PHYSICO-CHEMICAL STUDIES ON STRUCTURE AND STEREOCHEMISTRY OF ISOPRENOIDS

A Thesis submitted to
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for the degree of

DOCTOR OF PHILOSOPHY
(in Chemistry)

By
M. R. Sarma, M.Sc.,
National Chemical Laboratory,
Poona 8.

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1968

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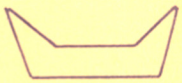
CHAPTER 1

CONFORMATIONAL MOBILITY OF THE B-RING IN SOME STEROIDS.

Although a simple cyclohexane molecule is capable of existing in several conformations, the important ones, which are well defined are the chair, the boat and twist conformations, I, II and III. Since the simple cyclohexane is very flexible and the energy of transformation from one to the other by rotation around the carbon-carbon single bond is not too high, it can pass through these conformations at room temperature, in solutions or in liquid state. However when bulky substituents are introduced into the ring or the cyclohexane ring is trans-fused to another, it rigidly holds its conformation. Thus in the normal tetracyclic and pentacyclic triterpenoids and steroids all the rings exist in chair conformation. However, an end ring like the A ring has some flexibility and in a transition state the ring can adopt a boat conformation, for purpose of a reaction although, normally it may be existing in the chair conformation.

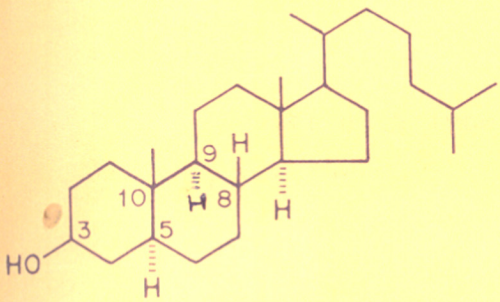
This however is not the case with the ring B. The B-ring of a usual steroid like cholestanol IV being trans-fused to the C-ring at C_8, C_9 on one side and again trans-fused to the A-ring at C_5, C_{10} on the other side, is more rigidly held and exists in a rigid conformation V. Hence values for the coupling constants of axial-axial, axial-equatorial etc. protons are often taken from substituents in ring B.

I



II

III



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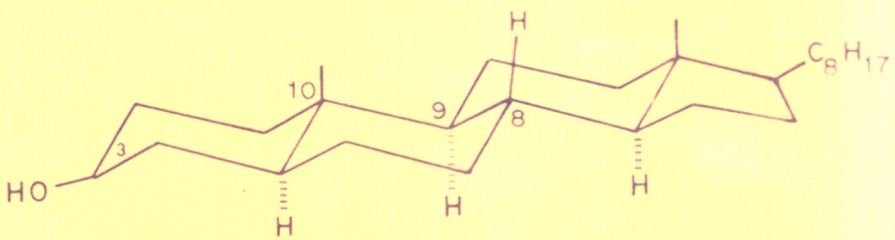
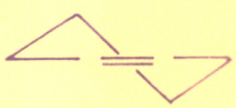


V

VI



VII

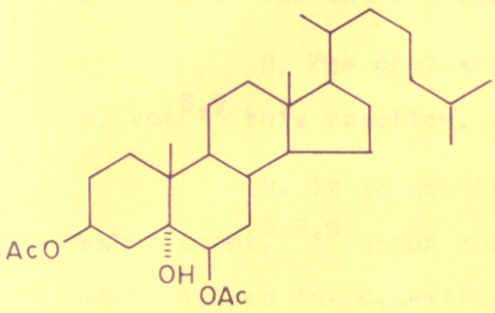
VIII

IX

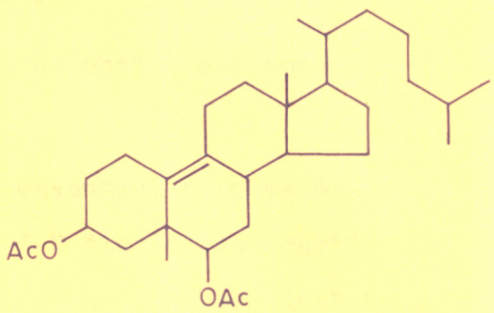
A double bond can be introduced into ring B, at six different positions, viz. 5,6 as in cholesterol, at 6,7 at 7,8 as in ergosterol and related compounds, at 8,9 as in triterpenes of the type euphol, lanosterol etc; and at 9,10 and 5,10 by the migration of C_{10} angular methyl group to an adjacent position.
The stable conformations in which a cyclohexene normally exists are the two alternate half chairs VI and VII and two alternate half boats VIII and IX. This is due to flexibility at the two carbon atoms further away from double bond, since the two carbon atoms involved in double bond and the two allylic carbon atoms are always rigidly held in one plane, due to the double bond. Applying this to the ring B of the steroid molecule, with 5,6 double bond, the carbon atoms that could be flexible are the ones at 8 and 9. But these being the positions of trans-fusion, between the B and C rings, are quite rigid and hence the cyclohexene formed by ring B with a 5,6 double bond can exist in one half chair conformation only.
In complete agreement with this, X-ray crystallographic studies have shown that cholesteryl iodide has its ring B existing in a half chair conformation[1].
Similarly with a 6,7 double bond, the positions capable of flexibility are 9 and 10 but these are the positions of trans-fusion with the C and the A-rings, and hence are inflexible. The same is also the case with a 7,8 double bond, the carbon atoms 10 and 5 being

positions of trans-fusion with the ring-A, with an 8,9 double bond 5 and 6 are the possible flexible positions, but 5 being the position of trans-fusion with ring A, both are inflexible. The same is the case with a 5,10 double bond, where 8 is a position of trans-fusion with ring C. Only in one position of the double bond, out of six possibilities, i.e. the one at 9,10, the carbon atoms involved are 6 and 7, both of which being not attached to any ring, have flexibility. Hence the cyclohexene formed by the introduction of this double bond is the only one in the B-ring which is potentially capable of a conformational flip from one to other of any of the half chair or half boat conformations.

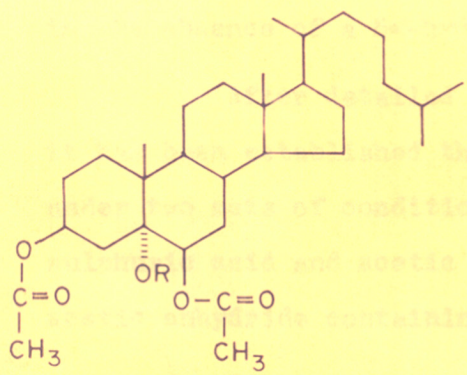
A suitable compound which has a double bond in the required 9,10 position is a rearranged compound which Westphalen[2] first obtained by treatment of cholestane 3β-5∝-6β-triol,3,6 diacetate (X) with acetic anhydride and sulphuric acid. After several attempts to elucidate its structure, this compound was finally shown to have the structure (XI). The position of the double bond being fixed on the basis of its U.V. absorption[4] which indicated that the double bond was tetra-substituted and doubly excocyclic. Since then several investigations have been made regarding the environment required for Westphalen rearrangement. These requirements may be summarised as follows:



X

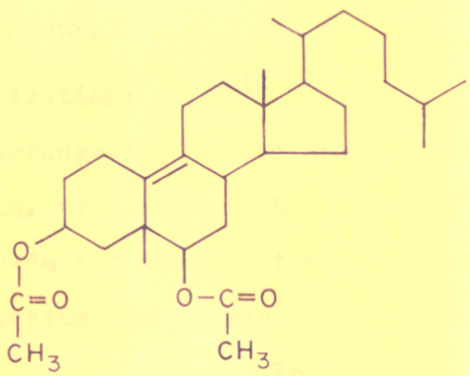


XI



XII

a) R = H
b) R = CH_3
e) R = $SO_2 \cdot O \cdot CO \cdot CH_3$



XIII

b) R = $CO\ CH_3$
d) R = SO_3H

1. The presence of a 3β-acetate is not essential[5] for the rearrangement.

2. The cholesterol side chain does not effect[6,7] this reaction.

3. It is absolutely essential to have 6β-substituent,[6,8,9] which through 1-3 diaxial interactions destabilises the C_{10}-methyl. It is however clear that the rearrangement is not restricted to the 6β-acetate[10].

4. It has been established by several experiments[11] that mere creation of a carbonium ion at C_5 is not sufficient for producing the rearrangement. It is significant that no rearrangement has been observed in the absence of a 5α-hydroxyl group.

After detailed investigations[12,13,14] it has been established that rearrangement occurs only under two sets of conditions, viz. treatment with sulphuric acid and acetic anhydride and treatment with acetic anhydride containing potassium bisulphate.

It has been unambigiously established[14] that in the first step there is sulphonation of the 5α-hydroxyl by either, SO_3, $-HSO_3$, $OH-SO_2-OAc$/is the one undergoing rearrangement. The reactions for the formation of these sulphonating agents and their subsequent reaction are represented below.

$H_2SO_4 + Ac_2O \rightarrow OH\text{-}SO_2\text{-}OAc + AcOH$

$OH\text{-}SO_2\text{-}OAc \rightarrow Ac\text{-}O\text{-}SO_2\text{-}OAc + AcOH$

Followed by breakdown of the mixed anhydride species.

either $HO\text{-}SO_2\text{-}OAc \rightarrow SO_3 + AcOH$

or $HO\text{-}SO_2\text{-}OAc \rightarrow HSO_3 + Ac^{\oplus}$

XII + sulphonating species → XIId or XIIe

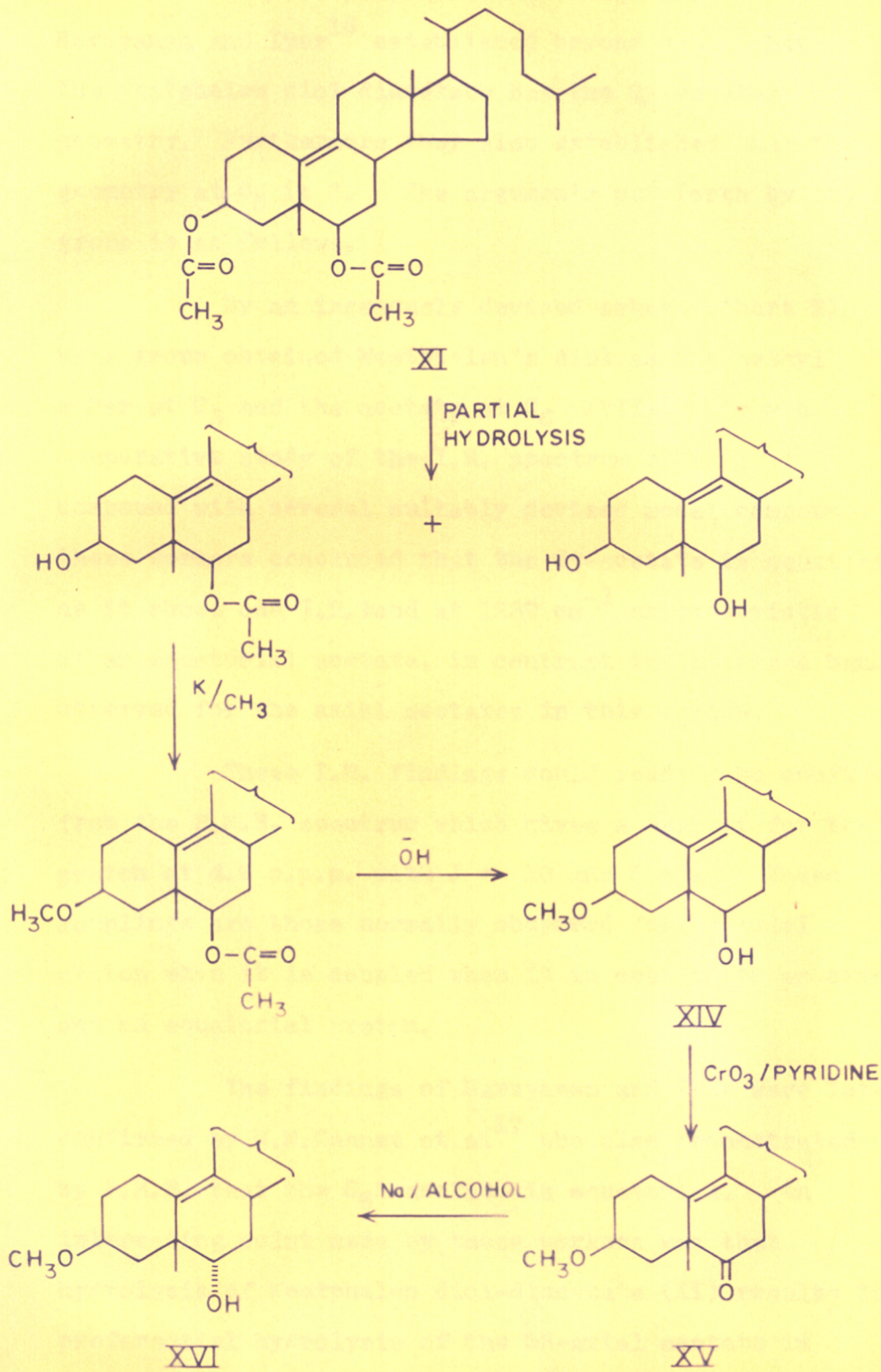
Then XIId → XII + HSO_4^- or

XIIe → XII + $AcO\text{-}SO_2\text{-}O^-$

Conformation of Westphalen diol-diacetate

In an elegant series of experiments Jones[15] and Summers converted Westphalen's diacetate (XI) by the scheme outlined in Chart I to Westphalen 3β-6β-diol 3-methyl ether (XIV). Oxidation of this compound furnished a ketone (XV) which on reduction with sodium and alcohol gave an oily product (XVI) different from the starting material. These workers presumed this to be an equatorial alcohol on the grounds that sodium alcohol reduction yields the more stable alcohol. As the Westphalen product has a β-hydroxyl group, the reduced compound (XVI) must therefore have the α-geometry. As the reduced product is α and equatorial the starting compound (XIV) must be β- and axial.

CHART-1



REACTION SCHEME OF JONES AND SUMMERS

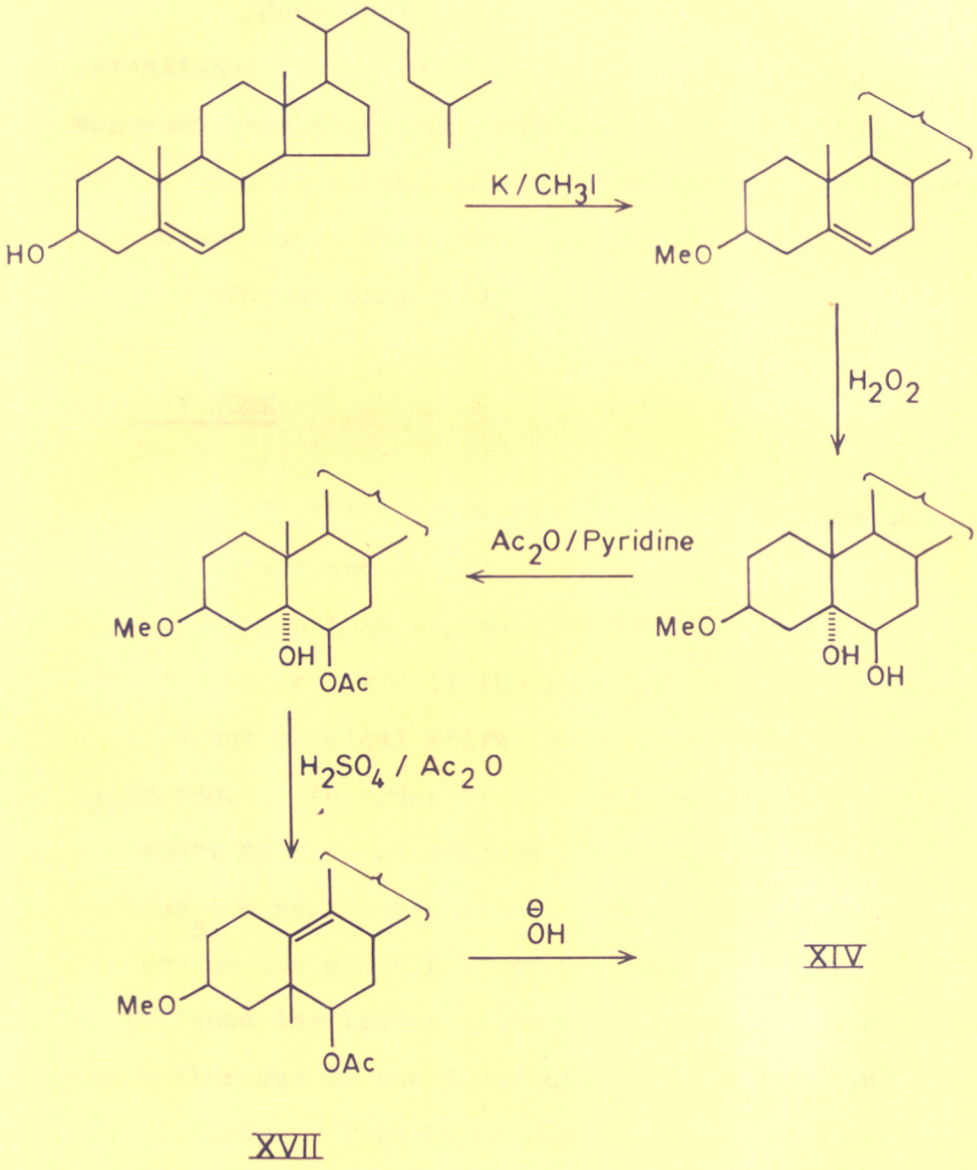
In a comprehensive investigation Narayanan and Iyer[16] established beyond doubt that the Westphalen diol diacetate has the $C_6\beta$-equatorial geometry. Furthermore they also established that the geometry at C_5 is β. The arguments put forth by this group is as follows.

By an ingenously devised scheme (Chart 2), this group obtained Westphalen's diol as the methyl ether at C_3 and the acetate at C_6 (XVII). From a comparative study of the I.R. spectrum of this compound with several suitably devised model compounds these workers concluded that the 6β-acetate is equatorial as it shows one I.R. band at 1239 cm^{-1} characteristic of an equatorial acetate, in contrast to the three bands observed for the axial acetates in this region.

These I.R. findings could readily be confirmed from the P.M.R. spectrum which gives a quartet for the C_6-proton at 4.8 p.p.m. with J = 10 and 5 cps. These couplings are those normally observed for an axial proton when it is coupled when it is counled to an axial and an equatorial proton.

The findings of Narayanan and Iyer were later confirmed by M.M.Cannet et al[17] who also demonstrated by P.M.R. that the $C_6\beta$-acetate is equatorial. An interesting point made by these workers was that hydrolysis of Westphalen diol-diacetate (XI) results in preferential hydrolysis of the 3β-axial acetate in

CHART-2



XIV

XVII

REACTION SCHEME OF NARAYANAN AND IYER

contrast to the normal rule of conformational analysis which would postulate a more easy hydrolysis of an equatorial isomer as compared to axial one.

Nevertheless one point remained to be established is that concern with the geometry of the compound resulting from sodium alcohol reduction. With a view to establish this, the present investigation was undertaken. This investigation revealed a very novel conformational mobility of the ring-B.

PRESENT WORK

As the conformation of the C_6-proton can be readily determined by the P.M.R. spectrum, it is necessary to distinguish this proton from that at C_3. This can be achieved if the substituent at C_3 would lead to a different chemical shift for the C_3-proton as compared to C_6-proton. In order to achieve this goal, it is necessary to have a methoxyl group at C_3. As mentioned earlier two procedures can be used, one that of Jones and Summers[15] or the other that of Narayanan and Iyer. We followed the latter procedure as the yields obtained are better due to the fact that no selective hydrolysis is necessary. Thus by the scheme outlined in Chart 2 the Westphalen diol 6β-acetate 3-methyl ether (XVII) was obtained. The spectral properties of this compound clearly showed that the acetate is equatorial.

Also the nature (narrow signal) of the C_3-proton revealed that this proton is equatorial. As the starting material has the C_3-proton axial, this indicated that the geometry of C_5 substituent has changed during rearrangement.

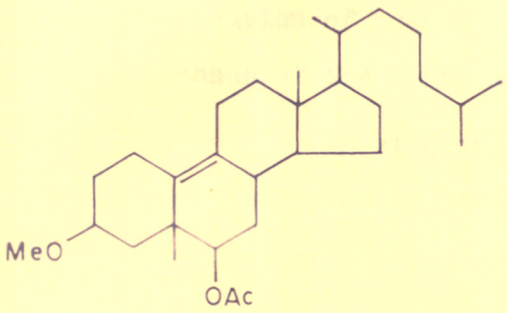
Hydrolysis of the Westphalen compound furnished the alcohol (XIV) (Chart 3) M.P. 107°; $(\alpha)_D$ 124° in agreement with that reported by Jones and Summers. The I.R. of this compound established the absence of an acetate group and displayed the hydroxyl absorption at 3550 cm^{-1}.

The P.M.R. spectrum of the alcohol could not be used to come to any conclusion regarding the geometry of the C_6- or C_3-proton as these were hidden by the methoxy methyl signal at 3.25 p.p.m.

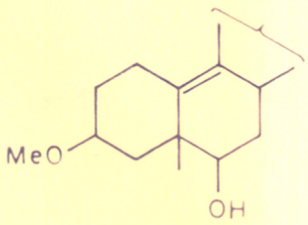
Oxidation of the alcohol (XIV) yielded the ketone (XV) whose properties were identical with those reported by Jones and Summers. The I.R. spectrum revealed ketone absorption at 1725 cm^{-1} while the P.M.R. spectrum showed essentially the same pattern for the C_3-proton (narrow signal at 3.48 p.p.m.). Its U.V. absorption at λmax 295 mμ shows a normal ε max of 55,[18] indicating thereby that there is no appreciable overlap between the C_9 double bond and the C_6-carbonyl.

When this ketone was reduced with sodium and alcohol, it furnished an oily product, the T.L.C. of which showed that it was homogenous. As the R_F value for this reduced product is identical with that of the Westphalen alcohol M.P. 107° (XIV), it was

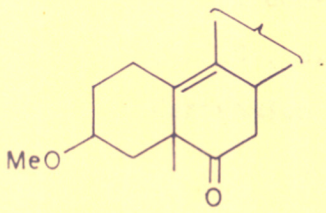
CHART-3



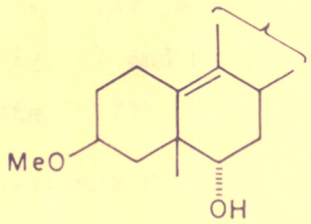
XVII



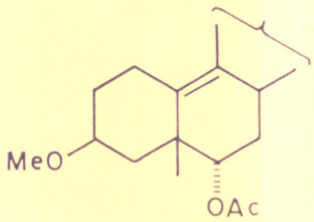
XIV



XV



XVI



XVIII

COMPOUNDS PREPARED IN PRESENT INVESTIGATION

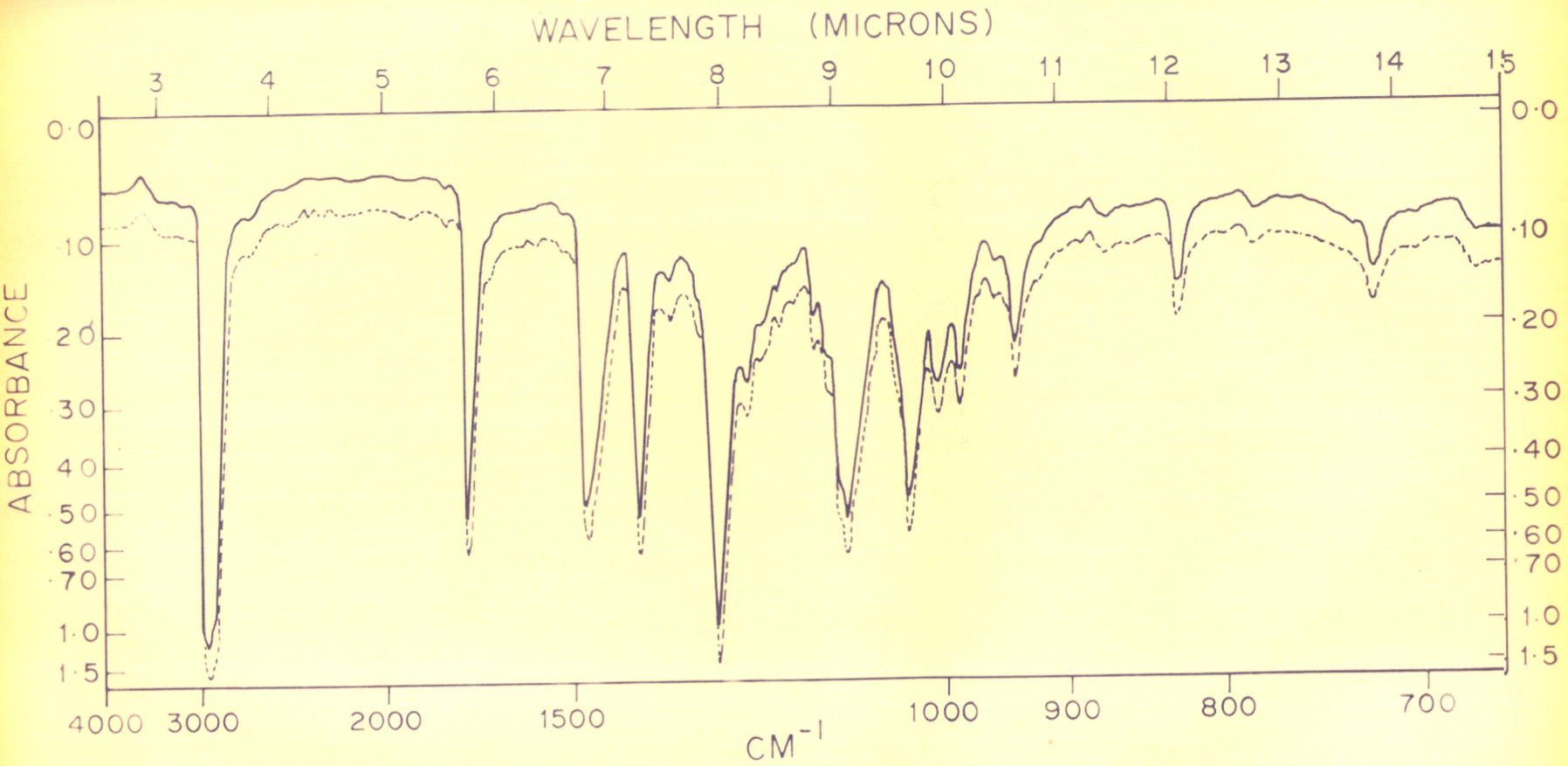
indeed doubted whether this compound was homogenous. The P.M.R. spectrum of this compound displayed its C_6-proton as a broadened signal at 3.4 P.P.M. the location of some of the methyl signals differed from those of the Westphalen alcohol.

In view of this we attempted to purify this compound through an acetate. The acetate obtained was an oil, but its T.L.C. (benzene + 5% pet. ether solvent R_f = 0.56, 0.53) showed that it was a mixture of two compounds, none of which corresponds to the starting alcohol. The faster moving spot corresponding to that of Westphalen's acetate (XVII).

A separation of this mixture using P.L.C. afforded both components in pure form. The faster moving compound M.P. 117° was identical in every respect (T.L.C., I.R. (Fig. 1) and N.M.R. (Fig. 2) with the Westphalen acetate (XVII).

The slower moving compound (XVIII) could not be obtained in crystalline form though its homogenity could be clearly shown not only by T.L.C. in several solvent systems but also by its P.M.R. spectrum.

In the first instance these experiments clearly establish that the reduced product of Jones and Summers in all probability is non-homogenous. The I.R. spectrum of this compound (XVIII, Fig. 3) showed the absence of a hydroxyl group and the presence of an acetate function (1730 cm^{-1}, 1239 cm^{-1}). It could normally be anticipated that this acetate would be the axial isomer,



Fig·1

——— I.R. SPECTRUM OF WEST PHALEN ACETATE

..... I.R. SPECTRUM OF WEST PHALEN ACETATE OBTAINED BY P.L.C. AFTER REDUCTION AND ACETALYATION

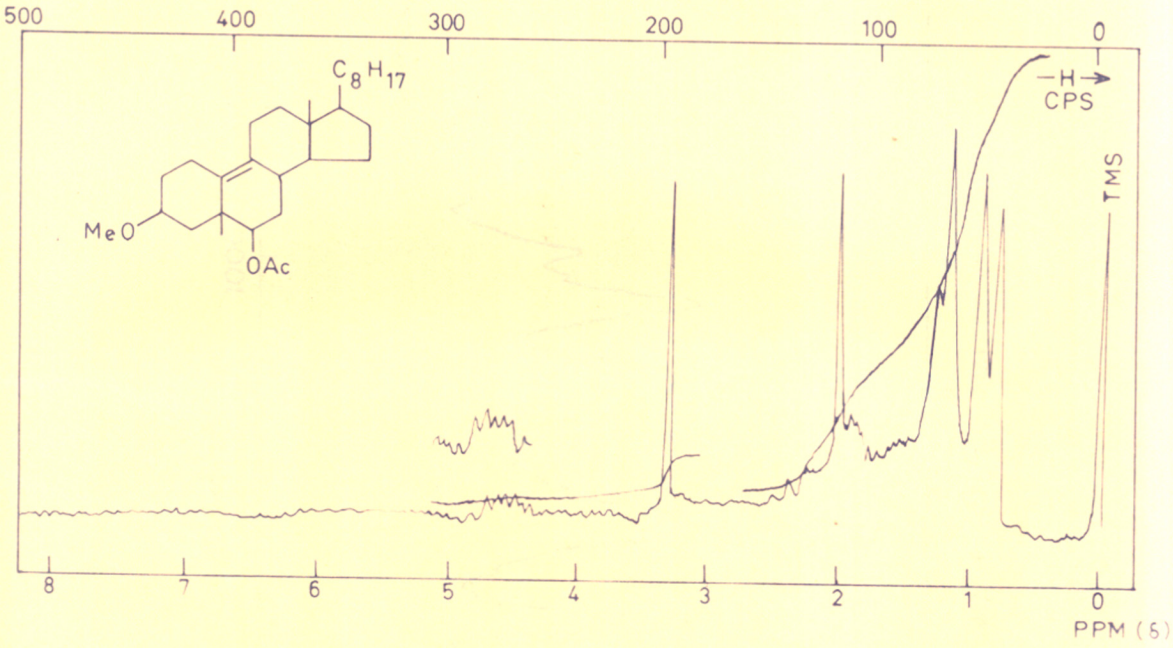


FIG. 2.

PMR SPECTRUM OF 3ß-METHOXY 5ß-METHYL 19 NOR - 5ß-CHOLEST 9(10) EN 6ß-ACETATE

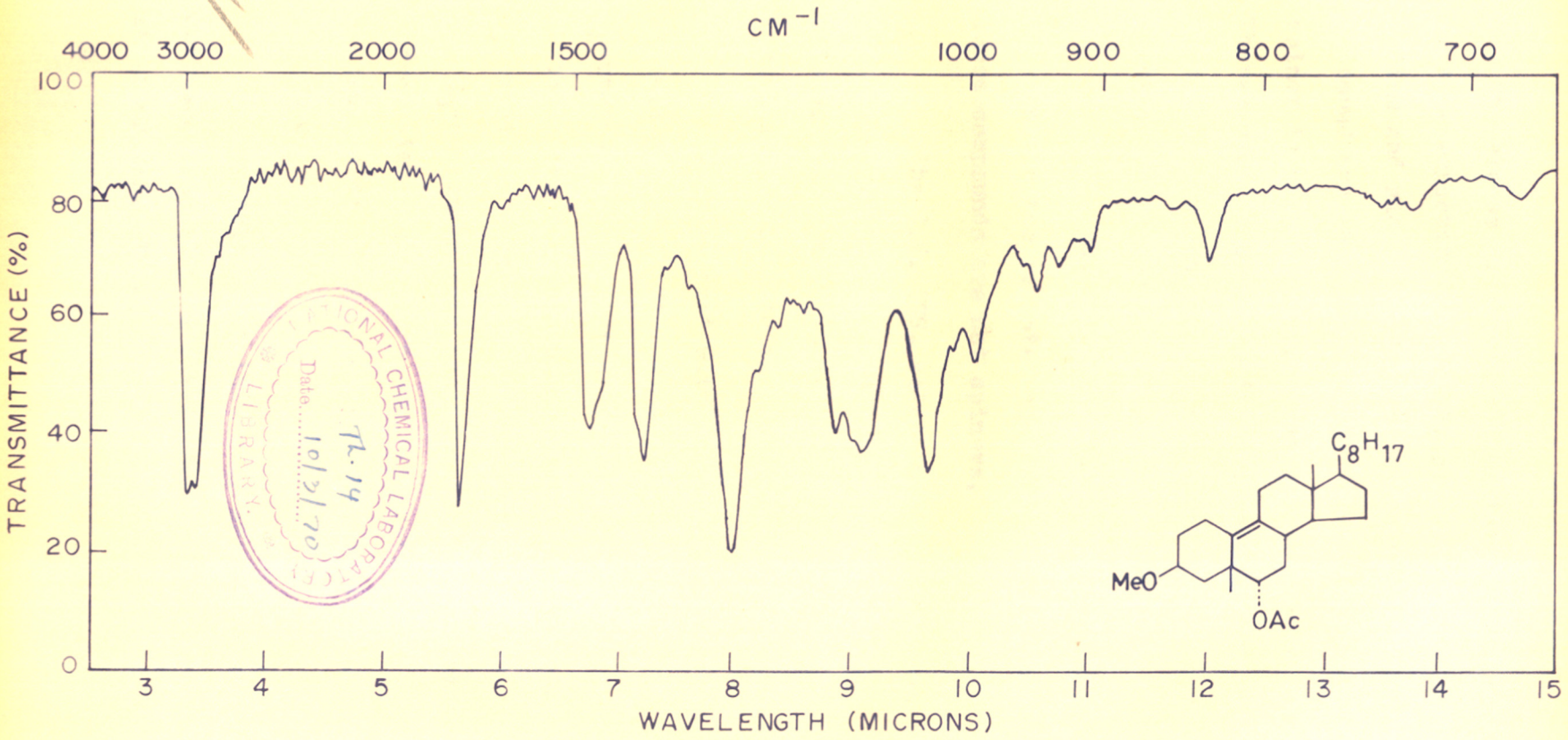


FIG. 3. I.R. SPECTRUM OF 3β-METHOXY 5β-METHYL 19 NOR 5β-CHOLEST 9(10) EN 6α-ACETATE

but two pieces of evidence immediately cast doubt on this expectation. The first of these is that sodium and alcohol reduction usually yields the equatorial compound and the secondly that in T.L.C. the axial epimer is usually faster moving than the equatorial one. Though neither of these is in itself a rigid proof that this compound is not equatorial, together they cast doubt on the nature of the oily product.

The large molecular rotation difference(267^{o}) between the two acetates also raised the suspicion that these compounds are not epimers.

The I.R. (CS_2 solution) (Fig. 4) of this oily acetate exhibits only one absorption band at 1239 cm^{-1} a fact which has been clearly shown by Narayanan and Iyer to indicate an equatorial acetate.

In agreement with this expectation the P.M.R. of this compound (Fig. 5) displays signals for the C_6-proton as a quartet at 4.6 p.p.m. J = 11 and 4 c.p.s. which is due to the coupling of the C_6-hydrogen with the C_7-axial and equatorial protons respectively. The C_3-proton in this spectrum can be recognised as a narrow signal at 3.45 p.p.m. indicating its equatorial nature and demonstrating that the AB rings are still cis fused.

Hydrolysis of this oily acetate gave the corresponding alcohol (XVI) $(\alpha)_D$ $+15^{o}$ which shows IR absorption at 3400 cm^{-1} (Fig 6)

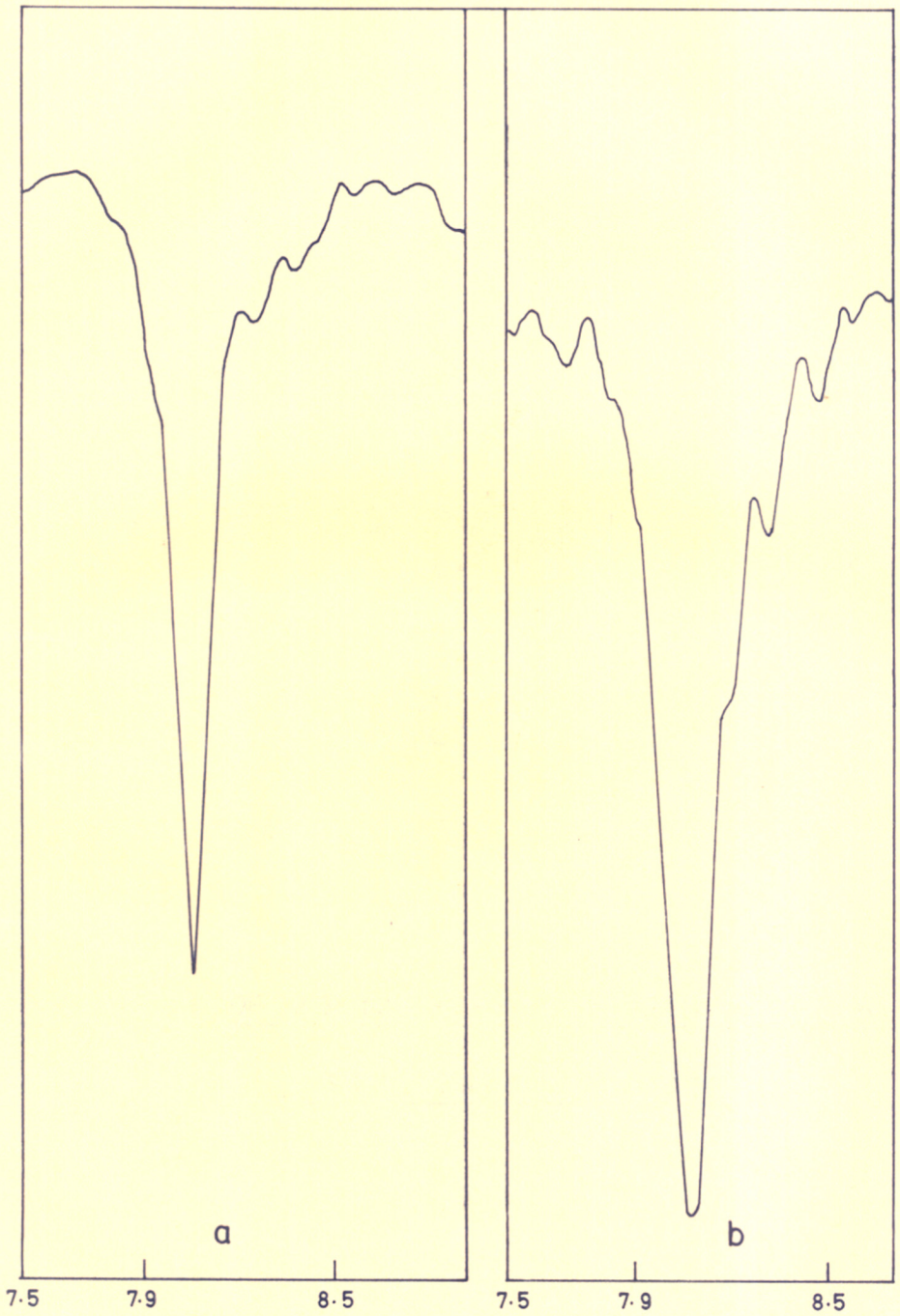


FIG. 4 a. IR SPECTRUM OF 3ß-METHOXY 5ß-METHYL 19 NOR 5ß-CHOLESTA 9(10) EN 6∝- ACETATE (CS_2)

b. IR SPECTRUM OF 3ß-METHOXY 5ß-METHYL 19 NOR 5ß-CHOLESTA 9(10) EN 6ß- ACETATE (CS_2)

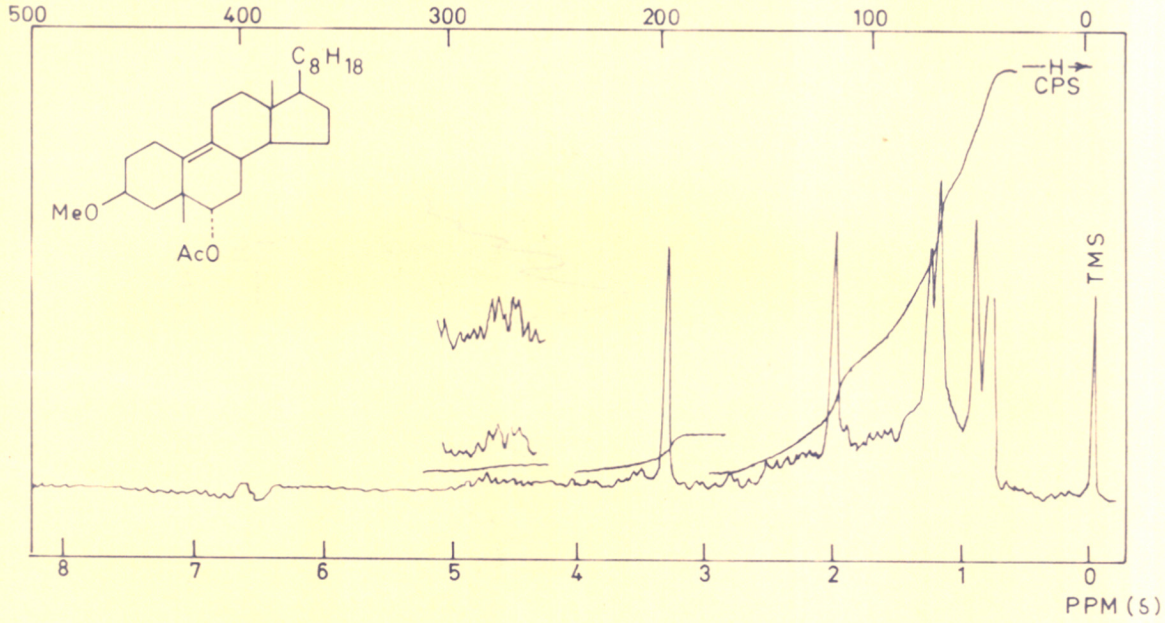


FIG. 5

PMR SPECTRUM OF 3 ß - METHOXY 5 ß - METHYL 19-NOR-5 ß - CHOLEST-9(10)-EN, 6 α - ACETATE

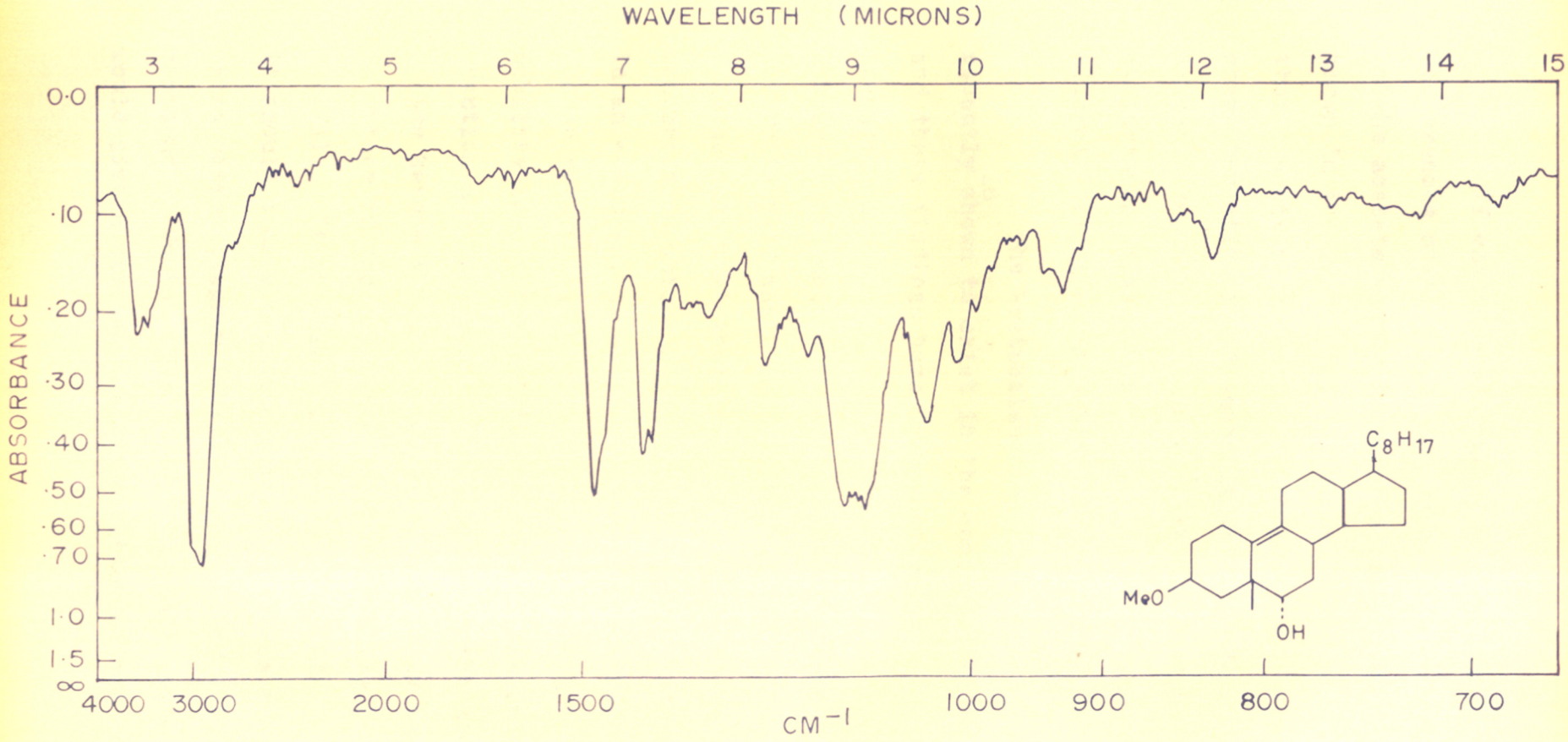


FIG. 6. IR SPECTRUM OF 3β-METHOXY 5β-METHYL 19 NOR 5β-CHOLEST 9(10) EN 6α-OL

If one compares the molecular rotation differences observed on passing from the alcohol to the acetate with the examples of 5β-compounds, known in the liaterature[19] (Table I), it became clear that a positive rotation differences is characteristic of a 6β-substituent - while a 6∝-substituent is associated with a negative rotation difference. This therefore establishes that the new alcohol (XVI) and the acetate (XVIII) are ∝-oriented.

As The Westphalen diol-diacetate has been recently[16] shown to exist in the conformation (XIX) and these findings have been confirmed in the present investigation. It seems that in oxidation or in subsequent reduction there is ring flip into an alternate half chair conformation (XX) and that the oily acetate must therefore be in this alternate conformation which would have its C_6-substituent in an ∝-equatorial position (XXI). This therefore can be regarded as a very novel example of ring mobility in a fairly rigid skeleton. The only question now remaining to be decided is the geometry of the ketone (XV). The ketone naturally can exist in either of the alternate half chair forms (XXII) or (XXIII). If it exists in conformation (XXII), it should show a positive cotton effect as revealed by an octant diagram (XXIV) of a model of this compound. On the other hand, the conformation (XXIII) would give a strongly negative cotton effect as

TABLE I

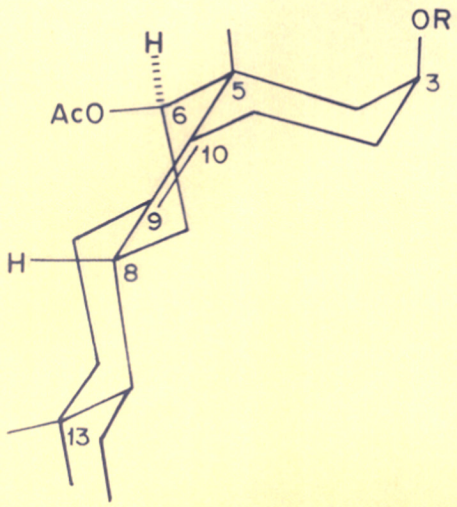
MD contributions of 6-substituted 5β-steroids

6α-alcohol	-100	6β-alcohol	+7
6α-acetate	-87	6β-acetate	-62
difference	-13	difference	+69

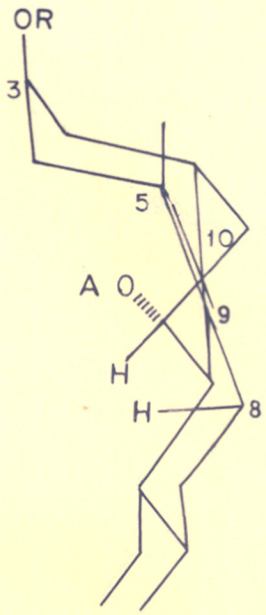
Molecular rotations observed

XVI	+65.4	XIV	+532.5
XVIII	+143.4	XVII	+401.5
difference	-78	difference	+131.0

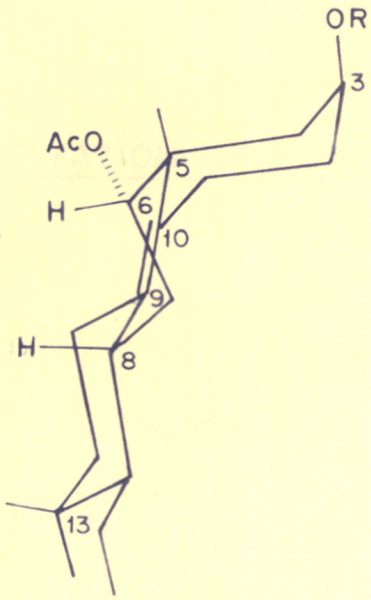
CHART-4



XX



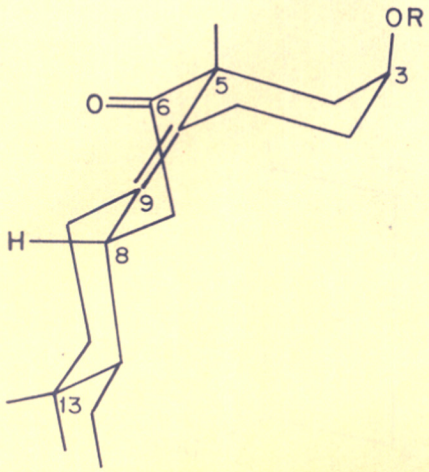
XIX



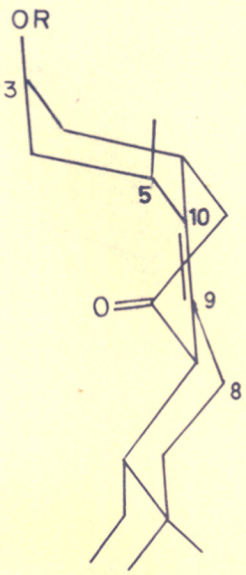
XXI

CONFORMATIONS OF ACETATES

CHART-5

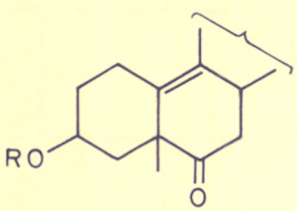


XXIII



XXII

CONFORMATION OF KETONE



XXVI R = Ac

XXVII R = H

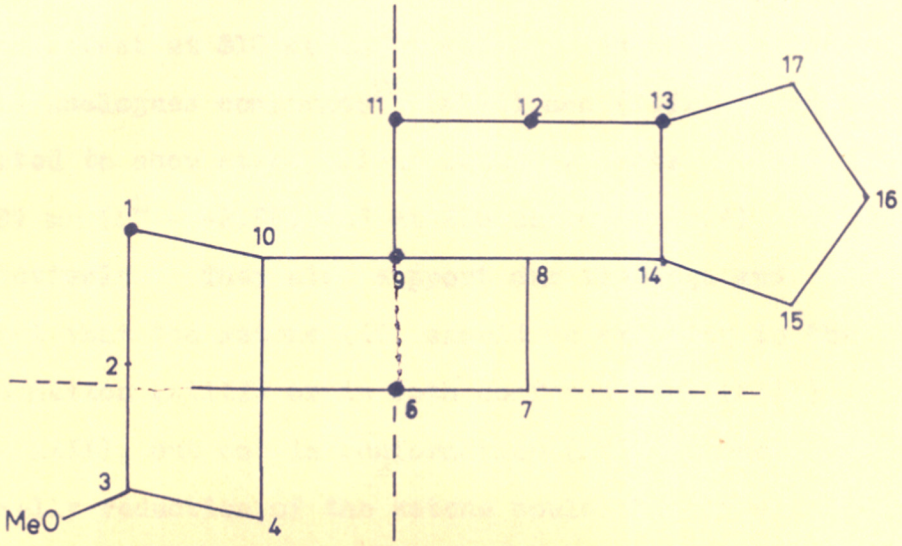


FIG. XXV. OCTANT PROJECTION FOR KETONE XXIII

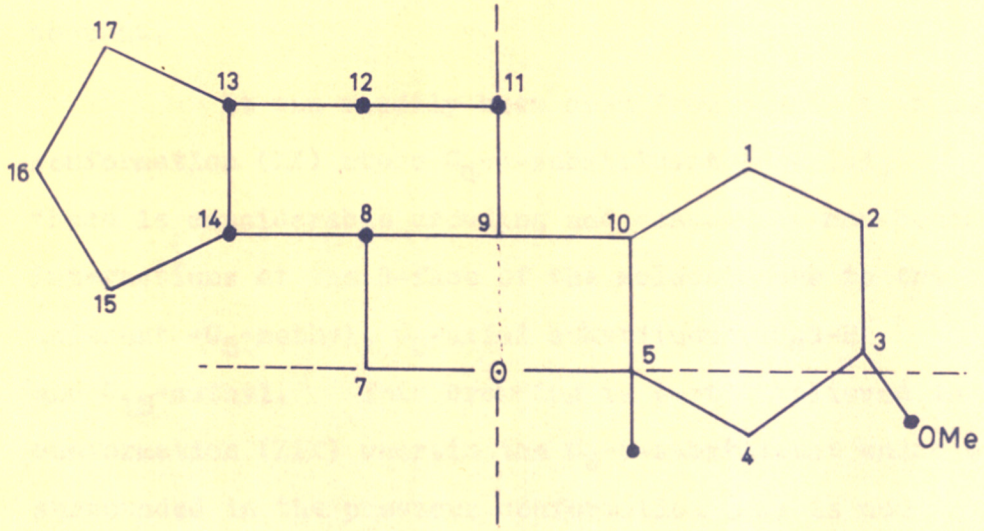


FIG. XXIV. OCTANT PROJECTION FOR KETONE XXII

represented in the octant diagram (XXV). The O.R.D. curve* of the ketone shown in methanol a strongly negative cotton effect at 310 mμ (Δ^E = -6.). As the C.D. curve of the analogues compounds[20] (XXVI) and (XXVII) have been reported to show strong minus cotton effects at 297 mμ (Δ^E = -2.98) and at 296 mμ (Δ^E = -2.8) respectively. They also support our findings and suggest that the ketone (XV) should be existing in the conformation (XXIII) or in both conformations (XXII) and (XXIII), but not in conformation (XXII) alone. Naturally reduction of the ketone could therefore give raise to either C_6-∝-equatorial alcohol or a mixture of equatorial alcohols from both possible half chair conformations. As both alcohols were obtained it may appear that the ketone can exist in both possible forms which have only slight difference in their energy content.

It can readily been seen from models that in conformation (XX) where C_6-β-substituent is axial, there is considerable crowding and consequent non-bonded interactions at the β-face of the molecule due to the adjacent -C_5-methyl, C_3-axial substituent, C_8β-H and C_{13}-methyl. This crowding is partly relieved in conformation (XIX) wherein the C_6-β-substituent which was surrounded in the previous conformation (XX) is now equatorial and no longer subject to these interactions.

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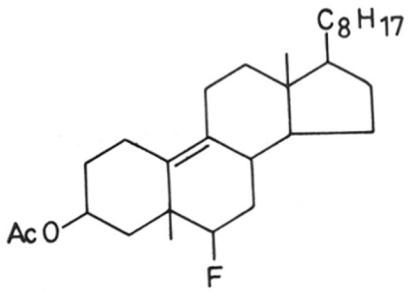
* We are indebted to Prof. W. Klyne.

When the oxidation to ketone occurs strain is relieved and there is very little to choose between either of the two conformations (XXII and XXIII) for the ketone.

After* the communication of our results[17] the French workers who had initially supported the findings of Narayanan and Iyer put forward evidence[22] to show that ring B exists in a boat conformation, the arguments put forth by these workers are summarised below:

(1) The sum of the couplings of the C_6-proton with the C_7-protons is 14 c.p.s. This suggested that ring B is in a boat rather than a chair form as in the case of the latter the sum of the coupling constants should be around 18.5 c.p.s. based on calculations made using Karplus' equation.



XXVIII

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* In fact though our communication[30] appeared in November 1966 whereas that of the French workers appeared in December 1966[22] and January 1967.[24] The dates of receipt of this first French communication was earlier than ours by a month.

(2) The fluorine spectrum of the Westphalen compound having a 6β-fluoro substituent (XXVIII) displays a doublet at 128.3 and 129.1 p.p.m.*+ Each component of this doublet appears as a sufficiently well resolved triplet. The total width at half height being 88 c.p.s. This width is made up besides a large coupling ($J\ gem_{HF}$ = 50 c.p.s.) and a minor coupling* (2 c.p.s.) of coupling with the vicinal 7α- and 7β-protons. The sum of these vicinal couplings being [88-(50+2)] 36 c.p.s. is maximum vicinal coupling in the case of several fluorinated cyclohexane derivatives in the chair conformation is 11.7 c.p.s. These authors believed that the maximum sum of the coupling constant with vicinal hydrogens can only be ~ 23.4 c.p.s. The observed value of 36 c.p.s. according to these authors is due to the ring B being in the boat conformation. In support of this argument they suggested that the sum of the coupling constants in this case is the same as that of compounds containing a fluorine atom in the five membered ring, in which

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*+ Signals are measured in p.p.m. values using trifluoromethyl toluene as the internal reference.

* The minor coupling is due to long range coupling of the C_5-β-methyl with fluorine atom. This coupling (2 CPS) can be seen by splitting of the methyl group in the proton spectrum.

J vic H_F values of the order of 20 and 15 c.p.s. are reported. These authors therefore concluded that as in the five membered ring compounds here also the dihedral angles between fluorine and adjacent hydrogens must also be 0° and 120°.

(3) These authors believe the boat conformation to be more stable than the half chair conformation as there is a heavy interaction between the 1∝ and 11∝ hydrogens which are separated by a distance of 1.4°A in the chair conformation. This distance increases to 2.0°A in the boat conformation.

(4) Conformation of ring B in 6 fluoro and 6-acetoxy derivatives is the same, as the shift of 18-methyl on preparation of Westphalen compound from the starting material is in all cases the same, that is 0.12 p.p.m. downfield as compared to the unrearranged starting material.

In our opinion none of these arguments may be regarded as convincing evidence to rule out the half chair conformation. In the subsequent paragraphs flaws in the arguments of these workers are presented.

(1) In a very detailed[23] analysis of several compounds whose conformations have been well established, it has been shown that the sum of the coupling constants of an axial hydrogen with its vicinal neighbours is around 18 c.p.s. in case of both chair and boat derivatives.

This makes it unreasonable to suppose that if the sum of the coupling constants is 14 c.p.s., it would indicate that the compound exists in boat conformation.

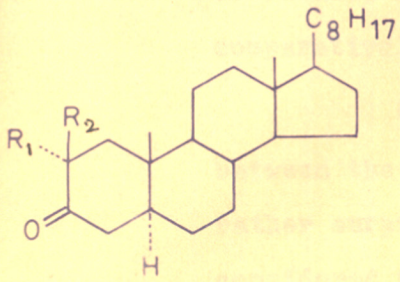
Also the same authors observed that the difference in the coupling constant is larger in compounds of chair conformation than those of boat conformation. These results are presented in the Table 2. It can also be pointed out that though observed coupling constants for vicinal coupling may have larger differences than the actual couplings examples in which the reverse is true are very rare indeed. Based on these arguments the observed coupling of 5 and 10 c.p.s. for the C_6 proton could necessarily imply that the actual couplings may be 5 and 10; 4 and 11; 3 and 12 etc. but not 6 and 10; 7 and 9; etc. Our observed coupling constants therefore favours the half chair in preference to the boat conformation.

(2) As regards to the fluorine P.M.R. spectrum two important objections can be raised namely that in fluorine spectra 1 mm corresponds to 13 c.p.s. In view of this and the fact that no expansion techniques were used by the French workers, it seems doubtful whether the line widths actually observed are in fact those reported. Thus a width of 6.7 mm. corresponds to a boat conformation whereas that of 6 mm. would suggest a chair conformation. In a slightly twisted conformation the difference would be still smaller. This argument/therefore cannot be considered significant.

Table 2

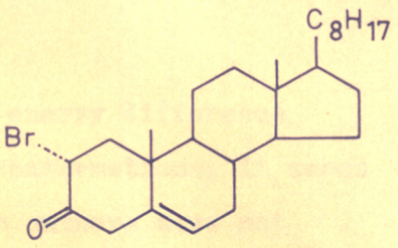
Chemical shifts and coupling constants of 2-bromo-3-ketones

Compound	Conformation	(p.p.m.) X	A	B	J_{AX}	J_{BX}	J_{AB}	Jaa + Jea
XXIX	Chair	5.12	2.68(1β)		5.7	13.4	-13.1	19.1
XXX	Boat	5.08	2.74(K)	2.19	11.1	8.1	-13.4	19.2
XXXI	Boat	4.54	2.68(1β)	2.07	7.6	12.4	-13.6	20.0
XXXII	Boat	4.37	2.66(1β)	2.07	8.0	12.4	-13.6	20.4
XXXIII	Chair	4.70	2.69(1β)	-	6.0	13.2	-13.0	19.2

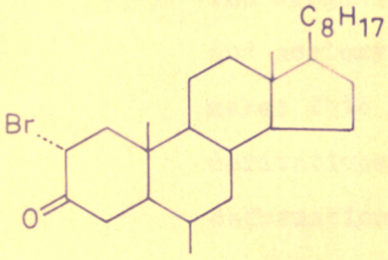


XXIX $R_1 = Br$, $R_2 = H$

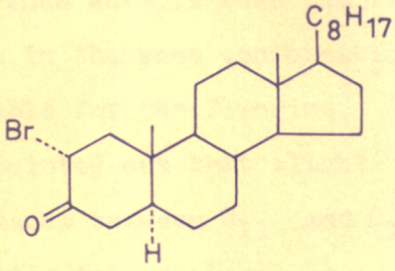
XXX $R_1 = H$, $R_2 = Br$



XXXI



XXXII



XXXIII

Seconldly the number of examples of vicinal coupling in rigid system of F-H couplings are comparatively few.

(3) As regards to the energy difference between the boat and half chair conformations, it seems rather surprising that the French authors have not considered the very important eclipsed interactions which exist in the boat form. As such interactions occur between C_6 and C_7, it can readily be realised that these interactions would considerably destabilise the boat form, furthermore as C_6-also bears a substituent, like the acetoxy group, these interactions will be more significant. The elegant proof given by the French authors that the fluoro and acetoxy compounds both exists in the same conformation, makes this argument equally tenable for the fluorine derivatives. Finally it can be pointed out that slight deformation can increase the distance between C_{11} and C_1 hydrogens in half chair conformation to a distance approaching 2°A. Of course this twisting would require some energy.

In a subsequent paper[24] the French workers have shown that epoxidation of the epimer of Westphalen's alcohol furnishes an α-epoxide which is hydrogen bonded. They argue that this is possible in the boat form of this compound, but not so in the case of the half chair form (XXI) in which this alcohol is equatorial. It can be very readily seen that if there is a gain in energy through hydrogen bonding this alcohol could easily pass over

into the alternate half chair form, when it would become axial and close enough to hydrogen bond.

In case of Westphalen's alcohol the French workers report that a mixture of β- and α-oxides are formed. Though they did not separate the mixture, they put forward arguments to show that the α-epoxide is not hydrogen bonded whereas the β-oxide is. In this case also, it can be seen that the half chair conformation in which this hydroxyl becomes axial (XX) would also be able to form a hydrogen bond with the β-epoxide but not the α-one. It is also seen that for the Westphalen alcohol the α-epoxide cannot be hydrogen bond in either of the conformations (XIX) or (XX).

In our opinion therefore these arguments in fact favour the half chair form, as in the boat conformation the distance between oxygen of the epoxide and the hydroxyl hydrogen is larger than in the case of the half chair forms where the hydroxyl group is axial. A change from one form to another due to the gain in the energy through hydrogen bonding is well documented.[31]

Another very important argument which has not been considered by the French workers is the fact that the I.R. spectrum indicatedthat the acetate group in the Westphalen compound and its epimer is equatorial a position only possible in the two alternate half chairs, but not in a boat, in which the epimer of Westphalen's compound would be

equasi axial, a finding in contrast to the observations. In summing up it can therefore be said that in all possibility, the Westphalen compound and its epimer exist in alternate half chair conformations rather than the boat conformation though further work would be necessary to completely rule out the latter.

EXPERIMENTAL

General remarks

Melting points are uncorrected and have been taken in a Gallenkamp melting point apparatus. Optical rotations were determined in 1% chloroform solution on a Perkin-Elmer spectropolarimeter or a Carl Zeiss polarimeter. Ultraviolet spectra (alcohol solvent) were taken on a Perkin-Elmer model 350 spectrophotometer. Infrared spectra were recorded as Nujol mull unless otherwise stated, on a Perkin-Elmer Infracord or Perkin Elmer Model 221 spectrophotometer, maxima are reported in cm^{-1}. Proton magnetic resonance spectra were recorded on a Varian A-60 spectrometer in carbontetrachloride solution, using tetramethyl silane as the internal standard. The chemical shifts are reported in p.p.m. (unless otherwise stated). Alumina used in chromatography was neutral and grade II[25]. Thin layer chromatography was carried out using the apparatus described by Gupta and Sukh Dev[26], on silica gel (200 mesh) mixed with plaster of paris 15% as binder. Spots were visualised by spraying with concentrated sulphuric acid.

Pet. ether refers to the fraction boiling between 60-80°C.

Cholesteryl methylether[16]

To a solution of cholesterol (2 g) in dry benzene (96 ml), potassium metal (1.1 g) was added and the mixture was refluxed for one hour with vigorous shaking at intervals to disperse the molten potassium into small globules. Methyl iodide (36 ml) was added and refluxing continued for three hours, when potassium iodide generally separated out. The reaction mixture was then cooled, methanol was added and the solvents were removed in vacuo. The residue was extracted 4-5 times with boiling pet. ether. The pet. ether eluates were filtered through a column of alumina (60 g).

The pet. ether eluates on crystallisation from acetone-methanol gave colourless long needles of cholesteryl methylether.

Yield 1.5 g.

M.P. 84° Lit.[27] 85°

$[\alpha]_D$ -45° Lit.[27] -45.8°

I.R. 1195 and 1108 cm^{-1} (aliphatic ether)

Elution of the column with ether gave cholesterol (0.4 g) which was crystallised and identified by m.p. and mixed m.p.

Cholestane-3β-5α-6β-triol-3-methylether[16]

To a suspension of cholesteryl methylether (1 g) in formic acid 80% (10 ml), hydrogen peroxide 33% (1.5 ml) was added slowly and the reaction mixture was kept for fifteen hours at room temperature. Hot water was added to the reaction mixture to destroy the excess of hydrogen peroxide,

cooling at 0° for 1 to 2 hours yielded a white mass which was filtered and refluxed with 2.5% methanolic potassium hydroxide (50 ml) for 6 hours. The reaction mixture was poured into water and extracted with ether. The ether layer was washed with dil. HCl, $NaHCO_3$ and distilled water, dried over sodium sulphate and evaporated. A white solid was obtained which on crystallisation from ether-methanol gave a crystalline compound.

Yield	0.6 g.		
M.P.	153°	Lit.[28]	154°
$[\alpha]_D$	-6°	Lit.[28]	-5.8°

I.R. 3500, 3300 cm^{-1} (hydroxyl)

3β-Methoxy-5β-methyl 19 nor-5β-cholest 9(10)en 6β-acetate[29] (XVII)

The triol methyl ether (250 mg) (XVIII) and acetic anhydride (2.5 ml) were heated on water bath for one and half hours. Then glacial acetic acid (10 ml) and acetic acid containing sulphuric acid (1 cc of acetic acid containing 12 mg of H_2SO_4, 0.5 ml) was added and kept at room temperature for one and half hours. Afterwards it was poured into crushed ice and left overnight. It was extracted with ether and the organic layer was washed with sodium bicarbonate and water, dried and evaporated to obtain an oily material which was chromatographed on alumina (8 g.)

Pet. ether, benzene (1:1) elution gave a white solid which was crystallised from methanol thrice to obtain the expected Westphalen acetate.

M.P.	120-121°	Lit.[28]	122°
$[\alpha]_D$	+84°	Lit.[28]	+84°

I.R. 1750 cm^{-1} (acetate)

3β-Methoxy 5β-methyl 19 nor 5β-cholest 9(10) en 6β-ol (XIV)[28]

The rearranged product was refluxed with 5% methanolic potassium hydroxide for six hours, when the corresponding 6β-hydroxyl compound was obtained.

Yield quantitative.

M.P. 107° Lit.[28] 108°

$[\alpha]_D$ +120° Lit.[28] +118°

I.R. 3500 cm^{-1} (hydroxyl)

3β-Methoxy 5β-methyl 19 nor 5β-cholest 9(10) en-6-one (XV)[15]

The above compound (450 mgs) in pyridine (4 ml) was treated with chromium trioxide (450 mgs) in pyridine (4 ml). After twenty-four hours at room temperature the product was worked up as usual to obtain oily material which was chromatographed over alumina. Benzene elution gave a white solid which was crystallised from methanol.

Yield 250 mgs.

M.P. 64° Lit.[15] 64°

$[\alpha]_D$ -4° Lit.[15] -4°

I.R. 1725 cm^{-1} (ketone)

Sodium-alcohol reduction of 3β-methoxy 5β-methyl 19 nor 5β cholest 9(10) en 6-one (XV)[15]

The methoxy ketone (400 mgs) in ethanol (10 ml) was refluxed with sodium (2.5 g) for two hours. Isolation of the product in the usual way gave an oil which was chromatographed on alumina (15 g). Elution with ether gave the product as an oil.

Yield 200 mgs

$[\alpha]_D$ $+20^\circ$ Lit.[15] $+22^\circ$

I.R. 3400 cm^{-1} (hydroxyl)

3β-Methoxy 5β-methyl 19 nor 5β cholest 9(10) en 6∝-acetate (XVIII)

The above material (300 mgs) was acetylated using pyridine (2 ml) and acetic anhydride (2 ml) in the usual way. Work up gave an oil which on T.L.C. (benzene + 5% pet. ether) showed two spots (R_F 0.53, 0.56) none of them corresponds to the starting material. The faster moving spot corresponds to the Westphalen acetate.

A separation of this mixture using preparative thin layer chromatography afforded both components in pure form. The faster moving compound (120 mgs) was identified as Westphalen acetate by its M.P., I.R. and N.M.R.

The slower moving compound (60 mgs) could not be obtained in crystalline form, though its homogenity could be clearly seen not only by T.L.C. in several solvent systems, but also by its P.M.R. spectrum.

Yield 60 mgs.

$[\alpha]_D$ $+30.4^\circ$

Analysis:

Found: C, 78.64; H, 10.87%

$C_{30}H_{50}O_3$ requires: C, 78.55; H, 10.99%

I.R. 1730 cm^{-1} (acetate)

3β-Methoxy 5β-methyl 19 nor 5β-cholest 9(10) en 6∝-ol (XVI)

Hydrolysis of 6∝-acetate (50 mgs) with 5% methanolic potassium hydroxide gave the pure alcohol as an oil.

Yield 30 mgs.

$[\alpha]_D$ +15.5°

Analysis

Found: C, 80.51%; H, 11.4%

$C_{28}H_{48}O_2$ requires: C, 80.7%; H, 11.6%

I.R. 3400 cm^{-1} (hydroxyl)

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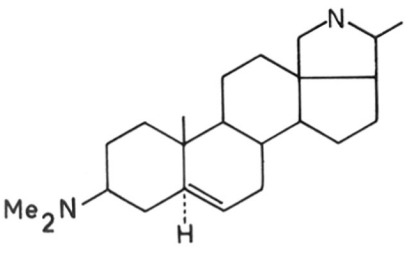
CHAPTER 2

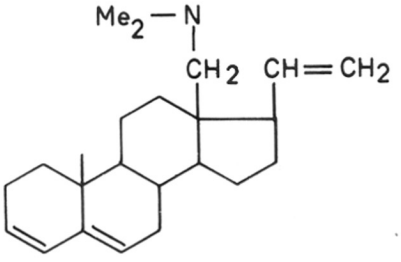
SUBSTITUTION OF THE N,N-DIALKYL GROUP BY AN ACETATE.

In several steroidal alkaloids a dimethyl amino group is usually present on the steroid nucleus. One of the requirements in the investigation of steroidal alkaloids is therefore to find a method of replacing the dimethylamine residue by a new group whose position would be helpful in locating this dimethyl amino function. A brief survey of different methods used for this purpose is given below. Only a few representative examples are chosen as illustrations.

The oldest procedure for the removal of the dimethyl amino function is Hoffman[1] degradation. The first use of this procedure in steroidal alkaloids was reported by Spath and Hromatka[2] who investigated the degradation of conessine derivatives. In a study Haworth, Mekenna and Whitfield were able to obtain the triolefin (2) from conessine (1) in 40% yield.

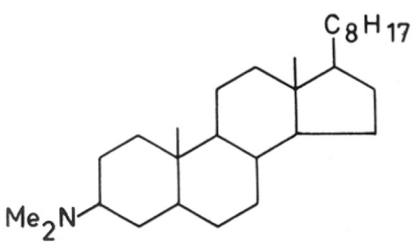


(1)

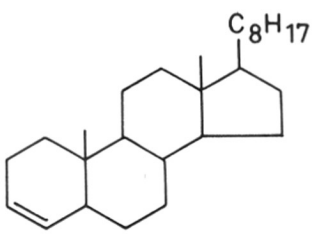


(2)

When this degradation was attempted[4] with 3β-dimethyl amine cholestane (3), an yield of 5% of the Δ^3-compound (4) was obtained. Even in the case of 3β-dimethyl amino allopregnanes a low yield (~ 5%) was obtained. In this reaction with 3β-dimethyl amino Δ^5-cholestane the yield was not mentioned. As the yield of the olefin is very high (50%) in the case of the 3α-dimethyl amino compounds, it was argued that this low yield of olefins in the case of 3β-dimethyl amino compounds arises from the fact that in these compounds the trans anti geometry required for elimination[5] is not present.

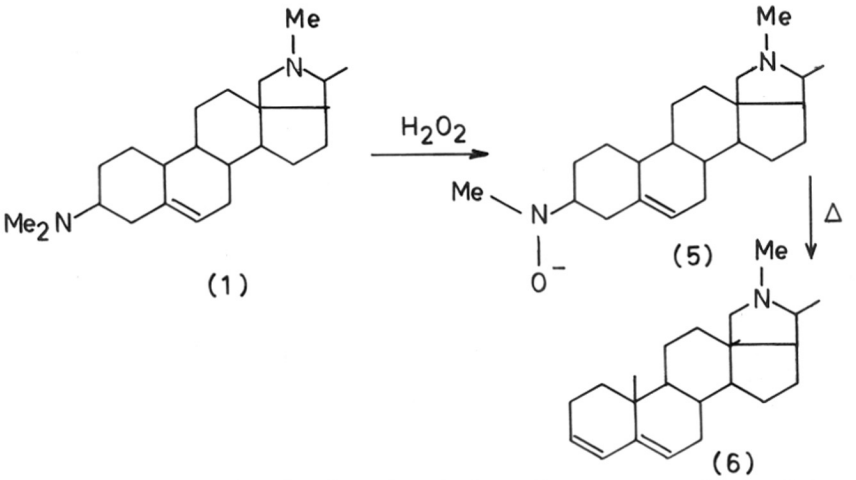


(3)



(4)

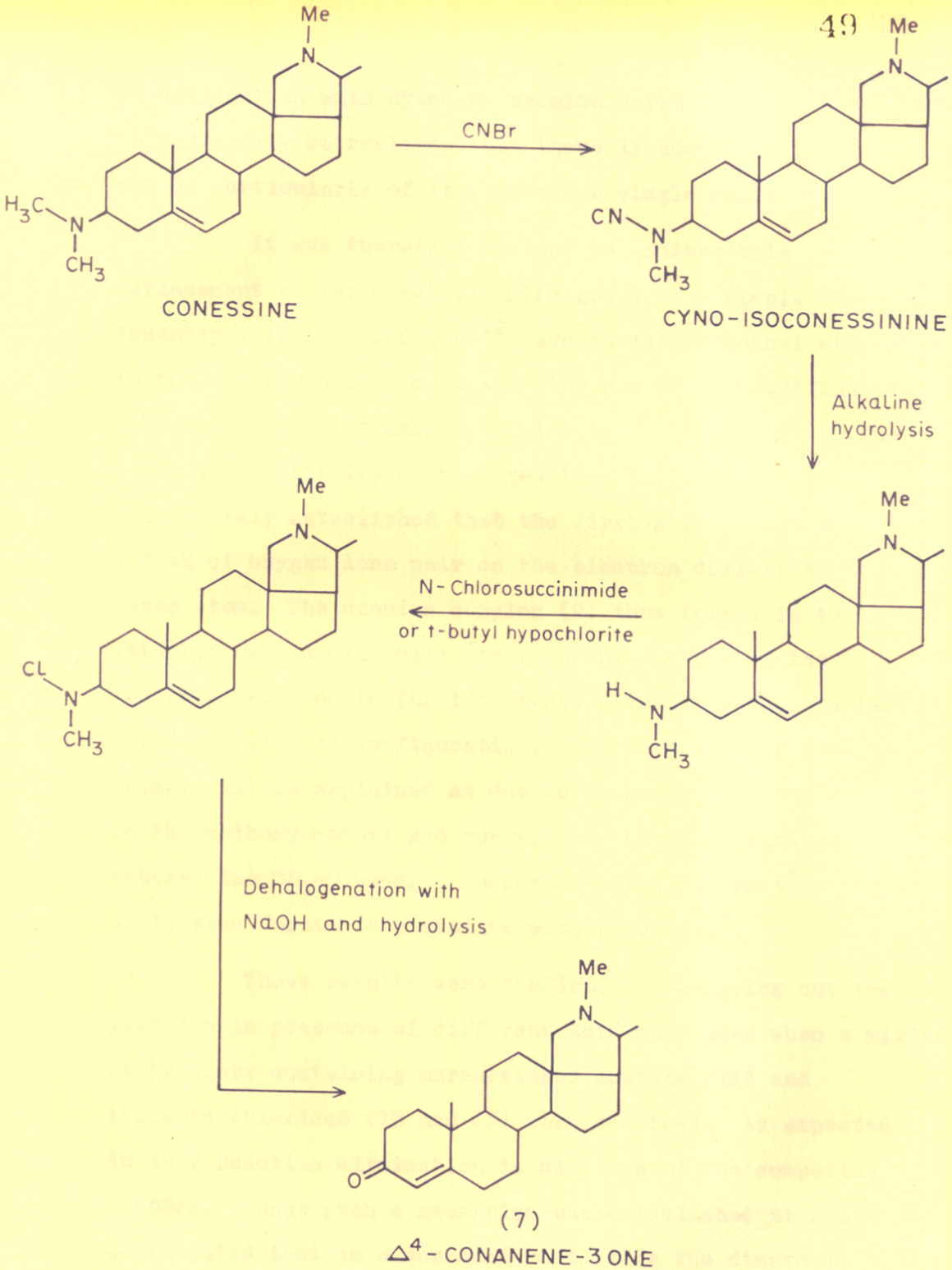
Recently Bhattacharyya[6] et al reported the pyrolysis of the N-oxide (5) from conessine (1) and obtained the diolefin (6). These workers however did not report the yield in this reaction.



(1) (5) (6)

A very standard method for the conversion of an amine to the ketone (7) is Ruschig's[7] procedure. In this procedure the amino function is first converted into a chloro-derivative, subsequent dehydrochlorination and hydrolysis of the enamine furnished the required ketone. One of the important requirements for utilising this procedure is the fact that the nitrogen atom must carry at least one hydrogen substituent. In such cases this method can be regarded as the method of choice. The scope of Ruchig's procedure can be extended by the use of cyanogen bromide[8] which results in the conversion of an N-dimethyl amino group into the methyl amino derivative. Actually this method of demethylation has been used by W.S.Johnson[9] et al who obtained Δ^4-conanene 3-one (7) from conessine (1) by the scheme outlined in the Chart I. Several other examples of the use of cyanogen bromide and the Ruschig procedure have been reported.[10-13]

From the above few examples it is clear that the best available method for replacing the dimethyl amino group by a suitable function (for the purpose of fixing the location of the dimethyl amino group) is the Ruschig procedure



(7)

Δ^4-CONANENE-3 ONE

CHART - I

CONVERSION OF A DIMETHYL AMINO GROUP TO A KETONE

in conjunction with cyanogen bromide demethylation. It can easily be realised that there is scope for a new method particularly of it involves a single reaction.

It was therefore decided to achieve this replacement of the dimethyl amino group by a simple process. Recently Narayanan and Iyer[14] have converted methyl ethers to the corresponding acetate by the use of borontrifluoride etherate and acetic anhydride mixture. In a study of the mechanism (Chart II) of this reaction these workers conclusively established that the first step involves the attack of oxygen lone pair on the electron deficient boron atom. The oxonium complex (9) thus formed is then attacked by a nucleophile $\bar{O}Ac$ with the rupture of the weakened C-O bond to furnish predominately the acetate (10) with inversion of configuration. The formation of the epimer (11) is explained as due to an attack by $\bar{O}Ac$ on the methoxy methyl and rupture of the ether linkage between the CH_3-O bond. Subsequent attack with $\overset{+}{A}c$ would then furnish the acetate with retention.

These results were confirmed by carrying out the reaction in presence of different acid chlorides when a mixture of products containing unrearranged acetate (11) and isomeric chlorides (12 and 13) were obtained. As expected in this reaction elimination is also one of the competing factors. Once such a mechanism was established it was anticipated that in a compound containing the dimethyl amine group, also all the conditions required for this

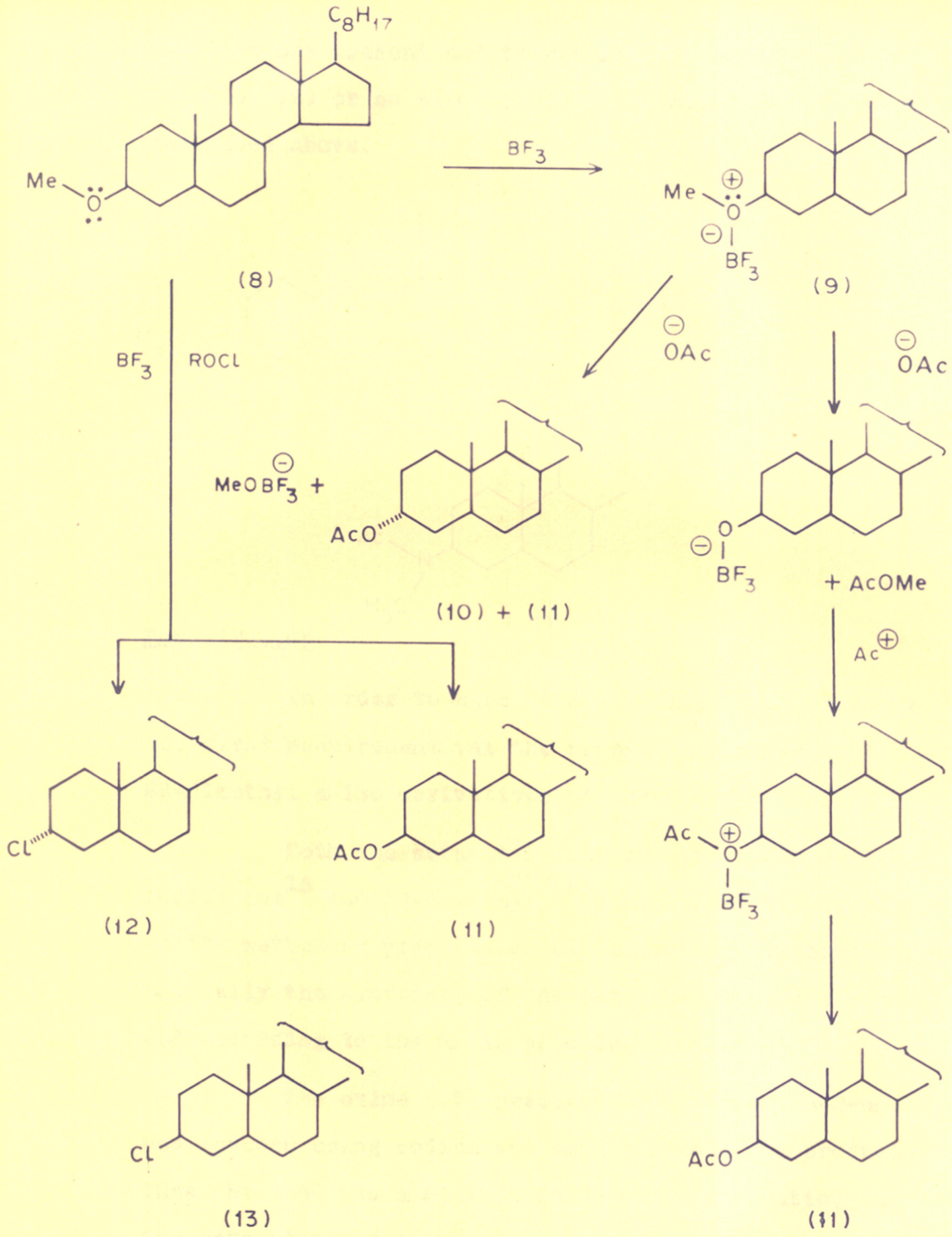
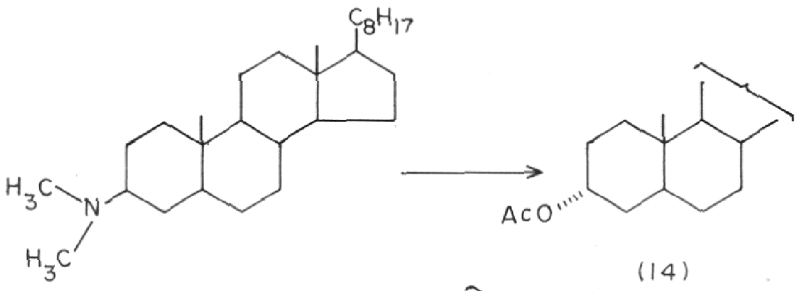


CHART - II

MECHANISM OF BF_3 - CATALYSED CONVERSION OF METHYL ETHERS TO ACETATE

reaction are present and therefore one should obtain an acetate (14) or an amide (15) by a similar mechanism as described above.



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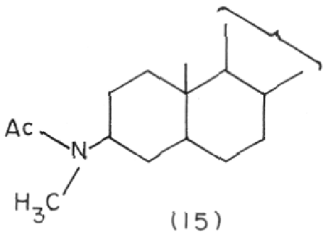


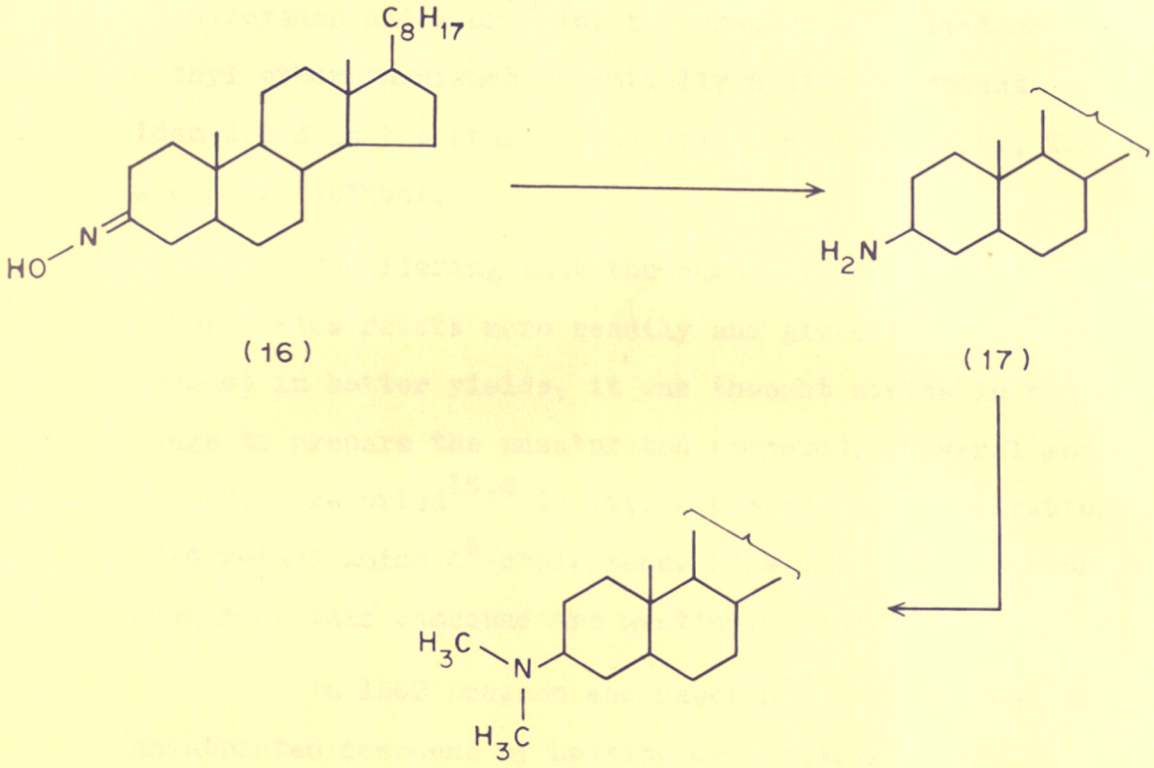
(15)

PRESENT WORK

In order to check the validity of these ideas the first requirement was the preparation of suitable 3β-dimethyl amino derivatives of steroids.

Both the saturated compound viz. 3β-dimethyl amino cholestane[15] and 3β-dimethyl amino Δ^5-cholestene[9] have been reported. As the method of preparation of the latter compound was tricky, initially the synthesis of the saturated compound was carried out according to the known procedure (Chart III).

The oxime (16) prepared from cholestan-3-one was reduced using sodium and amyl alcohol, the product (17) thus obtained was a mixture in which it was anticipated that the more stable β-amino compound (17) predominated. This was confirmed by the fact that crystallisation of the methylated product (18) afforded needles m.p. 104°C. $[\alpha]_D$ 20°.



(16) (17) (18)

CHART - III

PREPARATION OF 3β - DIMETHYL AMINO CHOLESTANE

These properties were in agreement with those reported[15] for the 3β-dimethyl amino cholestane*.

Treatment of this compound with boron trifluoride and acetic anhydride under the conditions described by Narayanan and Iyer[14] for the analogous cholestane 3β-methyl ether furnished essentially a single product identified as the starting material by usual (M.P., T.L.C. and I.R.) methods.

Considering that the homoallylic compound in the ether series reacts more readily and gives the described product in better yields, it was thought advisable at this stage to prepare the unsaturated compound. Several methods have been reported[15,4] in literature for the preparation of 3β-dimethyl amino Δ^5-cholestene. The different methods for preparing this compound are mentioned below.

In 1952 Dodgson and Haworth[15] had prepared the unsaturated compound by heating cholesteryl chloride (19) in 50% methanolic dimethylamine hydrochloride for 18 hours at 80°. This method afforded the amine (20) in an yield of 10% (See Chart IV).

Haworth, McKenna and Powell[4] had obtained the compound in slightly better yields (~ 15%) by heating cholesteryl tosylate (21) with dimethylamine at 100° for 8 hours. But they obtained two other compounds besides the required product.

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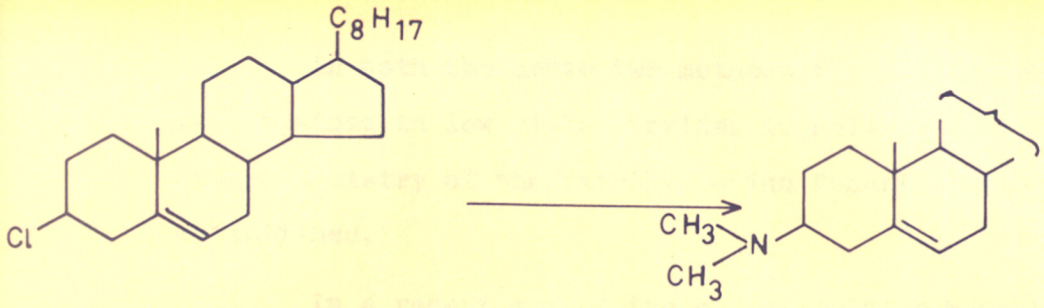
* The P.M.R. spectrum did not help in explaining the stereochemistry of the dimethyl amino function as the 6-proton singlet of the dimethyl amino group masked the signal due to the C-3 proton.



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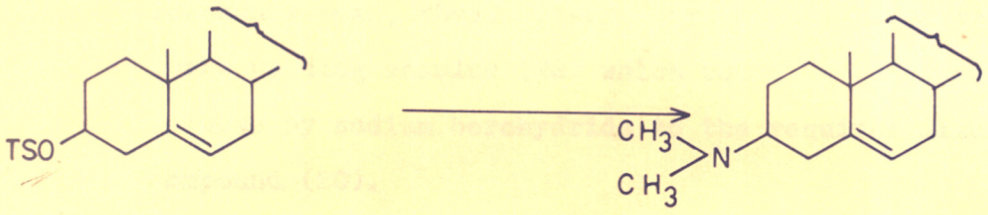

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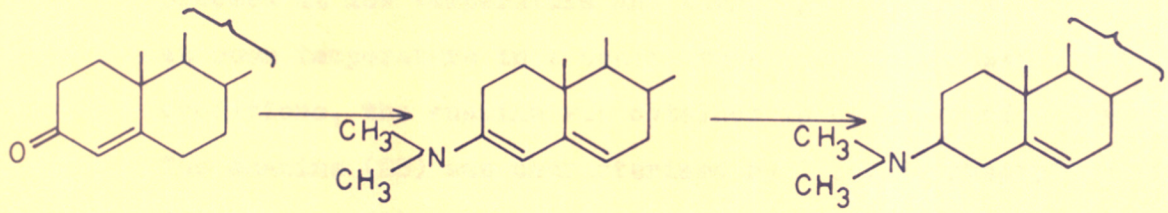

(22) (23) (20)

CHART - IV.

DEFFERENT METHODS OF PREPARIATION OF 3ß- DIMETHYL AMINO Δ5- CHOLESTENE

In both the above two methods the desired product was obtained in low yield, besides in neither case had the stereochemistry of the dimethyl amino function been established.

In a recent method these difficulties have been overcome by Johnson, Bauer and Franck[9] who obtained the required compound in good yield, by the reaction of Δ^4-cholestan-3-one (22) with dimethyl amine in presence of para toluene sulphonic acid, these workers were able to isolate the corresponding enamine (23) which could be conveniently reduced by sodium borohydride to the required dimethyl amino compound (20).

It was therefore decided to follow the above procedure in the present case. The major difficulty however was the absence of any detailed reaction conditions. After several trials it could be established that the reaction did not proceed at low temperature and therefore had to be conducted at room temperature in a sealed tube. Under these forcing conditions, the enamine was obtained in an yield of 40%. The enamine (23) was characterised by its U.V. absorption at 270 mμ (ε=1700).

The reduction of the enamine could be readily achieved using sodium borohydride. The product (20) thus obtained has no absorption above 220 mμ. In its P.M.R. spectra (Fig. 1) this material displayed a sharp 6-proton singlet at 2.3 p.p.m. characteristic of an N-dimethylamino group and an olefinic proton at 5.1 p.p.m., as a narrow multiplet.

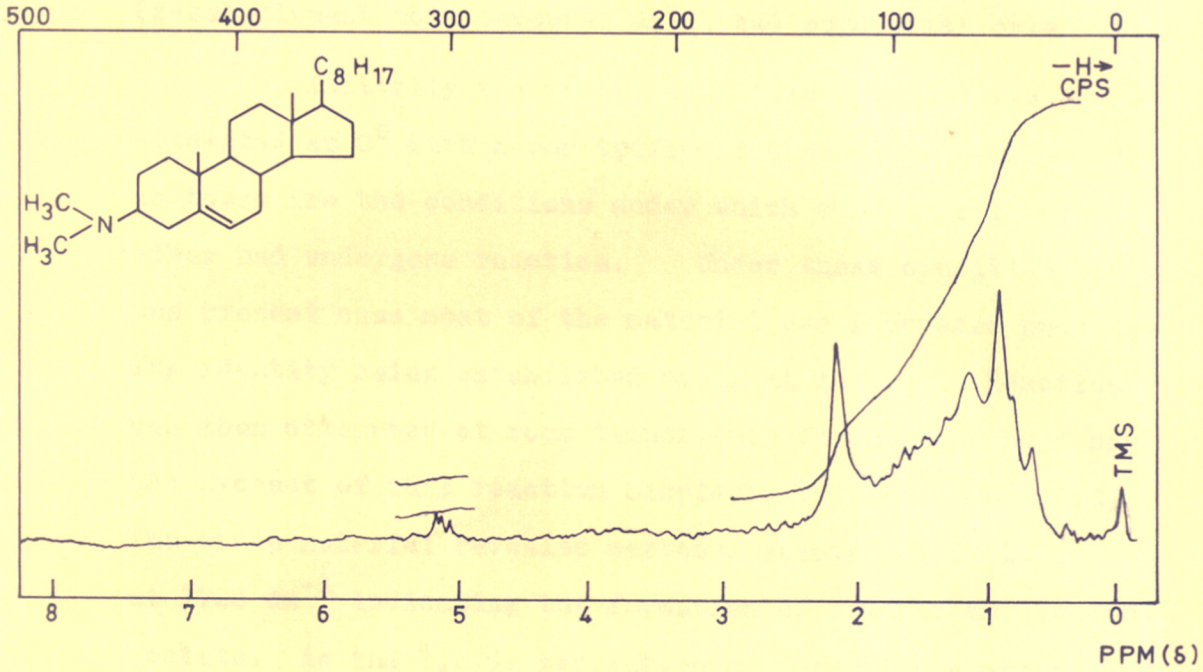

FIG. 1 . PMR SPECTRUM OF 3ß-DIMETHYL AMINO Δ^5-CHOLESTENE

The P.M.R. spectrum was not useful in assigning the stereochemistr of the dimethylamino function as the C-3 proton is not clearly visible. However as this compound has formed through borohydride reduction of the enamine it should have the β-orientation through both steric approach control (α-attack) and product development control.[16,17] (β-substituent more favoured as it had equatorial orientation).

Initially the reaction of this material was attempted at 0° with boron trifluoride and acetic anhydride as these are the conditions under which cholesteryl methyl ether had undergone reaction. Under these conditions in the present case most of the material was recovered unchanged. Its identity being established by usual methods. Reaction was then attempted at room temperature for one and half hours, the product of this reaction displayed two spots on T.L.C. The crude material revealed carbonyl absorption in I.R. at 1725 cm^{-1} indicating the formation of some of the desired acetate. As the T.L.C. separation of these two compounds was not good, it was anticipated that separation on column of this mixture would be very difficult. As the expected product is an acetate which has to be separated from the starting material, the separation would be facilitated if hydrolysis was carried out. T.L.C. of the hydrolysed product clearly bore out the effectiveness of this reasoning. A facile separation through alumina chromatography was then effected. The benzene eluate yielded needles m.p. 148°, $[\alpha]_D$ -33° which could be identified as the starting material. The benzene-10% ethyl acetate eluate provided a crystalline

material m.p. 148° identical in every respect (T.L.C., I.R., M.P. and mixed M.P.) with cholesterol.

As the yield of cholesterol is poor (5%) different reaction conditions were attempted. In order to improve the yield the reaction was carried out at room temperature for 30 hours. The T.L.C. and I.R. of the product indicated that there was no appreciable increase in the percentage of cholesterol.

Reaction was then attempted with heating on water bath for one and half hours. The product in this case was however tarry and no cholesterol could be isolated from it after hydrolysis.

All these experiments indicated that though reaction is taking place, according to expectation, the yield is not satisfactory to make it a practical procedure. However from a mechanistic angle these experiments demonstrate the validity of the earlier mechanism suggested by Narayanan and Iyer[14].

As mentioned earlier (Chart II) retention of configuration in the case of a saturated compound involves an attack by $\bar{O}Ac$ on the $-O-CH_3$ band with subsequent attack by $\overset{+}{Ac}$. In the case of the unsaturated compound an alternate route is also available for the formation of product with retention of configuration. In this alternate method the participation of double bond is invoked in order to aid the rupture of the steroid oxygen bond, this results in inversion. A second inversion through attack by $\bar{O}Ac$, then yields the product with retention.

In the present case the extent of these two factors in affording the product with retention can easily be judged because rupture by earlier mechanism (path A, Chart V) would give an amide whereas rupture by path B would yield the acetate. As no amide is formed in any of these reactions it is clear that path B is the only possible method for explaining the results. It is also clear that here path C involving attack by ^{-}OAc followed by rupture of steroid nitrogen bond is totally absent as the product does not contain any epicholesteryl acetate.

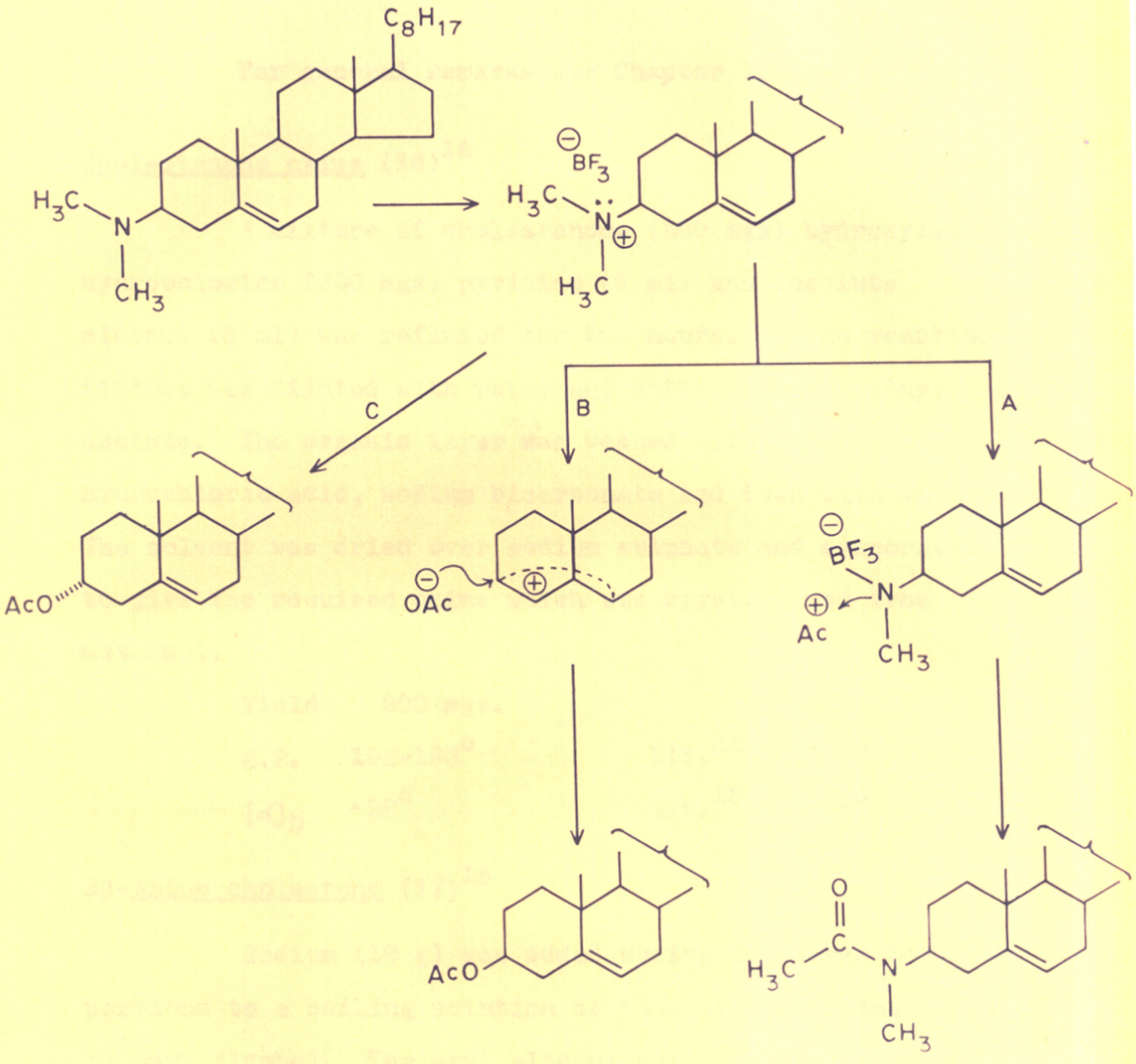

CHART IV

MECHANISM OF BF_3 - ETHERATE CATALYSED REACTION

3 β - DIMETHYL AMINO Δ^5 - CHOLESTENE

EXPERIMENTAL

For general remarks see Chapter I.

Cholestanone oxime (16)[15]

A mixture of cholestanone (300 mgs) hydroxylamine hydrochloride (300 mgs) pyridine (2 ml) and absolute alcohol (5 ml) was refluxed for two hours. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with dilute hydrochloric acid, sodium bicarbonate and then with water. The solvent was dried over sodium sulphate and evaporated to give the required oxime which was crystallised from methanol.

Yield	200 mgs.		
M.P.	192-193°	Lit.[15]	196°
$[\alpha]_D$	+38°	Lit.[15]	+38°

3β-Amino cholestane (17)[15]

Sodium (12 g) was added during two-three hours in portions to a boiling solution of cholestanone oxime (2 g) in amyl alcohol. The amyl alcohol solution was washed with water and then evaporated under reduced pressure. The residue was taken up in dry ether. The amine hydrochloride which precipitated on passing hydrogen chloride gas was filtered, basified with ammonia and extracted with ether. The ether layer was washed with water, dried and evaporated under vacuo to provide the required amine which was crystallised from alcohol.

Yield 1 g.

M.P. 101° Lit.[15] 106°-120°

$[\alpha]_D$ +20° Lit.[18] +29°.

3β-Dimethyl amino cholestane (18)[15]

3β-Amino cholestane (800 mgs) was heated on water bath for three hours with 90% formic acid (16 ml) and 40% formaldehyde (14 ml). After the addition of ethanol (13 ml) concentrated hydrochloric acid (6 ml) and sodium nitrite (260 mgs) the mixture was heated (on water bath) for one hour more. The product was taken up in ether and precipitated as the water insoluble hydrochloride. Usual work up yielded the dimethyl amine which was crystallised twice from ether.

Yield 300 mgs.

M.P. 104°. Lit.[15] 106°

$[\alpha]_D$ +20° Lit.[15] +23°

Action of BF_3-etherate on 3β-dimethylamino cholestane

Acetic anhydride (8 ml) and borontrifluoride etherate (3 ml) were added to an ice cold solution of 3β-dimethylamino cholestane (200 mgs) in dry ether (5 ml). The reaction mixture was allowed to stand for fifteen hours at 0°C. It was then poured in cold water and extracted with ether. The ether solution was washed with sodium bicarbonate and water, dried and evaporated. The white solid obtained was crystallised from ether-methanol.

Yield 180 mgs.

M.P. 104°

Mixed M.P. with 3β-dimethylamino cholestane was undepressed.

3β-Dimethylamino Δ^{3-5} cholestadiene (23)[9]

Δ^{4}-Cholestene 3-one (0.5 g) dimethylamine (15 ml), anhydrous magnesium sulphate (2 g) and a trace of p-toluene sulphonic acid were sealed in a pyrex tube and left at room temperature for sixteen hours. The reaction mixture was taken up in ether and the ether layer was washed with sodium bicarbonate solution and water, dried and evaporated. The white solid obtained was crystallised from acetone.

Yield 225 mgs.
M.P. 96°-97° Lit.[9] 97°-99°
I.R. 1613, 1647 cm^{-1} (olefin)

3β-Dimethylamino Δ^{5}-cholestene (20)[9]

To the stirred solution of the above enamine (200 mgs) and sodium borohydride (100 mgs) in dioxane (8 ml), acetic acid (8 ml) was added gradually at room temperature. The reaction mixture was refluxed for one hour and extracted with ethyl acetate. The organic layer was washed with sodium bicarbonate solution and then with water, dried over sodium sulphate and evaporated to afford the required dimethylamino Δ^{5}-cholestene.

Yield 100 mgs.
M.P. 148° Lit.[9] 150°
$[\alpha]_D$ -33° Lit.[15] -31.5°

Action of BF_3-etherate on 3β-dimethylamino Δ^{5}-cholestene

(a) At low temperature

Acetic anhydride (8 ml) and borontrifluoride etherate (3 ml) were added to an ice cold solution of 3β-dimethylamino Δ^{5}-cholestene (200 mgs) in dry ether (6 ml).

The reaction mixture was allowed to stand for fifteen hours at 0°C. After usual work up, the white solid obtained was crystallised from acetone.

Yield 180 mgs.

Mixed M.P. with that of 3β-dimethylamino-Δ^5-cholestene was undepressed.

(b) At room temperature

Acetic anhydride (8 ml) and borontrifluoride etherate (3 ml) were added to the solution of 3β-dimethyl amino cholestene (200 mgs) in dry ether (5 ml). After keeping one and half hours at room temperature the reaction was worked up in the usual way to afford a sticky material (183 mg). To facilitate separation the mixture obtained was hydrolysed with 5% methanolic potassium hydroxide (20 ml) by keeping overnight at room temperature. Usual work up gave a product (150 mgs) which was chromatographed on silica gel column (5 g).

Benzene + 5% ethyl acetate gave a white solid (80 mgs) which was found to be starting material by M.P. and mixed M.P.

Ether elution gave a white solid (20 mgs) which was crystallised from ether-methanol. This solid was identified as cholesterol.

M.P.	148°	Lit.[19]	149°
$[\alpha]_D$	-40°	Lit.[19]	-39°

I.R. 3400 cm^{-1} (hydroxyl)

The above experiment was repeated by keeping the reaction mixture at room temperature for thirty hours. Usual work up, hydrolysis and chromatography gave cholesterol in essentially the same yield (23 mgs).

When the reaction was carried out at steam bath temperature. Usual work up, hydrolysis and chromatography failed to yield any cholesterol.

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CHAPTER 3

CARBONYL SPLITTINGS IN INFRARED SPECTRA OF ESTERS OF STEROIDAL ALCOHOLS.

Splitting in carbonyl absorption of steroidal lactones was observed by Jones et al[1,2] in several compounds containing hydroxy and acetoxy functions in addition to the lactone ring. To establish that this splitting is not due to interaction between the lactone and the functional groups they later[3] studied the spectra of simple model compounds and came to the conclusion that this splitting is probably due to Fermi resonance. The authors were however not satisfied that this provides the full explanation and in their opinion intramolecular vibration effects are definitely responsible.

Bond et al[4] observed similar splitting in ∝-β-unsaturated ϒ-lactones and suggested that Fermi resonance involving the carbonyl stretching frequency and the first overtone of the =C-H out of plane deformation affords a satisfactory explanation in the case of ∝,β-unsaturated lactones. However this explanation cannot explain splittings observed in the case of saturated ϒ-lactones. No explanation for the latter splitting was proposed.

Among the other compounds in which such splitting was observed are five membered cyclic anhydrides in which maleic anhydride[5] should be specially mentioned. Splittings have also been observed in the Raman[6] and infrared[7] spectra of benzoyl chloride.

In the present investigation carbonyl splitting was observed in the case of hydroxy esters when the ester group has the axial orientation but not when the ester group has the equatorial orientation.

Present Work

Before discussing the infrared spectra, preparations of certain new compounds used in this investigation have been described.

Preparation of 11β-hydroxy 5∝-pregnane

This compound was prepared starting from 3,11,20-triketo 5∝-pregnane (2), the preparation of which has been reported[8,9] earlier starting from 3β-acetoxy-5∝-pregn 16-en 11-one (1)*. Chart I summarises the reactions involved in this preparation.

Wolfkishner reduction of 3,11,20-triketo 5∝-pregnane (2) was expected to afford 11-keto 5∝-pregnane (3) as the 11-keto function would not form a hydrazone because of its hindered position. The 11-keto pregnane (3) was characterised by its infrared spectrum (1700 cm^{-1}) and P.M.R. spectrum (Fig. 1) in which the position of the C_{10} and C_{13} methyls agree with the values expected on the basis of Zurcher's rule[10].

Calculated	1.00 and 0.52 p.p.m.
Observed	1.00 and 0.53 p.p.m.

Reduction of the 11-keto compound (3) with lithium aluminium hydride was expected to give 11β-hydroxy 5∝-pregnane (4) because of approach of hydride from less hindered side.[11,12]

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* This compound was obtained from Messrs. Cipla Private Ltd., Bombay.

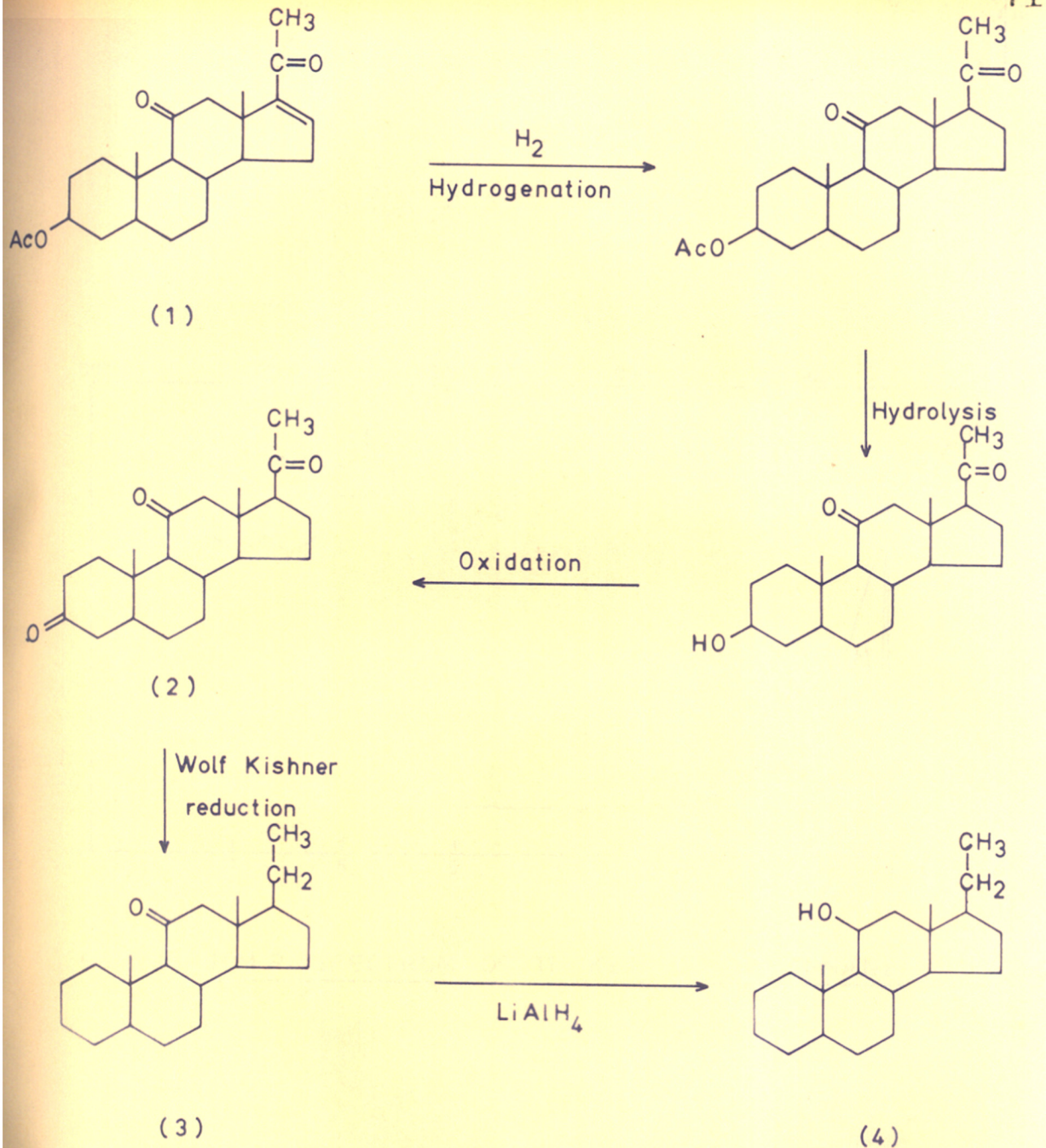


CHART - I.

PREPARATION OF 11 ß- HYDROXY PREGNANE

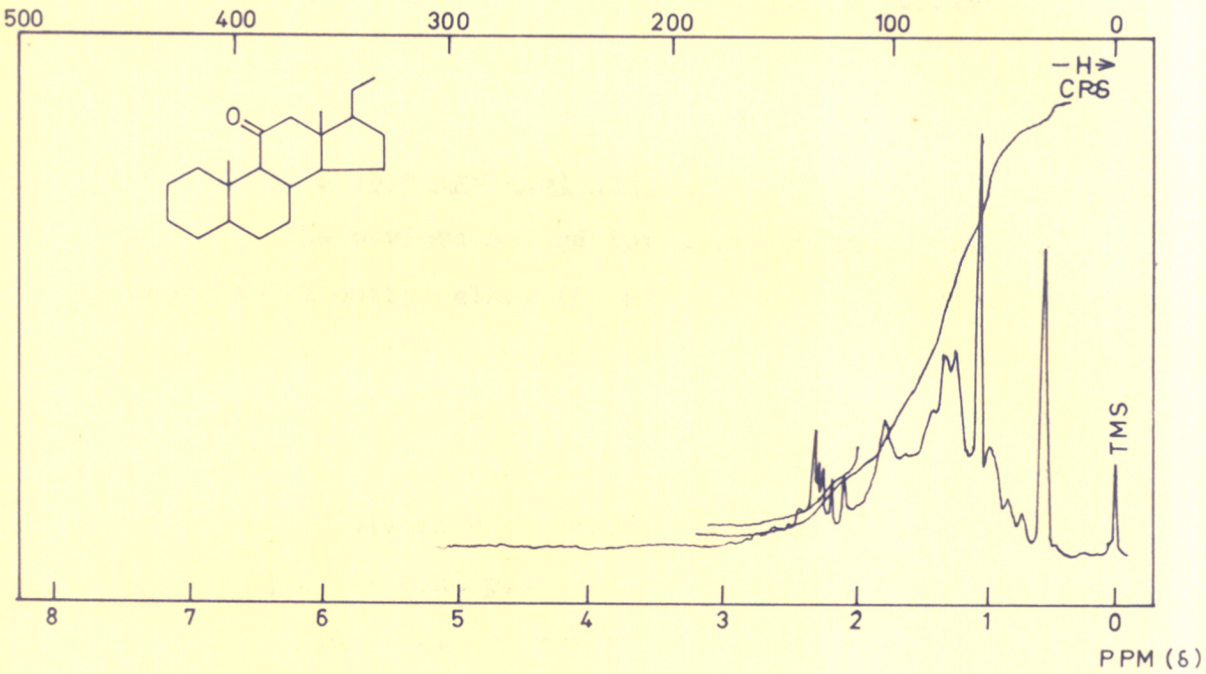


FIG. 1. P.M.R. SPECTRUM OF 11-KETO 5α-PREGNANE

In accordance with this expectation the infrared spectrum revealed the presence of hydroxyl at 3500 cm^{-1} and the absence of a keto function. The P.M.R. spectrum (Fig. 2) had a downfield signal at 4.4 p.p.m. as a narrow multiplet in agreement with the axial orientation of the hydroxyl group. The position of the C_{10} and C_{13} methyls also agree with those expected on the basis of Zurcher's addivity observation[10].

Calculated	1.042,	0.812 p.p.m.
Observed	1.030,	0.78 p.p.m.

3β-Methoxy 5∝-hydroxy cholestane

The obvious method for this compound would involve cholesteryl methyl ether (5) as a starting material (Chart 2). The epoxide of this compound could be obtained by perbenzoic acid epoxidation[13]. The 3β-methoxy cholestane 5∝,6∝-epoxide (6) was characterised by its P.M.R. spectrum which had no olefinic proton. The methoxy methyl appeared as a singlet at 3.33 p.p.m. and the C_3 proton as a broad signal at 3.0 p.p.m. whereas the C_6 proton is seen at 4.2 p.p.m. as a narrow signal.

Reduction of epoxide with lithium aluminium hydride provided as expected 3β-methoxy 5∝-hydroxy cholestane (7) whose infrared spectrum showed hydroxyl frequency at 3500 cm^{-1}. The P.M.R. spectrum (Fig. 3) of this compound shows the C_3 proton as a broad signal at 3.61 p.p.m. and the methoxy methyl at 3.33 p.p.m.

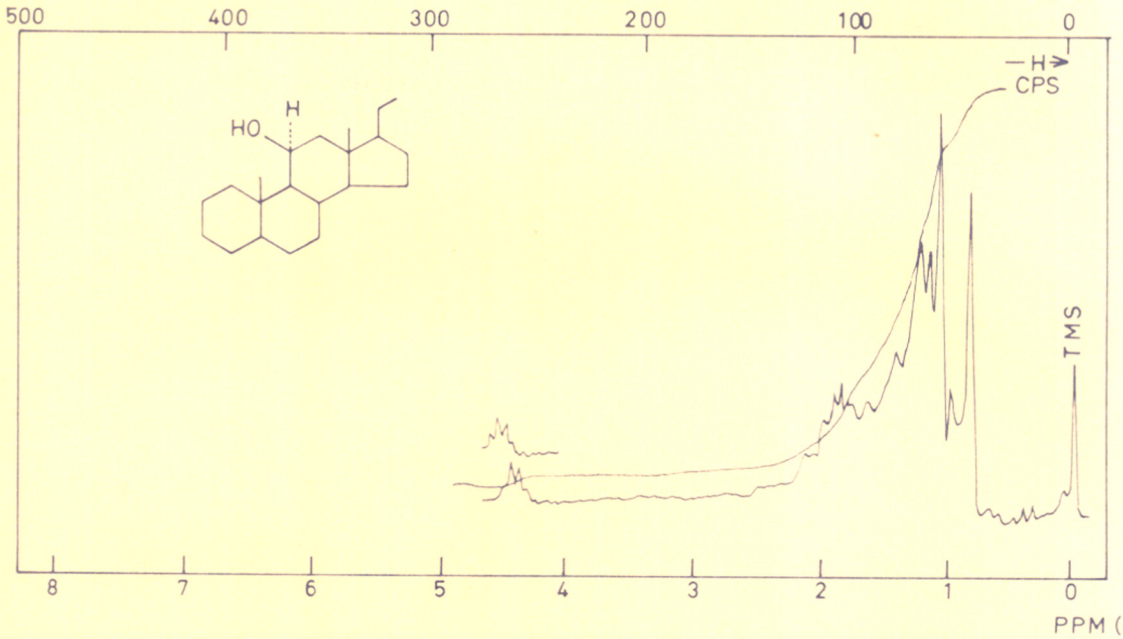
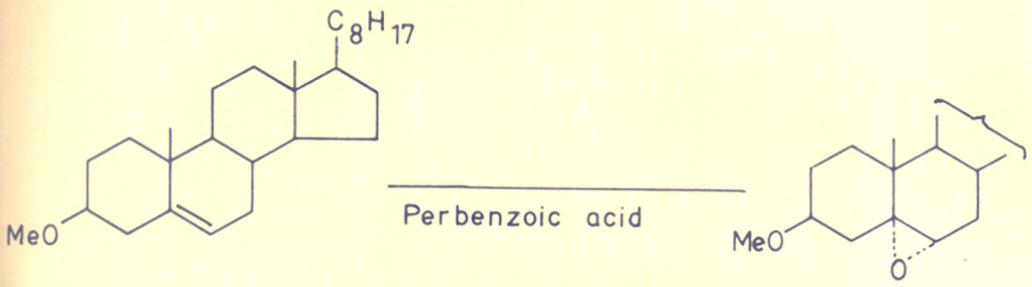


FIG. 2. PMR SPECTRUM OF 11β-HYDROXY-5α-PREGNANE ($CDCl_3$)



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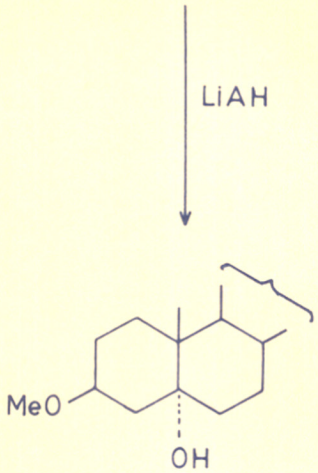

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CHART - 2.

PREPARATION OF 3 ß - METHOXY 5 ∝ - HYDROXYCHOLESTANE

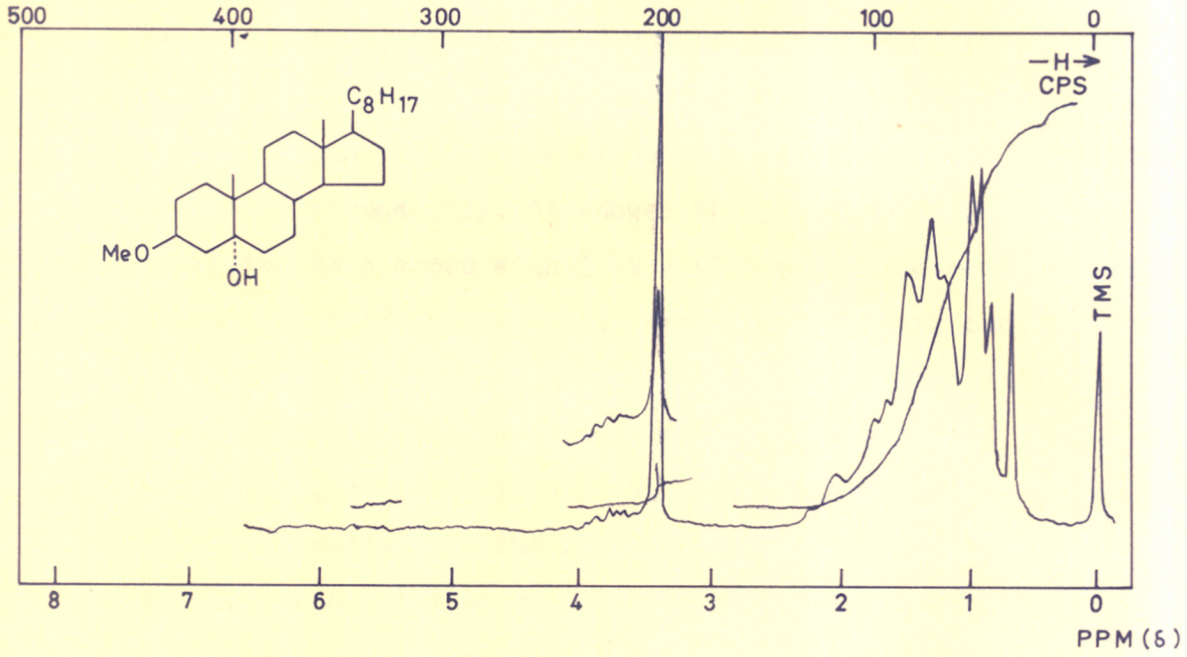

FIG. 3. PMR SPECTRUM OF 3ß-METHOXY 5α-HYDROXY CHOLESTANE

Cholestane 6∝-acetate

A very convenient route for the preparation of this compound was hydroboration-oxidation followed by acetylation (Chart 3). The product obtained from hydroboration-oxidation of Δ^5-cholestene (8) was shown by T.L.C. to be a mixture of two major products. Chromatography over silica gel afforded both compounds in pure form. The more polar of these is expected* to be the required 6∝-hydroxy compound (9). Its infrared spectrum indicated the presence of hydroxyl at 3400 cm^{-1} and the absence of olefinic linkage. The P.M.R. of this compound (Fig. 4) showed the presence of an axial C_6 proton as a broad signal at 3.41 p.p.m. The melting point and rotation of this product were identical with that reported[5] for this compound. The acetate (11) of this alcohol in its infrared spectrum showed no hydroxyl absorption, but had typical absorptions (1750 cm^{-1}) of an acetate function. The P.M.R. spectrum (Fig. 5) exhibited the C_6 proton at 4.65 p.p.m. as a broad signal confirming that the acetate function was equatorial. The physical constants of this acetate also agreed with the reported values[14].

The less polar compound in its infrared spectrum had hydroxyl absorption at 3350 cm^{-1}. The P.M.R. spectrum of this compound exhibited a narrow signal at 3.75 p.p.m. characteristic of an axial hydroxyl. Based on mechanistic

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* On mechanistic grounds it is expected that the other compound would be coprostan 6β-ol. In this compound the C_6-hydroxyl is axial whereas in the desired compound the hydroxyl is equatorial. In chromatography axial alcohols move faster than equatorial ones.

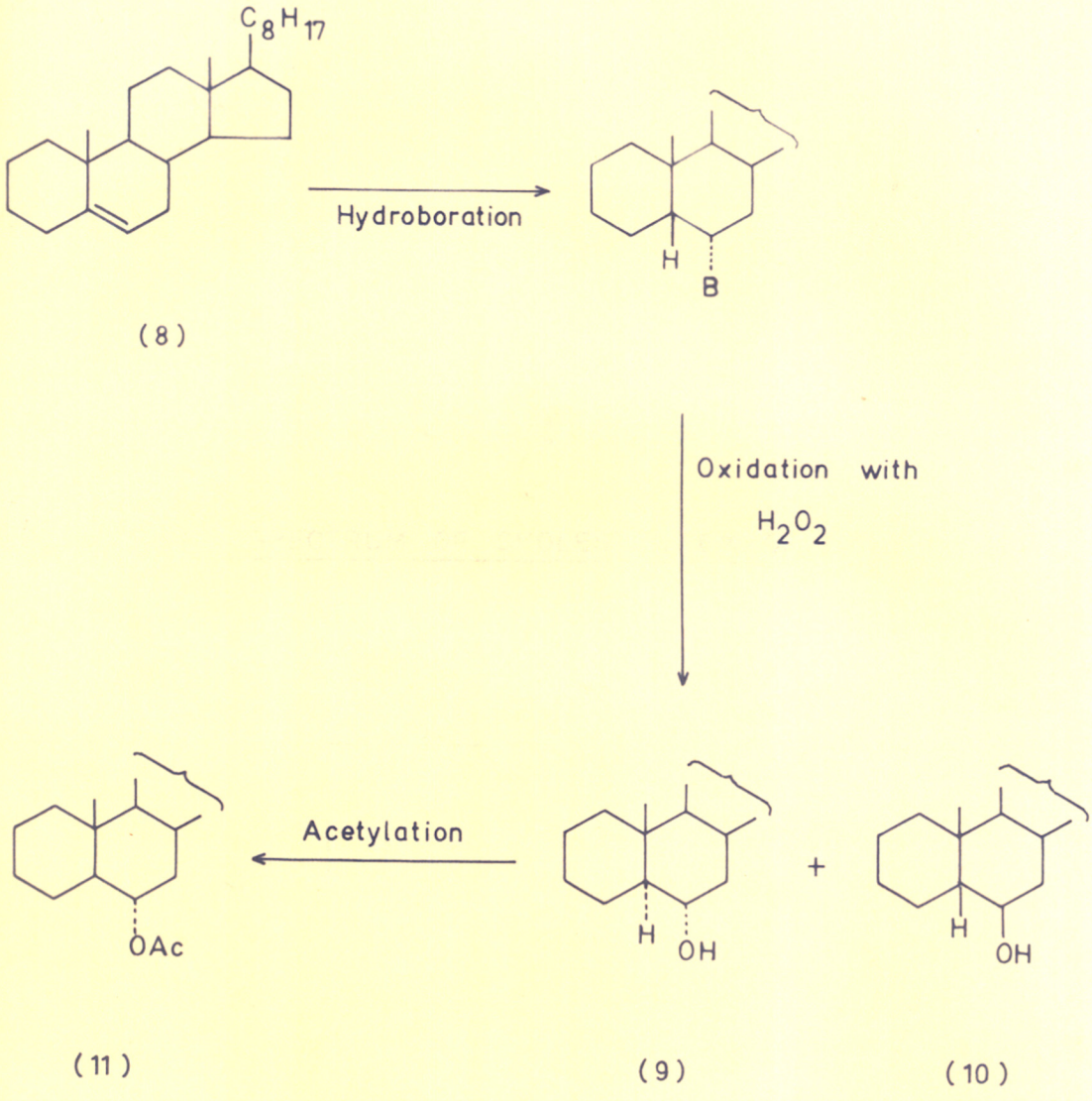

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CHART - 3

PREPARATION OF CHOLESTANE 6α - ACETATE

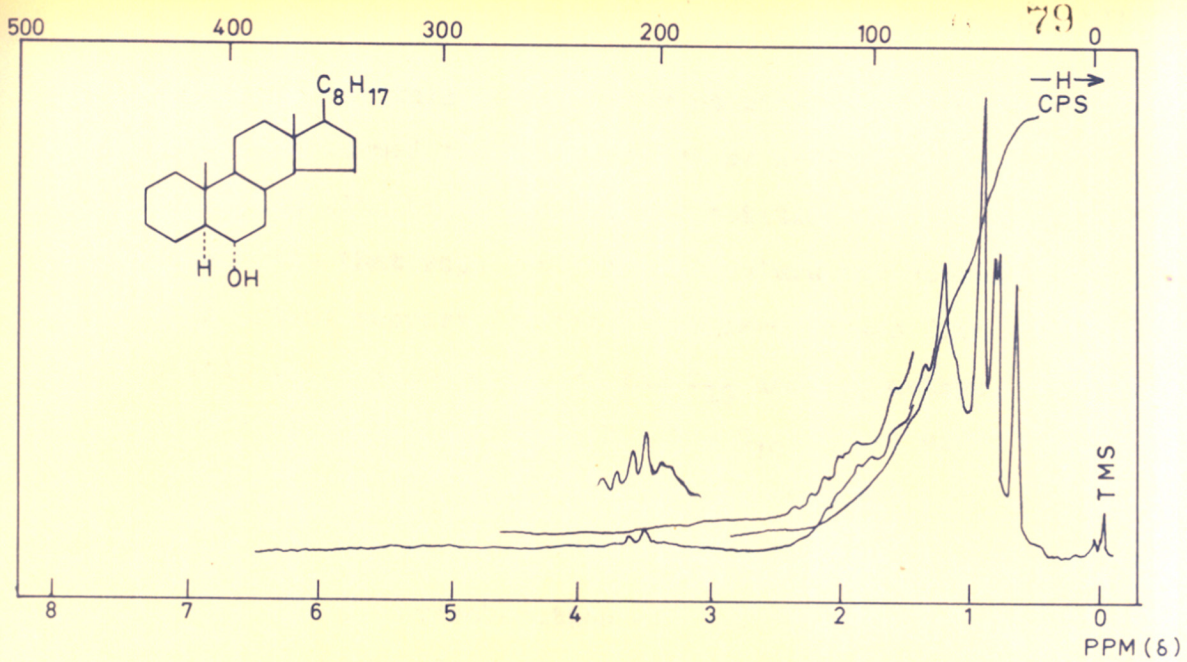


FIG. 4. PMR SPECTRUM OF CHOLESTAN 6α-OL

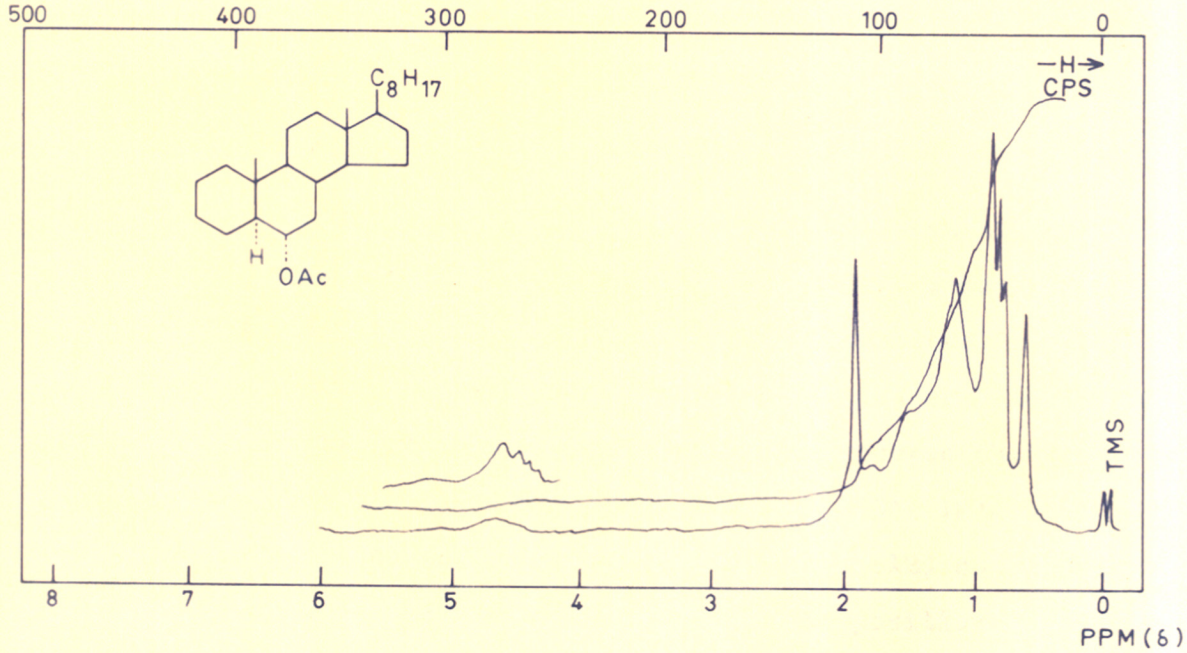


FIG. 5. PMR SPECTRUM OF CHOLESTAN 6α-ACETATE

considerations this compound may be considered to coprostan 6β-ol (10) formed through β-attack of hydroborating agent. In keeping with this the M.P. and rotation of this compound agrees with that reported[15] in literature for coprostan 6β-ol. An important support for this assignment stems from positions (1.09 and 0.68) of the C_{10} and C_{13} methyl, in which it is anticipated that the C_{10} methyl would be strongly deshielded. The positions calculated for these signals on the basis of Zucher's rule are 1.1 and 0.68 p.p.m.

Though hydroboration of cholesterol has been reported to give cholestan 3β-6α-diol in a very high yield 78%), in the present case, cholestan 6α-ol was obtained in comparatively moderate yield (50%). Though it could be anticipated that the absence of 3β-hydroxyl would reduce the amount of the 6α-hydroxy compound formed, the rather large difference cannot be easily explained.

Spectral Studies

During the study of Westphalen rearrangement the infrared spectrum of cholestane 3β-5α-6β-triol, 3 methyl ether 6 acetate (12) and its C_6-epimer (13) were examined in carbon disulphide solution to determine the conformation of the acetate group. It was found (Fig. 6) as expected that the 6β-(axial) epimer displayed three bands at 1261, 1242 and 1223 cm^{-1} whereas the equatorial (6α) epimer (13) showed only (Fig. 7) a single band at 1233 cm^{-1}. The position of carbonyl

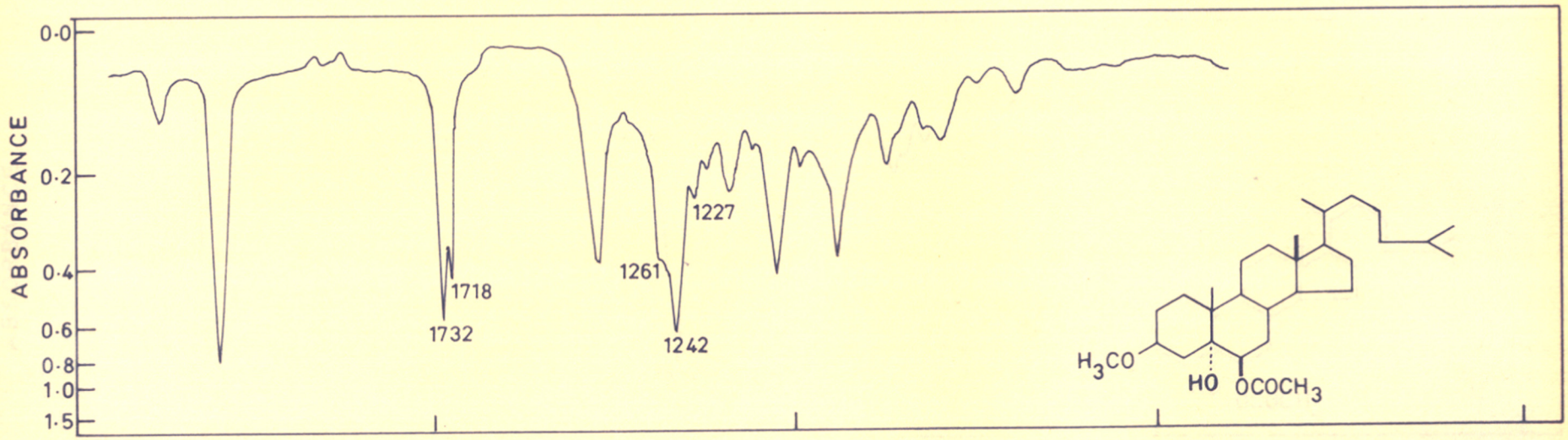


FIG. 6. IR SPECTRUM OF CHOLESTANE 3β-5α-6β-TRIOL, 3-METHYL ETHER 6-ACETATE (12) (CS_2)

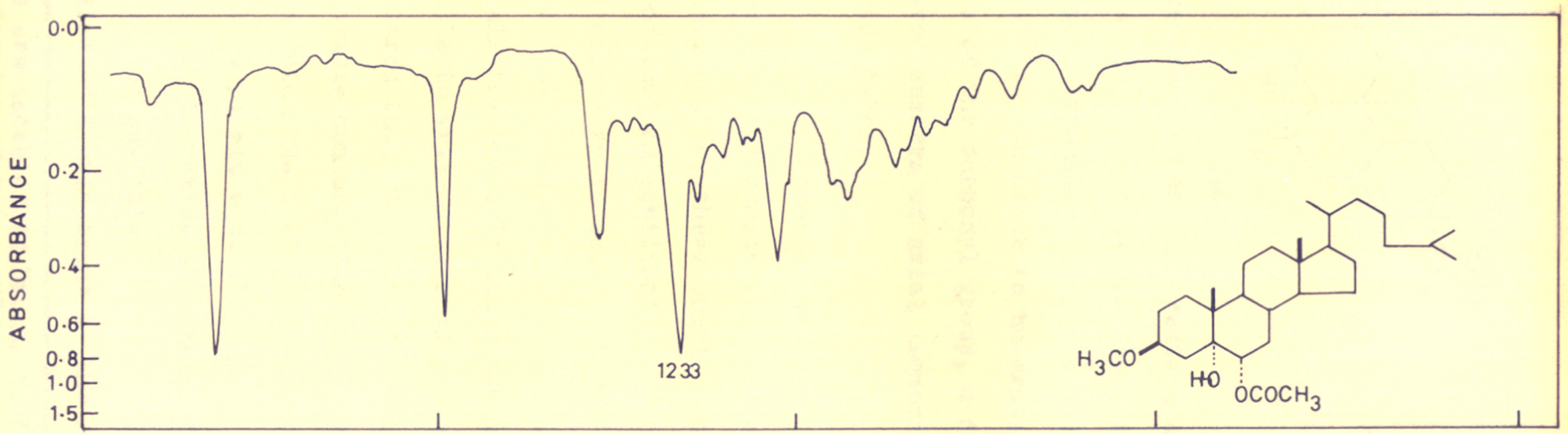
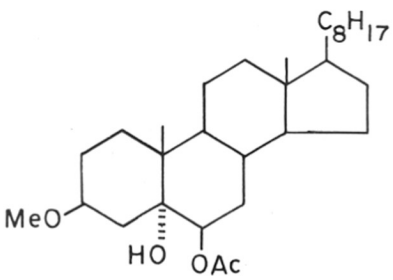


FIG. 7. IR SPECTRUM OF CHOLESTANE 3β-5α-6α-TRIOL, 3-METHYL ETHER 6-ACETATE (13) (CS_2)



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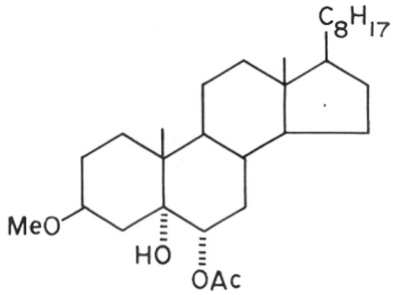

(13)

absorption was more interesting as the ∝-epimer* revealed only a single band at 1732 cm^{-1} whereas in the β-epimer two equal intensity absorptions were noticed at 1732 and 1718 cm^{-1}. In order to understand this difference in the nature of the carbonyl group, a detailed examination of infrared spectra of axial isomers which show split carbonyls was undertaken.

R. N. Jones et al[3] have pointed out that in case of monocarbonyl compounds which exhibit two bands in carbonyl region, these doublets have often been attributed to conformation equilibria. But, in order to prove this it is necessary to show that the two peaks are temperature and solvent dependent, but independent of concentration. When all these requirements are met it should also be possible to write a pair of conformationally isomeric structures that will permit a significant amount of the less stable conformation to be present at room temperature and in which the barrier to interconversion must be small enough to permit equilibration. It is also necessary that mesomeric, inductive and field effects of the carbonyl must differ sufficiently in the two forms to induce a significant displacement of the carbonyl frequency.

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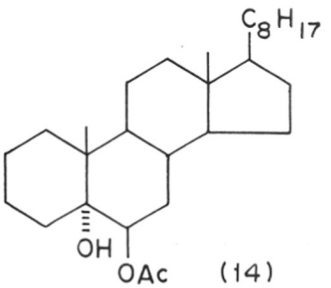
* We are indebted to Dr. R. V. Pachhapurkar of this Laboratory for a sample.

In order to check these different requirements, the first necessity is the study of solvent effect (Table 1). In carbon tetrachloride solution the 6β-epimer exhibited two bands at 1733 and 1721 cm^{-1} in which the latter was of lower intensity. In the case of chloroform spectrum not only were the intensities reversed, the band at lower intensity being now more intense, but there was also a shift in the frequencies particularly the low frequency absorption. Such frequency changes have been observed in several[16] cases (Fig. 8).

The next phase of the investigation, now rested on a study of the effect of concentration. In carbon tetrachloride solution, the intensities of the two bands vary with concentration and at dilution 0.005M only a single absorption band was observed at 1732 cm^{-1}. (Fig. 8) In the case of chloroform spectrum the position was quite different, change in concentration surprisingly did not reveal any change in the intensities of these absorptions. In view of these peculiarities and the known importance of concentration effects we were lead to examine the spectra of other derivatives, in order to determine, whether these are actually concentration dependent or not.

The first such example that we studied was that of cholestane 5α-6β-diol 6-acetate (14). The selection of this



(14)

TABLE I

Effect of solvent on cholestane 3β,5∝,6β-triol 3-methyl ether-6-acetate

Concentration	Carbonyl frequency in cm^{-1}		
	CCl_4	$CHCl_4$	CS_2
0.1M	1721	1726*	1715
	1733*	1734(Sh)	1730*

* This represents the stronger of the two peaks.

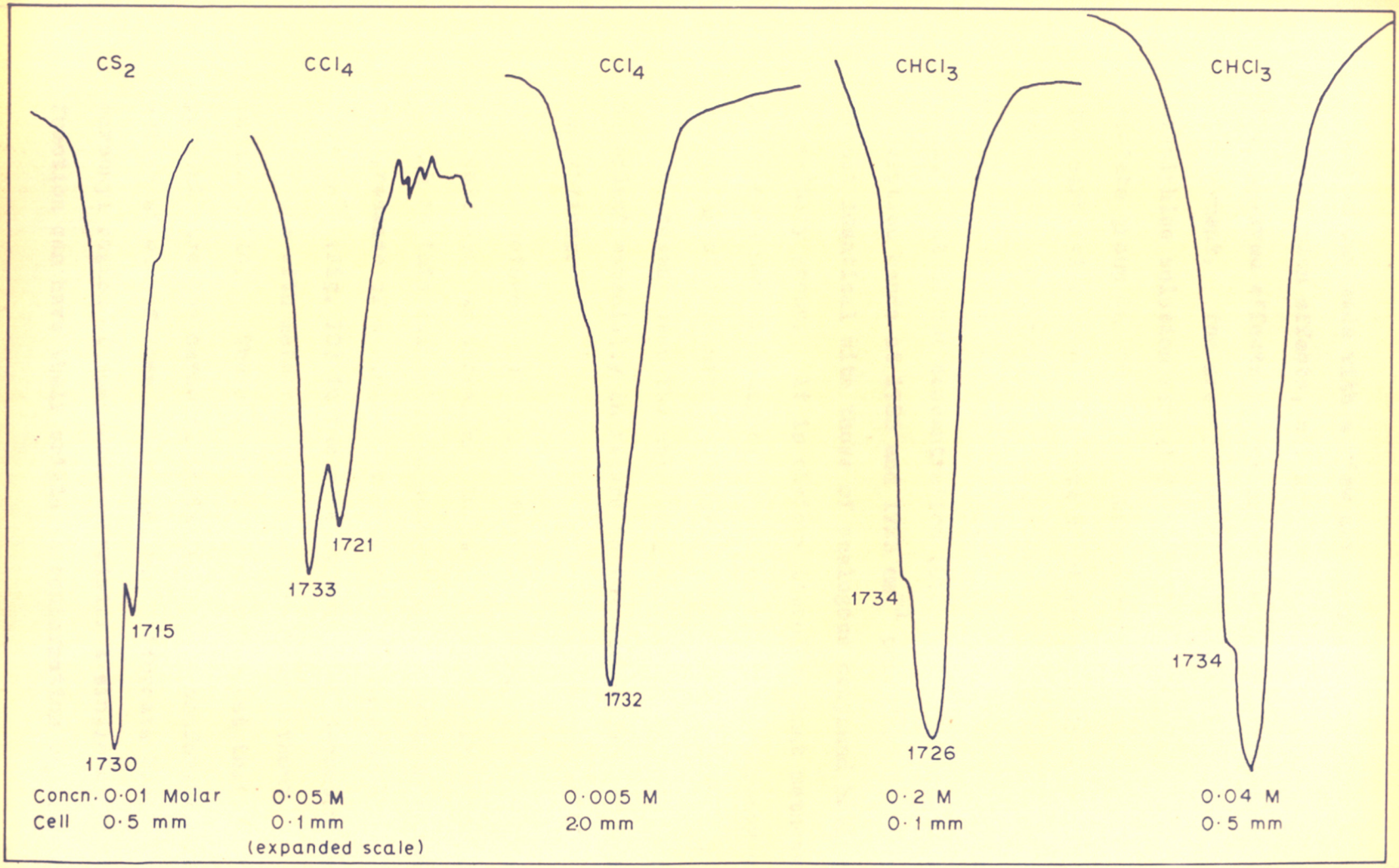


Fig. 8. CARBONYL FREQUENCIES OF COMPOUND 12 IN DIFFERENT SOLVENTS

compound was made with a view not only to check the concentration effects, but also to clearly establish that the observed effects do not arise due to the methoxyl substituent. The spectrum of this compound in carbon disulphide solution revealed the presence of an axial acetate group due to single absorption at 1240 cm^{-1}. The carbonyl region in dilute solution (0.045M) showed essentially a single absorption at 1726 cm^{-1} with a slight influxion at 1710 cm^{-1} (Fig. 9). The carbon tetrachloride solution at higher concentration (0.2M) clearly showed two absorption bands at 1733 and 1723 cm^{-1} positions which are almost identical with those of analogous compound having the 3β-methoxy group. It is obvious therefore that methoxyl group does not have any effect on either the frequency or the splitting of the carbonyl absorption. Thus it appears that in the present case the splitting of the carbonyl is solvent dependent especially in regard to the intensities of these absorptions.

Before considering other examples, effects of other solvents such as dioxan had to be examined. Significantly enough in this solvent even concentrated solutions revealed the presence of a single carbonyl absorption band at 1728 cm^{-1} (Fig. 10) in the case of both the 6β-compounds (i.e.) one with methoxyl (12) and the other without methoxyl (14). These results seem to suggest that in the present case the carbonyl splitting is not conformational, but an effect of some other factor. Bifurcate bands in the carbonyl region of compounds containing a single carbonyl function can have their origin in conformation equilibria[17],

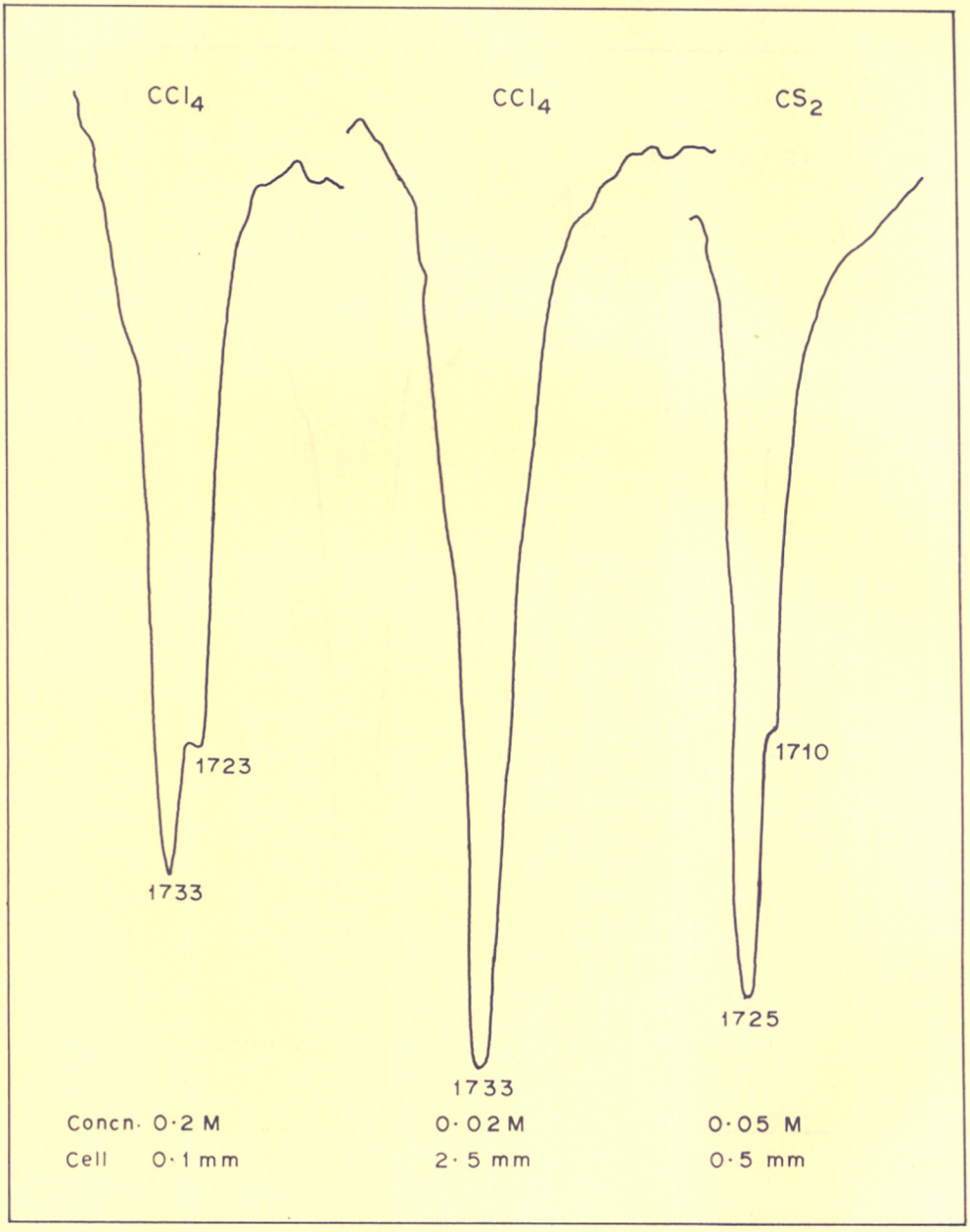


Fig. 9. CARBONYL FREQUENCIES OF COMPOUND (14) IN DIFFERENT SOLVENTS

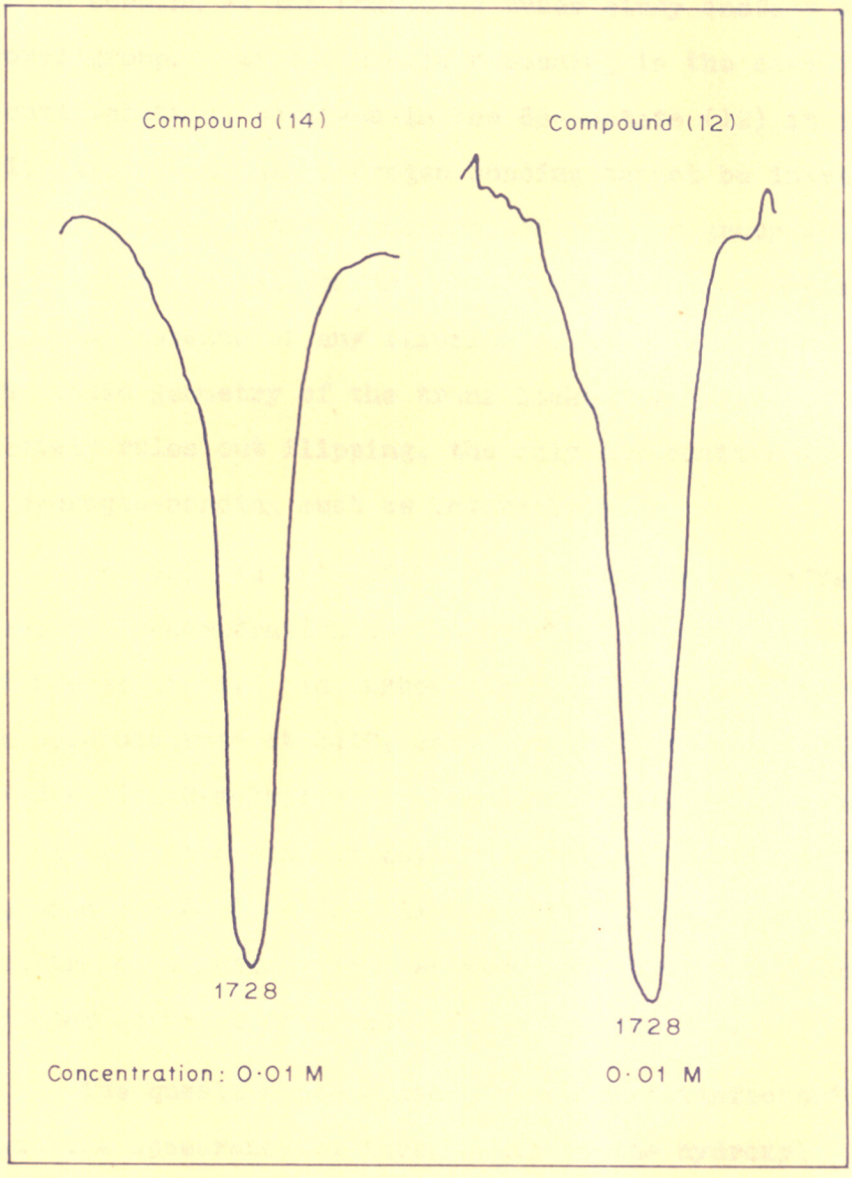


Fig. 10. CARBONYL FREQUENCIES IN DIOXANE SOLUTION

solvent-solute interactions[10], hot transitions[18], Fermi resonance[19] or vibrational coupling[20].

The first possibility that can be considered is hydrogen bonding as the compounds under study contain a hydroxyl group. If the hydrogen bonding is the case for the carbonyl splitting observed in the 6β-acetate (12) it is fairly clear that this hydrogen bonding cannot be intramolecular as the acetate function and hydroxy group are trans diaxial, a position in which they cannot hydrogen bond in the absence of any flipping of the cyclohexane ring. As the rigid geometry of the trans locked A, B rings completely rules out flipping, the only alternative is that this hydrogen-bonding must be intermolecular.

In order to establish this contention the effect of solvent and concentration on the hydroxyl stretching vibration was first examined. In carbon disulphide solution three bands were observed at 3612, 3580 and 3510 cm^{-1} in cholestane 3β-5α-6β-triol 3-methyl ether 6-acetate (12). The carbon tetrachloride solution displayed stretching vibrations at 3612, 3598 and 3520 cm^{-1}. As the absorption at 3520 cm^{-1} disappears on dilution, it does seem that hydrogen bonding is present in these compounds (Table II, Fig. 11).

The question now arises is how to satisfactorily explain the appearance of three bands in the hydroxyl stretching region. It can be assumed that the band at high frequency (3612 cm^{-1}) will correspond to the free hydroxyl group while the other may represent the hydrogen bonded forms wherein the hydroxyl hydrogen is bonded either to the hydroxyl oxygen or to the carbonyl oxygen. It is wellknown[22]

TABLE II

Hydroxyl stretchings of cholestane 3β,5∝,6β-triol 3-methyl ether, 6-acetate (12) and cholestane 5∝,6β-diol, 6-acetate (14)

Compound	CCl_4	$CHCl_3$	Dioxan	CS_2
	3622	3613	3430	3612
	3598	3592		3580
12	3520	3495		3510
	3619		3440	3610
14	3599			3580
	3525			3515

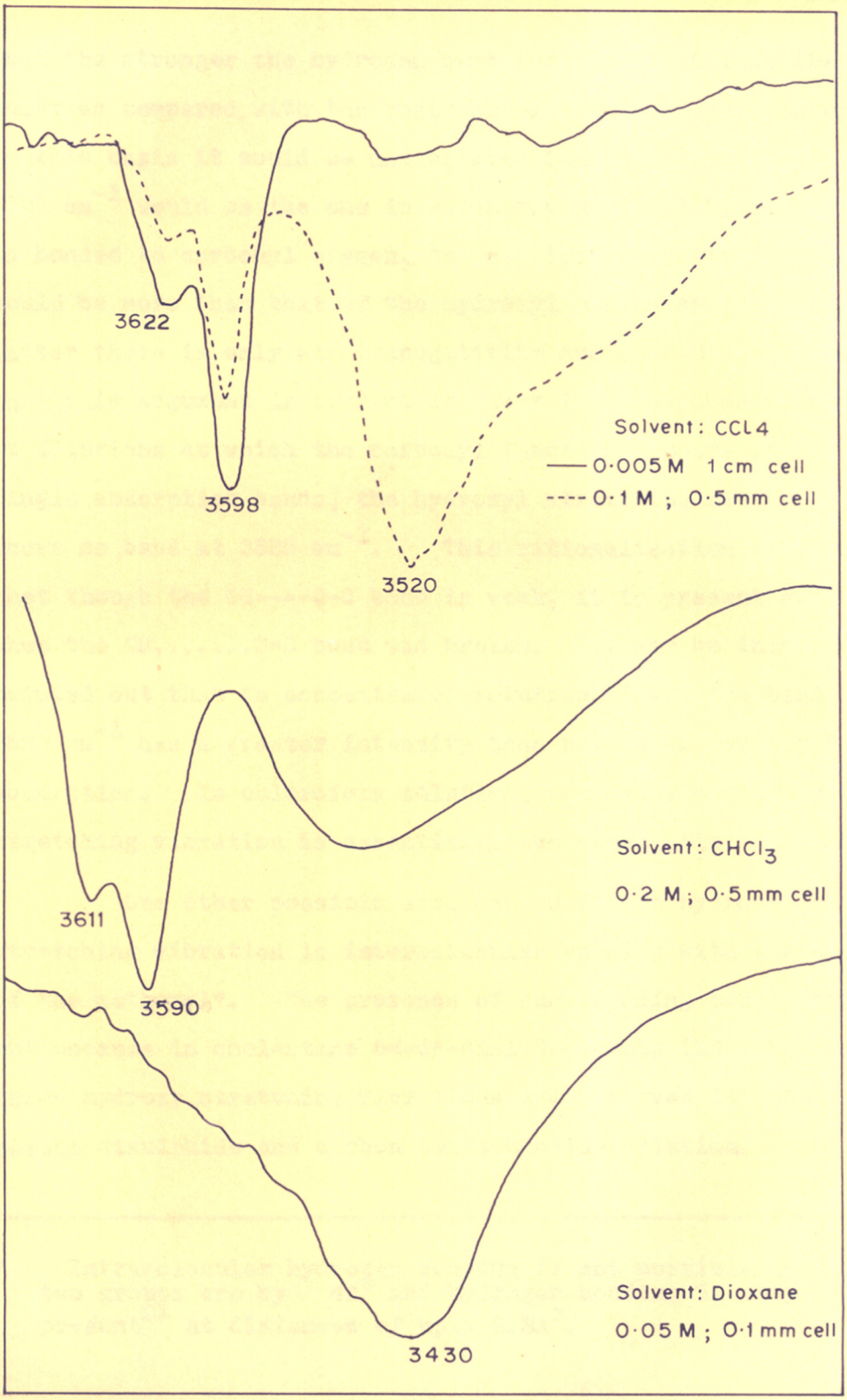


Fig. 11. EFFECT OF SOLVENTS ON HYDROXYL STRETCHING FREQUENCY OF COMPOUND 12.

that the stronger the hydrogen bond the larger will be the shift as compared with the position of the free hydroxyl group. On this basis it could be anticipated that the band at 3520 cm^{-1} would be the one in which the hydroxyl hydrogen is bonded to carbonyl oxygen, the electronegativity of which would be more than that of the hydroxyl oxygen as in the latter there is only electronegativity due to C-O single bond. That this argument is correct is clear from the observation that at dilutions at which the carbonyl function appears as single absorption bands, the hydroxyl stretching region shows no band at 3520 cm^{-1}. This rationalisation implies that though the OH----O-C bond is weak, it is present even when the OH.....O=C bond was broken. It may be incidentally pointed out that in concentrated solution (0.1M) the band at 3520 cm^{-1} has a greater intensity than any other hydroxyl absorption. In chloroform solution, the behaviour of hydroxyl stretching vibration is essentially the same. (Fig. 11, Table II).

One other possible explanation of the hydroxyl stretching vibration is intermolecular bonding with the oxygen of the methoxyl*. The presence of such bonding can be ruled out because in cholestane 5α-6β-diol 6-acetate (14) the same three hydroxy stretching vibrations are observed in both carbon disulphide and carbon tetrachloride solution.

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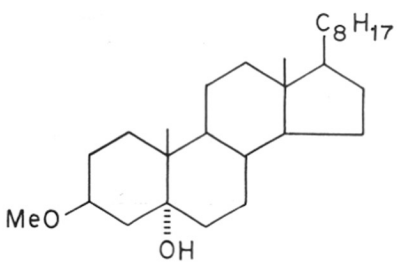
* Intramolecular hydrogen bonding is not possible as these two groups are by 4A^{o} and hydrogen bonding is only present[21] at distances of upto 3.3A^{o}.

In dioxan solution hydroxyl hydrogen is surrounded by dioxan molecules with the result that the hydroxyl can no longer bond with the carbonyl and only the carbonyl band corresponding to free carbonyl would naturally be observed. Due to polarity of the medium this free carbonyl absorption does not occur at the same position as the free carbonyl in carbon tetrachloride, but at a lower frequency (1733 cm^{-1} in carbon tetrachloride, 1728 cm^{-1} in dioxane). The correctness of these arguments can be seen from the hydroxyl stretching region which has now a single absorption band at 3430 cm^{-1} corresponding to bonding between the oxygens of dioxan and the hydroxyl hydrogen.

In order to fix the different bands in the hydroxyl stretching region, the infrared spectrum of 3β-methoxy 5α-hydroxy cholestane (7) was examined in fairly concentrated

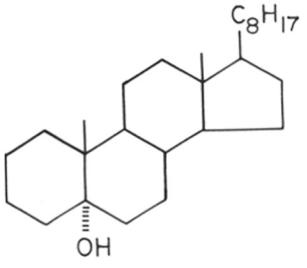


(7)

solution (CCl_4 0.2M). Under these conditions this compound revealed the presence of three bands at 3625, 3610 and 3490 cm^{-1}, the latter was generally a broad absorption band. In dilute solutions however (0.01M) the spectrum still showed the presence of two hydroxyl stretchings located at 3625 and

3610 cm^{-1} (Table III). Two explanations are possible for this splitting of hydroxyl stretching vibration - namely intermolecular hydrogen bonding (C-O....H-O-C) or splitting of the hydroxyl due to different spatial orientations. The persistence of this splitting even in fairly dilute solution (0.01M) would suggest the preference for the latter factor. However it is difficult to visualise firstly whey there should be different orientations as one is staggered and hence should predominate, and secondly how even if two orientations exist they should result in different locations as this group (OH) does not feel different inductive mesomeric or field effects depending on its orientation. The broad absorption at 3490 cm^{-1} can however arise by hydrogen bonding which is intermolecular in nature as it disappears on dilution. However there are two functions to which this intermolecular hydrogen bonding can take place, one being the ether oxygen and the other, the hydroxyl oxygen.

With a view to avoid this complication, it was decided to examine the infrared spectrum of 5∝-hydroxy cholestane (15).



(15)

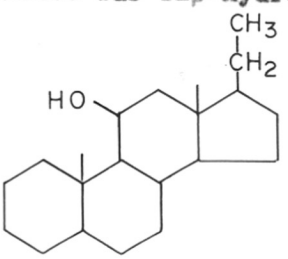
TABLE III

Hydroxyl stretching vibrations of alcohols

Compound	Concentration	CCl_4
(7)	0.2M	3625 3610 3410
	0.02M	3625 3610
(15)	0.2M	3630 3610 3490 (w,b)
	0.02M	3630 3610
	saturated	3630 3610 3540
(4)	0.05M	3645 3622
	0.25M	3645 3622
	saturated	3647 3615 3500

In concentrated solution (CCl_4, 0.2M) this compound had two major absorption bands at 3630, 3610 cm^{-1} and a weak, but broad absorption at 3490 cm^{-1}. The intensity of this absorption was considerably enhanced in more concentrated solution (saturated). In dilute solution (0.02M) however the spectrum had absorption only at 3630 and 3610 cm^{-1}.

It is clear from this spectrum that the methoxy oxygen has no significant role on the hydroxy absorption. The third alcohol examined was 11β-hydroxy 5∝-pregnane (4)



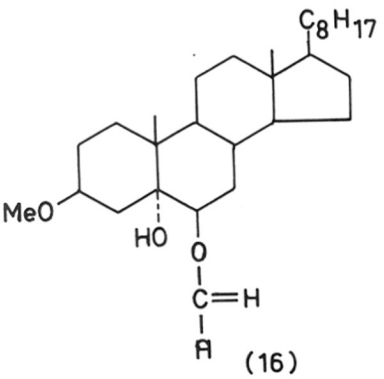
(4)

in which the hydroxyl is in more sterically crowded position. Because of the crowded location, it was anticipated that the capacity for intermolecular hydrogen bond will be reduced. In accordance with this argument, it was found that the intensity of this absorption (3490 cm^{-1}) is extremely low under conditions in which in other examples this band had fairly good intensity. However in saturated solution it also displayed three absorptions at 3645, 3625, and 3490 cm^{-1}. In this case also the spectrum in dilute solution (0.05M) displayed only two absorption bands at 3645 and 3625 cm^{-1}.

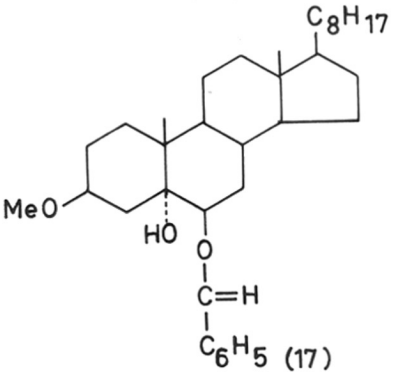
The appearance of two bands (for example at 3645 and 3625 cm^{-1} in compound 4) in the very dilute solution

spectra of all hydroxyl compounds studied therefore had the same basis which we assume is essentially conformational.

In the next phase of the investigation it was decided to check whether the splitting of the carbonyl frequency is seen in the case of other acetylated compounds. In this connection the spectrum of cholestane 3β-5∝-6β-triol-3-methyl ether, 6-formate (16) and 6-benzoate (17)



(16)



(17)

were also examined (Table IV). In carbon tetrachloride the benzoate showed equal intensity carbonyl absorptions at 1718 and 1700 cm^{-1}. On dilution however the intensity of the 1700 cm^{-1} vibration was greatly reduced. In carbon disulphide solution also two absorption bands of almost equal intensity were visible. The frequencies being now at 1710 and 1691 cm^{-1}. An interesting feature of this compound was the dioxan spectrum which apart from a prominent vibration at 1712 cm^{-1} had an inflexion at 1716 cm^{-1} which is difficult to rationalise. This compound in the hydroxyl stretching region had the usual three bands of which that at lowest wave number was reduced in intensity and of greater width.

TABLE IV

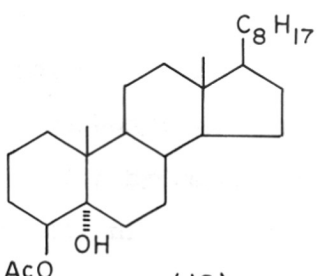
Carbonyl and Hydroxy stretching vibrations of cholestane 3β,5α,6β-triol, 3-methyl ether 6-formate (16) and 6-benzoate (17)

Compound	CCl_4		CS_2	
	OH region	C=O region	OH region	C=O region
16	3636,3596	1720	3610,3582	1720
	3530,3436		3530,3440	
17	3626,3590	1718	3610(sh)	1710
	3515	1700	3580,3510	1691

The formate ester displayed in both carbon tetrachloride and carbon disulphide only a single absorption band at 1723 and 1720 cm^{-1} respectively. As the dioxan spectrum indicated single absorption (1718) it was clear that the band at 1723 cm^{-1} must represent the free carbonyl. In order to detect the hydrogen bonded carbonyl the spectrum was examined in chloroform. In this solvent the splitting of the carbonyl could be observed the two bands appearing at 1719 and 1710 cm^{-1}. This failure of the formate to be resolved into two carbonyl absorptions in carbon tetrachloride solution is unique.

The possible rationalisation of this spectrum can be the weakness of the hydrogen bond in the formate ester. This weakness of the hydrogen bond is apparent from chloroform spectrum which generally had a low frequency vibration of greater intensity. In the formate even in chloroform the band assignable to free carbonyl is of greater intensity. It is therefore quite natural that in carbon tetrachloride the hydrogen-bonded carbonyl would be of even lower intensity and therefore it is not detected.

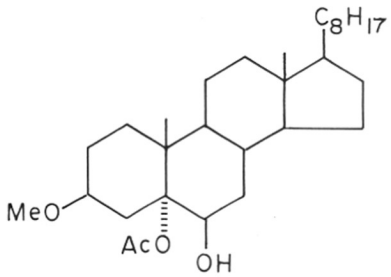
In the next phase, the examination of the infrared spectra of other axial acetates was undertaken. As cholestane 5α-4β-diol 4-acetate (18) and cholestane 5α-6β-diol 6-acetate (12) have similar structural features



(18)

similarities in infrared spectra could be anticipated. In agreement with this the hydroxyl region of these compounds has the usual three absorption bands (see Table V). The carbonyl region also reveals the usual splitting in which dilution resulted in the disappearance of absorption at lower frequency.

The other acetate studied was the cholestane 3β-5∝-triol 3 methyl ether 5-acetate (19). The hydroxyl stretching of this compound had also three bands both in carbon tetrachloride



(19)

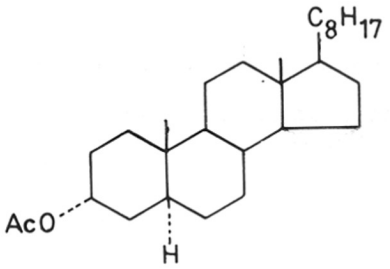
and carbon disulphide. In dioxan solution however as anticipated there was a single absorption corresponding to hydrogen bonding of dioxan oxygen with hydroxyls. In the carbonyl region in both carbon disulphide and carbon tetrachloride solutions two absorption frequencies (1727, 1713 cm^{-1}) are seen of which the lower frequency one disappears on dilution. Dioxan solution had only one absorption frequency (1725 cm^{-1}) corresponding to free carbonyl. These features are therefore consistent with the hypothesis that carbonyl splitting in the present examples is due to intermolecular hydrogen-bonding of the carboxy with the hydroxy function.

TABLE V

Hydroxyl and carbonyl frequencies in CCl_4 of some axial acetates

Compound	Concentration	Carbonyl	Hydroxyl
18	0.1M	1735	3625
		1724	3600
			3520
	0.01M	1735	3625
			3600
19	0.1M	1727	3630
		1713	3545
			3450
	0.01M	1727	3630
			3545

The confirmation for this argument arises from an examination of the spectrum of 3∝-hydroxy cholestane acetate*(20) which has even in concentrated solution (0.2M)



(20)

in chloroform only a single carbonyl absorption at 1733 cm^{-1}. The location of the carbonyl also suggests that 1733 cm^{-1} band observed in the hydroxy acetates studied must have been due to the free rather than bonded carbonyl.

If hydrogen bonding is the explanation for the carbonyl splitting in axial acetates, then the question that has to be answered, is why the splitting is not present in equatorial acetates such as cholestane 3β-5∝-6β-triol, 3 methyl ether 6-acetate (12). The most easy explanation would be that in this particular case hydrogen bonding is intramolecular and therefore giving rise to a frequency corresponding to bonded hydroxyl. Two arguments however tend to disprove this reasoning. The first of these is that intramolecular hydrogen bonding would require the formation of

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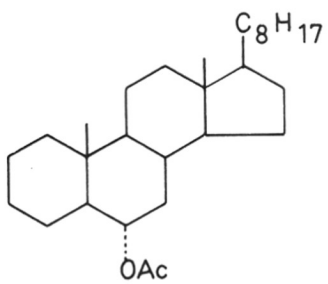
* The sample was kindly given by Mr. N. R. Bhadane of this laboratory.

a seven membered ring complex which is not usually favoured. The second important argument arises from the observation that this carbonyl absorption occurs at 1730 cm^{-1} and in the corresponding axial epimer, it has been possible to show that the corresponding carbonyl frequency is actually due to free carbonyl. A rationalisation of this behaviour can be offered if it is suggested that the equatorial acetate would have a higher carbonyl frequency than the axial acetates.

In order to completely rule out any such possibility it was necessary to examine the spectrum of an equatorial acetate which has no possibility of intramolecular of intermolecular hydrogen bond formation. An obvious choice for this purpose was cholestane 6∝-ol acetate (11),



(11)

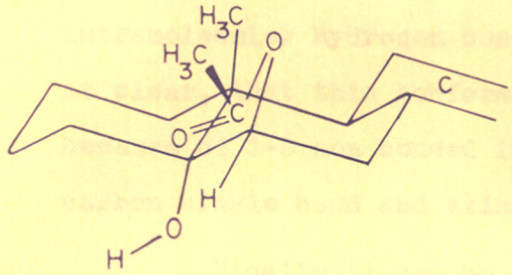
in which the absence of hydroxyl would completely rule out any inter or intramolecular hydrogen bonding possibility.

Cholestane 6∝-acetate (11) had in its infrared spectrum a single absorption band at 1732 cm^{-1} (CCl_4 solution) and even with different dilutions or change of solvent no splitting of this band was observed. As in this particular case this carbonyl frequency must correspond to free carbonyl frequency of cholestane 3β,5∝,6∝-triol, 3 methyl ether,

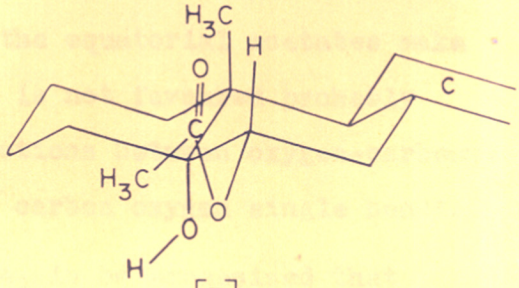
6-acetate (13) also represents a free carbonyl and not a hydrogen bonded carbonyl.

The next point remaining to be explained is the failure of the equatorial acetate to have intermolecular hydrogen bonding whereas the axial acetate has such bonding. This particular feature must reflect the differences in conformations of axial and equatorial acetates.

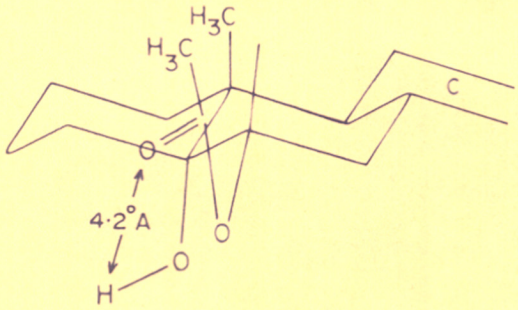
Jennings, Mose and Scopes[23] have recently demonstrated by O.R.D. measurements that in cholestan 6β-acetate, the acetate group has a conformation such that the carbonyl function eclipses the C_6-hydrogen. In this conformation 'A' (Chart 4) for the axial epimer the carbonyl is held away from C_{10} methyl and can therefore easily intermolecularly hydrogen bond with a hydroxyl. In 6α-acetate, on the other hand, the carbonyl in the preferred conformation 'B' would be pointing towards the C_{10} methyl and is therefore in a more crowded enviornment which prevents approach of the hydroxyl from a different molecule. The result is intermolecular hydrogen bonding is not possible. It can also be pointed out that in conformation 'B' the carbonyl is farther than 4A° from the hydroxyl hydrogen. This therefore can explain the absence of intramolecular hydrogen bonding in this compound. One important observation that must however be made is that in order to gain energy through intramolecular hydrogen bonding, a molecular can exist in a conformation which is not the normally preferred one. Several examples of this type have been recorded[24]. If this argument holds good in the present case though intramolecular hydrogen bonding cannot



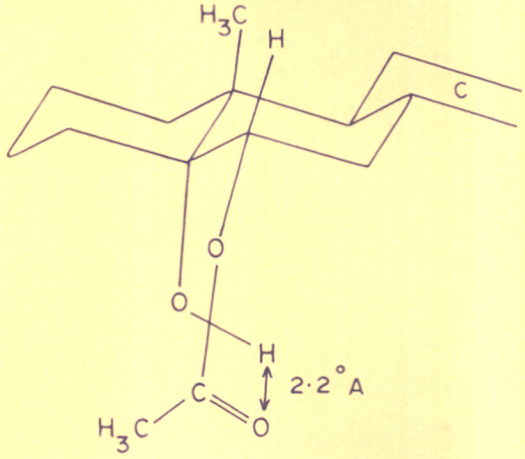
[A]



[B]



[C]



[D]

CHART-4-CONFORMATIONS OF C_6 —ACETATE GROUP

occur in conformation 'B' or 'C' (distance between carbonyl oxygen and hydroxyl hydrogen 4.2A°) it can however occur in a conformation such as 'D'. The absence of any intramolecular hydrogen bond in the equatorial acetates make it clear, that this conformation is not favoured probably because of 1-3 non bonded interactions between oxygen-carbonyl carbon single bond and axial, C_5 carbon oxygen single bond*.

Finally it can be once again be emphasised that in the case of axial acetates intramolecular hydrogen bonding is not possible because the closest distance between the carbonyl oxygen and hydroxyl hydrogen is 4A° + in the preferred conformation (i.e. A).

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* A small interaction with axial C_7 hydrogen is also present.

\+ Normal intramolecular hydrogen bonding occur if the distance between the hydroxyl hydrogen and carbonyl oxygen is 3.3A°.

EXPERIMENTAL

For general remarks see Chapter I.

All I.R. spectra recorded in this chapter were measured on a Perkin Elmer spectrophotometer model 221, by Dr. T. K. K. Srinivasan.

The preparation of the following compounds are reported in Chapter I.

Cholestane 3β-5∝-6β-triol, 3-methyl ether 6-acetate (12), and 6-formate (16).

Cholestane-3β-5∝-6β-triol, 3-methyl ether 5-acetate (19).

The following compounds' preparation are reported in Chapter 4.

Cholestane-5∝-6β-diol, 6-acetate (14)

Cholestane-4β-5∝-diol, 4-acetate (18).

3β-Acetoxy 5∝-pregnane 11-20 dione[8]

3β-Acetoxy-5∝-pregna 6-ene 11-20 dione (2 g) dissolved in ethyl acetate (70 ml) was hydrogenated with 5% palladised charcoal (250 mgs) for three hours at atmospheric pressure. The hydrogenated product was filtered and filtrate was evaporated to dryness. The white solid obtained was crystallised from aqueous methanol to furnish the desired product.

Yield 1.7 g.

M.P.	126°	Lit.[8]	128°
$[\alpha]_D$	+88°	Lit.[8]	+89°

I.R. 1725 (acetate) 1700 cm^{-1} (ketone).

3β-Hydroxy 5∝-pregnane 11-20 dione[8]

The above saturated dione (1.5 g) was kept with 5% methanolic potassium hydroxide overnight at room temperature. It was then poured into water, extracted with ether, washed well with water, dried over sodium sulphate and evaporated to dryness. The compound obtained was crystallised from acetone.

Yield 1.2 g.

M.P. 193° Lit.[8] 196°

$[\alpha]_D$ +111° Lit.[8] +110°

I.R. 3400 cm^{-1} (hydroxyl), 1710 cm^{-1} (ketone)

5∝-Pregnane 3,11,20 trione[9]

A mixture of chromium trioxide (500 mg) in acetic acid (60 ml) was added to a solution of 3β-hydroxy 5∝-pregn 11-20 dione (800 mgs) in acetic acid (60 ml) and the solution was stirred for some time and then kept at room temperature for five hours. The solution was diluted with ice cold water and extracted with ether. The organic layer was washed with sodium bicarbonate solution and water, dried over sodium sulphate and evaporated to dryness. The product was crystallised from ethyl acetate-ether.

Yield 400 mgs.

M.P. 210° Lit.[9] 212°

$[\alpha]_D$ +130° Lit.[9] +133°

I.R. 1700 cm^{-1} (ketone)

11-Keto 5∝-pregnane (3)

Potassium hydroxide (1.25 g) and hydrazine hydrate (2 ml) were added to a solution of the trione (1.5 g) in triethylene glycol (15 ml). The reaction mixture was heated on metal bath at 130° for one hour. Then the condenser was removed and the temperature was allowed to rise to 190°. Heating was continued for three hours at 190°C. The reaction mixture was poured into water and extracted with ether, washed with water, dried and evaporated to dryness. The crude product gave three spots on T.L.C. (benzene solvent system) and it was chromatographed over silica gel (50 g). Pet. ether elution (2x100 ml) gave a white crystalline material which was crystallised from acetone. This was found to be 5∝-pregnane.

Yield 120 mgs.

M.P. 80°

$[\alpha]_D = 20°$

Lit[9] = 85°

Lit[9] = 19.6°

Pet. ether and benzene (9:1) mixture gave the desired compound. On crystallisation from acetone-methanol white needles were obtained.

Yield 700 mgs.

M.P. 106°

$[\alpha]_D$ +46°

I.R. 1700 cm^{-1} (ketone.

Analysis:

Found: C, 83.1; H, 11.6%

$C_{21}H_{34}O$ requires: C, 83.4; H, 11.25%.

11β-Hydroxy pregnane (4)

A solution of 11-keto pregnane (300 mgs) in ether (20 ml) was added slowly to a refluxing solution of lithium aluminium hydride (600 mgs) in ether (100 ml) and refluxing was continued for six hours. After usual work up, a white solid was obtained which was crystallised from methanol.

Yield 150 mgs.

M.P. 123-124°

$[\alpha]_D$ +38°

I.R. 3500 cm^{-1} (hydroxyl)

Analysis:

Found: C, 82.87; H, 11.91%

$C_{21}H_{36}O$ requires: C, 82.83; H, 11.92%.

Cholestane 3β-methoxy 5α,6α-enoxide (6)

A chloroform solution of perbenzoic acid (0.015N, 20 ml) was added to a solution of 3β-methoxy Δ^5-cholestene (1 g) in chloroform (40 ml) and kept for three days at 15°.
The reaction product was extracted with ether, washed well with sodium bicarbonate and water, dried and evaporated.

T.L.C. of the crude product showed two spots, one corresponding to that of starting material. The crude material was loaded on silica gel (30 g) and chromatographed.

Pet. ether and benzene (4:1) mixture gave the starting material which was identified by M.P., mixed M.P. and T.L.C.

Benzene and ether (1:1) mixture gave the required epoxide which was crystallised from ether metahnol.

Yield 600 mgs.

M.P. 78°

$[\alpha]_D$ +32°

I.R. 1248, 880 cm^{-1} (epoxide oxygen) oxirane ring

Analysis:

Found: C, 80.65; H, 11.4%

$C_{28}H_{48}O_2$ requires: C, 80.71; H, 11.61%.

Cholestane 3β-5α-diol-3-methyl ether (7)

A solution of the above epoxide (600 mgs) in ether (30 ml) was added to a solution of lithium aluminium hydride (12 g) in ether during refluxing. The reaction mixture was refluxed for a further six hours. Usual work up and crystallisation from methanol afforded a product which showed two spots in T.L.C. The crude product was chromatographed on silica gel (17 g).

Pet. ether and benzene (1:11) mixture gave a material (50 mgs) which was identified (by the usual methods) as the starting epoxide.

Benzene elution gave the required compound which was crystallised from ether-methanol.

Yield 450 mg.

M.P. 126°

$[\alpha]_D$ +20°

I.R. 3500 cm^{-1} (hydroxyl)

Analysis:

Found: C, 80.45; H, 11.8%

$C_{28}H_{50}O_2$ requires: C, 80.32; H, 12.04%.

Cholestan-6∝-ol[25] (9)

Diborane gas was generated by adding sodium borohydride (500 mgs) in diglyme (25 ml) to a mixture of boron trifluoride (10 ml) and diglyme (15 ml) according to the procedure of Brown and Subba Rao[26]. Diborane gas was passed through a solution of Δ^5-cholestene (1 g) in tetrahydrofuran (20 ml) for one hour and after that the reaction product was left overnight at room temperature. The solvent was evaporated at reduced pressure and the crude product was treated with 5% ethanolic potassium hydroxide (20 ml) and hydrogen perioxide (3.3%, 30 ml) and left at room temperature for one and half hours. The reaction product was poured into water, extracted with ether, washed with water, dried over sodium sulphate and evaporated to dryness. The crude product on T.L.C. (benzene solvent) shows three spots. Top spot corresponds to starting material,, the middle spot is expected to be β-alcohol and the lower one to be an equatorial ∝-alcohol. All the three compounds were separated by chromatography over silica gel column (30 g).

Pet. ether elution gave a white crystalline material (100 mgs) which was identified by T.L.C., I.R., M.P. and mixed M.P. as starting material (i.e. Δ^5-cholestene). Benzene elution gave an oily material (400 mgs) which did not crystalise even in different solvent systems. This product was identified by N.M.R., I.R. and rotation.

$[\alpha]_D$ $+20^\circ$ Lit.[15] $+21^\circ$

I.R. 3400 cm^{-1} (hydroxyl)

Benzene and ether (40%) mixture elution gave a white solid (400 mgs) which was crystallised from methanol. It was identified (by the usual methods) as cholestan-6∝-ol.

M.P.	130°	Lit.[14]	130°
$[\alpha]_D$	33°	Lit.[27]	34.8°
I.R.	3500 cm^{-1} (hydroxyl)		

Cholestan 6∝-acetate[14] (11)

A mixture of cholestane 6∝-ol (200 mgs) pyridine (4 ml) and acetic anhydride (4 ml) was left overnight at room temperature. The reaction mixture was poured into water and extracted with ether, washed with 2N hydrochloric acid, sodium bicarbonate solution and water. The ether layer was dried and evaporated to obtain the required product, which was crystallised from ether-methanol.

Yield	150 mgs.		
M.P.	92°	Lit.[14]	96°
$[\alpha]_D$	+70°	Lit.[14]	+69°
I.R.	1710 cm^{-1} (acetate)		

Cholestane 5∝,6∝-epoxide[28]

A chloroform solution of perbenzoic acid (0.015N, 50 ml) was added to a solution of Δ^5-cholestene (2 g) in chloroform (100 ml). After keeping for three days at 15°C the reaction mixture was worked up as usual and crystallised from ether-methanol.

Yield	1 g.		
M.P.	75°	Lit.[28]	
I.R.	1240, 880 cm^{-1} (epoxide oxygen)		

Cholestan-5α-ol (15)[29]

A solution of the above epoxide (200 mgs) in ether (30 ml) was reduced with lithium aluminium hydride (600 mgs). After usual work up, crystallisation from methanol gave the required product.

Yield 100 mgs.

M.P. 100°C. Lit.[29] 109°

$[\alpha]_D$ +9° Lit.[29] +11.2°

I.R. 3500 cm^{-1} (hydroxyl)

Cholestane-3β-5α-6β-triol 3-methyl ether 6-benzoate (17)

Benzoyl chloride (2 ml) was added to a solution of cholestane 3β,5α,6β-triol 3-methyl ether (300 mgs) in pyridine (2 ml). The reaction mixture was left at room temperature overnight and poured into ice cold water. After extraction with ether the organic layer was washed with 2N hydrochloric acid, sodium bicarbonate solution, and water, dried and solvent was evaporated to yield the required product which crystallised from methanol.

Yield 200 mgs.

M.P. 154°

$[\alpha]_D$ -26°

I.R. 3400 (hydroxyl), 1725 cm^{-1} (ester carbonyl)

Analysis:

Found: C, 78.0%; H, 10.2%

$C_{35}H_{54}O_4$ requires: C, 78.02; H, 10.1%.

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CHAPTER 4

DESHIELDING EFFECT OF NEIGHBOURING PROTONS ON THE ESTERIFICATION OF A HYDROXYL GROUP.

It has been known since long that the proton attached to the carbon holding the hydroxyl group shows an appreciable downfield shift on acetylation[1] and in fact this has been regarded as one of the methods whereby a secondary hydroxyl group may be detected. It has been presumed that this shift may involve the inductive effect due to the acetate which may perhaps be strengthened by some stereochemical effect by the newly created carbonyl of the acetate.

This argument is supported by the fact that the conversion of an alcohol to the methyl ether[2] results in an upfield shift of the secondary proton. This upfield shift was being rationalised by regarding it as a result of the positive inductive effect of the methoxy methyl. In this connection it may be pointed out that Narayanan and Iyer[2] have demonstrated that this shift also can be considered as a method of locating a secondary hydroxyl.

Though much work has been done on the examination of the P.M.R. spectra of numerous steroids,[3,4] no generalisation regarding shifts observed by acetylating an hydroxyl on a proton of an adjacent carbon atom has so far been made. In a very exhaustive study Tori and Komeno[3] have examined the spectra of numerous steroids having functional groups on adjacent carbons.

Their examination failed to reveal any generalisation of this nature. This is probably because most of the multi-functional compounds were ring A derivatives in which there is certain amount of flexibility.

Very recently Takeda et.al[5] in a study of some anisatin derivatives (Fig. 1) reported a shift in the signal of the secondary proton by 1.1 p.p.m. on acetylation of the hydroxyl of the adjacent carbon atom. As the system was very complex consisting of β- and γ-lactones with 7-oxygen functions in the molecule, these workers could not assign any reason for the observed shift, though they presumed that this downfield shift was due to the tertiary hydroxyl which has a 1-3 diaxial relationship with another oxygen*.

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* This work was published after our results were ready for communication. As no generalisation was made for the shift observed, this work has no special significance.

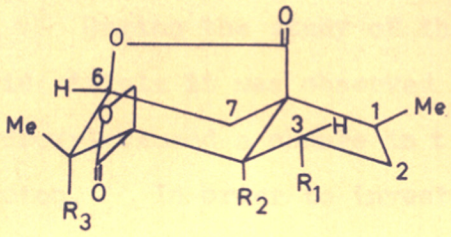


FIG. 1.

Noranisatin $R_1 = R_2 = OH$ $R_3 = OH$

Noranisatin diacetate $R_1 = R_3 : OAc$; $R_2 = OH$

Norneo-anisatin $R_1 = H$; $R_2 = R_3 = OH$

Norneo-anisatin monoacetate $R_1 = H$, $R_2 = OH$; $R_3 = OAc$

Noranisatin carbonate $R_1R_2 = -O.CO.O-$; $R_3 = OH$

Noranisatin carbonate acetate $R_1R_2 = -O.CO.O-$; $R_3 = OAc$

PRESENT WORK

During the study of the chemistry of steroid triols it was observed that acetylation of the C_5-hydroxyl caused a change in the location of the C_6-proton. In order to investigate this shift, several alcohols and their acetates were prepared. In order that the location of the adjacent proton should be visible, these compounds naturally should have substituents on adjacent atoms whereby these signals can readily be detected. For this purpose the vic-glycols and their acyl derivatives would be suitable; by having one of the hydroxyls on a tertiary carbon atom, the location of the secondary proton poses no difficulty. The most satisfactory compounds therefore are the steroid glycols situated at positions 5 and 6. The choice of the steroids is necessary as these have fixed well determined configurations. Furthermore, these 5,6-substituted derivatives are easy to prepare through cholestane epoxide.

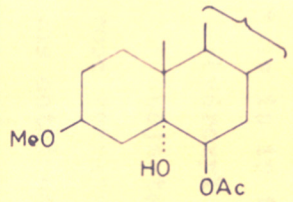
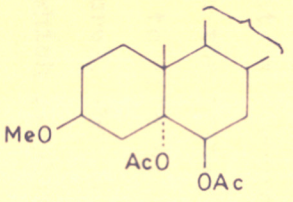
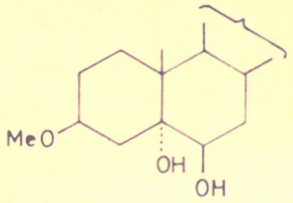
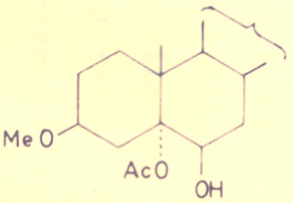
Tables (1-7*) summarise the chemical shifts of only those protons which can be easily detected and identified. Thus in compound 1 (Table 1) the methoxyl protons were seen as a 3-proton singlet at 3.2 p.p.m.

---

* Tables 1-7 show only partial formulae. All these compounds belong to cholestane series.

TABLE - 1

COMPARISON OF SPECTRAL CHANGE CAUSED BY ACETYLATION

S. NO.	COMPOUND	C_3-H	C_6-H	C_3-OMe	C_6-OAc	C_5-OAc
1			4·63	3·2	1·96	—
2		3·13	5·83	3·33	2·06	2·06
3			3·55	3·36	—	—
4		3·13	4·67	3·33	–	3·33

whereas the acetate methyl protons are seen as expected at 1.96 p.p.m. The proton ∝ to the methoxyl group (C_3-proton) is not easily detected and is probably hidden below the signal at 3.2 p.p.m. (Fig. 2). It can be pointed out that this C_3-proton being axial will have a broad signal having a large coupling with the axial 2β and 4β protons and smaller couplings with equatorial 2∝ and 4∝ protons. The signal at 4.63 p.p.m. can be attributed to the C_6-proton from its chemical shift as also its narrow nature which suggests that this proton has only small couplings with its neighbours, which is consistent with its equatorial disposition.

This compound (1) was acetylated at C_5 with acetic anhydride and p-toluene sulphonic acid at room temperature. The P.M.R. spectrum (Fig. 3) of this compound (2) exhibited signals whose assignments (based on previous arguments) are presented in Table 1. The only novel feature of this spectrum is the downfield shift of C_6-proton. This downfield shift cannot at first instance be rationalised as acetylation has been carried out on the vicinal carbon. In order to check whether this is in fact is a common feature of acetylating a C_5-hydroxyl in the trans-fused system, compound (2) was subjected to mild hydrolysis (5% methanolic KOH at room temperature), when a product different from (1) was obtained which displayed in its I.R. spectrum the presence of a hydroxyl group (3500 cm^{-1}) and an acetate

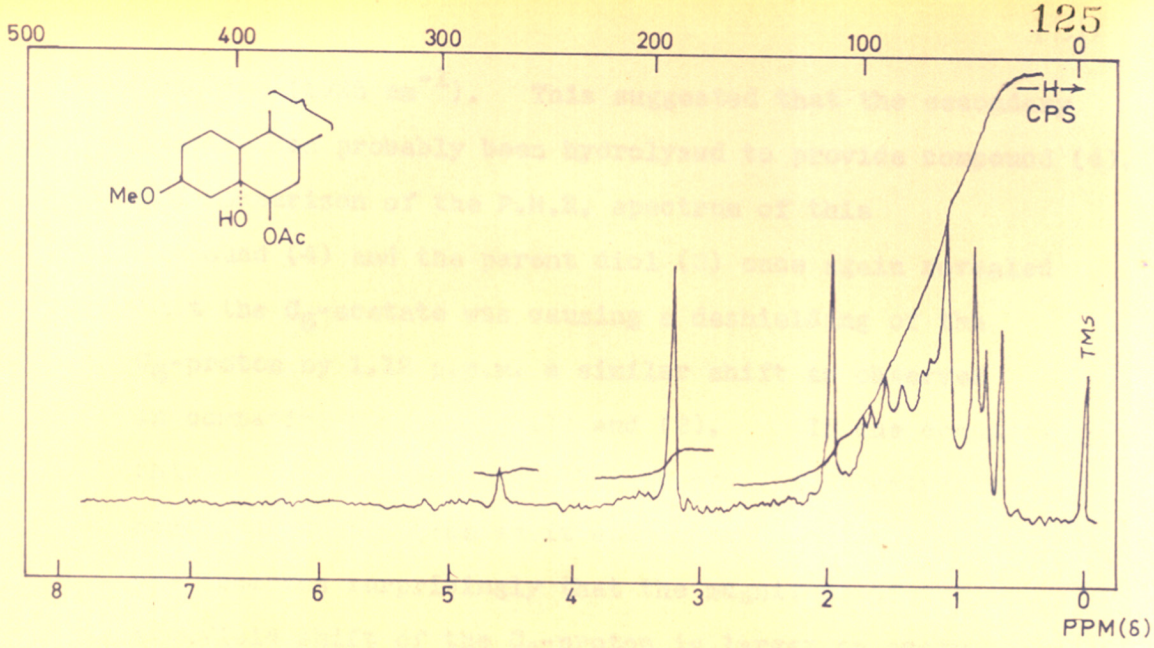


FIG. 2. PMR SPECTRUM OF

CHOLESTANE 3β-5α-6β-TRIOL, 3 METHYL ETHER, 6-ACETATE

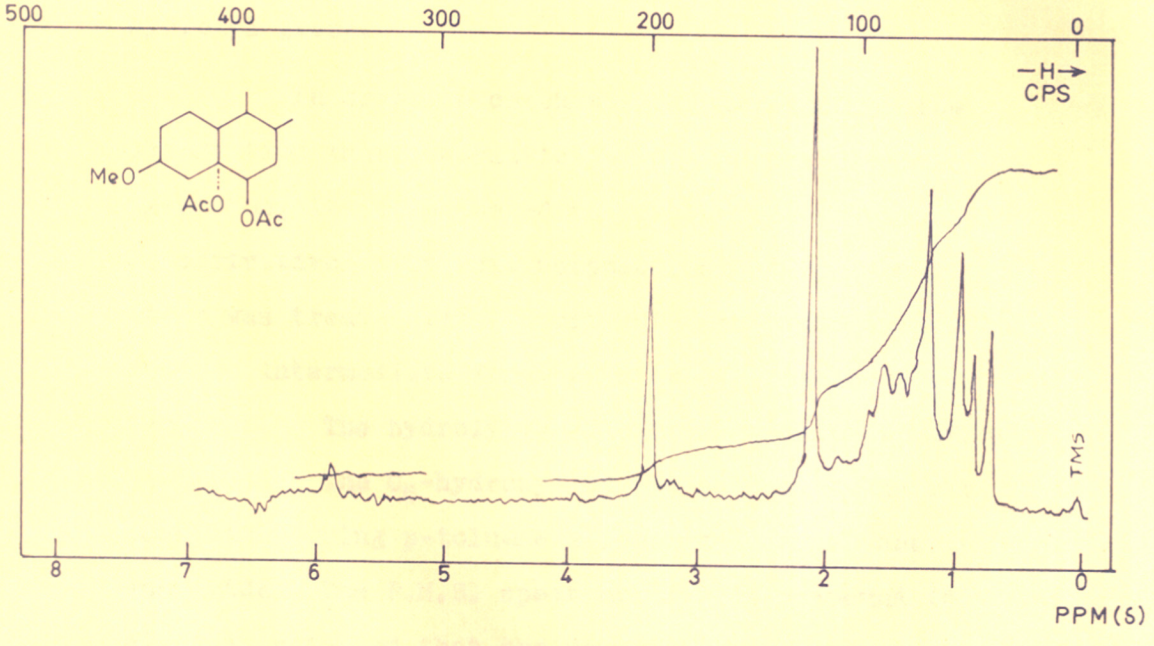


FIG. 3. PMR SPECTRUM OF

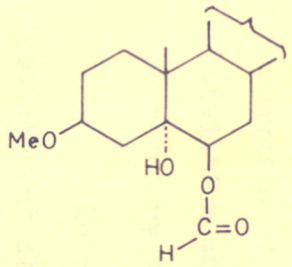
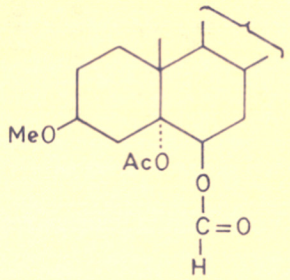
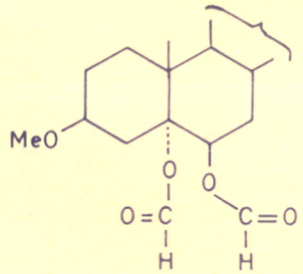
CHOLESTANE 3β-5α-6β-TRIOL, 3 METHYL ETHER, 5,6 DIACETATE

function (1725 cm^{-1}). This suggested that the secondary acetate has probably been hydrolysed to provide compound (4). The comparison of the P.M.R. spectrum of this compound (4) and the parent diol (3) once again revealed that the C_5-acetate was causing a deshielding of the C_6-proton by 1.12 p.p.m. a similar shift as observed in comparing compounds, (1) and (2). If one compares this downfield shift with that observed in passing from compounds 3 to 1 (the shift due to acetylation), one observes surprisingly that the magnitude of the downfield shift of the C_6-proton is larger on acetylating the C_5-hydroxyl than the C_6-hydroxyl (1.2 p.p.m. as compared to 1.08 p.p.m.). This clearly indicates that the inductive effect does not have a significant role in the downfield shift observed on acetylating a secondary hydroxyl group.

In order to check the significance of the observation that acetylation of C_5-hydroxyl effects the C_6-proton, it was proposed to prepare the formylated C_5-derivative. For this purpose cholesteryl methyl ether was treated with formic acid and hydrogen peroxide and the intermediate formate ester (5) (Table 2) was isolated. The hydrolysis of this formate ester affords diol (3). The C_5-hydroxyl of the formate ester was acetylated using p-toluene sulphonic acid and acetic anhydride. The P.M.R. spectrum of this compound (6) clearly indicated that the formate ester group was still intact (signal at 8.1 p.p.m.). Comparison of the

TABLE - 2.

COMPARISON OF SPECTRAL CHANGE CAUSED BY ACETYLATION AND FORMYLATION

S. NO.	COMPOUND	C_3 – H	C_6 – H	C_3 – OM	C_6 – FORMATE	C_5 – SUBST
5			4·86	3·34	8·3	—
6		3·08	5·96	3·31	8·1	2·06
7		3·05	5·88	3·3	8·1	8·1

P.M.R. spectrum of compound (5) with that of compound (6) (Table 2) (Fig. 4 and 5), once again revealed that the C_6-proton has been shifted downfield by 1.10 p.p.m. The compound 5 was then converted to compound 7 (formic acid and phosphorus pentoxide). The presence of two formyl groups in this derivative was indicated by the two proton signals at 8.1 p.p.m. in the P.M.R. spectrum (Fig. 6), as also the absence of hydroxyl in the I.R. spectrum. Comparison of the P.M.R. spectra of compounds (5) and (7) revealed a downfield shift of the C_6-proton by 1.02 p.p.m. This clearly indicated that though the C_5-formate deshields the C_6-protons, the deshielding is not as large as shown by C_5-acetate, though the difference in the deshielding is quite small.

That this kind of deshielding is general, is shown by a study of other derivatives (8-11). Thus comparison (Table 3) of the spectra of 8, 9 and 10 revealed that acetylation at C_6 causes a downfield shift of the C_6-proton by 1.17 p.p.m., whereas acetylation of C_5 causes a downfield shift of 1.03 p.p.m. Comparison of spectra of (10) and (11) showed that acetylation of C_5 has caused a downfield shift of the C_6-proton by 1.05 p.p.m.

To demonstrate clearly that the C_3-substituent plays no role in shifts so far discribed, it was proposed to prepare compounds having no substituents at this carbon atom. Δ^5-Cholestene,

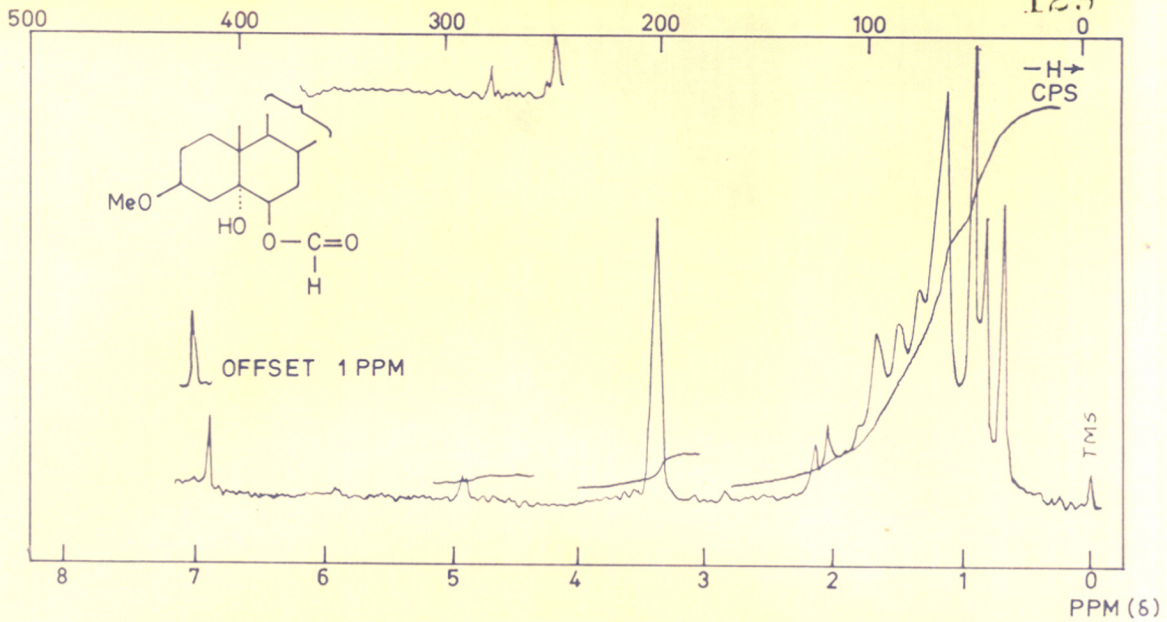


FIG. 4. PMR SPECTRUM OF

CHOLESTANE 3β-5α-6β-TRIOL, 3 METHYL ETHER, 6-FORMATE

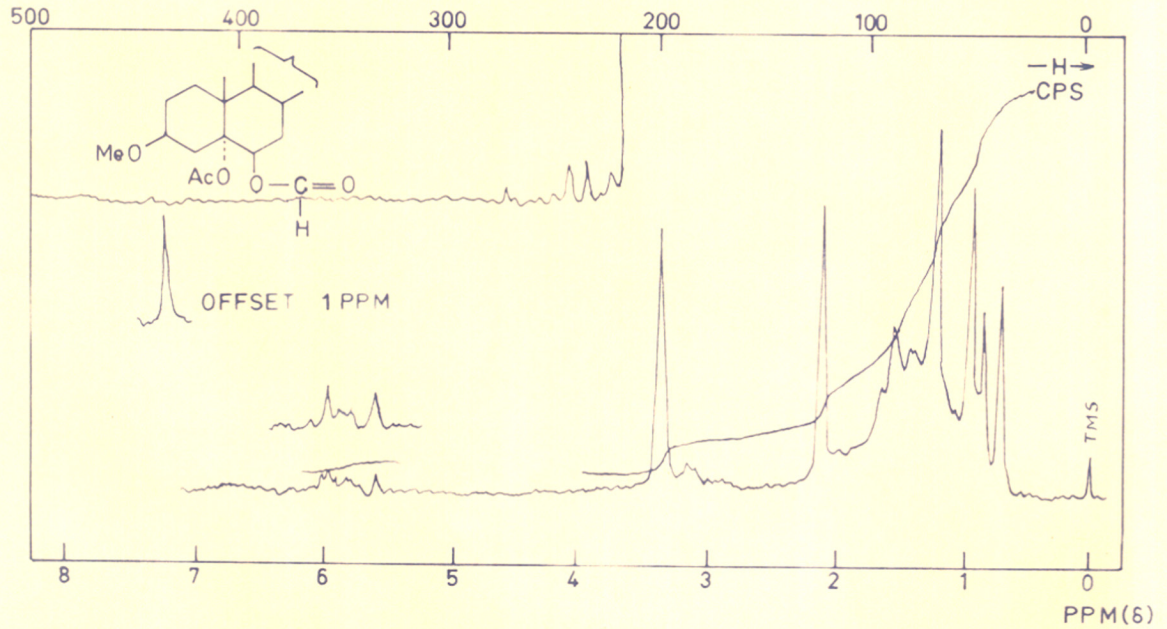


FIG. 5. PMR SPECTRUM OF

CHOLESTANE 3β-5α-6β-TRIOL, 3 METHYL ETHER, 5 ACETATE

6-FORMATE

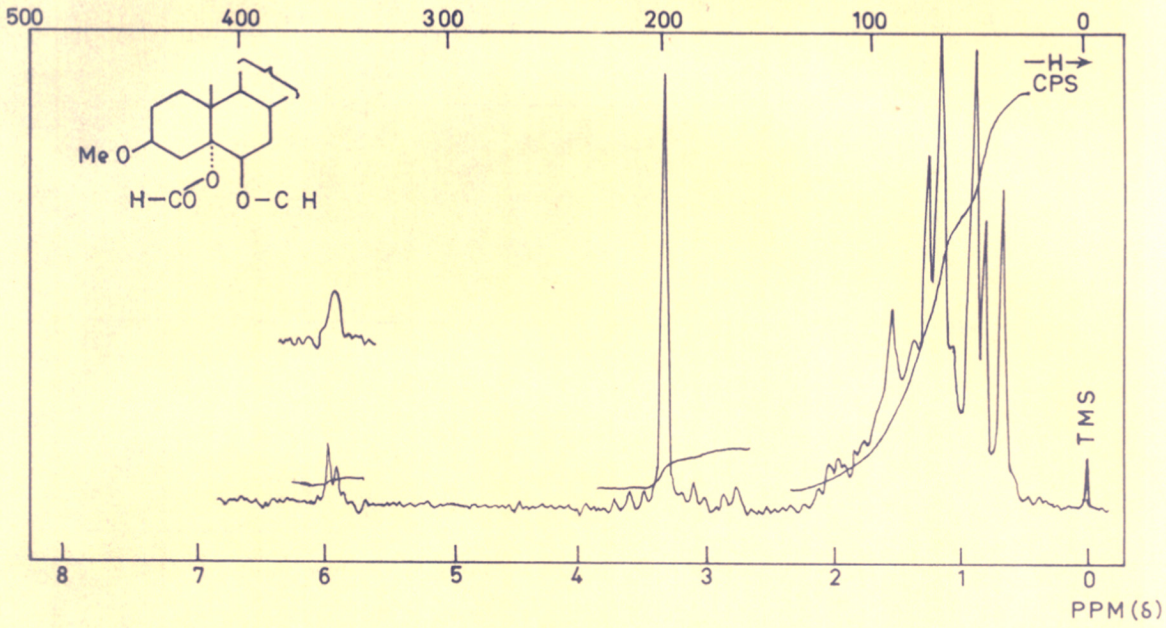
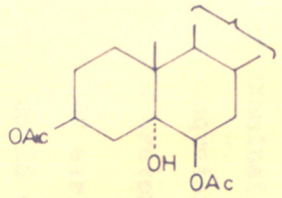
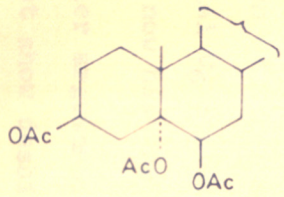
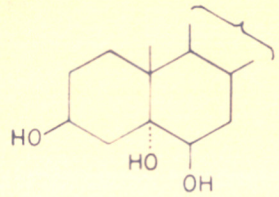
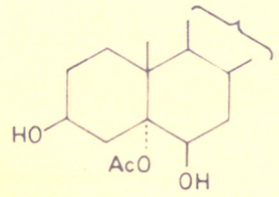


FIG. 6. PMR SPECTRUM OF

CHLESTANE 3β-5α-6β TRIOL , 3 METHYLETHER 5,6 DIFORNATE

TABLE – 3

SPECTRAL CHANGES CAUSED BY ACETYLATION OF 3-ACETATE SERIES

S.NO.	COMPOUND	C_3 – H	C_6 – H	C_3 – OAc	C_6 – OAc	C_5 – OAc
8			4·72	2·01	2·06	—
9		4·65	5·75	2·06	2·06	2·06
10			3·55	—	—	—
11			4·6	—	—	2·01

prepared by treating cholesteryl chloride with sodium and alcohol[6], was hydroxylated through its epoxide to furnish cholestane 5∝- 6β-diol (14). From which the 5∝- 6β-diol 6-acetate (12) and 5∝- 6β-diacetate (13) were prepared by known procedures. A comparison of the P.M.R. spectra (Table 4) of these three compounds showed a 1.2 p.p.m. shift for C_6-H by acetylating $C_6\beta$-OH, and a shift of 1.11 p.p.m. on acetylating the 5∝-OH group. The magnitude of these shifts is similar to that observed in the earlier compounds and clearly establishes that the function at C_6 has no role in the downfield shifts of the C_6-proton.

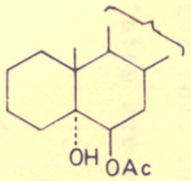
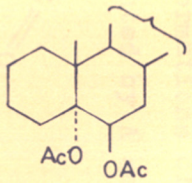
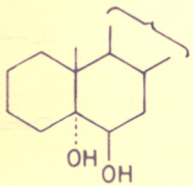
In summing up the above results it may be pointed out that acetylation of $C_5\alpha$-OH or $C_6\beta$-OH causes essentially the same downfield shift on the signal of the C_6-equatorial proton. These facts can be visualised as arising through deshielding of C_6-proton by the carbonyl of the C_5 acyl group whose deshielding cone must include the C_6-equatorial hydrogen.

A ready check for this suggestion can arise from an examination of the spectra of compounds having an equatorial C_6-function in which case the detectable proton now has the β-geometry.

If earlier arguments are correct such compounds would not show deshielding when the C_5-hydroxyl group is acetylated. That this is indeed

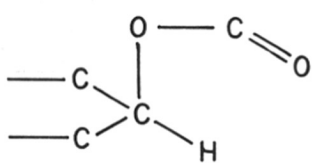
TABLE - 4.

SPECTRAL CHANGES CAUSED BY ACETYLATION IN COMPOUNDS WITHOUT C_3-SUBSTITUENT

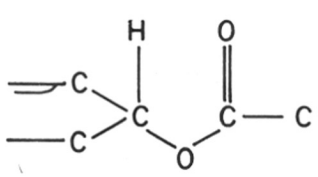
S. NO.	COMPOUND	C_6 – H	C_6 – OAc	C_5 – OAc
12		4·71	2·05	
13		5·82	2·05	2·05
14		3·51		

the case is seen by comparing the spectra of compounds (15 and 16) (Table 5; Fig. 7 and 8). These spectra clearly showed that there is no significant deshielding (0.1 p.p.m.) arising through acetylation of the C_5-OH. The comparison of the spectra of (15) and (17) clearly reveal the usual downfield shift (1.4 p.p.m.) of the C_6-proton on acetylation of C_6-OH.

The first requirement for explaining these results is that the C_6-acetate should have a fixed geometry. The existence of preferred conformations for acids and ester has been recently suggested by different groups of workers[7] using different methods. Very recently Jennings Mose and Scopes[8] have demonstrated from O.R.D. measurements that acetates in the B-ring of steroids have preferred conformations in which the acetate carbonyl eclipses the hydrogen carried by the carbon bearing the acetate. These findings which pertain to measurements in solutions had earlier been proposed by Mel Mathieson[9] who had shown by X-ray analysis of the solids that the conformations of axial and equatorial acetates are represented by A and B respectively.



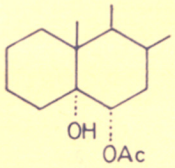
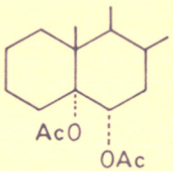
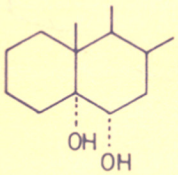
A



B

TABLE - 5.

EFFECT OF ACETYLATION ON C_6 - AXIAL PROTON

S. NO.	COMPOUND	C_6-H	C_6-OAc	C_5-OAc
15		5·0	2·06	—
16		5·1	2·06	2·01
17		3·6	—	—

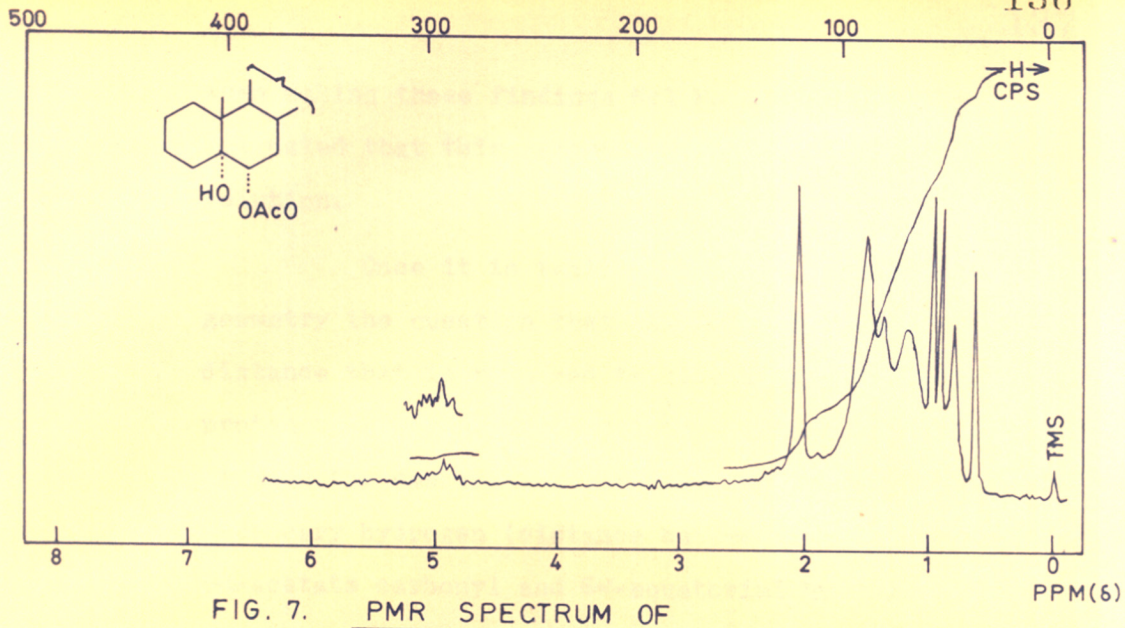


FIG. 7. PMR SPECTRUM OF

CHOLESTANE - 5α - 6α - DIOL, 6 - OCETALE

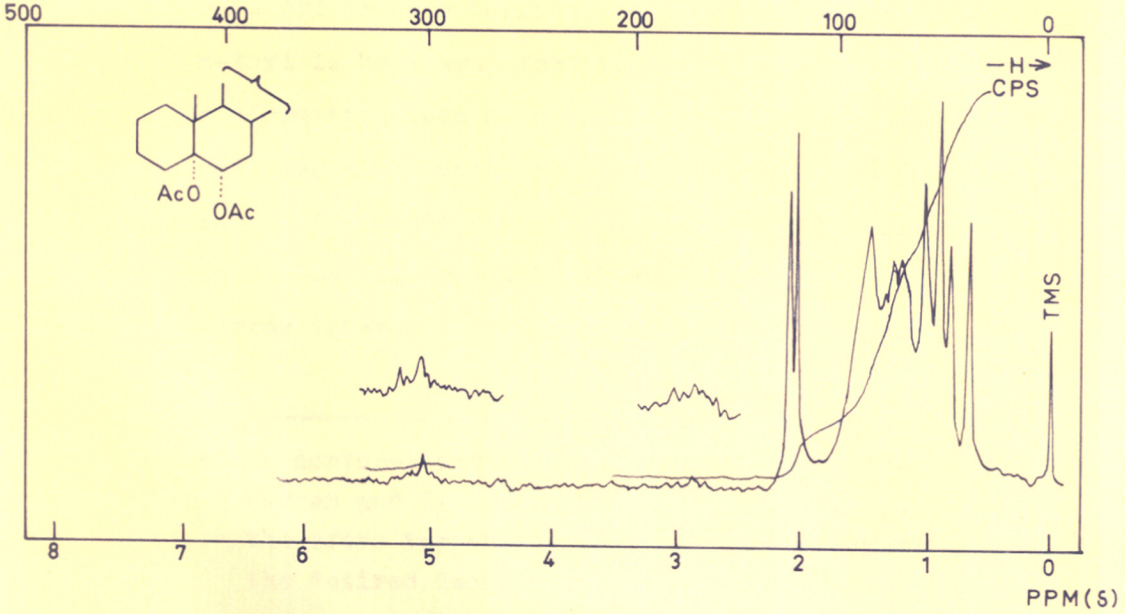


FIG. 8. PMR SPECTRUM OF

CHOLESTANE - 5α - 6α - DIOL, 5, 6 DIACETATE

When making these findings Mcl Mathieson had also suggested that this geometry would also hold in solution.

Once it is realised that an acetate has a fixed geometry the question that has to be decided is the distance that is required to have deshielding of a proton by a carbonyl. It is clear that this distance between the deshielding carbonyl and the concerned secondary hydrogen (distance between 6β-acetate or 5α-acetate carbonyl and 6α-equatorial hydrogen) must be approximately the same to account for the identical deshielding by these groups. An examination of Drieding models showed that this is only possible in one of the two (C and D) orientations* i.e. C where a carbonyl is held towards the steroid nucleus while the methyl is held away from it. Though several such conformations can be made, the best conformation (C) would have the carbonyl group bisecting the C_6 and C_4 equatorial protons. In any of the other possible conformations the carbonyl or the methyl would have larger interactions with either C_6 or C_4 hydrogens.

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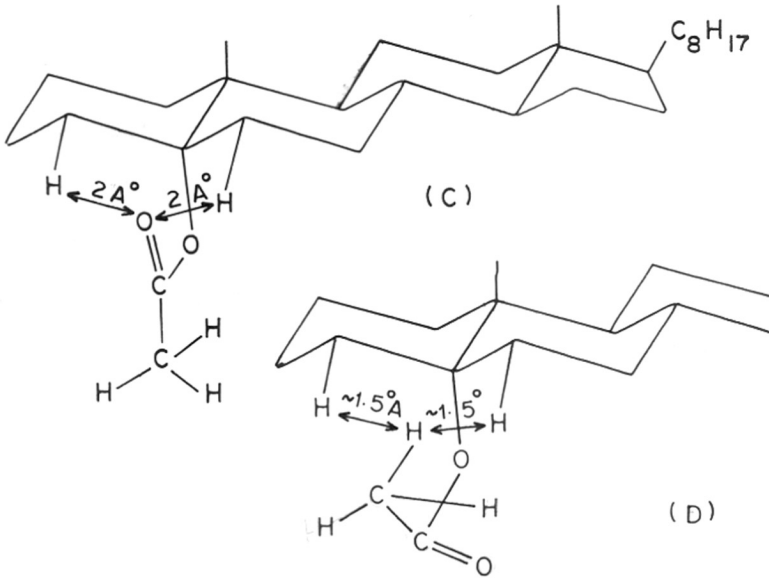
* In conformation D the distance between the carbonyl oxygen and C_6 and C_4 hydrogens is > 3.5A°, it is therefore improbable that such a conformation would give the desired deshielding.



An independent proof for the preference of conformation C as compared to D is forthcoming from the examination of solvent induced shifts observed in the case of compound (4), when the solvent is changed from $CDCl_3$ to benzene. According to the theory of Bhacca and Williams[10] if a plane is held perpendicular to the carbon oxygen double bond and passing through the carbon of the carbonyl then those groups on the same side of the plane as the oxygen atom are deshielded whereas those on the other side are shielded on passing from $CDCl_3$ to benzene as solvent. According to this argument the C_6-equatorial hydrogen would be deshielded whereas the acetate methyl would be shielded in conformation (C). In conformation (D) both C_6-proton and acetate methyl should be shielded.

It is observed that the C_6-α-H is deshielded (-0.1 p.p.m.) while the acetate methyl is shielded (+0.28 p.p.m.) (Table 6; Fig. 9 and 10). The findings therefore are a clear indication that the conformation of the C_5-acetate is as depicted in (C).

When one compares the conformations (C) and (D) it can indeed be seen that (D) is an unfavoured conformation as compared to (C), because of the interactions of the acetate methyl with the C_6 and C_4 hydrogens which are only 1.5°A from each other in (D); whereas in (C) these are very well separated, in (C) however the distance between the carbonyl oxygen and the C_6 and C_4 hydrogen is about 2°A.

In the conformation (C) the carbonyl group is shown as being equidistant from the C_4 and C_6 equatorial hydrogens. No evidence for this has been presented, but symmetry considerations require that there is no reason, why oxygen-carbonyl carbon single bond should be held closer to C_6 than to C_4. An experimental demonstration of this would require an examination of the P.M.R. spectra of 4β-substituted derivatives in which case the 4α-proton could be located and the shift observed in passing from the C_5-alcohol to C_5-acetate can then be compared to the corresponding shift of the earlier

TABLE - 6

SOLVENT INDUCED CHEMICAL SHIFT OF 3ß - 5α - 6ß - TRIOL, 3 METHYL ETHER 5 ACETATE

SOLVENT	C_6 - PROTON	ACETATE METHYL
$CDCl_3$	4·67	2·03
BENZENE	4·77	1·75
Δ $CHCl_3$ - BENZENE	0·1	0·28

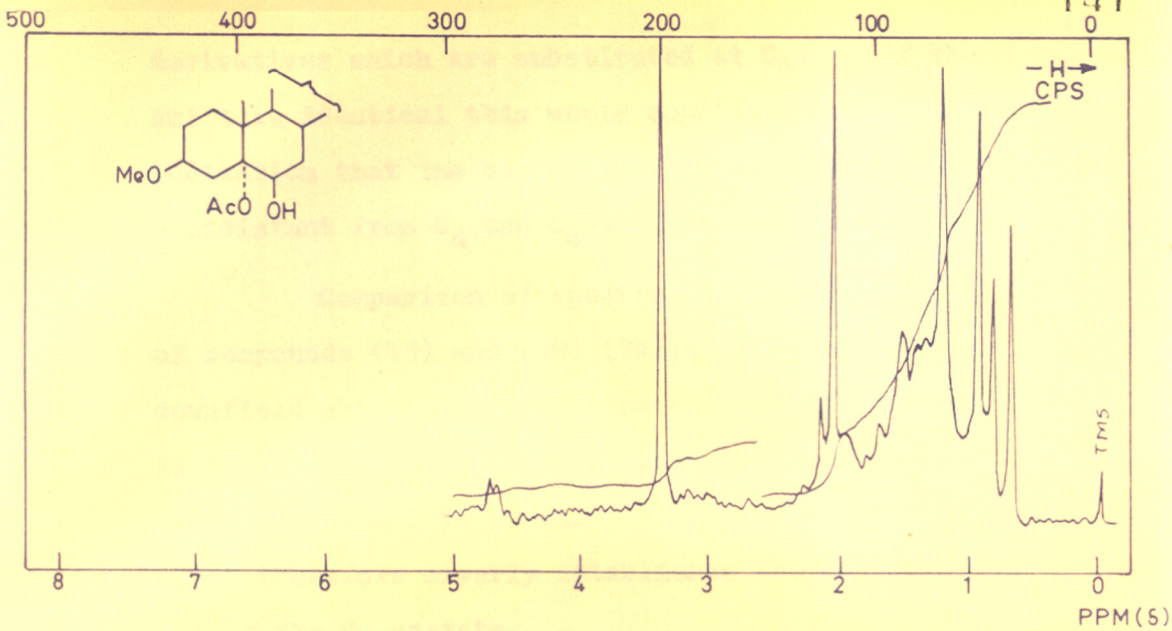


FIG. 9.
PMR SPECTRUM OF
CHOLESTANE 3 β - 5 α - 6 β - TRIOL 3 METHYL ETHER
3 ACETATE (IN $CDCl_3$)

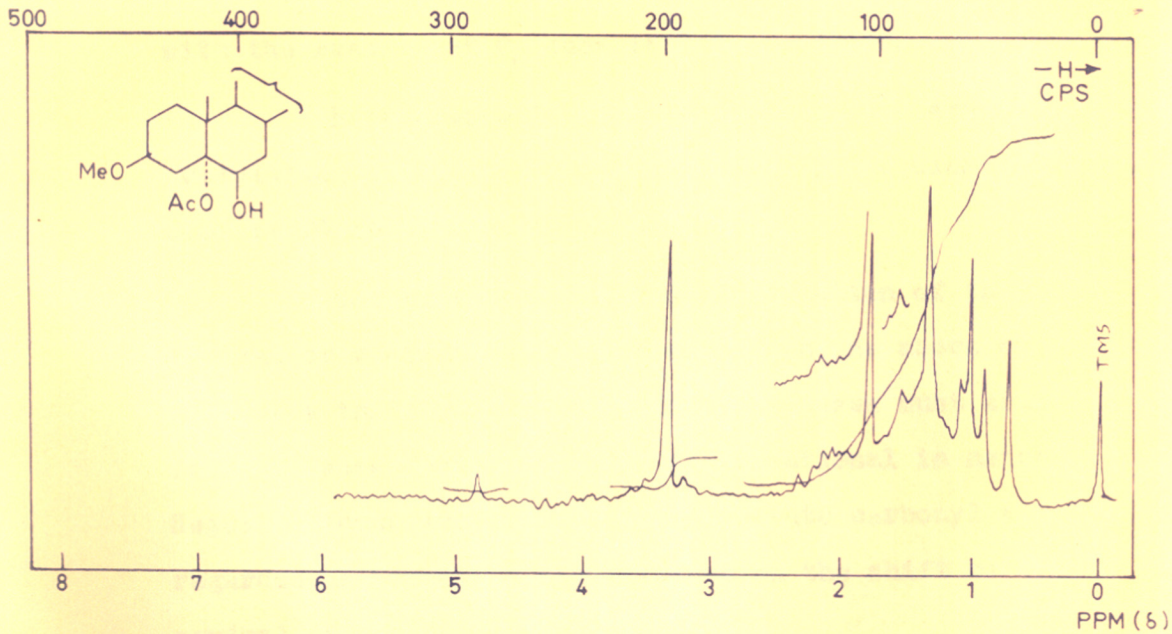


FIG. 10.
PMR SPECTRUM OF
CHOLESTANE 3β - 5α - 6β - TRIOL 3METHYL ETHER 5 ACETATE
(IN BENZENE)

derivatives which are substituted at C_6. If the shift is identical this would constitute an indication that the carbonyl of the C_5-acetate is equidistant from C_4 and C_6-equatorial hydrogens.

Comparison of spectra (Fig. 11 and 12) of compounds (18) and (19) (Table 7) showed that the downfield shift of the C_4 proton on acetylation of C_5 is 1.03, whereas for the C_6-proton (compare 12 and 13) this shift was 1.11 p.p.m. The near identity of this shift therefore clearly established the conformation (C) of the C_5-acetate.

The shift of C_4-equatorial proton on converting (18 to 19, acetylation of C_5) or converting (20 to 18, acetylation of C_4) is almost of the same magnitude (1.03 to 1.19). These results are in keeping with the results of C_6-substituted compounds.

Furthermore identical shifts are observed on acetylation in the C_4 and C_6-series. (1.19 against 1.2 p.p.m. compare 20 to 18 against 14 to 12).

The fact that shift on acetylation of the geminal or vicinal carbons is equal* in the above cases, indicates that this shift in both the cases must arise from the same factor which is conformational in nature. Negative inductive effect of the acetate carbonyl may be regarded as having no significance on the shift of the geminal proton during acetylation.

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* When the geometry is the correct one.

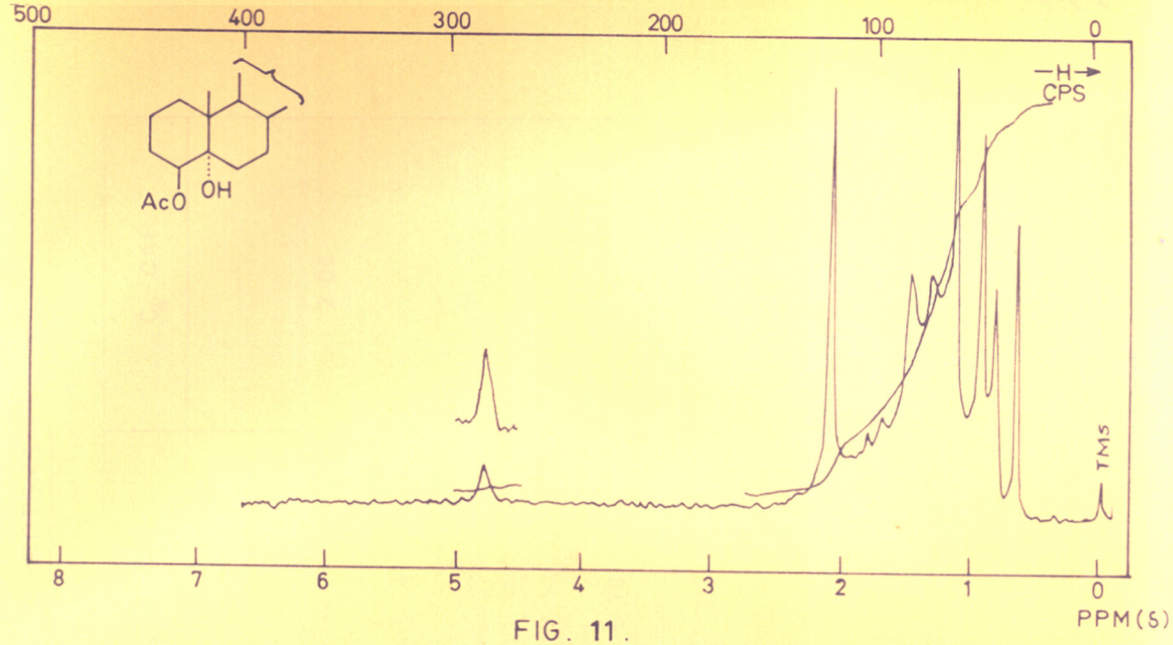


FIG. 11.
PMR SPECTRUM OF
CHOLESTANE 4β-5α-DIOL, 4 ACETATE

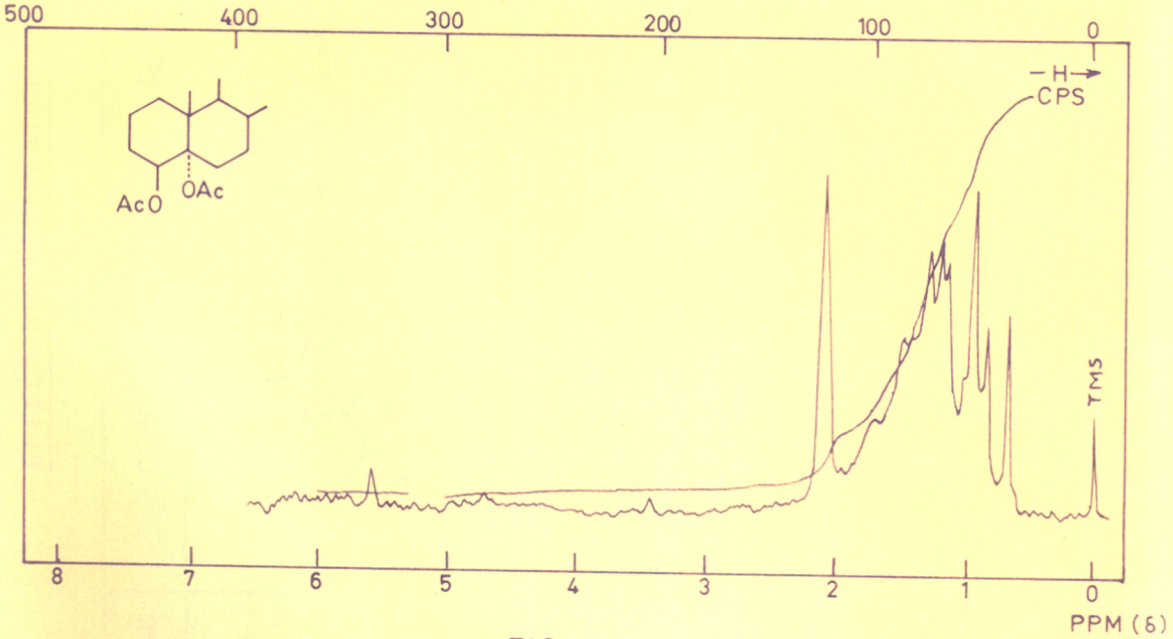
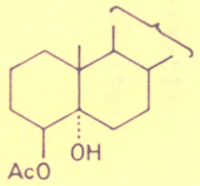
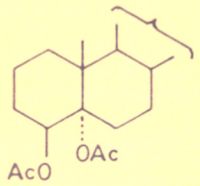
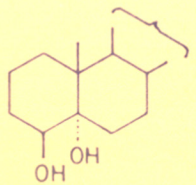


FIG. 12.
PMR SPECTRUM OF
CHOLESTANE – 4β–5α–DIOL, 4,5 DIACETATE

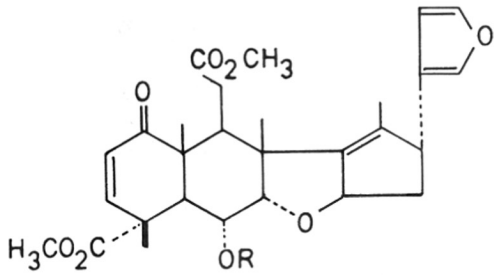
TABLE - 7

EFFECT OF ACETYLATION ON C_4 - EQUATORIAL PROTONS

S. NO.	COMPOUND	C_4 - H	C_5 - OAc	C_4 - OAc
18		4·75	—	2·06
19		5·78	5·78	5·78
20		3·56		

It is also clear that the axial C_3-acetate for example cannot have a similar deshielding effect on equatorial protons of the adjacent carbon atoms (C_2 or C_4) as the spatial relationship is no longer similar. In fact such shieldings can only occur for tertiary axial acetates common to two rings provided, the acetate conformation is fixed in the geometry mentioned earlier. If one examines Dreiding models of steroids with an AB cis fusion, then it could be anticipated that the C_5-acetate will deshield the equatorial protons at C_4 and C_6. The deshielding of the C_4-proton in this case can be regarded as deshielding by an equatorial acetate of an axial proton. However the deshielding of C_6-proton may be considered as deshielding of an equatorial proton by equatorial acetate. Thus it is anticipated that the real factor which would govern the deshielding would be the spatial relationship, and in a suitable location deshielding by an equatorial acetate can be anticipated.

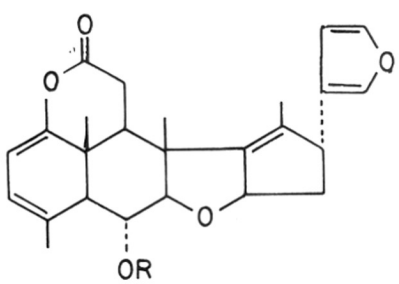
One such group of examples is that of nimbin derivatives (21 and 22)[11] where acetylation of the C_6-equatorial hydroxyl deshields the C_5-



(21)

		5∝-axial H at ppm	7∝-equatorial H at ppm
Desacetyl nimbin	21 R = H	3.37	4.01
Nimbin	21 R = Ac	3.67	4.03
Pyronimbic acid	22 R = OH	2.76	4.15
Pyronimbic acid acetate	22 R = oAc	3.21	4.2

axial proton by 0.3 (compare 21 R = H and R = Ac) and 0.45 p.p.m. (compare 22 R = H and R = Ac), whereas it has a negligible effect (0.02 and 0.05 p.p.m.) on the 7∝-equatorial proton.



(22)

EXPERIMENTAL

For general remarks see Chapter 1.

All P.M.R. spectra were recorded in chloroform solution.

The preparation of cholesteryl methyl ether, cholestane-3β-5α-6β-triol-3-methyl ether (3), cholestane-3β-5α-6β-triol-3-methyl ether 6-acetate (1) are reported in Chapter I.

Cholestane-3β-5α-6β-triol-3-methyl ether-5,6-diacetate (2)

Acetic anhydride (2 ml) and p-toluene sulphonic acid (200 mgs) were added to a solution of cholestane-3β-5α-6β-triol-3-methyl ether 6-acetate (1 :200 mgs) in acetic acid (10 ml). After allowing to stand at room temperature for 15 hrs. the mixture was poured into cold water and then kept aside for 5 hours to decompose excess acetic anhydride. Extraction with ether, washing with saturated sodium bicarbonate and water and drying over sodium sulphate afforded the product which on crystallisation from ether-methanol gave the desired compound.

Yield = 120 mgs.

M.P. 106°-108°

$(\alpha)_D$ -53°

Analysis: Found: C, 75.2; H, 10.69

$C_{32}H_{54}O_5$ requires: C, 74.09; H, 10.49%

I.R. showed the absence of hydroxyl and displayed bands at 1750 and 1240 cm^{-1} (acetate).

Cholestane-3β-5α-6β-triol-3-methyl ether 6-acetate (4)

The above diacetate (230 mgs) was kept overnight with 5% methanolic potassium hydroxide (25 ml) at room temperature. The solution was poured in to water and extracted with ether. The organic layer was washed with 2N.hydrochloric acid, saturated $NaHCO_3$ and distilled water, dried over sodium sulphate and evaporated. The white material obtained was crystallised from methanol.

Yield = 150 mgs.

M.P. 171°

$(\alpha)_D$ = -11

Anslysis: Found: C, 75.87; H, 10.96%

$C_{30}H_{52}O_4$ requires: C, 75.58; H, 11%

I.R. 3500 (hydroxyl), 1725, 1260, 1280 cm^{-1} (acetate).

Cholestane-3β-5α-6β-triol-3-methyl ether-6-formate (5)

Cholesteryl methyl ether (500 mgs) was left overnight with formic acid (5 ml) and hydrogen peroxide (0.7 ml) at room temperature). Addition of hot water to the solution followed by cooling at 0° gave a white solid which was filtered and crystallised from ether methanol.

Yield = 350 mgs.

M.P. 152°

$(\alpha)_D$ = +28

Analysis: Found: C, 77.86; H, 11.09%

$C_{29}H_{50}O_4$ requires: C, 77.97; H, 11.28%.

I.R. 3400 (hydroxyl), 1750, 1270 and 1290 cm^{-1} (formate).

Cholestane-3β-5α-6β-triol-3-methyl ether 5-acetate-6-formate (6)

A mixture of the above compound (480 mgs) glacial acetic acid (25 ml), p-toluene sulphonic acid (480 mgs) and acetic anhydride (5 ml) was allowed to stand at room temperature for fifteen hours. The reaction mixture was poured into cold water and then (after 4 hrs.) was extracted with ether. The ether extract was washed with saturated solution of sodium bicarbonate and distilled water and dried over sodium sulphate and evaporated. An oily material was obtained which on standing in acetone-methanol furnished crystals.

Yield = 200 mgs.

M.P. 154°C.

$(\alpha)_D$ = -34

Analysis: Found: C, 73.52; H, 10.25%

$C_{31}H_{52}O_5$ requires: C, 73.76; H, 10.38%.

I.R. 1725 cm^{-1} (acetate and formate)

Cholestene-3β-5α-6β-triol-3-methyl ether 5,6-diformate (7)

Cholestene-3β-5α-6β-triol-3-methyl ether 6-formate (500 mgs) was suspended in formic acid (98%; 6 ml) and then phosphorus pentoxide (400 mgs) was added slowly with shaking. During the reaction there was a rapid release of CO_2 with decomposition phosphorus pentoxide. The substance dissolved slowly giving a dark brown colour. After the completion of addition, the solution was kept at room temperature for one hour. It was then poured into cold water and extracted with ether. The ether layer was washed with sodium bicarbonate and water to remove acidic impurities, dried and evaporated to give an oily material.

T.L.C. of the oil in benzene, ethyl acetate (9:1) showed the presence of two components (R_f 0.23 and 0.63). The more polar of these corresponded to the starting material and the other less polar spot was expected to be the diformate.

Both the compounds were separated by P.L.C. using the above solvent system. The diformate did not crystallise even in different solvent systems.

Yield = 200 mgs.

$(\alpha)_D$ = -35.

Analysis: Found: C, 75.6; H, 10.4.

$C_{31}H_{52}O_5$ requires: C, 75.9%; H, 10.6%

I.R. 1725 cm^{-1} (formate)

Cholestane-3β-5α-6β-triol (10)

This was prepared by the method of Fieser and Rajagopalan[12]. A suspension of cholesterol (1 g) in formic acid (10 ml) was heated at 80° for five minutes when an oil separated. The resulting mixture was cooled to room temperature and then treated with 30% hydrogen peroxide (1.8 ml). After occasional swirling, the solid dissolved to give a pale blue fluorescent solution. After standing at room temperature for fifteen hours hot water was added to the solution, when a granular white solid separated out. The solid was filtered, dried and refluxed for an hour with methanolic potassium hydroxide (5%). The solution was acidified and diluted when cholestane-3β-5α-6β-triol separated as a fine solid. The solid was collected by filtration, washed free of acid, dried and crystallised from acetone-methanol.

Yield = 0.8 g.

M.P. 234-236°. Lit.[12] 237-239°

$(\alpha)_D$ = +2°. Lit.[12] +3°.

I.R. 3500 cm^{-1} (hydroxyl).

Cholestane-3β-5α-6β-triol 3,6-diacetate (8)

Following the method of Plattner and Lang[13] cholestane-3β-5α-6β-triol (200 mg) was allowed to stand overnight in a mixture of dry pyridene (1 ml) and acetic anhydride (1ml). After usual work up and crystallisation from methanol, the trans triol diacetate

was obtained as stout needles.

Yield = 170 mgs.

M.P. 163-165°. Lit.[13] 166°

$(\alpha)_D$ = -42° Lit.[13] -44.9°

I.R. 3500 (hydroxyl); 1725 cm^{-1} (acetate)

Cholestane-3β-5α-6β-triol triacetate (9)[14]

1. The triol (200 mgs) was dissolved in glacial acetic acid (10 ml). Acetic anhydride (2 ml) and p-toluene sulphonic acid (200 mgs) were added to the above solution and the mixture left overnight at room temperature. The reaction mixture was then poured into ice cold water. After 4 hours the aqueous layer was extracted with ether. The ether layer was washed with $NaHCO_3$ solution and water, dried and evaporated to provide a white solid which was crystallised from acetone-methanol.

Yield = 140 mgs.

M.P. = 146°-147° Lit.[14] 148-149°C

$(\alpha)_D$ = -100

I.R. 1750 cm^{-1} (acetyl) showed absence of hydroxyl.

2. The procedure using dimethyl aniline and acetyl chloride[16] in chloroform solution provided the same triacetate in essentially the same yields.

Cholestane-3β-5α-6β-triol-5α-acetate (11)[15]

The above triacetate was hydrolysed with 5% methanolic KOH by keeping the reaction overnight at room temperature. The material was worked up as usual.

Yield = quantitative.

M.P. 168° Lit.[15] 170°

I.R. 3400 (hydroxyl); 1725 cm^{-1} (acetate)

Cholestane-5α-6β-diol (14)[16]

To a suspension of Δ^5-cholestene (2 g) in formic acid (25 ml; 88%), hydrogen peroxide (2 ml) was added. The mixture was stirred at room temperature overnight. Some wax like material remained undissolved. After addition of water (200 ml) the mixture was extracted with ether and the ether solution was washed with 2N sodium hydroxide and water, and then dried and evaporated. The residue was refluxed with 2.5% methanolic potassium hydroxide solution (25 ml) and the solution was acidified, diluted with water and extracted with ether. After washing with sodium bicarbonate solution and water, the ether solution was dried and evaporated.

The residue was chromatographed on alumina (6 g). The fraction eluted with pet. ether gave the starting material (400 mg) and the benzene-ether eluate gave the 5α-6β-diol as a white solid which

was crystallised from ether-methanol.

Yield = 1 g.

M.P. 120° Lit.[16] 125°C

$(\alpha)_D$ = -5 Lit.[16] -3

I.R. 3571, 3448 cm^{-1} (hydroxyl).

Cholestane-5α-6β-diol 6-acetate (12)[16]

Cholestane-5α-6β-diol (200 mg) was dissolved in pyridine (2 ml) and acetic anhydride (2 ml). The mixture was kept at room temperature overnight. The reaction mixture was poured in water and extracted with ether and worked up as usual. The product was crystallised from acetone-methanol.

Yield = 170 mgs.

M.P. 110° Lit.[16] 112-114°C

$(\alpha)_D$ = -40°

I.R. 3500 (hydroxyl) 1725 cm^{-1} (acetate).

Cholestane-5α-6β-diol-diacetate (13)[16]

Cholestane-5α-6β-diol (150 mgs) was refluxed with acetyl chloride (3 cc) and dimethylaniline (3 cc) in alcohol-free chloroform (25 ml) for 4 hrs. Usual work up gave an oil which was purified by elution from alumina with pet.ether to give the required diacetate which crystallised from acetone.

Yield = 100 mgs.

M.P. 72° Lit.[16] 76°

$(\alpha)_D$ -30 Lit.[16] -33

I.R. 1740 cm^{-1} (acetyl)

Cholestane-5α-6α-diol (17)[16]

Δ^5-Cholestene (1 g), in dry dioxane (20 ml) was treated with osmium tetroxide (600 mgs) and pyridine (2 ml). The solution was kept at room temperature for 48 hrs. in darkness. After H_2S gas was passed for 30 minute the mixture was filtered, and the residue was washed thoroughly with ether. The filtrate together with the washings was diluted with water and extracted with ether. The organic layer was washed with dil. 2N hydrochloric acid and sodium bicarbonate solution and then with water. After drying over anhydrous sodium sulphate and solvent removal under vacuo, the product was crystallised from ether-methanol.

Yield - 800 mgs.

M.P. 174° Lit.[16] *180-181°

$(\alpha)_D$ +12° Lit.[16] +15°

Cholestane-5α-6α-diol 6-acetate (15)[16]

Cholestane-5α-6α-diol was acetylated in the usual way with pyridine and acetic anhydride. The acetate was crystallised from acetone-methanol.

M.P. 130-131° Lit.[16] 117-118°

$(\alpha)_D$ +20 Lit.[16] +24

I.R. 3440 (hydroxyl); 1724 cm^{-1} (acetate).

Cholestane-5∝-6∝-diol diacetate (16)[16]

Cholestane-5∝-6∝-diol (100 mg) was refluxed with dimethyl aniline (2 ml) and acetyl chloride (2 ml) in alcohol free chloroform (20 ml) for 4 hrs. Usual work up furnished an oily product which could not be crystallised, though TLC in different solvent systems showed it to be homogenous.

$(\alpha)_D$ +40° Lit.[16] +43°

I.R. 1730 cm^{-1} (acetate)

Cholestane-4β-5∝-diol (20)[16]

Δ^4-Cholestene (1 g) was stirred with formic acid (20 ml) and benzene (10 ml) whilst hydrogen peroxide was added slowly. The mixture was left overnight, poured into water and extracted with ether. The ether extract was washed thoroughly with $NaHCO_3$ solution and water. The extract was dried over sodium sulphate and evaporated to give a residual oil. The oily material was refluxed with 5% methanolic KOH (25 ml) for 2 hrs. and the reaction product was worked up in the usual way. The product was chromatographed over alumina. Elution with pet. ether gave white crystalline Δ^4-cholestene (200 mg) which was crystallised from methanol. Whilst elution with ether:benzene (1:1) gave cholestane-4β-5∝-diol which was crystallised from methanol.

Yield = 600 mgs.

M.P. 170-171°. Lit.[16] 171-172°C.

$(\alpha)_D$ +20° Lit.[16] +27

I.R. 3400 cm^{-1} (hydroxyl)

Cholestane-4β-5α-diol-4-acetate (18)[16]

The 4β-monoacetate was obtained by use of acetic anhydride and pyridine at room temperature for 16 hrs. The product crystallised from acetone in needles. Yield was almost quantitative.

M.P. 175-176°. Lit.[16] 175-176°

$(\alpha)_D$ +40° Lit.[16] -38°

I.R. 3400 (hydroxyl); 1725 cm^{-1} (acetate).

Cholestane-4β-5α-diol diacetate

The 4β-5α-diol (100 mgs) was refluxed with acetyl chloride (1.5 ml) and dimethyl aniline (0.9 ml) in alcohol free chloroform (20 ml). 4β-5α-Diacetoxy cholestane (75 mgs) was isolated as an oily product in the usual way, but chromotography on alumina (5 g) and elution with benzene pet. ether (1:1) gave 4β-5α-diacetoxy cholestane which could be crystallised from aqueous acetone.

Yield = 50 mgs.

M.P. 145° Lit.[16] 147°-148°C.

$(\alpha)_D$ = +58 Lit.[16] = +60

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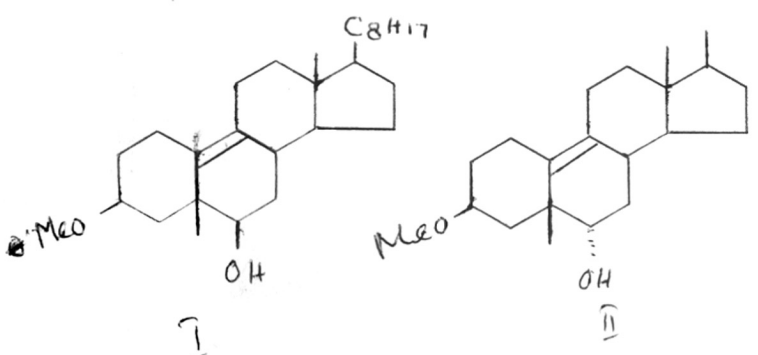
S U M M A R Y

:: SUMMARY ::

Chapter I describes a novel example of the mobility of the B ring of steroids. In this study it has been established that the Westphalen alcohol I on oxidation and reduction affords two alcohols both of which have been shown to have the equatorial orientation. In one of these, which is identical with the Westphalen alcohol, the hydroxyl is β oriented whereas in the other it is ∝- oriented II. these findings lead to the postulate that in the case of the ∝-isomer II the molecule must exist in the alternate half chair conformation.

After the publication of these results[1] French workers[2] have suggested on the basis of several arguments that the Westphalen alcohol I exists in the boat conformation. However none of the arguments can be considered as conclusive and it appears that the Westphalen alcohol I exists in the half chair conformation, though the boat conformation cannot altogether be ruled out.



In Chapter II a simple method for determining the location of a dimethyl amino group in a steroidal alkaloid was studied. Though this method was not useful for the purpose for which it was investigated, it shed light on the mechanism of the boron fluoride-acetic anhydride conversion of methyl ethers to acetates[3].

The splitting of carbonyl bands in the infrared spectra of diol monoacetates in steroids was examined. This splitting which is observed in axial acetates but not equatorial acetates is due to intermolecular hydrogen bonding with the hydroxyl group. The difference in behaviour of the axial and equatorial acetates arises from the difference in conformations of these acetates which causes the carbonyl of the equatorial acetates to occupy a hindered position which prevents intermolecular hydrogen bonding. These studies are presented in Chapter III.

Chapter IV deals with the deshielding of a vicinal hydrogen caused on acetylation. As this deshielding is similar to that observed for a geminal proton, the carbonyl groups in both acetates must be equidistant from the concerned hydrogen. These studies therefore establish the conformations of acetates. As these shifts of vicinal proton are not observed in the case when the concerned hydroxyl is equatorially oriented, these studies can help in determining the configuration also.

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M. R. Sama
(M. R. SAMA)
Candidate

Poona.