ref. 9.

* Only the major paths are included here. Excluded are inertion-type reactions[10].

(c) A Wagner-Meerwein-type shift of some neighbouring group can occur to give a new carbonium ion, which can react as in (a) or (b).

This shift will occur in such a manner as to produce a new carbonium ion of lesser energy[11]. For example, a primary-carbonium ion is generally transformed by an alkyl shift into a tertiary-carbonium ion, but not the reverse.

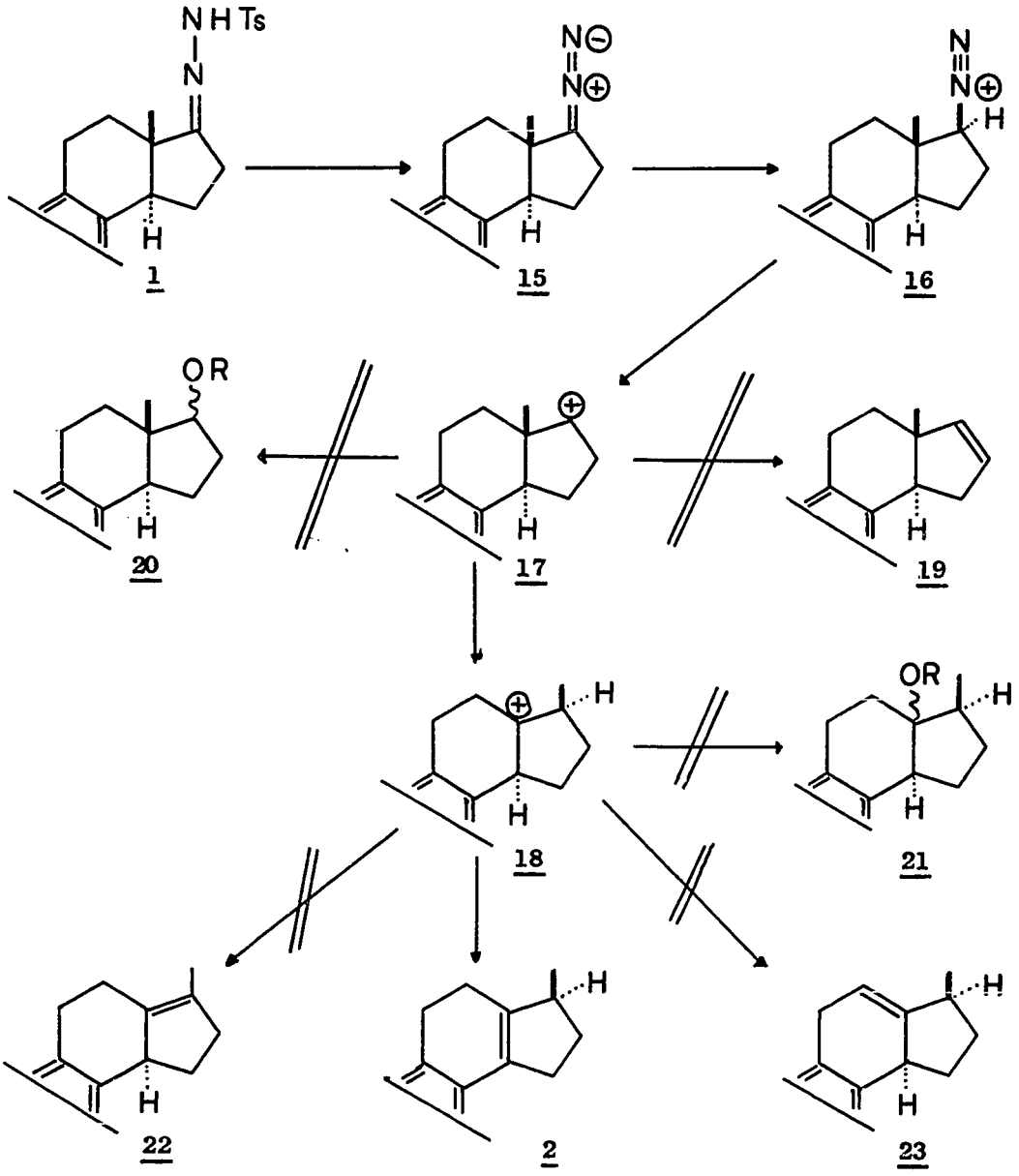
If this general mechanism is applied to the protic base decomposition of (1), the following steps can be envisaged (See figure 3):

The tosylhydrazone (1) decomposes in the usual manner to diazo compound 15. This is protonated by the solvent, probably from the less hindered α-side, to give the diazonium ion (16), which then decomposes into nitrogen and a secondary-carbonium ion at C-17. The β-methyl group at C-13 migrates in a 1,2-Wagner-Meerwein shift to C-17, resulting in a more stable tertiary-carbonium ion at (18). Finally, elimination of the 14α-hydrogen yields the product (2).

Several other reactions are known which initially resulted in the formation of a carbonium ion at C-17. These will be discussed together with the Bamford-Stevens reaction of equilenin methyl ether tosylhydrazone in an effort to elucidate the mechanisms which are

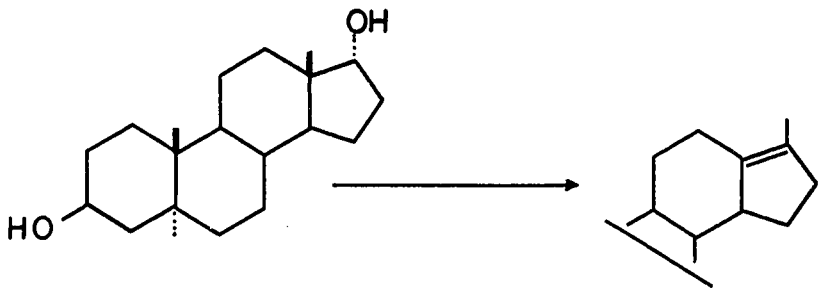
FIGURE 3

Reaction Scheme for the Bamford-Stevens Reaction of Equilenin Methyl Ether Tosylhydrazone (1)



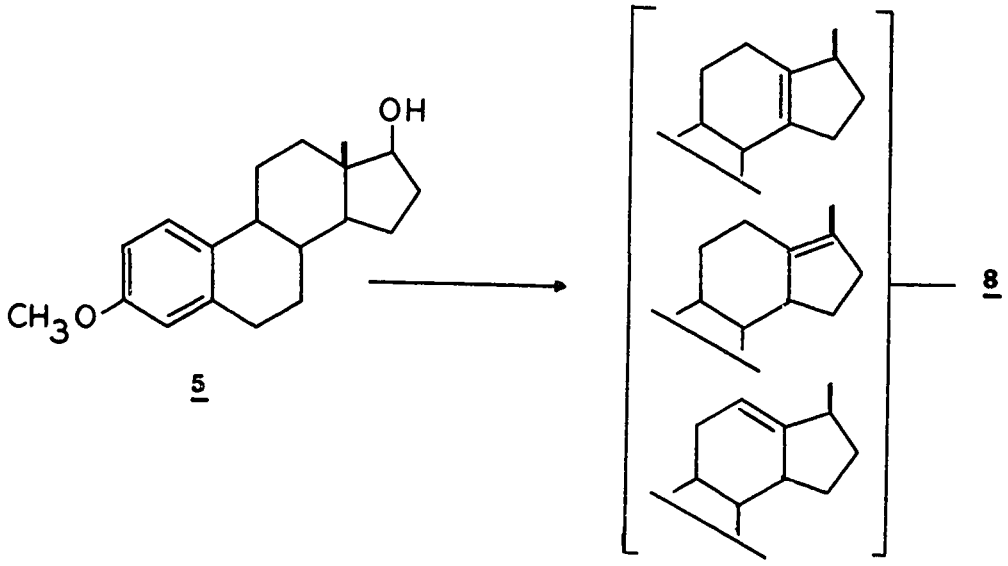
common to all, in more detail.

When compound 24 was refluxed for two hours in formic acid product 25 resulted[12].



24 25

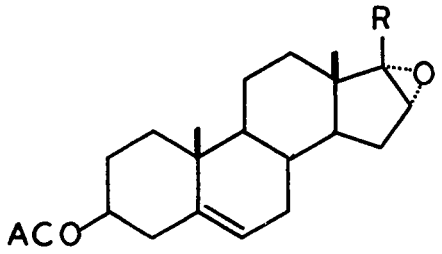
Compound 5, when heated in anhydrous zinc chloride for ½ hour at 170°-180° gave a mixture of olefin isomers 8 which were not separated[3].



5

When compound 26 was heated in acetic anhydride at 170° for 3 hours, 27 resulted[14].

R = CH_3CO-



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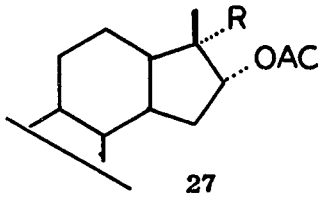
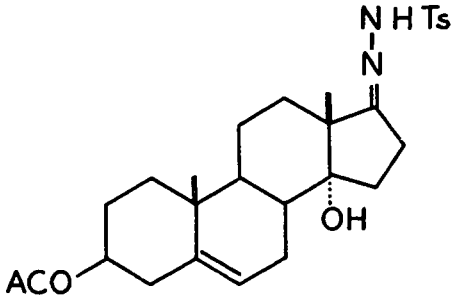
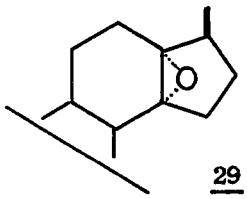


27

Decomposition of tosylhydrazone 28 led to 29[41]. Here, a 13,14-oxide was the product instead of an olefin.



28



29

All these reactions can be postulated to proceed through carbonium ion intermediates[15]; indeed, it is difficult to explain the Wagner-Meerwein methyl shifts from C-13 to C-17 in any other manner.

Like the protic decomposition of equilenin methyl ether tosyl-

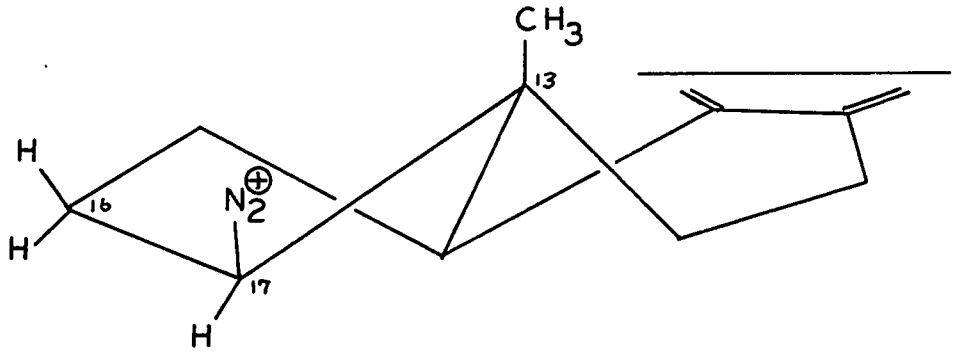
hydrazone (1) all these reactions generated their carbonium ions at a high temperature and in relatively poorly solvating media. This would be expected to yield carbonium ions with fairly high energies,+ which would attempt to stabilize themselves quickly by methyl migration. Because of this effective means of intramolecular stabilization and the lack of strongly nucleophilic solvents, no products of solvent attack upon a carbonium ion are observed (e.g. 20 and 21, figure 3). However, in some Bamford-Stevens reactions, solvent attack can be significant[6,18].

It is also noteworthy that in no case was a Δ^{16}-olefin (e.g. 19 from 17 in figure 3) corresponding to an elimination of a 16-hydrogen from the un-rearranged carbonium ion was isolated. This is so because the diazonium ion is not trans-diaxial to either 16-hydrogen (see figure 4), a condition which is necessary if facile elimination is to occur[20]. But neither is it trans-diaxial to the C-13 methyl group (see figure 4), which is also the usual requirement for 1,2-methyl shifts[36]. However, a certain degree of driving force for methyl migration is present in the natural strain due to the trans-junction of rings C and D[33], which is relieved when C-13 takes up the sp^2 trigonal configuration of a carbonium ion[19]. Artificially synthesized C-D cis-steroids do not rearrange, at least during an elimination at C-17, because there is comparatively little strain[33].

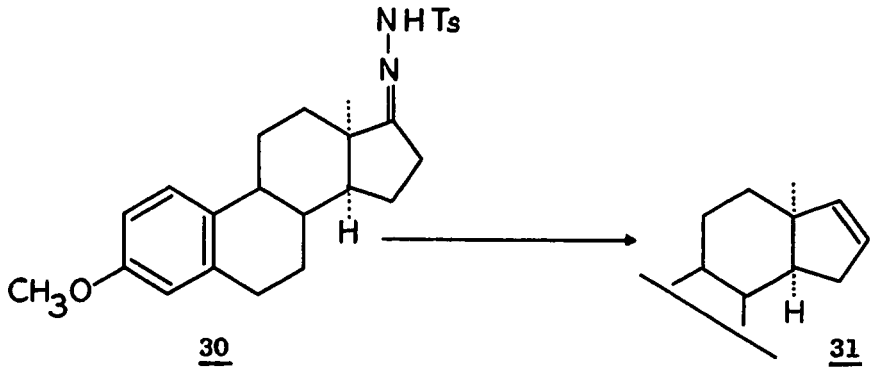
+ Diazonium ion decomposition normally yields carbonium ions with higher energies than those from solvolysis reactions because of the driving force to give stable nitrogen molecules[16,17].

FIGURE 4

Three-Dimensional Drawing of Diazonium Ion 16



By this token, the Bamford-Stevens decomposition of the lumiestrone derivative (30); should yield mostly the Δ^{16}-olefin (31); this hypothesis awaits experimental verification.

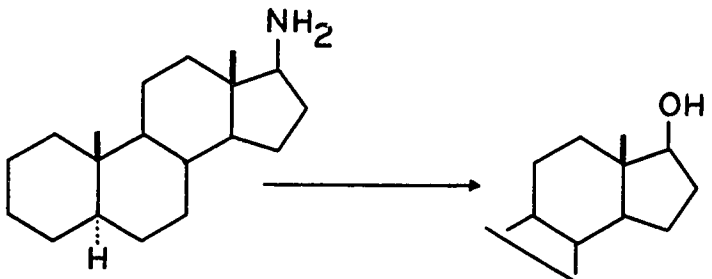


30 31

The other reactions which gave rise to carbonium ions at C-17 with the resulting methyl shift, have analogous mechanisms to the protic Bamford-Stevens reaction, once the carbonium ion is formed. The relative amounts of the final olefins vary greatly, in some cases because the experimenter did not investigate his crude product in any detail, and in others possibly because of subtle differences in the reaction conditions. However, the tendency of a C-17 carbonium ion in a C-D trans steroid formed at a high temperature to stabilize itself by a methyl shift is still evident.

In vivid contrast to the aforementioned examples, is the deamination of 17-androstanylamine (32) in aqueous solution, which yields only the product of solvent attack upon the first-formed carbonium ion at C-17 (33)[21].

This example shows that the temperature at which the carbonium ion is produced and the medium in which it finds itself, have a



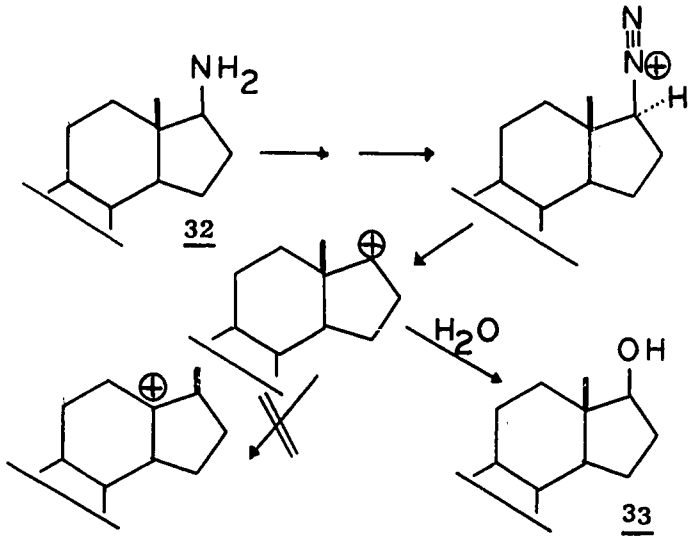
32 33

determining effect upon its subsequent reaction.

Deamination is known to occur through a diazonium ion which decomposes to a carbonium ion[22], in a similar manner to the protic Bamford-Stevens reaction (See figure 5).

FIGURE 5

Partial Mechanism of the Deamination of 17β-Amine 32

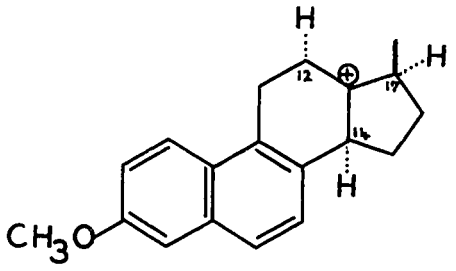


32 33

Only in this case, the decomposition takes place at a much lower temperature (0°C. vs. 120°-180° for the Bamford-Stevens reaction), and in water, which is also a good solvator and a good nucleophile. Thus the first-formed carbonium ion at C-17 is attacked by solvent before it can rearrange, since it does not have enough energy to do so quickly.

Position of Double Bond Formed

In carbonium ion 18, there are three axial hydrogens which can be eliminated to form an olefin: the 12α-, 14α-, and 17α-hydrogens.



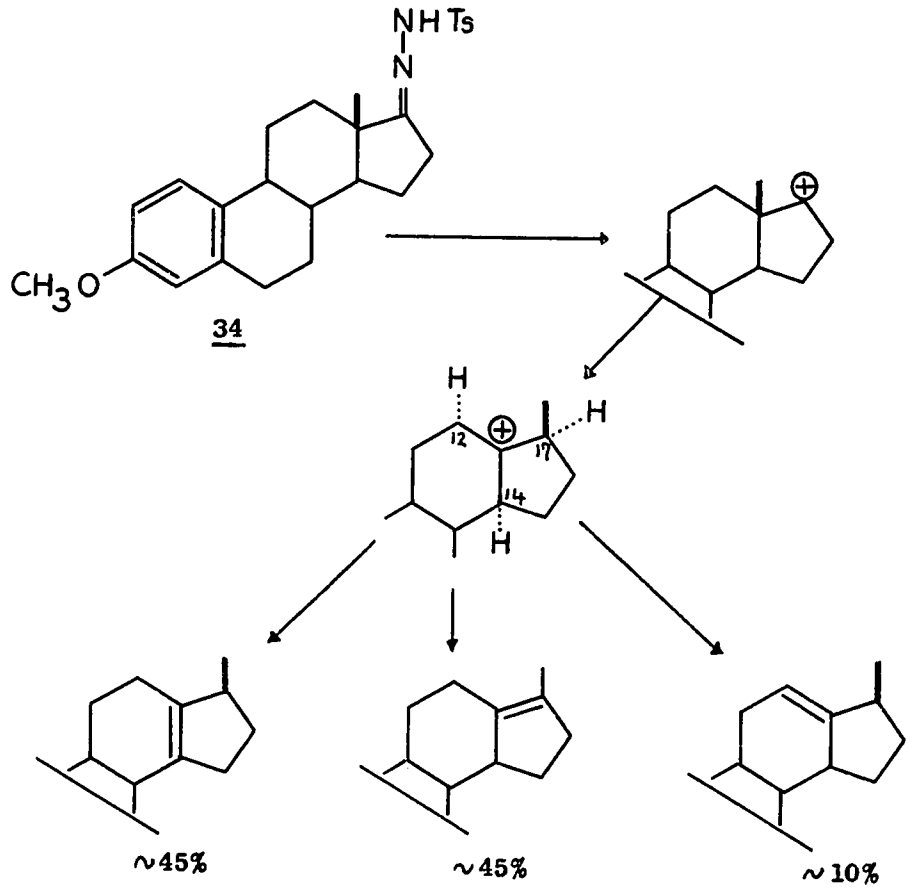
18

The preponderant elimination will be of the 14α-hydrogen, since it is α to the naphthalene system, and the resulting Δ^{13}-olefin will be conjugated with it. In addition, this double bond relieves the torsional strain present, when a 5-membered ring is fused to a 6-membered ring[23,24]. The 17α-hydrogen will be the next most likely to be eliminated because it will relieve the partial eclipsing of the

17β -methyl group with the 16β -hydrogen. The least likely hydrogen to go will therefore be the 12α.

In actual fact, the conjugation of the resulting Δ^{13}-double bond is the deciding factor; only very small amounts of olefins 22 and 23 (figure 3) could have been formed.

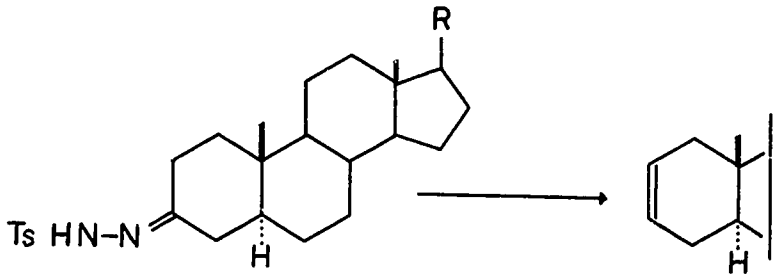
When the possibility of conjugation is removed, as in the protic decomposition of estrone-3-methyl ether tosylhydrazone (34), the probability of elimination becomes almost equal for the 14α- and 17α-hydrogens, with the 12α-hydrogen still less[1,2].



Implicit in these statements, is the hypothesis that the hydrogen eliminated in the protic Bamford-Stevens reaction is the one which will lead to the most stable olefin. In at least two cases this is justified:

1. Decomposition of cholestanone tosylhydrazone (35) gives only the Δ^2-olefin (36)[1,38], which is more stable than the other possibility, the Δ^3-olefin[39].

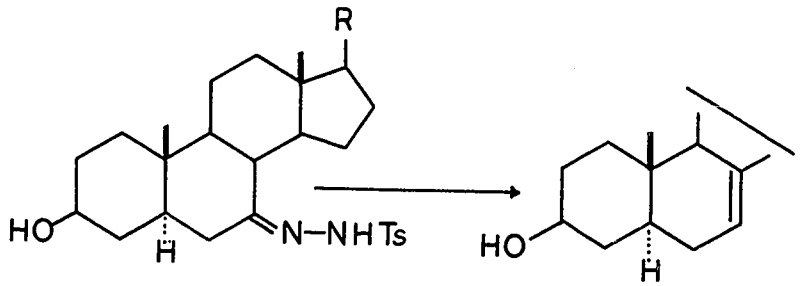
R = C_8H_{17}



35 36

2. 3-Hydroxycholestan-7-one tosylhydrazone (37) gives only the more stable Δ^7-olefin (38) and not the Δ^6-olefin[40].

R = C_8H_{17}



37 38

Configuration of the C-17 Methyl Group in Compounds (2) and (3)

A. Mechanistic Derivation:

It has been shown in the previous sections that the β-methyl group at C-13 in the starting equilenin system (1) moves to the electron deficient C-17 in a typical Wagner-Meerwein 1,2-shift. It is difficult to imagine such a rearrangement resulting in an α-methyl group at C-17, as this would involve movement from the top position of one carbon atom to the bottom of an adjacent one. Since no such examples have ever been found, it is safe to assign the β-configuration to this methyl group on mechanistic grounds.

B. Derivation from Optical Rotations:

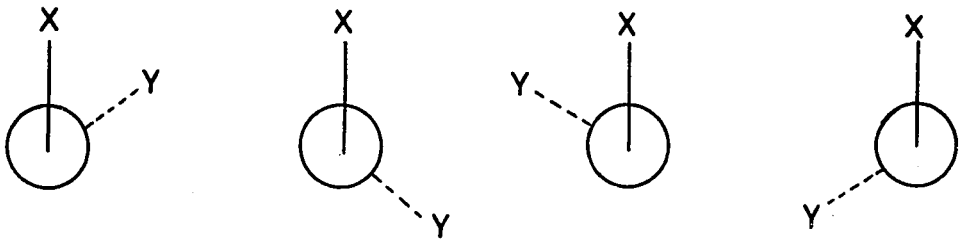
Optical rotation measurements have been used frequently by Barton and others[25] to determine the configuration at various asymmetric centres in steroids, but the rules formulated were empirical ones. The first calculations of the sign (and usually the value) of optical rotations in simple dissymmetric compounds from a more solid theoretical footing were made in a series of papers by Brewster [26-29]. For a detailed explanation of the principles involved, one must turn to the original publications, but some of the basic rules will be given here in order to explain their application to the determination of the configuration about C-17 in phenanthrene derivative (3) (and hence also in (2)).

According to Brewster[29], any twisted chain of four atoms is dissymmetric, since it cannot be superimposed on its mirror image, and

therefore will rotate the plane of plane-polarized light. This is called a "conformational unit". The following conformational units, in Newman projection, are postulated to contribute to the overall rotation in the directions indicated (See figure 6). This has been derived from a consideration of the theory of optical rotation[26].

FIGURE 6

Conformational Units and their Rotatory Contribution



I	II	III	IV
+	+	-	-

The amount of the contribution ($\triangle M_D$) is a multiplicative function of the atomic or group polarizabilities of X and Y in the form

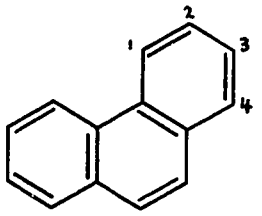
$$\triangle M_D = k(X)(Y)$$

with sign depending upon the conformation. Naturally those conformational units which include strongly polarizable groups as X and Y substituents, will contribute the most to the total optical rotation. Brewster has ranked several substituents in order of their polarizability[26,28]; only four will be used.

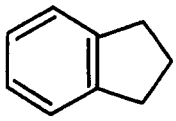
$$C{=}C \gg Me \approx CH_2 \gg H$$

It is also necessary to include the aromatic bond in this ranking. It should be placed somewhere between C=C and Me- depending upon its environment.

For instance, it has been determined theoretically as well as from experimental results that the 3,4-aromatic bond in phenanthrene (39) has more "double-bond character" than the 2,3-bond[30].



39



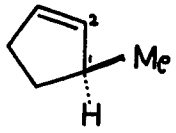
40

In indane (40) all the aromatic bonds are equivalent[30].

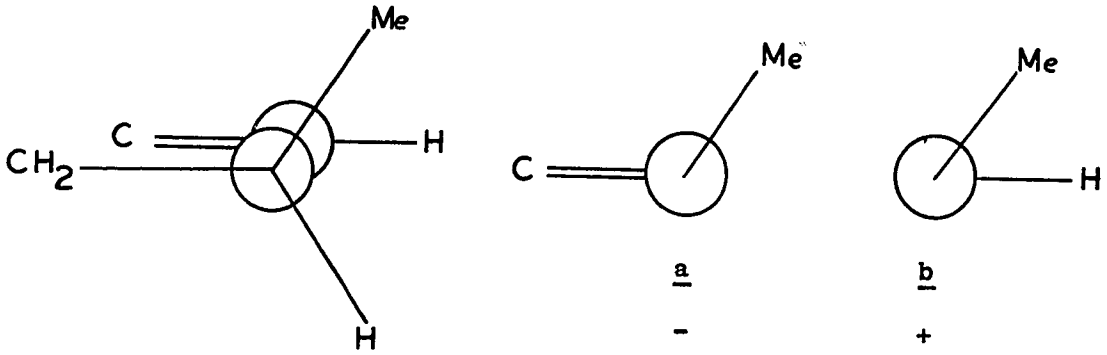
Since the polarizability of a bond rests, to a major extent upon the π-electron cloud, the following hypothesis is, at least, intuitively logical: A conformational unit which contains an aromatic bond of greater "double-bond character", will make a greater rotatory contribution than an otherwise similar conformational unit containing an aromatic bond of lesser "double-bond character".

This theory may be applied to (R) -1-methylcyclopent-2-ene (41).

If a Newman projection of this compound is viewed along the 1,2-bond, two conformational units can be seen: (a) and (b).+



41



a

-

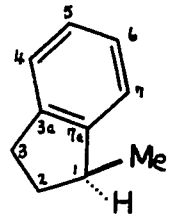
b

+

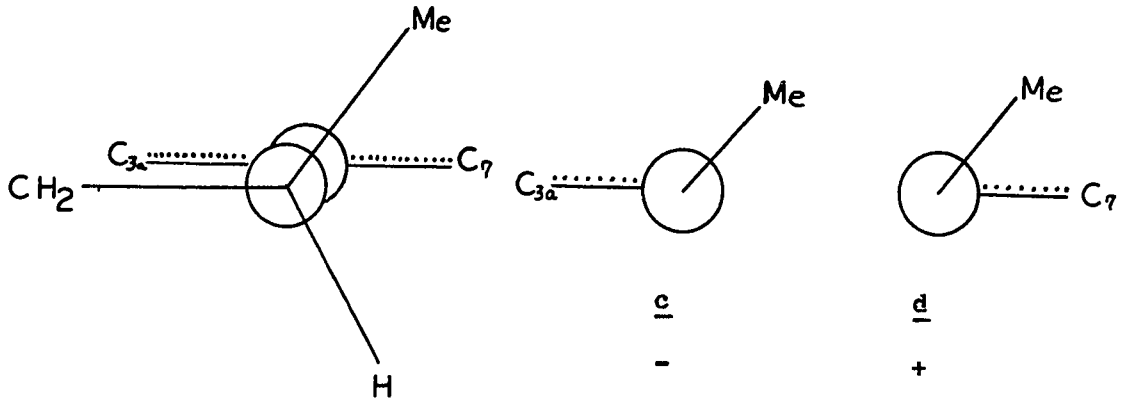
Conformational unit (a) corresponds to IV (figure 6) and will make a negative contribution; conformational unit (b) corresponds to I (figure 6) and will make a positive contribution. However, (a) contains the highly polarizable double bond, and so its negative contribution should far override the positive contribution of (b). In fact (R)-1-methylcyclopent-2-ene has $[M]_D$ -64^o.[28]

+ Other conformational units can be picked out, but they will not make significant contributions[28].

A Newman projection of (S)-1-methylindan (42) along the 1,7a-bond shows the same type of conformational units IV and I ((c) and (d)) with opposite rotary contributions.



42



c

\-

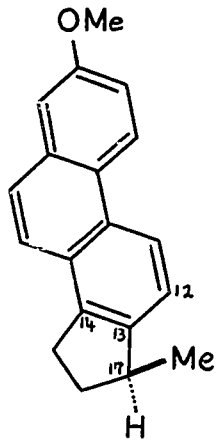
d

\+

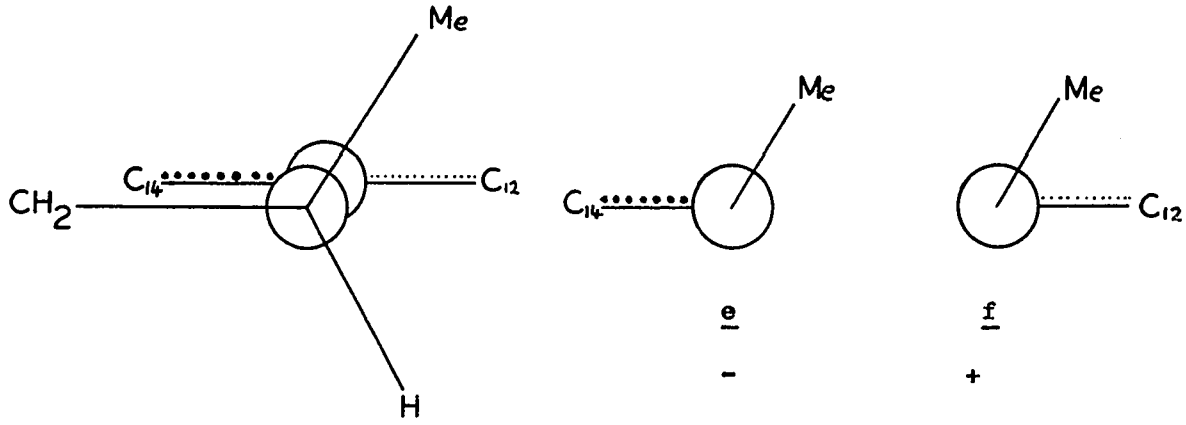
In this case neither (c) nor (d) will have an overriding effect since all the aromatic bonds in indane (40) have the same amount of "double-bond character." Therefore, (S)-1-methylindan should have a zero or small molecular rotation; in fact $[M]_{D}^{\sim+5^{\circ}}$ [31] *.

* Optical rotations in ref. 31 were taken only up to 556 mμ on (R)-1-methylindan; extrapolation to 589 mμ and change of sign gave a value of $[M]_{D}^{\sim+5}$ for (S)-1-methylindan.

We may now consider the dehydrogenation product (3). A Newman projection along the 17, 13-bond reveals two conformational units, (e) which is negative, and (f) which is positive. These are similar to (c) and (d) in the previous example, in that both involve aromatic bonds as part of their conformational units.



3



e

-

f

+

However, the 13,14-bond will have a higher polarizability since it is equivalent to the 3,4-bond of phenanthrene (39), and therefore, conformational unit (e) will make the predominant contribution to the overall rotation. Thus 3 should have a negative rotation.+ In fact, $[M]_D$ is -22°, which is not as large as for (s)-1-methylcyclopent-2-ene (41), but then the difference between conformational units is not as marked.

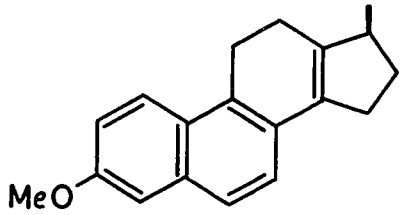
In this manner, Brewster's treatment, when applied to (3), confirms the β-configuration of the C-17 methyl group. (Therefore, it is also β in the Bamford-Stevens reaction product (2)). Since there is no reason why this cannot be applied to other aromatic systems with one optically active centre, it may be of value in predicting or confirming absolute configurations. Conversely, since the value of the optical rotation is dependent upon the relative polarizability of aromatic bonds, it may be possible to calculate the relative amounts of π-bond character from optical studies.

Chirality of the C-Ring of the Bamford-Stevens Product (2)

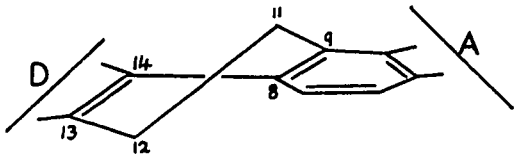
Molecular models of 2 indicated that the Δ^{13}-double bond would not be coplanar with the naphthalene ring system, and thus would contribute an element of optical dissymmetry to the molecule[29].

This represents, in principle, a special case of the skewed styrene chromophore, which has been discussed by Crabbe[34]. Only five examples have been studied, but preliminary results indicated that the

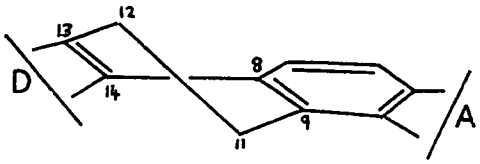
+ The rest of the molecule ie. rings A and B, are planar and will make no rotatory contribution.



2



43a



43b

direction of skewness or chirality could be corelated with the optical rotatory dispersion curve of a compound, a right-handed helix giving rise to a negative Cotton effect[+].

However, the optical rotatory dispersion curve of 2 taken down to 240 mμ in dioxane, showed no Cotton effect. This can be attributed to either of two reasons:

(a) Skewed vinylnaphthalene systems such as 2 give no Cotton effect; or

+ This is apparently opposite to the effect of skewed dienes[35].

(b) There is an equilibrium between the two skewed conformations (43a) and (43b), which cancels out any Cotton effects. Measurements of the non-bonded interactions of these conformations indicate that neither form should be especially favoured, and therefore, this may be the correct explanation.

EXPERIMENTAL

1. All melting points were made on a Gallenkamp melting point apparatus, and are corrected.
2. Infrared spectra were taken on a Perkin Elmer Infracord or 337 spectrophotometer in potassium bromide pellets or carbon tetrachloride solution. Absorption intensities are listed: (vs) very strong, (s) strong, (m) medium, (w) weak, and (sh) shoulder.
3. Nuclear magnetic resonance spectra were taken on a Varian A-60 spectrometer in deuterochloroform solution, and are recorded in p.p.m. with tetramethylsilane as external reference.
4. Ultraviolet spectra were taken on a Unicam SP-800 or Perkin Elmer-350 spectrophotometer in ethanol solution.
5. Optical rotations were made on a Carl Zeiss .005° spectropolarimeter in chloroform solution.
6. Optical rotatory dispersion curves were taken on a JASCO recording spectropolarimeter.
7. Elemental analyses were performed by Dr. C. Daessle, Montreal, Canada.
8. All solutions were dried over anhydrous magnesium sulphate prior to evaporation.
9. Purity of all compounds was checked by thin-layer chromatography on microscope slides coated with silica gel.

Equilenin Methyl Ether

10 ml. of 50% aqueous potassium hydroxide and 10 ml. of dimethyl sulphate were added over 1 hour to a stirred solution of 0.96 gm. of equilenin in 20 ml. of ethanol. After stirring for a further 10 minutes and refluxing for 10 minutes, the solution was cooled, and the precipitate washed well with water and dried to give equilenin methyl ether, m.p. 197.5°-198.5°. Literature m.p. 197°-198°. Yield: 0.98 gm. (97%).

Equilenin Methyl Ether Tosylhydrazone (1)

To a solution of 0.94 gm. of equilenin methyl ether and 1.2 gm. of tosyl hydrazine in 100 ml. of 1:1 ether-methanol at reflux, was added 1 drop of concentrated hydrochloric acid. The refluxing was continued for 3 hours, the solution was cooled, and evaporated to 50 ml., and the resulting solid recrystallized from ethanol to give 1.4 gm. of 1 (Yield: 93%) m.p. 201°.

Infrared Spectrum: (KBr)(cm^{-1}) 3220 (s), 3050 (m), 3000 (sh), 2960 (s), 2890 (sh), 2840 (s), 1670 (w), 1630 (s), 1605 (s), 1578 (m), 1515 (m), 1492 (s), 1415 (s), 1385 (s), 1350 (s), 1245 (s), 1170 (vs), 1100 (m), 1025 (s), 1005 (s), 933 (s), 916 (sh), 856 (s), 815 (s), 718 (s).

Analysis calc. for $C_{26}H_{28}N_2O_3S$: C, 69.66 ; H, 6.25; N, 6.24%.

Found: C, 69.97; H, 6.01; N, 6.06%.

Equilenin Methyl Ether

10 ml. of 50% aqueous potassium hydroxide and 10 ml. of dimethyl sulphate were added over 1 hour to a stirred solution of 0.96 gm. of equilenin in 20 ml. of ethanol. After stirring for a further 10 minutes and refluxing for 10 minutes, the solution was cooled, and the precipitate washed well with water and dried to give equilenin methyl ether, m.p. 197.5°-198.5°. Literature m.p. 197°-198°. Yield: 0.98 gm. (97%).

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Infrared Spectrum: (KBr)(cm^{-1}) 3220 (s), 3050 (m), 3000 (sh), 2960 (s), 2890 (sh), 2840 (s), 1670 (w), 1630 (s), 1605 (s), 1578 (m), 1515 (m), 1492 (s), 1415 (s), 1385 (s), 1350 (s), 1245 (s), 1170 (vs), 1100 (m), 1025 (s), 1005 (s), 933 (s), 916 (sh), 856 (s), 815 (s), 718 (s).

Analysis calc. for $C_{26}H_{28}N_2O_3S$: C, 69.66 ; H, 6.25; N, 6.24%.

Found: C, 69.97; H, 6.01; N, 6.06%.

Protic Bamford-Stevens Reaction of Equilenin Methyl Ether Tosylhydrazone (1)

The compound 1 (1.4 gm.) was suspended in 60 ml. of ethylene glycol in which 1.2 gm. of sodium had previously been dissolved. The mixture was slowly heated to 170° over one hour and held there for a further 30 minutes. Nitrogen evolution commenced from the orange-coloured solution at 130° and ceased at 170°. The solution was cooled, diluted with three times its volume of water, and extracted three times with ether. The ethereal extracts were washed four times with water, once with saturated sodium chloride solution, dried, and evaporated under reduced pressure, and the residue crystallized from ethanol to give 0.69 gm. of pure 2 with one mole of water of crystallization. Chromatography of the mother liquors on Grade III Woelm neutral alumina raised the total yield to 0.76 gm. (89%) of 3-methoxy-18-nor-17β-methyl-estra-1,3,5(10),6,8,13-hexaene (2) m.p. 121°-122°, $[\alpha]_D$ + 24.3°.

Infrared Spectrum: (CCl_4) ($cm.^{-1}$) 3050 (m), 3020 (sh), 2958 (s), 2864 (sh), 2840 (s), 1624 (s), 1605 (m), 1488 (m), 1467 (m), 1424 (m), 1381 (m), 1326 (m), 1298 (m), 1172 (s), 1149 (vs), 1043 (m).

Ultraviolet Spectrum: See figure 1.

Nuclear Magnetic Resonance Spectrum: 1.1 (doublet), C-17 methyl; 2.1 (singlet), H_2O of crystallization; 3.9 (singlet), OCH_3; 7.0-8.0 (multiplet), aromatic protons.

Analysis calc. for $C_{19}H_{20}O \cdot H_2O$: C, 80.81; H, 7.85%.

Found: C, 80.80; H, 7.72%

Dehydrogenation of 3-Methoxy-18-nor-17β-Methylestra-1,3,5(10),6,8,13-hexaene (2)

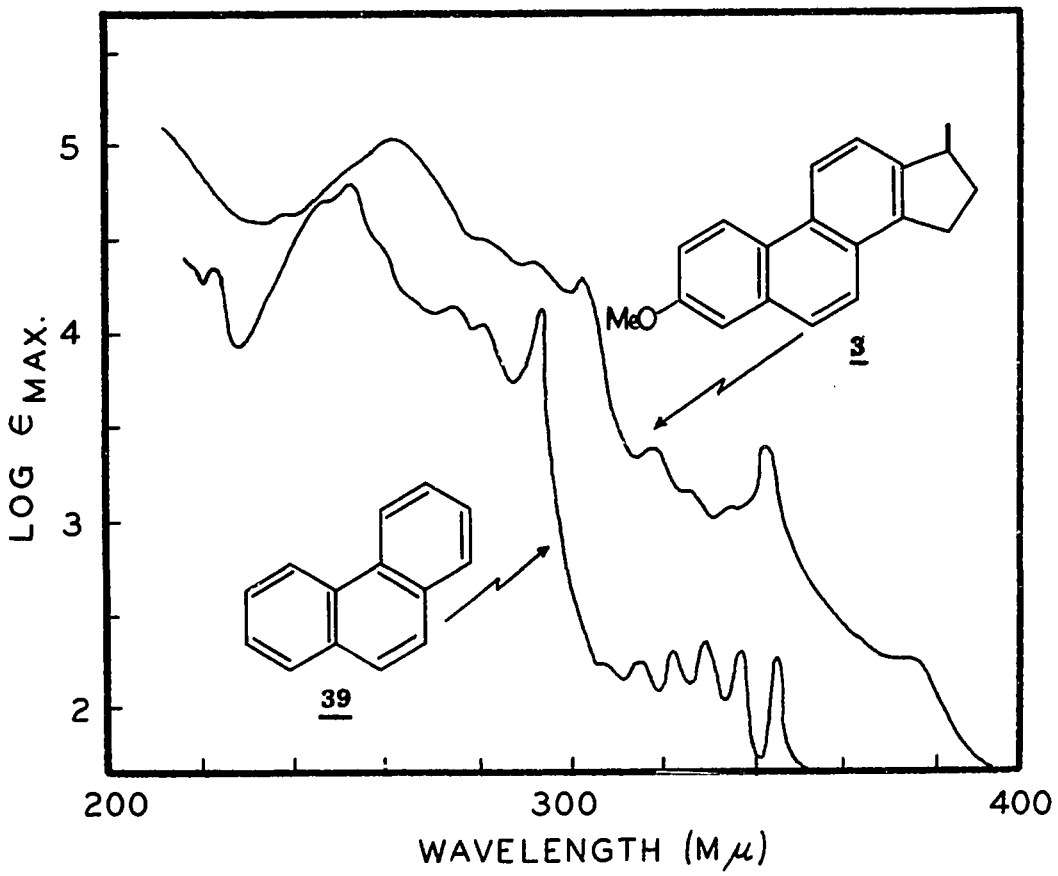
The compound 2 (27 mg.) and 180 mg. of 5% palladium-on-charcoal were added to 3 ml. of xylene, which was then refluxed for 3 hours with a slow stream of nitrogen passing through it. The solution was cooled, filtered, concentrated under reduced pressure, and crystallized from ethanol to give a virtually quantitative yield of 3-methoxy-18-nor-17β-methylestra-1,3,5(10),6,8,11,13-heptaene (3)[32], m.p. 149°-151°. Literature m.p. 146.5°-148.5°[13]; $[\alpha]_D$ -7.4°, $[M]_D$ -22°.

Ultraviolet Spectrum: See figure 7.

The sym. trinitrobenzene complex was made by heating equimolar amounts of 3 and sym. trinitrobenzene in ethanol, and allowing the complex to crystallize, m.p. 133°-133.5°. (Literature m.p. 135.5°-136.5°)[13].

FIGURE 7

The Ultraviolet Spectra of Dehydrogenation Product (3) and Phenanthrene (39)[+]



+ Ref. 42

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CLAIMS TO ORIGINAL RESEARCH

1. 10α-Cholesterol, the first epimer of cholesterol, has been synthesized.

2. Conformational and stereolectronic analysis of polycyclic systems was extended to Inhoffen's ketone, and dehydro-Inhoffen's ketone.

3. The alkylation of 2-methylcyclohexanone by 1,3-dichlorobut-2-ene was repeated and was found to give 80% of the 2-alkylated product and 20% of the 6-alkylated product. Previous results had indicated that only the former product was formed.

4. Reasons for the preferred alkylation of Inhoffen's ketone at C-6 were advanced.

5. A new route to the anthrasteroids was described.

6. A new method for production of dehydro-Inhoffen's ketone was described.

7. Dehydro-Inhoffen's ketone was alkylated with 1,3-dichlorobut-2-ene, and the position of alkylation was proven to be C-10.

8. An improved procedure for alkylation by methyl vinyl ketone was applied to dehydro-Inhoffen's ketone.

9. Reasons were proposed for the facile dehydration of 10β-cholest-9(11)-en-5α-ol-3-one by base, and the resistance of the 10α- analogue to the same reaction. Practical use was made of this in separating the 10α- and 10β-epimers.

10. An explanation was advanced for the extensive hydrogenolysis during the hydrogenation of 10α-cholest-9(11)-en-5α-ol-3-one.

11. The configuration of 10α-cholestan-5α-ol-3-one was proved by optical rotatory dispersion.

12. A mechanism for the elimination of water from 10α-cholestan-5α-ol-3-one by reflux in benzene with para-toluenesulphonic acid was proposed.

13. An explanation was advanced for the tendency of the conjugated 4,5-double bond in unsaturated 10α-3-keto-steroids to migrate to the unconjugated 5,6-position (relative to the 10β-analogues).

14. The stereochemistry of the C-3 hydroxyl group in 10α-cholesterol was proven to be β by the nuclear magnetic resonance spectrum, and the reasons for its formation in this configuration were given.

15. Melting points and optical rotations of C-10 epimers were compared, and general "rules of thumb" regarding their differences were advanced.

16. The following compounds were synthesized for the first time:

    6α-(3'-Chlorobut-2'-enyl)-10α-des-A-cholestan-5-one

    6α-(3'-Oxobutyl)-10α-des-A-cholestan-5-one

    10α,6β-Anthracholest-4-en-3-one

    10α,6β-Anthracholest-4-en-3-one-2,4-dinitrophenylhydrazone

    10-(3'-Chlorobut-2'-enyl)-des-A-cholest-9(11)-en-5-one

10α-Cholest-9(11)-en-5α-ol-3-one

Cholesta-4,9(11)-dien-3-one

5β-Cholest-9(11)-en-3α-ol

5β-Cholest-9(11)-en-3α-ol acetate

10α-Cholestan-5α-ol-3-one

10α-Cholestan-5α-ol

10α-Cholest-4-en-3-one

10α-Cholest-4-en-3-one-2,4-dinitrophenylhydrazone

10α-Cholest-5-en-3-one

10α-Cholesta-3,5-dien-3-ol acetate

10α-Cholesterol

10α-Cholesterol acetate

APPENDIX

1. The protic Bamford-Stevens reaction of equilenin methyl ether tosylhydrazone was described, and the structure of the product proved by spectral and chemical evidence. A compound which was claimed by Johns to have this structure therefore cannot be this.

2. A reaction mechanism leading to this product was proposed, and a comparison was made between the protic Bamford-Stevens reaction and several related reactions.

3. A hypothesis was advanced to predict the olefins formed in the protic Bamford-Stevens reaction on the basis of product stabilities.

4. Brewster's theory of optical rotation was extended to more complicated aromatic systems, and from this, the stereochemistry of the C-17 methyl was deduced to be β in 3-methoxy-18-nor-17β-methylestra-1,3,5(10),6,8,11,13-heptaene and in the Bamford-Stevens reaction product. This was confirmed by a study of the mechanism.

5. The chirality of the C-ring of the Bamford-Stevens reaction product was studied by optical rotatory dispersion, and reasons were advanced for the lack of a detectable Cotton effect.

6. The following compounds were synthesized for the first time:

   Equilenin methyl ether tosylhydrazone

   3-Methoxy-18-nor-17β-methylestra-1,3,5(10),6,8,13-hexaene

Synthesis of 100%-Cholesterol: Detailed Reaction Scheme