PART I

CHAPTER I - INTRODUCTION
OPENING OF STEROID 4,5 AND 5,6-EPOXIDES
INTRODUCTION

Although trimethylene oxide is sometimes called 1,3-epoxide and tetrahydrofuran as 1,4-epoxide, the term oxirane or epoxide is more generally used to mean a 1,2-epoxide consisting of a three-membered ring. Ethylene oxide I is the simplest example of an epoxide. All other epoxides can be considered as derivatives of it. The bond lengths and bond angles of ethylene oxide as confirmed by electron diffraction measurements \(^1\) are shown in Fig.1. Each CH\(_2\) group is in a plane at right angles to the plane of the ring and the angle between each CH\(_2\) plane and the plane of the carbon carbon bonds is \(159^0, 25'\). The dipole moment of ethylene oxide is 1.32D in benzene solution and 1.91D in the gas phase \(^2\). Infrared \(^3\), \(^4\) and NMR spectral measurements \(^5\) show that the electron density at the oxygen atom is unusually low, compared to those in acyclic and larger ring ethers. The strain energy has been found to be 13 Kcal/mole \(^6\). It can conjugate with an attached unsaturated group when the \(\pi\) orbitals of the unsaturated group orient themselves with their axes parallel to the plane of the ethylene oxide ring \(^7\). Hence for purposes of conjugation, the orbitals of the epoxide ring must lie in the plane of the ring \(^7\) and not above or below it as in benzene.

The representation of a "bent bond" structure for ethylene oxide like that of cyclopropane is given in Fig.2 \(^8\).
1,2-epoxy cyclohexane can be regarded as an extension of the ethylene oxide structure to a ring system. Electron diffraction studies have given it the dimensions given in Fig. 3.

The four carbon atoms nearest to the oxygen, namely $C_1$, $C_2$, $C_3$ and $C_6$ are co-planar and the angles $C_1,C_2$, $C_3$ and $C_2,C_1,C_6$ are 118.5°. The other angles and bond lengths are as given in the figure. The 3-membered ring gives rigidity to the system as is the case with a double bond and only the far away carbon atoms of the cyclohexane ring have conformational flexibility (see II). It will be useful to bear this structure in mind while considering the reactions of cyclohexane-1,2 epoxides in steroids, terpenoids, alkaloids, sugars etc. The cyclohexane ring can normally acquire the usual chair or boat conformation only on the cleavage of the epoxide ring. But since the substituent and the hydroxyl groups produced are trans diaxial, the transition state of the ring must be approaching a chair conformation.

**Orientation of ring opening**

An unsymmetrically substituted epoxide III can open in two ways giving two different products IV and V. Generally the reagent attaches itself to the carbon atom holding the larger number of hydrogen atoms as in IV and
this product is called the normal product.

Products formed by additions violating this rule as in V are termed abnormal products\(^\text{10}\). Under neutral and basic conditions attack at the less substituted carbon takes place leading to the normal isomer IV which is the only or major product isolated in these cases. This is strong evidence for an $\text{SN}_2$ attack of the reagent on the epoxide ring carbon atom giving rise to a transition state shown in Fig.4.

These reactions are subject to steric effects and the normal isomer IV formed by reaction at the less alkylated position is due to the steric hindrance of the alkyl group $R$. However, if a $\text{SN}_1$ mechanism is followed the rate determining step would carry a partial positive charge on the more substituted carbon atom as shown in Fig.5. This positive charge would be stabilised if the $R$-group contains electron releasing atoms or groups. A third mechanism is a modified $\text{SN}_2$ attack on the more substituted carbon leading to V as shown in Fig.6, in which the reagent is further away from the usual seat of attack.

The driving force for the reaction is the partial transfer of electrons from carbon to oxygen than from reagent to carbon. Thus both the partial bonds of the transition state are longer than usual and hence the $R$-group is less subject to steric hindrance, though the
FIG. 4

FIG. 5

FIG. 6
positive charge is stabilised by it. Reaction of propylene oxide with halogen acids provide examples for this type of reaction. Here bond breaking is more important than bond making and hence it is called a "modified SN$_2$ mechanism" different from a "true SN$_2$ mechanism". In a true SN$_2$ mechanism substitution takes place at the less substituted carbon.

Stereochemistry of ring opening

Since steroids are conformationally homogeneous, examples of ring opening of their epoxides would clearly show the stereochemical effects involved. A study of their epoxides have shown that they normally open to give diaxial products. It is well known that generally there is inversion of configuration at the point of attack. These facts are illustrated by the reactions of p-toluene-sulphonic acid on 2,3-epoxy cholestanes as shown to IX. The 2β, 3β-epoxide (VI) gives 2β-hydroxy 3α-tosyloxy cholestane VII with both groups diaxial and inversion at C$_3$, the point of attack. Similarly the 2α, 3α-epoxide (VIII) gives the 2β-tosyloxy, 3α-hydroxy cholestane IX again with both groups axial and with inversion at C$_2$, the point of attack. It is assumed that the stable chair conformation of the cyclohexane ring is partially reestablished in the transition states. The diaxial transition state is more stable than the diequatorial one since the
reagent is in the same plane as the epoxide ring in the diaxial transition state, whereas it is not so in the diequatorial one.

However, the reaction of hydrobromic acid with lanostane 2β, 3β-epoxide (X) gives the diequatorial 2α-bromo-3β hydroxy lanostane XI. Barton, Lewis and McGhie explain this by assuming distorted boat form of ring A in the epoxides, but since carbon atoms 1, 2, 3 and 4 are co-planar, it is doubtful whether there is any significant difference between a distorted boat or chair form for this ring for a substitution reaction at C₂. An ultimate explanation would be that attack of the reagent at C₃ to give the diaxial products would be considerably hindered sterically by the C₄-methyl groups and this may raise the energy of the diaxial transition state above that of the diequatorial one.

Opening of steroidal 4,5 and 5,6-epoxides

The simplest way to open up an epoxide is reduction with lithium aluminium hydride (LAH) which adds on a hydride ion at the site of attack and opens up the epoxide to an alcohol.

Reduction of a simple cyclohexane epoxide can illustrate the stereochemical points involved. Reduction following a cis and trans epoxide XII and XIII respectively show that
the addition of the hydride ion and opening up of the epoxide ring to give a hydroxyl group are both diaxial giving the diols XIV and XV respectively\(^{13}\). However, simple cyclohexanes are not rigid and they can exist in the alternate conformations also. The cyclohexane systems are rigidly fused in the steroid skeleton and hence the course of LAH reduction of some typical steroid epoxides may give more reliable information on the course of the reaction. Shoppe found that cholestane 4,5\(\alpha\)-epoxide XVI on treatment with LAH gives 5\(\alpha\) axial alcohol XVII by the addition of hydride from the 4\(\beta\) axial side\(^{14}\). Presence of a 3\(\beta\) acetoxy\(^{15}\) XVIII or 3\(\beta\) chloro group\(^{16}\) (XIX) or 3\(\alpha\)-hydroxy\(^{15,17}\) (XX) or a 7\(\beta\) acetoxy group\(^{19}\) XXI does not affect this mode of diaxial opening and give the 5\(\alpha\) hydroxy cholestane XXII, XXIII, XXIV and XXV respectively with the respective substituent.

A 4,5\(\beta\)-epoxide XXVI can open diaxially in two different ways that is either as XXVII or as XXVIII. In the trans fused product (XXVII) the incoming group P has added \(\alpha\)-axial at C\(_5\) and the epoxide opened up to \(\beta\)-axial hydroxyl group at C\(_4\). In the other compound XXVIII the A/B rings are cis fused and hence the incoming group has added axial at C\(_4\) \(\alpha\) and the epoxide has opened \(\beta\)-axial at C\(_5\) to ring A. Any other way of opening would not be
trans diaxial. It has been found that the simple 4,5$\beta$-epoxy cholestane XXIX on LAH reduction opens trans diaxial to XXX with a $\beta$-hydroxyl group at C$_5$ by the addition of a hydride $\alpha$-axial at C$_4$ to the cis fused ring A$^{14}$. The addition of a $\beta$ or $\alpha$-hydroxyl group at C$_3$ as in XXXI$^{15,17}$ or a $\beta$-acetate at C$_6$ as in XXXII$^{18}$ or at C$_7$ as in XXXIII$^{19}$ produces only the C$_5$$\beta$ hydroxy cholestanes (XXXIV) to (XXXVI) with the corresponding substituents show that these substituents do not affect the mode of cleavage of the epoxide.

In the case of the 3$\beta$-hydroxy 4,5$\beta$ and 14,15$\beta$ di-epoxy steroid (XXXVII), the LAH reduction product is its 5$\beta$,14$\beta$ dihydroxy derivative (XXXVIII)$^{20}$. In the cis fused C, D rings it may be noted that addition of a hydride at 15$\alpha$ is actually quasi-axial and the 14$\beta$-OH is also quasi-axial to ring D. However, addition of an $\alpha$-hydride at C$_{14}$ and opening up of the 14,15$\beta$-epoxide ring to give the 15$\beta$-quasi axial secondary alcohol would also have been both diaxial. The product formed, however, is only the former one with the tertiary alcohol as has been found in all the cases referred to above$^{21}$. In all these compounds although there are two possible ways of opening diaxially as in the cases of (XXVII) or (XXVIII) only the type XXVIII giving the tertiary alcohol is observed. As the other possibility of diaxial opening to give the secondary alcohol
like (XXVII) is not readily encountered in any of these examples, it could be suspected whether LAH reduction of a secondary, tertiary epoxide would tend to give only a tertiary alcohol. The following example of LAH reduction of 5,6\(\beta\) -epoxy cholestane (XXXIX) to 5\(\beta\) -hydroxy cholestane supports this view. For what is produced in this case also is the same tertiary 5\(\beta\) -hydroxy steroid by LAH reduction, but the addition of the hydride at C\(\_6\) -position and the opening up of the epoxide at C\(\_5\) -position are not diaxial, but diequatorial to ring B, to which the epoxide was attached. Hence it is not very clear whether in LAH reduction, of a secondary, tertiary epoxide, formation of a tertiary alcohol is more important than the normal mode of diaxial reagent addition and epoxide opening. It would therefore appear worthwhile to see the opening of steroid 4,5 & 5,6 epoxides with other reagents.

Cholestane 4,5\(\alpha\) -epoxide (XVI) on treatment with aqueous mineral acid is found to give the diaxial 4\(\beta\),5\(\alpha\) diol (XL). Here the epoxide undergoes the normal opening by addition of a hydroxyl group at the less substituted carbon atom C\(\_4\) axially and with inversion and the epoxide opens up to give the axial alcohol at C\(\_5\) (XL). Similarly reaction with hydrobromic acid in acetic acid the epoxide (XVI) gives the 4\(\beta\) -bromo-5\(\alpha\) -hydroxy cholestane (XLI). Presence of a \(\beta\) -acetoxy group at C\(\_6\) does not affect this epoxide cleavage
as seen from the examples (XLII) to (XLIII)\textsuperscript{18}. In the same way epoxide (XLII) taken in methanol and on treatment with p-toluene sulphonlic acid undergoes the same type of opening to give 4\textbeta-methoxy steroid (XLIV)\textsuperscript{18}. Similarly 3\textbeta-acetoxy 4,5\textlambda-epoxy cholestane (XLV) on treatment with hydrazoic acid gives the corresponding 5\textlambda-hydroxy 4\textbeta-N\textsubscript{3}-substituted cholestane (XLVI) by diaxial opening as before\textsuperscript{22} or 23. In an analogous way treatment of 4,5\textbeta-epoxy 6\textbeta-hydroxy (XLVII) or acetoxy (XLVIII) cholestane on treatment with methanol and p-toluene sulphonlic acid gives the 4\textlambda-methoxy, 5\textbeta-hydroxy cholestane with the C\textsubscript{6}\textbeta-hydroxy (XLIX) or acetoxy (L) substituent was was originally present. In these cases the reagent addition in, and the opening up of, the epoxide to form the hydroxyl group are both axial to ring A to which the epoxide was attached. It may also be noted that the reagent attack in these cases is at C\textsubscript{4}, away from the point of attachment of the substituent that is C\textsubscript{6}\textsuperscript{18,21}.

Similarly a 6\textlambda-hydroxy 4,5\textbeta-epoxy cholestane (LI) on treatment with p-toluene sulphonlic acid in methanol gives the corresponding 6\textlambda-5\textbeta-dihydroxy-4\textlambda-methoxy steroid (LII) as the sole product\textsuperscript{18}. It may thus be noted that in both the 4,5\textlambda and 4,5\textbeta-epoxides with C\textsubscript{6} substituents, the opening of the epoxide is at the less substituted carbon at C\textsubscript{4} and away from the substituents.
(XVI) $\xrightarrow{R\cdot H} (XL, R=OH)\, (XL\text{I}, R=Br)$

(XLII) $\xrightarrow{?\cdot III} (XL\text{III}, R=Br)\, (XLIV, R=OMe)$

(XLV) $\xrightarrow{?\cdot III} (XLVI)$

(XLVII) $R=\text{H}$

(XLVII) $R=\text{Ac}$

(XLIX) $R=\text{H}$

(XLIX) $L=\text{H}$

(XLIX) $L=\text{Ac}$
4β-acetoxy 5,6α-epoxy cholestane (LIII) on treatment with HBr gives as expected 4β-acetoxy 5α-hydroxy 6β-bromo cholestane (LIV) by opening up at the less substituted C₆ carbon atom. But 4α-acetoxy 5,6β-epoxy cholestane (LV) on treatment with p-toluene sulphonic acid in methanol gives by diaxial opening 4α-acetoxy 5α-methoxy-6β-hydroxycholestane (LVI)\(^1\). In this case the epoxide ring is opened at the more substituted C₅ carbon and near the C₄ substituent probably since that is the only way to cleave the 5,6β-epoxide diaxially.

It has been observed that 4,5β-epoxy 6β-hydroxy cholestane (XLVII) on treatment with p-toluene sulphonic acid and methanol gives besides the methoxy diol (XLIX) cholest-4ene-6-one (LVII). The same product is also obtained by treating (XLVII) with acid washed alumina. This would require an opening up of the epoxide at C₅ and going through the intermediates (LVIII) to (LX) and finally to (LVII). Had there been a symmetrical intermediate it would have also given cholest-5-ene-4-one (LXI) as shown below. However, ketone (LXI) is not obtained in the above reaction, but is obtained by the treatment of 4β-hydroxy 5,6β-epoxy cholestane (LXII) with methanol and p-toluene sulphonic acid or alumina. This reaction does not give cholest-4-ene-6-one (LVII) as a by-product as would have been the case.
with a symmetrical intermediate. These results would thus indicate that with each hydroxy epoxide the reaction is converted and only one unique product can be obtained in any one case as shown in (LXII) to (LXI). It may however be noted that the cleavage in both these cases has been from C₅ and to give axial intermediates. However in the case of the 4,5β-epoxide (XLVII) the cleavage at C₅ is rather unusual, because C₄ used to be the point of cleavage with other reagents e.g. with p-toluene sulphonic acid in methanol see e.g. (XLVII) or (XLVIII) to (XLIX) and (L).

In contrast to all these reactions cleavage of 5,6β-epoxy-4β-acetoxycholestane (LXII) with HBr in acetic acid is found to give 4β-acetoxy 6α,bromo-5β-hydroxy cholestane (LXIII) as the sole product wherein the functional groups at C₅ and C₆ are both equatorial to ring B, which was holding the epoxide. It has been argued that the bulky bromonium ion may find it difficult to approach from the α-side at C₅ of the cis fused A/B rings especially in the presence of the neighbouring 4β-acetoxy group. However, 4α-acetoxy 5,6β-epoxy cholestane (LV) under the same conditions gives only 30% of the product (LXIV) by diequatorial opening as before, but produces 45% of 4α-acetoxy-5α-bromo-6β-hydroxy cholestane (LXV) by diaxial opening. The steric hindrance for approach of a bromonium ion from the α-side at C₅ should be much more with a 4α-acetoxy group.
than with a 4\(\beta\)-acetoxy group and still the product by such an approach is formed in this case and that too 50% more than the one obtained by the approach of the bromonium ion at C\(\delta\). Thus the factors determining the exact mode of cleavage of these epoxides do not seem to be very clear.

**Neighbouring group participation**

Substituents on neighbouring carbon atoms have sometimes been found to influence the rate and course of reaction of epoxides. Henbest and Wrighey found that cholestane 5,6\(\lambda\)-epoxide (LXVII, \(R=H\)) without any substituents at C\(3\) is cleaved to the corresponding 5\(\beta\)-H 6 one (LXVIII, \(R=H\))\(^{23}\) to a maximum yield of 30% in two minutes time with BF\(_3\) etherate in benzene. In the presence of 3\(\lambda\) substituent (LXVII, \(R=OAc\)) the corresponding ketone is obtained (LXVIII, \(R=OAc\)) but it takes 14 hours with the same reagents to get the maximum yield of 61%\(^{24}\). However, in the presence of a 3\(\beta\)-acetate the same epoxide (LXIX, \(R=OAc\)) with the same reagents cleaves to give 6\(\beta\)-fluoro-5\(\lambda\)-hydroxy cholestane (LXX, \(R=OAc\)) in 5 minutes to a maximum yield of 62%\(^{24}\). When instead of the acetate group a 3\(\beta\)-chloro group is present at C\(3\) the epoxide (LXIX, \(R=Cl\)) takes 18 minutes for completion of the cleavage and gives only a maximum yield of 45% of the fluorohydrin (LXX, \(R=Cl\))\(^{24}\). Similarly cholestane 5,6\(\beta\)-epoxide (X\(\lambda\).XIX)
with the same reagents cleave to give 5\(\Delta\)-cholestane-6-one (LXXI) in 2 minutes to a maximum yield of 90% when 3\(\beta\)-acetoxy 5,6\(\beta\)-epoxide (LXXII) is subjected to the same reagents, what is obtained is the corresponding 5\(\Delta\)-fluoro-6\(\beta\)-hydroxy derivative (LXXIII) the maximum yield being about 70% in 30 minutes. The longer time taken for the cleavage with the substituents is explained as due to the inductive effect of the electronegative groups at C\(3\) impending the development of a positive charge at C\(5\) during the cleavage. There is no satisfactory explanation for the difference in the type of products obtained with the change in substitution pattern at C\(3\). One generalization that could be made is that with no substituent, steroid 5,6\(\Delta\)- or \(\beta\)-epoxides rearrange to a C\(6\)-ketone, and with a C\(3\)\(\beta\)-substituent they rearrange to diaxial fluorohydrins.

Coxon and coworkers have recently found examples of more direct participation by neighbouring groups. When 3\(\beta\)-acetoxy 4,5\(\Delta\)-epoxy cholestane (XLV) was treated with BF\(_3\) etherate in benzene it was found converted to 4\(\beta\)-acetoxy-3\(\beta\),5\(\Delta\)-dihydroxy cholestane (LXXIV)\(^{25}\). This requires a participation of the 3\(\beta\)-acetate group as shown in (XLV) to (LXXV) to (LXXIV). Compound (LXXIV) is precipitated after an hour. However, if left as such, the precipitate gradually dissolves and on working up after six hours gives
3β-acetoxy 4-oxo-5β-cholestan-5-one (LXXVI) (yield 43.5%).
This requires a reversal of (LXXV) to (LXV) and then cleavage without participation as shown in (LXXV) to (LXXVI).
It is not clear why after keeping for a longer time the reaction reverses and reforms compound (XLV), and why in the reformed (XLV) no more participated cleavage at C₄ occurs, but only simple cleavage at C₅ takes place. By a similar participation 3β-acetoxy 4,5β-epoxy cholestane (LXXVII) on treatment with BF₃ etherate gives 3β-acetoxy 4β,5α-dihydroxy cholestane (LXXVIII). The intermediate (LXXIX) is trapped by treating it with lithium borohydride to form the crystalline acetal (LXXX) which was fully characterised. This acetal on treatment with p-toluene sulphonic acid gave 3α,4β,5α-trihydroxy cholestane (LXXXI) thus proving the participation²⁵. Keeping the reaction mixture for a longer time without working up, however did not give in this case any 4-keto compound as in the case of (XLV); cleavage at C₅ and shift of an α-hydride from C₄ to C₅ does not take place. Reaction of 3β-acetoxy 4,5β-epoxy cholestane (LXXXII) with BF₃ etherate under the same conditions, neither gives a diol nor a ketone as in the previous analogous cases, but the fluorohydrin (LXXXIII)²⁵. Although the 3β-acetoxy group cannot form a bridge and participate in the cleavage as in the two previous cases, it is not clear why cleavage from C₅
and shift of an $\mathcal{L}$-hydride from C$_4$ to form 3$\beta$-acetoxy 5$\mathcal{L}$-H cholestan-4-one does not take place.

Morrison and coworkers have found that cleavage of 3$\beta$-methoxy 4$\beta$-acetoxy 5,6$\mathcal{L}$-epoxy cholestane (LXXIV) on treatment with BF$_3$ etherate gives a mixture of 3$\beta$-methoxy 4$\beta$,5$\beta$-dihydroxy 6$\mathcal{L}$-acetoxy cholestane (LXXV) and 3$\beta$-methoxy 4$\beta$-acetoxy 5$\beta$,6$\mathcal{L}$-dihydroxy cholestane (LXXXVI). An interesting point in this reaction is that the functional groups produced, namely the 6$\mathcal{L}$-acetate or hydroxyl group and the 5$\beta$-hydroxyl group are both equatorial to ring B, to which the 5,6$\mathcal{L}$-epoxide was attached. Thus the epoxide opens diequatorial instead of the usual diaxial. The production of the 5$\beta$-hydroxy 6$\mathcal{L}$-acetate (LXXV) from the 4$\beta$-acetate (LXXXIV) requires the involvement of a bridged intermediate as (LXXXVII). An acetal of structure (LXXXVII) has been isolated as proof of this intermediate by NaBH$_4$ reduction of the intermediate (LXXXVII). Cleavage by participation of the adjacent acetoxy group occurs in the absence of the 3$\beta$-methoxy group as would be expected. Thus 4$\beta$-acetoxy 5,6$\mathcal{L}$-epoxide opens up with BF$_3$ etherate diequatorially to give the corresponding mixture of diols (LXXXIX) and (XC) by participation. But on treatment with HBr where no participation appears to take place, the product obtained (XCI) is by diaxial opening. It may, however, be noted
that this diaxial opening is not a reagent specificity of HBr. For 4β-acetoxy, 5,6β-epoxy cholestane (LXIIA) on treatment with HBr, has given 4β-acetoxy 5β-hydroxy 6α-bromo cholestane (LXIII), product of exclusive diequatorial opening. Similarly treatment of 6β-acetoxy 4,5α-epoxy cholestane (XLII) the alternate epoxide, with BF₃-etherate has been found to give a mixture of 4α-acetoxy 5β, 6β-dihydroxy cholestane (XCII) and 4α,5β-dihydroxy 6β-acetoxy cholestane (XCIII) by participation of the neighbouring 6β-acetoxy group in the opening up of the 4,5α-epoxide. In all these cases the neighbouring acetoxy group participates in the cleavage of the adjacent terminus of the epoxide (at C₅) which has the opposite stereochemistry although that is the more substituted end of the epoxide.

However, treatment of the same epoxides with HBr in acetic acid takes a different turn, the 4β-acetoxy 5,6α-epoxy steroid (LIII), giving the 5α-hydroxy 6β-bromo derivative (LIV). Similarly 6β-acetoxy 4,5α-epoxy cholestane (XLII) on treatment with HBr gives the corresponding 5α-hydroxy 4β-bromo derivative (XCIV). Both of these epoxides are opened up at the less substituted end and further away from the acetate group. However, 3β-methoxy 4α-acetoxy, 5,6β-epoxy cholestane (XCV) under the same reaction conditions produces the fluorohydrin (XCVI) by diaxial opening. In this case it is not clear why the acetate group which is
placed at the neighbouring carbon at the sterically favourable position, does not participate as before in the epoxide cleavage to get a $5\alpha,6\beta$-dihydroxy steroid.

Reaction of $3\beta$-methoxy $4,5\Delta$-epoxy cholestane (XCVII) with hot 2N sulphuric acid gives the hydroxy compound (XCVIII) by elimination, the A/B trans $C_4$ ketone (XCIX) by hydride shift and isomerisation and the $3\beta$-methoxy $4\beta,5\Delta$-diol (C) by normal diaxial opening at $C_4$. Generally with one reagent, the ring opening used to be in one way only. In this case the $4,5\Delta$-epoxide ring is found opened both at the more alkylated site $C_5$ and the less alkylated site $C_4$. The product (XCVIII) formed has interestingly the equatorial hydroxyl group at $C_4$. It is unlikely that a $\beta$ hydroxyl group was attached at $C_5$ (so that both the $5\beta$-OH and the $4\Delta$-OH are axial) and then the later eliminated under the acid conditions, because under the same conditions, the $5\Delta$-axial hydroxyl group has survived. After the ring opening at $C_5$, elimination of the $6\beta$-H has taken place to produce the $5$-ene thus making the $4\Delta$-OH equatorial.
CHAPTER II
OPENING OF STEROID 4,5-EPOXIDES BY RITTER
REACTION
Certain drugs of antitubercular activity and some important antibiotics such as penicillins and modified penicillins contain a benzamido function or analogous acylamino function. Recently a simple method was evolved to introduce a benzamido group into a molecule by a one-step reaction. Thus the 5,6β-epoxides of cholesterol and cholestane Ia and Ib were converted to the corresponding 5β-benzamido 6β-hydroxy steroids IIa and IIb by treatment with benzonitrile and perchloric acid.

Similarly cholesterol 5,6α-epoxide III is converted to 6β-benzamido 3β,5α, dihydroxy cholestane (IV) by the same reaction. These epoxides are also converted to the corresponding acetamido compounds by treatment with acetonitrile the usual reagent for the Ritter reaction, in place of benzonitrile.27

The French workers Ryan and Julia28 studying the Ritter reaction with the usual reagent acetonitrile on a series of cholesterol 4,5α and 4,5β-epoxides having a hydroxyl or acetate group at C3, as V to VIII, found that the epoxide opened invariably at C5 as shown in structures IX to XV. There was no attack and opening at C4 by a "true SN₂ mechanism". They explained these results on the basis of the development of a partial positive charge at the more alkylated carbon C5 in such reactions, which would lead to substitution only at C5 by a "modified SN₂ reaction".
Ref 27 C. R. Narayanan, A. K. Kulkarni, A. B. Landge & M. S. Wadla
Synthesis 35 (1977)
However, this did not appear to be consistent with the diverse type of openings of 4,5 and 5,6 epoxides as described in the previous Chapter.

A steroid 4,5 $\beta$-epoxide XVI can open diaxially in two different ways as shown in XVII and XVIII. In XVII A/B rings are transfused and the reagent has attacked and attached itself at C$_5$ and the hydroxyl group at C$_4$, both trans diaxial. In XVIII, A/B rings are cis fused and the reagent X$^-$ attacked and got attached at C$_4$ and the hydroxyl at C$_5$ again with trans diaxial to ring A, to which the epoxide was attached. Similarly a steroid 4,5 $\alpha$-epoxide can open diaxially in two different ways as shown in XIX to XX and XXI. In XX, the A/B rings are trans, the reagent attack and attachment is at C$_4$ and in XXI A/B rings are cis and the reagent attack and attachment is at C$_5$ the groups in all cases being trans diaxial to ring A to which the $\alpha$-epoxide was attached.

In the case of Ryan and Julia$^{28}$, their $\beta$-epoxides V to VII opened to form the more stable A/B trans fused rings as in XVII, but the $\alpha$-epoxide VIII opened to form the less stable cis fused rings as in XXI the common factor in both the cases being attack at the more alkylated position C$_5$. From this they concluded that the partial development of an initial positive charge at C$_5$ was the cause of this type of opening in all the cases.
An alternate explanation is possible for these results. An electronegative substituent at C_3 is likely to reduce the chances of ring opening in its vicinity at C_4 \textsuperscript{10} both by electronic and steric factors. Hence C_5 here should be considered as that end of the epoxide further away from the steric and the electronegative effect of an adjacent substituent, rather than merely a more alkylated position. If that is really the case the same epoxide without such a substituent at C_3 and with a substituent in its vicinity, namely C_6 should be expected to open differently under the same reaction conditions.

Accordingly 6 β-acetoxy 4,5 β-epoxy cholestane \textsuperscript{XXII} was prepared and subjected to Ritter reaction. Two new products, one m.p. 186-38°,$[\alpha]_D = +4.5°$ and another m.p. 142-44°,$[\alpha]_D = -26°$ were obtained from the reaction in 32 and 40% yield respectively. The former was assigned structure \textsuperscript{XXIII} for the following reasons. It showed infra red bands at 3400 (OH, NH), 1740, 1240 (O-acetate) and 1650 cm\textsuperscript{-1} (NHAc). Its PMR spectrum in CDCl\textsubscript{3} displayed signals at 6 0.55 (s, 3H, 18-H), 1.05 (s, 3H, 19-H), 1.98, 2.38 (s, 3H each, NHAc, OAc), 4.2 (1H, m, 4 β-H, the signals sharpen to a narrow triplet on $D_2O$ exchange), 5.32 (1H, narrow triplet, 6 β-H) and at $6 6.1$ (1H, d, J = 9 Hz, exchangeable with $D_2O$ that is removed on shaking.
with D$_2$O). The last signal was characteristic of the NH proton of the secondary amide on a secondary carbon, the coupling being between the anti-parallelly oriented NH proton and secondary methine proton$^{29}$. In accordance with this, on D$_2$O treatment, the doublet signal of the NH proton at 6.1 vanished and the broad multiplet of the methine proton at C$_4$ sharpened to a narrow triplet by loss of coupling to diaxial like NH proton. These spectral characteristics coupled with the mode of formation would clearly require this compound to be represented by structure XXIII. If it had the alternate structure (XXV) wherein the epoxide had opened at C$_5$, as in the case of Ryan and Julia and introduced a 5$\alpha$-NHCOCH$_3$, then, the NH would have shown itself as a singlet and not with a coupling of 9 Hz with the adjacent CH, removable by D$_2$O exchange. Trans structure XXVI and cis structures XXVII and XXVIII are not readily feasible from the 4,5$\beta$ epoxide$^{30}$. Besides structure XXVI has a 4$\beta$-NHCOCH$_3$ which would have pushed the C$_{19}$-H signal to 6 1.3 or even lower field, and in XXVII and XXVIII, the 4$\beta$-H and 4$\alpha$-H respectively are axial and would have shown large coupling constants on decoupling from the NH, all contrary to what is observed. Thus there is no alternate structure and hence the new compound m.p. 186-38$^\circ$ has to be represented by structure XXIII.

The second product m.p. 142-44$^\circ$ showed M$^+$ m/e 462
Ref: C. R. Natoyaan, A. K. Kulka, A. S. Lunde, S. M. S. Wadia
for $C_{29}H_{50}O_4$ and displayed infra red bands at 3500-3400 (OH), 1730 and 1240 cm$^{-1}$ (OAc) and exhibited PMR signals at 0.67 (s, 3H, 18-H), 1.03 (s, 3H, 19-H) 2.07 (s, 3H, OAc) 3.63 (1H, C 4/3-H) and 4.37 (1H, C 6/2-H). These spectral features along with its mode of formation suggest structure XXIV for the compound. On acetylation at room temperature with acetic anhydride and pyridine it afforded a diacetate m.p. 160-162$^\circ$ $C_{31}H_{52}O_5$ whose spectral features $\nu_{max}$ 3450 (OH), 1730, 1240 cm$^{-1}$ (OAc), $\delta$ 0.68 (s, 3H, 18-H), 1.02 (s, 3H, 19-H), 2.07 (s, 6H, 4/3 and 6/2-OAc), 4.77 (2H, $H_2$ = 6$H_2$, 4$H$ and 6$H$ equatorial protons) is entirely consistent with the structure proposed. Had it been a 5$H$, 4$H$-hydroxy compound, it would not have readily acetylated at the usual conditions, due to steric hindrance as reported in similar cases.

Thus both the compounds XXIII and XXIV are formed by opening of the epoxide at $C_4$ with inversion, that is by a "true SN$_2$ reaction".

These results are different from those of Ryan and Julia who had cleavage at $C_5$ in all the four cases they studied. This cleavage at $C_5$ has therefore to be due to the presence of a substituent at $C_3$ which sterically and electronically hinders the approach of the reagent at $C_4$ and therefore pushes the cleavage to the far end at $C_5$ in their case.
In the present example (XXII) there is no substituent present at C₃. On the other hand there is present an acetate group at the other end C₆, which would sterically and electronically hinder cleavage and substitution at the neighbouring carbon C₅. Thus both factors are favourable for the cleavage at C₄. It therefore appears that it is the neighbouring group that determines the point of cleavage and not necessarily the degree of alkylation at any particular position.

When however, 4,5β-epoxy 6β-hydroxy cholestane (XXIX) was subjected to Ritter reaction the product obtained is the 4β-hydroxy 5α-cholestan-6-one (XXX), identified by its m.p. 124-26°C (α)D +44°, M+ 402 and other spectral features. Such a hydroxy epoxide is set for a concerted opening and hydride shift as shown in (XXIX) to give the unique product (XXX). Had the ring opening produced the symmetrical carbonium ion (XXXI) without a concerted hydride shift from C₆, 6β-hydroxy 5α-cholestan-4-one (XXXII) m.p. 110-111°C (α)D -16° would also have been produced in this reaction. Absence of the latter ketone supports the concerted mechanism for this ring opening in the presence of the 6β-hydroxyl group instead of the acetoxy group.

When 4,5α-epoxy cholestane 6β-acetate (XXXIII) was subjected to the Ritter reaction the product obtained m.p. 198-200°C is assigned structure namely 6β-acetoxy, 4α,5α-
dihydroxy cholestane (XXXIV). It showed $\nu_{\text{max}}$ 3350 ($-\text{OH}$), 1720, 1260 cm$^{-1}$ (OAc) $\delta$ 0.92 (19-H), 2.08 (OAc), 3.2 (exchangeable with D$_2$O $\text{OH}$) 3.87 (narrow triplet 4\textbeta-H), 5.3 (6\textalpha-H); in complete agreement with structure (XXXIV). 5\textbeta,4\textalpha-Hydroxy 6\beta-acetoxy cholestane would have shown a larger downfield shift for the 19-H.

Thus, both the 4,5\textbeta and 4\textalpha-epoxy cholestanes with a 6\beta-acetoxy group has opened up at the further end namely C$_4$ under the usual Ritter reaction conditions. The 6\beta-acetate did not participate in the opening as was the case with BF$_3$ etherate in ether solution to produce the 5\beta,6\textalpha-hydroxy steroid as observed by Campion et al.$^{26}$ Probably such internal participation of a neighbouring group for ring opening requires less polar solvent than acetonitrile as ether and absence of aqueous mineral acid.

As acetonitrile did not participate in the reactions, the above reaction was repeated in acetonitrile with only a drop of perchloric acid instead of the usual 4 or 5 drops. However, the product obtained was not the one in which acetonitrile had participated in the reaction, but one in which the epoxide had rearranged into a ketone as in (XXXV). Consistent with this, the infrared spectra of the product was transparent in the hydroxyl region and showed absorptions at 1725, 1250 (OAc) and 1695 cm$^{-1}$ (ketone carbonyl). As
PMR spectra displayed signals at $\delta$ 0.7 (3H, 18-H), 0.96 (19-H) 1.97 (OAc) and 5.3 (narrow triplet, 6$\alpha$-H deshielded by the C$_4$ carbonyl in the cis A/B rings). The NMR spectrum of the compound in benzene solution showed a downfield shift to $\delta$ 5.71 for the 6$\alpha$-H, in complete conformity with its position in front of the carbonyl group in the cis A/B rings. Probably with insufficient aqueous mineral acid instead of substitution, internal rearrangement has taken place.

It is now found that under normal Ritter reaction conditions, a 4,5$\alpha$- or $\beta$-epoxide with a 3$\beta$-acetate opens at its farther end namely C$_3$ and with a 6$\beta$-acetate also opens at its farther end which is now C$_4$. It was then considered interesting to see the mode of cleavage of a 4,5$\alpha$-epoxide with acetate groups at both C$_3$ and C$_6$. Therefore 4,5$\alpha$-epoxy cholestane, 3$\beta$,6$\beta$-diacetate (XXXVI) was prepared and subjected to Ritter reaction. Purification of the complex mixture and separation by Preparative layer chromatography gave two crystalline products F$_2$ and F$_3$. Compound F$_2$ displayed infrared bands for OH and acetate and in the PMR spectra it showed signals at $\delta$ 0.72 (18-H), 0.93 (19-H), 2.07, 2.18 (two -OCOCH$_3$) 3.89 (1H, d, J, 3 Hz) 4 equatorial H coupled with 3$\alpha$-axial H) 4.98 (1H, a sharp singlet 6$\alpha$-H), 5.11 (1H, v.b. 3$\alpha$-axial H). This compound has to be characterized as cholestane, 3$\beta$,4$\beta$,5$\alpha$,6$\beta$-tetrol, 3,6-diacetate (XXXVII). The other product showed in the
infrared absorptions in the hydroxyl and acetate carbonyl regions. The PMR spectra of the compound showed all the signals mentioned above for compound (XXXVII) and besides showed signals at $\delta$ 3.73 (1H, b.d, J = 5 Hz, 4\(\phi\) equatorial H coupled with 3\(\psi\) -equatorial H) 4.62 (1H, multiplet 3\(\phi\) -equatorial H coupled with 4\(\phi\) -equatorial H and the two C\(_2\)-protons) and two additional acetate methyl signals at 2.06, 2.11. This has then to be a mixture consisting of compound (XXXVII) and another compound 3\(\beta\),4\(\beta\),5\(\beta\),6\(\beta\)tetra-hydroxy cholestane 3,6-diacetate (XXXVIII). The second compound could not be separated and as the amount was small further chromatography was not attempted. Steroidal compounds are known sometimes to form sharp melting mixed crystals\(^{33}\). The percentage of pure (XXXVII) obtained by the normal opening at C\(_4\) was only 4%. The mixture of (XXXVII) and (XXXVIII) comes to about 12.5% of which the integration show that the proportion of the former to the latter is as 3:2 which will make about 7.5% (XXXVII) and 5% of (XXXVIII). Thus the total pure products (XXXVII) and (XXXVIII) are obtained in a ratio of 11.5 : 5 or about 2:1. Because of the acetate groups on either side, epoxide cleavage is inhibited on either side, but as the epoxide cannot stay unreacted in the strong acid solution, fragmentations or rearrangements have largely taken place. On the usual cleavage of the epoxide, the normal cleavage from
the less alkylated position C₄ by a true SN₂ attack is more than double the abnormal cleavage from C₅ by a "modified SN₂ attack". To have an idea of the effect of a strong electronegative group on the epoxide cleavage one of the acetates was substituted by a chlorine atom by preparing 6β-acetoxy 3β-chloro-4,5α-epoxy cholestane (XXXIX). When this was subjected to Ritter reaction, the product obtained was transparent in the hydroxyl region and showed absorption at 1740, 1220 (OAc), 1710 (νC=O) and at 777 cm⁻¹ (ν.s., C-Cl next to a ketone) in its infra red and at δ1.02 (19-H), 2.07 (OAc), 3.84 (1H, narrow q, 3J equatorial H) and at δ 4.93 (1H, n.t., 6α-H) in its PMR spectra. The signal of the 6α-H as required for its position in front of the carbonyl group in the cis A/B rings, was shifted downfield from δ 4.93 to 5.28 in benzene solution. The compound has therefore to be assigned structure (XL). In complete confirmation of this it showed ultraviolet absorption at λ max 280 mμ με max 160. In the keto A/B cis steroid the 3J-H and 6J-H are in front of the carbonyl group. In agreement with this, in benzene solution, the 3J-H is shifted down from 3.84 to 3.92 and the 6J-H from 4.93 to 5.28 32,33. The relatively strong electronegative chloro group has therefore inhibited cleavage in its vicinity at C₄ and hence directed it to take place from C₅.
It therefore appears, though the number of examples taken are limited, that an epoxide can be made to cleave at will from either side, by proper manipulation of electronegative substituents in its vicinity. Whether the product obtained is by substitution or rearrangement depends on many factors as facility for a concerted reaction, polarity of the solvent etc. It is not clear why acetonitrile which was invariably employed as the solvent in all the reactions, participated in a few, but did not participate in many other substitution reactions.
CHAPTER III - EXPERIMENTAL
EXPERIMENTAL

General Remarks

Melting points are uncorrected and have been taken on a Gallenkamp melting point apparatus. Optical rotations were determined by using 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, Beckman DK 2 ratio recording spectrophotometer. Infrared spectra were recorded in carbon tetrachloride solution, unless otherwise stated on a Perkin-Elmer Infracord or Perkin-Elmer Model 221 spectrophotometer and maxima are reported in cm⁻¹. Nuclear magnetic resonance spectra were recorded in chloroform on a Varian A-60, T-60 or FT ¹³C spectrometer using tetramethylsilane as internal standard. The chemical shifts are reported in δ ppm. Silica gel used for chromatography was grade II. Thin layer chromatography was carried out on silica gel (200 mesh) mixed with plaster of Paris (10%) as binder. Spots were visualised by spraying with concentrated sulphuric acid or in iodine chamber.

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

5a-benzoylamino-cholestan 3β,6β-diol (IIa)

5β,6β-epoxy cholestane 3βol was prepared by the method described by Rowland and Nace³⁴ by known series of
reactions (Please see Chart I).

To a solution of this epoxide (Ia, 500 mg) in methylene chloride (10 ml), benzonitrile (10 ml) was added. After addition of perchloric acid (0.1 ml, 72%), the reaction mixture was kept at room temperature for 48 hrs, poured into sodium bicarbonate solution (10%) and extracted with ether. The ether layer was washed with brine and dried. After distilling off ether and excess benzonitrile in vacuum, the crude material was chromatographed on neutral alumina (15 g). From pet. ether-benzene (1:3) and benzene eluates the starting material was obtained (100 mg). Further elution with benzene, ethyl acetate (7:3) afforded the required benzyolamino compound which was crystallized from methanol.

Yield 180 mg (36%), m.p. 212° [α]_D -12.5°
IR: 3350 cm⁻¹ (OH/NH) 1650 and 1520 cm⁻¹ (NHCOPh), 1575 cm⁻¹ (phenyl).
NMR in CDCl₃ δ 0.68, (18- H) 0.93 (19- H), 2.63 (–OH), 3.66 (3- α- H), 4.13 (6- β- H) 6.2 (NH).
Analysis: Found: C, 77.80; H, 10.34; N, 3.23.

C₃₄H₅₃O₃N requires: C, 77.96; H, 10.20; N, 2.87%.

5α-benzylamino-cholestan-3β,6β-diol diacetate (IIa')

On usual acetylation with pyridine, acetic anhydride the above diol (100 mg) gave crude product which was crystallized from ether-methanol. Yield: 72 mg (62%).
m.p. 92°, $\mu D-38.3°$.

IR: 3400 cm$^{-1}$ (NH), 1720, 1260, 1240 cm$^{-1}$ (acetate),
1650, 1510 cm$^{-1}$ (NH-CO-CH$_3$) and 1600 cm$^{-1}$ (phenyl).

NMR: 0.67 (singlet, 3H, 18-H), 0.91 (singlet, 3H, 19-H),
2.0 (singlet, 3H, 0-CO-CH$_3$), 2.06 (singlet, 3H,
0-CO-CH$_3$), 4.75 (broad multiplet, 1H, C$_3$-H), 5.1
(narrow multiplet, 1H, C$_6$-H), 6.17 (1H, NH) 7.29
(aromatic protons 5H).

Analysis: Found: C, 74.92; H, 9.42; N, 2.41.

C$_{38}$H$_{57}$O$_5$ requires: C, 75.08; H, 9.45; N, 2.30%.

5,6-acylamino cholestan 3β, 6β-diol (II'a)

To a solution of 5β,6β-epoxy cholestan 3β-ol
(500 mg) in methylene chloride (10 ml), acetonitrile (8 ml)
and perchloric acid (0.1 ml, 72%) were added. The resulting
reaction mixture was left overnight at room temperature,
poured into sodium bicarbonate solution (10%) and extracted
with ether. Ether layer was washed with brine and dried.
Ether was distilled off, the crude material was crystallized
from methanol, yield 280 mg (48%).

m.p. 252-53° Lit.$^{35}$ 255-57°

$\mu D$ +21° Lit.$^{35}$ +18°

IR: 3490 cm$^{-1}$ (-OH), 1650 and 1500 cm$^{-1}$ (NH-CO-CH$_3$).

NMR: 1.99 (singlet COCH$_3$), 3.7 (broad multiplet 1H
C$_3$-H), 4.54 (narrow multiplet 9 Hz 1H C$_6$-H),
three exchangeable protons at 2.96, 4.2 and 5.3 for
OH and NH. 5.3 (NH) does not undergo exchange readily.

**5-acylamino-cholestan 3β,6β-diol diacetate (II'a')**

The above diol (200 mg) on usual acetylation with acetic anhydride, pyridine gave the corresponding diacetate which was crystallized from methanol.

Yield 150 mg (68%)

m.p. 215-216°C Lit. m.p. 222.5 - 223.5°C

$[\alpha]_D^{18}$ -18 Lit. -21

IR: 3325 cm⁻¹ (NH) 1740 and 1240 cm⁻¹ (acetate) 1650 and 1520 cm⁻¹ (NH COCH₃).

NMR: 0.7 (singlet, 3H, H-13) 0.92 (singlet, 3H, H-19), 2.00 (singlet 6H, COCH₃), 2.07 (singlet 3H (OCH₃)), 4.75 (broad multiplet 1H, C₃-H), 5.79 (narrow multiplet $\frac{9}{2}$ Hz, 1H, C₆-H), 5.26 (1H, exchangeable, NH).

**Cholestan 5α,6β-diol**

To a suspension of $\Delta^5$-chestene (5 g) (Chart II) in formic acid (30 ml, 88%), hydrogen peroxide (20 ml, 30%) was added. The mixture was stirred at room temperature for about 24 hours. A little chloroform (3-4 ml) was added, to the mixture to dissolve insoluble sticky mass during stirring. The reaction mixture was then poured into hot water (200 ml) allowed it to cool to room temperature and extracted with ether. Ether layer was washed with 2N sodium hydroxide solution and water. Dried the solution, ether was distilled off. The residual material was refluxed with methanolic
potassium hydroxide solution (50 ml, 3%) for half an hour, cooled, poured in ice-water, acidified with dil. hydrochloric acid and extracted with ether. Ether layer was washed with sodium bicarbonate and water, dried and ether distilled off. Residual material was chromatographed over silica gel. Pet. ether and benzene eluents gave starting material (800 mg). Elution with benzene-ethyl acetate (9:1) afforded the 5\(\alpha\), 6\(\beta\)-cholestane diol as a colourless solid which was crystallized from ether-methanol.

Yield 2.6 g (58%)  
m.p. 122-124\(^\circ\)  \quad \text{Lit.}^{37} 125\(^\circ\)  
\([\alpha]_D\) \(-5\(^\circ\)\)  \quad \text{Lit.}^{37} \ -3\(^\circ\)  
IR: 3570, 3448 cm\(^{-1}\) (hydroxyl).

**Cholestanol-5\(\alpha\), 6\(\beta\)-diol diacetate**

To a solution of above diol (2 g) in glacial acetic acid (100 ml), acetic anhydride (20 ml) and p-toluene sulphonic acid (2 g) were added. The reaction mixture was left overnight at room temperature. Usual work up and crystallization from acetone furnished the required diacetate.

Yield 1.5 g (60%)  
m.p. 72-73\(^\circ\)  \quad \text{Lit.}^{37} \text{m.p.} 75-76\(^\circ\)  
\([\alpha]_D\) \(-30\(^\circ\)\)  \quad \text{Lit.}^{37} \ -33\(^\circ\)  
IR: 1740 cm\(^{-1}\) (acetate carbonyl).
5β,6β-epoxy cholestane (Ib) (Chart II)

The above diacetate (1 g) was added to a solution of sodium ethoxide in ethanol (prepared from sodium (1 g) and absolute ethanol (50 ml)). The mixture was refluxed for 3.5 hours and the reaction was quenched by the addition of water. The solvent was removed under reduced pressure. The residue obtained was extracted with ether and washed with brine, dried and ether was distilled off. Residue on crystallization from ether-methanol yielded the pure epoxide. Yield 0.670 g (84%).

m.p. 52° Lit.38 m.p. 53°

IR showed the absence of acetate carbonyl.

5α-acvlamino-6β-hydroxy cholestane (IIb)

5β,6β-Epoxy cholestane (500 mg) on Ritter reaction as described for II'a by using acetonitrile (8 ml) afforded a product which on crystallisation from pet. ether gave the pure title compound.

Yield: 190 mg (33%)

m.p. 198-199° \[ \mu_D +12.3° \]

IR: 3450 cm⁻¹ (NH/ OH) 1615 and 1510 cm⁻¹ (NHCOCH₃).

NMR: 0.71 (singlet, 3H, H-18), 0.91 (singlet, 3H, H-19), 1.29 (singlet, 3H, COCH₃), 4.56 (narrow multiplet \[ 9 Hz, 1H, H-6 \]), 3.45 and 5.24 (exchangeable 1H each for OH and NH respectively).
5α-acylamino 6β-acetoxy cholestane (IIb') Fig 13

On usual acetylation of the above compound (IIb) (100 mg) with acetic anhydride and pyridine the title compound was obtained which was crystallised from ether-methanol. Yield: 70 mg (63%); m.p. 201-203°C; [α]D 27.9° IR: 3350 cm⁻¹ (NH), 1750, 1245 and 1235 cm⁻¹ (acetate and 1650, 1525 cm⁻¹ (NHCOCH₃)).

NMR: 0.63 (singlet, 3H, H-18), 0.87 (singlet, 3H, H-19), 1.95 (singlet, 3H, COCH₃), 2.00 (singlet, 3H, COCH₃), 5.18 (1H, exchangeable, NH), 5.68 (narrow multiplet, 1H, H-6).

Analysis: Found: C, 76.63; H, 10.59; N, 2.97.

C₃₁H₅₃O₃N requires: C, 76.33; H, 10.95; N, 2.87%.

5α,6α-Epoxy cholestane 3p-ol.

A solution of monoperphthalic acid in ether (30 ml, 14%) was added to a solution of cholesterol (5 g) in dry ether (50 ml). The mixture was kept at 0°C for 12 hours and then at room temperature for about 16 hours. The ether extract was washed with sodium bicarbonate solution and water and dried. After removal of ether, residue obtained was crystallized from ethyl acetate, giving the required epoxide.

Yield: 3.2 g (61%).

m.p. 140-141° Lit.39 m.p. 141-143°
[α]D -42° Lit.39 -44.5°
IR: 3300 cm⁻¹ (-OH).
6\beta benzoylamino cholestane 3\beta,5\alpha-diol (IV) Fig 14

5\beta,6\alpha-epoxy cholestane-3\beta-ol (500 mg) was treated with benzonitrile (10 ml) as described earlier. On usual work up, a crude product containing a mixture of two compounds was obtained. It was chromatographed over neutral alumina (15 g). Elution with pet.ether/benzene (1:3) and benzene gave 60 mg starting material. Further elution with benzene/ethyl acetate (3:1) afforded the title compound which was crystallized from ether-methanol. Yield 220 mg (38%). m.p. 106°. [\alpha]_D -20.5°.

Analysis: Found: C, 77.61; H, 10.09; N, 2.76. 

C_{34}H_{53}NO_{3} requires: C, 77.96; H, 10.26; N, 2.67%.

IR: 3600 cm\(^{-1}\) (NH/OH), 1665 and 1525 cm\(^{-1}\) (NH-CO-Ph) and 1585 cm\(^{-1}\) (Phenyl).

NMR: 0.81 (singlet, 3H, H-18), 0.92 (singlet, 3H, H-19), 4.30 (broad multiplet 2H, C\(^\alpha\)-H and C\(^\gamma\)-H), 6.23 (doublet, N-H), 7.3 (singlet, aromatic protons).

6\beta-benzoylamino cholestane 3\beta,5\alpha-diol-3\beta-acetate (IV') Fig 15

The above diol (IV, 100 mg) was acetylated using acetic anhydride and pyridine. The crude material did not crystallize even after passing through a column of alumina (5 g). Yield: 62 mg (57%). [\alpha]_D -46.2°.

IR: 3300 cm\(^{-1}\) (OH/NH), 1710 and 1240 cm\(^{-1}\) (acetate), 1650 and 1500 cm\(^{-1}\) (NHCOPh) and 1575 cm\(^{-1}\) (phenyl).

NMR: 1.96 (singlet, COCH\(_3\)_), 5.16 (broad multiplet C\(^\alpha\)-H),
4.28 (narrow multiplet C₆-H), 6.33 (doublet, N-H).

Analysis: Found: C, 76.28; H, 9.67; N, 2.55.

C₃₆H₅₅O₄N requires: C, 76.41; H, 9.80; N, 2.48%.

6β-acvlamino-cholestan-3β,5α-diol (IVA) Fig 16

5α,6α-epoxy cholestane-3β-ol (500 mg) on treatment with acetonitrile (3 ml) as described before, gave a crude compound, which on crystallisation from ethyl acetate afforded pure 6-acyl amino compound. Yield: 260 mg (45%).

m.p. 254-256° Lit. 40 m.p. 260°
[α]D -24° Lit. 40 -26°

IR: 3300 cm⁻¹ (OH/NH), 1640 and 1500 cm⁻¹ (NHOCH₃).

NMR: 6 0.83 (singlet, 3H, 18-H), 0.94 (singlet, 3H, 19-H), 2.0 (singlet, 3H, COCH₃), 3.01 and 5.66 (exchangeable protons -OH and -NH respectively, N-H proton as a doublet), 4.07 (broad multiplet C₃ and C₆ -H).

6β-acvlamino-cholestan-3β,5α-diol-3β-acetate (IVA') Fig 17

Usual acetylation of the diol (IVA 180 mg) with pyridine-acetic anhydride furnished the title compound which was crystallised from methanol. Yield 120 mg (61%).

m.p. 194-196° Lit. 40 m.p. 195-196°
[α]D -7.9° Lit. 40 -11.6°

IR: 3450 cm⁻¹ (NH/OH), 1725 and 1240 (acetate), 1670 and 1520 cm⁻¹ (NHOCH₃); 4.13 (narrow multiplet, 1H, C₆-H), 5.16 (broad multiplet 1H, C₃-H).
Cholestane-5\(\lambda\)-6\(\beta\)-diol-6\(\beta\)-acetate (Chart III)

Cholestane-5\(\lambda\), 6\(\beta\)-diol (2 g) (as described earlier) on acetylation with pyridine and acetic anhydride in the usual manner gave the title compound, which was crystallized from ether/methanol.

Yield 1.8 g

m.p. 110-112\(^\circ\) Lit.\(^{37}\) m.p. 112-114\(^\circ\)

IR: 3500 (OH), 1725 (-OAc)

NMR: 0.80 (singlet, 3H, C\(_{18}\)-H), 0.9 (singlet, 3H, C\(_{19}\)-H), 2.03 (singlet, 3H, -OCOC\(_3\)), 4.66 (triplet 1H, 6\(\lambda\)-H).

Cholest-4 en, 6\(\beta\) acetate Chart III

The above compound (1.6 g) was dissolved in dry pyridine (25 ml) and the resulting solution was cooled to 0\(^\circ\)C. To this solution thionyl chloride (3 ml) was added dropwise. The mixture was shaken well maintaining the temperature at 0\(^\circ\)C throughout the addition and was then kept at 20\(^\circ\)C for half an hour. The reaction mixture was poured into cold water and extracted with ether. Ether layer was washed with 2N hydrochloric acid, aq. sodium bicarbonate and water, dried and distilled off ether. Residual material crystallised from ether-methanol. Yield 1.3 g.

m.p. 75-76\(^\circ\) Lit.\(^{37}\) m.p. 76-77\(^\circ\)

[\(\alpha\)]\(_D\) +72\(^\circ\) Lit.\(^{37}\) +74\(^\circ\)

IR: 1740, 1245 (acetate), 1650 (olefin).
NMR: $\delta$ 0.8 (singlet, 3H, 18-H), 0.91 (singlet, 3H, 19-H), 2.03 (singlet, 3H, COCH$_3$), 4.55 (narrow triplet 6-$\alpha$-H), 6.28 (triplet, 1H, 4-$\beta$-H).

**Cholest-4-en, 6$\beta$-ol**

6$\beta$-acetoxy cholest-4-en (1.25 g) was hydrolysed with ethanolic sodium hydroxide solution (5% 30 ml) by keeping the reaction mixture at room temperature for 16 hrs. Crude product obtained by usual work up was crystallised from acetone. Yield 1.0 g.

m.p. 86-88$^\circ$ Lit.$^3$ m.p. 86-87$^\circ$  
$[\alpha]_D^\circ$ +60.5$^\circ$ Lit.$^3$ +62$^\circ$

IR: 3400 (-OH), 1740 and 1240 (-OAc), 1660 (allylic double bond).

NMR: $\delta$ 0.81 (singlet 3H, 18-H), 0.9 (19-H), 4.17 (narrow triplet 1H, 6$\alpha$-H), 5.5 (triplet 1H, 4$\beta$-H).

4$\beta$,5$\beta$-epoxy cholestane-6$\beta$-ol (XXIX), Chart III. Fig 18

To a solution of cholest 4-en-6$\beta$-ol (1.0 g) in ether (25 ml) a solution of monoperphthalic acid (15%, 15 ml) was added. The reaction mixture was kept at 5$^\circ$ for about 15 hours, extracted with ether, washed with sodium bicarbonate solution and water. After drying over sodium sulphate ether was distilled off. Crude product crystallised from methanol. Yield 0.8 g.

m.p. 103-110$^\circ$ Lit.$^8$ m.p. 104-105$^\circ$  
$[\alpha]_D^\circ$ +1.4$^\circ$ Lit.$^8$ +0.8$^\circ$
IR: 3450 (-OH)
NMR: δ 3.05 (1H, triplet C₄-H), 3.32 (1H triplet C₆-H).

4β-5β-epoxy cholestane 6β-ol acetate (XXII)

On acetylation of the above epoxide (0.75 g) with acetic anhydride and pyridine in the usual manner, the title compound was obtained which was crystallised from ethanol. Yield 0.7 g.

m.p. 86-87° Lit.¹⁸ m.p. 85-87°
D -29.6° Lit.¹⁸ -32°

IR: 1725 and 1240 cm⁻¹ (acetate).
NMR: δ 2.05 (3H -OCOC₃H₃), 3.01 (triplet 1H, C₄-H), 4.45 (triplet 1H, C₆-H).

4β-α-Cyano-δ-hydroxy-cholestane-6β-acetate (XIII)

A solution of 4,5β-epoxy cholestane-6β-acetate (0.5 g) in methylene chloride (10 ml) was treated with acetonitrile (8 ml) in presence of perchloric acid (0.1 ml 72%). The reaction mixture was kept at room temperature for about 15 hours. On usual work up a crude product was obtained which was chromatographed on a silica gel column (15 g). It was eluted with (i) pet. ether (200 ml) (ii) pet. ether: benzene (1:1) 200 ml, (iii) benzene (200 ml), (iv) benzene: ethyl acetate (9:1) (200 ml), (v) benzene:ethyl acetate (8:2) (200 ml), (vi) benzene: ethyl acetate (1:1) (200 ml), (vii) Ethyl acetate (200 ml), (viii) Methanol (100 ml).
The fractions obtained in the eluent 10 to 20% ethyl acetate in benzene were combined, solvent was distilled off under reduced pressure and the residue obtained was crystallised from methanol, yield 150 mg.

m.p. 142-44° [\(\mu\)]_D -26°

IR: 3500-3400 cm\(^{-1}\) (-OH), 1730 and 1240 cm\(^{-1}\) (acetate).

NMR: \(\delta\) 1.03 (3H singlet, 19-H), 2.07 (3H, s, O-COCH\(_3\)), 3.63 (1H, C\(_4\)-H) and 4.87 (1H, C\(_6\)-H).

This compound was characterised as 4\(\Delta\),5\(\beta\)-dihydroxy cholestane-6\(\beta\)-acetate (XXIV).

The product obtained in ethyl acetate eluent was crystallised from methanol.

Yield: 120 mg. m.p. 186-88°; [\(\mu\)]_D +4.5°

IR: 3400 (OH/NH) 1740 and 1240 (-OAc), 1650 cm\(^{-1}\) (NHAc).

NMR: \(\delta\) 1.05 (singlet 3H, C\(_{19}\)-H) 1.98 and 2.08 (singlet, 3H each -NHAc and OAc), 4.2 (1H multiplet 4H), 5.02 (1H narrow triplet 6\(\Delta\)-H), 6.1 (1H doublet, J = 9 Hz exchangeable -NH).

This compound was characterised as 4\(\Delta\)-acylamino-5\(\beta\)-hydroxycholestane-6\(\beta\)-ol-acetate (XXIII).

Cholestane 4\(\Delta\),5\(\beta\),6\(\beta\)-triol 4\(\Delta\),6\(\beta\)-diacetate (XXIV)

4\(\Delta\),5\(\beta\)-dihydroxy cholestane 6\(\beta\)-acetate (XXIV, 100 mg) obtained in the above reaction was acetylated with acetic anhydride-pyridine in the usual way. Product obtained was crystallised from methanol. Yield 90 mg. m.p. 160-62°; [\(\mu\)]_D -5°.
IR: 3450 (-OH), 1730 and 1240 cm⁻¹ (-OAc).

NMR: δ 1.02 (singlet, 3H, C₁⁹-H), 2.07 (singlet 6H, C₄-H and C₆β - OCOCH₃), 4.77 (doublet, 2H, \(\Delta = 6\) Hz, 4β and 6α-H).

4α,5β-epoxy cholestane-6β-acetate (XXXIII) Chart III Fig 23

Cholest-4en-6β-ol-acetate (5 g) in ether (75 ml) was treated with monoperphthalic acid (15% 50 ml). The solution was kept at room temperature for about 48 hours. On usual work up, it furnished the title compound by crystallisation from ethanol. Yield 2.6 g.

\[
\begin{align*}
\text{m.p.} & \quad 101-103° \quad \text{Lit.}^{18} 102-103° \\
[\alpha]_D & +38° \quad \text{Lit.}^{18} [\alpha]_D +36°
\end{align*}
\]

IR: 1725 cm⁻¹ (-OAc).

NMR: δ 2.05 (singlet, 3H, C₆ OCOCH₃),
3.13 (triplet, 1H C₄-H), 4.13 (triplet, 1H, C₆-H).

4α-acylamino cholestane 5β,6β-diol (XXIII)

A solution of 4α acylamino cholestane 5β,6β-acetate (XXIII, 100 mg) in methanol (10 ml) was hydrolysed with methanolic sodium hydroxide (2%, 5 ml) by keeping the solution at room temperature for about 12 hours. The product obtained was crystallised from methanol. Yield 50 mg, m.p. 126-128°; 
\[
[\alpha]_D +37.3°
\]

IR: 3450 (OH/NH), 1720 (acyl carbonyl).

NMR: δ .83 (singlet, 3H, C₁₈-H), 0.94 (singlet, 3H, C₁⁹-H), 1.99 (singlet, 3H, -COCH₃), 4.2 (1H, multiplet, C₄-β H),
5.01 (narrow triplet, 1H, C₆₋H), 6.1 (doublet, J = 8 Hz, 1H, exchangeable with D₂O -NH).

4β-hydroxy, 5α-cholestan-6-one (XXX) Fig. 2.5

4β,5β-epoxy cholestane-6β-ol (250 mg) was dissolved in methylene chloride (5 ml). The solution was treated with acetonitrile (4 ml) and perchloric acid (72% 1 to 2 drops). The reaction mixture was kept at room temperature for about 15 hours. On usual work up, the product obtained was crystallised from methanol. Yield: 150 mg.

m.p. 124-26°C      Lit.31 m.p. 124-26°C
[α]D +44.0           Lit.31 +44°C

IR: 3400 (-OH), 1725 (-CO)
NMR: δ 0.80 (singlet, 3H, C₁₈-H), 0.93 (singlet, 3H, C₁₉-H)
3.85 (narrow triplet, 1H, 4α-H).

4β,5α,6β-cholestanetriol-6β-acetate (XXXIV) Fig. 2.6

A solution of 4α,5α-epoxy cholestane-6β-acetate (500 mg) in methylene chloride (10 ml) was treated with acetonitrile (8 ml) and perchloric acid (0.1 ml, 72%). The reaction mixture was kept at room temperature for about 16 hours. After usual work up a residue was obtained which was crystallised from ether-methanol, yield: 150 mg.

m.p. 198-200°C

IR: 3340 (–OH), 1712, 1260 (acetate).

NMR: δ 0.81 (C₁₈-H), 0.92 (C₁₉-H), 2.08 (singlet, 3H, –O-CO-CH₃), 3.2 (exchangeable with D₂O -OH), 3.87
Since there was no introduction of amide function, the above reaction was repeated with acetonitrile and only a drop of perchloric acid. However, the product obtained was found to be 5β-cholestan-4 one-6β-acetate (XXXV) crystallised from ether, yield 115 mg. m.p. 124-26°. IR: 1725, 1250 (-OAc), 1695 (ketone carbonyl).

NMR: 0.79 (3H, 18-H), 0.96 (19-H), 1.97 (-O-CC-CH₃), 5.3 (narrow triplet, 6β-H).

**Cholest-4ene-3β,6β-diol diacetate**

3β,5α,6β-cholestan triol 3β,6β-diacetate (10 g)

(This compound was prepared by known method given in the literature Chart 1) was dissolved in pyridine (150 ml) and cooled to 0°C. Thionyl chloride (20 ml) was added dropwise with occasional shaking maintaining the temperature at 0°C. The reaction mixture was kept for half an hour, poured into ice-cold water, and worked up in the usual manner. Crude product was crystallised from methanol. Yield 7.8 g.

m.p. 135-136°  
Lit.₄¹ m.p. 136°

[α]D -10°  
Lit.₄¹ [α]D -13°

IR: 1740, 1240 (acetate carbonyl).

NMR: δ 0.81 (C₁₈-H), 0.91 (C₁₉-H), 1.97, 2.0 (3H each for -OCO CH₃), 5.18 (broad multiplet, 2H, C₃ & C₆-H), 5.5 (1H, C₄).
42,5α-epoxy cholestane 3β,6β-diol diacetate (XXXVI)

Cholest-4 en-3β-6β-diol-diacetate (5 g) was dissolved in an ether solution of petbenzoic acid (20%, 50 ml). The reaction mixture was kept at room temperature for five days. After usual work up crude product was chromatographed over silica gel. Pure 4-epoxide was obtained. It was crystallized from ether-methanol, yield: 1.0 g.

m.p. 155-157°

Lit.42 m.p. 158-160°

$[\alpha]_D +15°$

Lit.42 $+17°$

IR: 1745 cm$^{-1}$, 1235 (acetate carbonyl)

NMR: 0.93, (C$_{17}$-H), 2.06 (6H, -O-CO-CH$_3$), 3.08 (C$_4$-H), 4.2 (multiplet C$_6$α-H), 4.83 (multiplet, 1H, C$_3$α-H).

Ritter Reaction on 42,5α-epoxy cholestane 3β,6β-diol diacetate (XXXVI)

To a solution of above epoxide compound (200 mg) in methylene chloride (4 ml), acetonitrile (3 ml) and perchloric acid (.05 ml, 72%) were added. The reaction mixture was kept at room temperature for about 16 hours and worked up in the usual manner. Crude product was obtained. The compounds were separated on preparative layer chromatography by using benzene-ethyl acetate (9:1) solvent system. Two pure compounds were obtained.

Fraction F$_3$ - Yield 8 mg, m.p. 150-51°.
IR: 3240 (–OH), 1700, 1265 (Acetate).

NMR: 0.78, 0.90 (C_{19}H), 2.06 and 2.11 (2-0.00CH_{3}), 3.73 (broad doublet, J = 5 Hz, 1H, C_{3}H), 4.52 (1H, C_{3}L-H)

The compound was found to be a mixture of 3β,4β, 5β, 6β-tetrol 3,6-diacetate and 3β,4β,5β,6β-tetrol 3,6-diacetate (XXXVIII). Fig 3.


IR: 3240 (–OH), 1700 and 1262 (acetate carbonyl).

NMR: 0.72 (18-H), 0.93 (19-H), 2.07 and 2.18 (two OCOCH_{3}), 3.39 (doublet J = 3 Hz, 1H, 4L-H), 4.93 (singlet, 1H, 6L-H), 5.11 (broad multiplet, 1H, 3L-H).

The compound was characterised as 3β,4β,5α,6β-cholestane tetrol 3,6 diacetate (XXXVII). Fig 3.

**Cholesteryl chloride**

Thionyl chloride (8 ml) was added to cholesterol (10 g azeotropically dried) shaked well the reaction mixture and kept at room temperature for about 12 hours. Excess of thionyl chloride was removed under reduced pressure. Residual product crystallised from acetone, yield 9.2 g. m.p. 95-96° Lit. 43 m.p. 95-96°

**Cholestan 3β,5α,6β-diol**

Cholesteryl chloride (8 g) was dissolved in formic acid (90%, 80 ml) and a little chloroform (10-15 ml). To this solution hydrogen peroxide (30%, 10 ml) was added.
The reaction mixture was kept at room temperature for 16 hours, poured in water, extracted with ether. After usual work up, the residue obtained, was refluxed in methanolic potassium hydroxide (2%, 150 ml) for half an hour, cooled and acidified with hydrochloric acid (2N). The reaction mixture was poured in cold water, filtered and washed with water, crystallised from ether-pet. ether. Yield: 5.2 g.

[α]D -5°

IR: 3400 (-OH), 770 (-C-Cl).

Cholestane 3β-chloro 5β,6β-dihydroxy 6β-yl acetate

The above diol (5 g) was acetylated with acetic anhydride-pyridine in the usual way. The product obtained was crystallised from methanol to furnish the required compound. Yield 4.3 g.

Cholestane 3β-chloro 4ene 6β-acetate

Cholestane 3β-chloro 5β,6β-diol 6-acetate (4 g) was dissolved in pyridine (60 ml) and cooled to 0°C. Thionyl chloride (6 ml) was added dropwise into the solution.
The reaction mixture was allowed to stand for 15 minutes at 0°C, poured into ice cold water and extracted with ether. Ether layer was washed with 2N hydrochloric acid, sodium bicarbonate and water. After drying, ether was distilled off. Crude product crystallised from acetone. Yield 3.1 g.

\[ [\mathcal{L}]_D^{+5.7} \quad \text{Lit.}^{44} [\mathcal{L}]_D^{+3.4} \]

IR: 1740, 1240 (acetate carbonyl), 1650 (olefinic double bond), 745 (C-Cl).

NMR: 0.75 (C\(_{13}\)-H), 0.93 (C\(_{17}\)-H), 1.38 (-O-CO-CH\(_3\)), 4.35 (broad multiplet C\(_3\)-H), 5.2 (1H C\(_6\)-H), 5.7 (C\(_4\)-H).

**Cholestane 3\(\beta\)-chloro 4\(\alpha\),5\(\alpha\), epoxy 6\(\beta\)-acetate (XXXIX)** Fig. 31

The above compound (2 g) was treated with perbenzoic acid (15%, 50 ml). The solution was kept at room temperature for about 24 hours. After usual work up required epoxide was obtained. It was crystallised from ether-methanol. Yield: 1.5 g. m.p. 130-320; [\mathcal{L}]_D^{+23.5}.

IR: 1730 (-OAc), 782 (-C-Cl)

NMR: 0.71 (C\(_{13}\)-H), 0.99 (C\(_{17}\)-H), 2.95 (3\(\alpha\), -O-CO-CH\(_3\)), 3.35 (1H C\(_4\)\(\beta\)-H ), 3.90 (1H b, 3\(\alpha\) -H ), 4.2 (1H C\(_6\)\(\alpha\)-H ).

**Ritter reaction on 3\(\beta\)-chloro, 4\(\alpha\),5\(\alpha\), epoxy cholestane 6\(\beta\)-acetate (XXXIX)**

To a solution of the above epoxide (400 mg) in
methylene chloride (8 ml), acetonitrile (6.4 ml) and perchloric acid (72%, 0.1 ml) were added. The reaction mixture was kept at room temperature for 16 hours and worked up in the usual manner. The crude product did not crystallize. It was chromatographed over silica gel, eluted with (i) pet. ether (100 ml) (ii) benzene-pet.ether (1:3) 100 ml (iii) benzene-pet. ether (1:1) (100 ml) (iv) benzene (100 ml), (v) benzene-ethyl acetate (9:1), 100 ml, (vi) benzene-ethyl acetate (3:1) (100 ml), (vii) benzene-ethyl acetate (1:1), (viii) Ethyl acetate (100 ml). A product obtained from pet.ether-benzene (1:1) eluent showed single spot on TLC. Yield 55 mg. m.p. 134-36°, $[\alpha]_D^{25} -66$.

IR: 1740, 1220 (-OAc), 1710 (\(\_\_C=O\)) and 777 cm\(^{-1}\) (C-Cl).

NMR: 1.02 (19-H), 2.07 (-OAc), 3.34 (1H, C\(_3\)-H), 4.93 (1H, narrow triplet, 6\(_2\)-H).

The product characterised as 3\(\beta\)-chloro-5 -\(\beta\) cholestane-4-one-6\(\beta\)-acetate (XL). Fig.32 & 33.
**Chart V**

1. $(\text{CH}_3\text{CO})_2\text{O}$, Pyridine → $\text{SOCl}_2$, Pyridine → Perbenzoic acid

2. $(\text{CH}_3\text{C}==\text{N}/H^+)$, $\text{CH}_2\text{Cl}_2$ → $(\text{XXXIV}) + (\text{XXXVII})$

**Chart IV**

1. $\text{SOCl}_2$ → $\text{HCOOH} / \text{H}_2\text{O}_2$, NaOH

2. Perbenzoic acid → $\text{SOCl}_2$, Pyridine

3. $(\text{CH}_3\text{CCl}_3)$, Pyridine
CHAPTER IV

THE ELECTRON IMPACT INDUCED FRAGMENTATION OF
STEROIDAL N-ACYL AND N-BENZOYL AMINES
INTRODUCTION

The mass spectra of various kinds of aliphatic amides have been reported by Gilpin. These results are consistent with the operation of processes which are characteristic of aliphatic carbonyl compounds and amines. Ionisation of primary amides can be visualized as going through the removal of one of the lone pair electrons of either oxygen or nitrogen and then an $\alpha$-fission to give a resonance stabilised ion (m/e 44).

$$R-\text{c}^{-}\text{O}^{+} \rightarrow R-\text{c}^{+}$$

Simple $\beta$-fission does not occur, but evidence for $\gamma$-cleavage is found in many amide spectra. When a $\gamma$-hydrogen atom is available, $\beta$-cleavage with transfer of this $\gamma$-hydrogen predominates in primary amides resulting in the ion m/e 59. This is the well known McLafferty rearrangement of carbonyl compounds. This fragmentation is shown by secondary and tertiary amides also. The other mass spectral features
characterising these amides are the double $d$ and C-N cleavage with hydrogen rearrangement analogues to secondary and tertiary amines$^{47}$.

**Cycloalkyl amides** - The mass spectra of cycloalkyl amides generally appear more complex than those of cycloalkyl amines or aliphatic amides. The mechanism involves a double hydrogen rearrangement. The origin of which has been established by deuterium labelling$^{47}$. In saturated polycyclic monosubstituted rings abundant ions correspond to cleavage at ring substitution$^{48}$.

The fission of the carbon-carbon bond next to nitrogen is one of the key operations. In amides, if the acyl group could be lost by keten elimination, this process occurs giving rise to the more stable amine ion. It is obvious that after acylation of the amine, the nitrogen atom cannot stabilize the positive charge so readily. Therefore, non-specific skeletal fragmentation also takes place.
J. Richter et al.\textsuperscript{59} have given the mass spectrometric fragmentation of acylated cyclic amines, resulting in ring contraction upon electron impact. The mechanism was confirmed by deuterolabelling.
Steroidal amides

Steroidal amides possess a stable polycyclic nucleus and give predominantly ions produced by fission of the bond between the ring and the nitrogen atom rather than fission of the ring. The spectra of these compounds are comparable to those of the cyclic amides. Djerassi et al. have studied the mass spectra of numerous deuterated analogs of simple aliphatic cycloalkyl and steroidal N-acyl amines and compared them with those of the non-labelled parent substances. Fragmentation pattern of 3α and 3β-N-acetyl aminoandrostanes are very similar giving rise to the (M-59)^+ peak involving the familiar six membered ring transition state. Extensive labelling of ring A hydrogen atoms show that in over 80% of all cases this peak involves a hydrogen transfer from C-2, C-4 or C-5. Transfer of C-5 hydrogen could be rationalized as illustrated in Chart I. The ion may rearrange to a less strained structure with a double bond. But it is more difficult to visualize this transfer when the amide group is β. The peak at m/e 204 by a Retro Diel's Alder rearrangement, loses methyl group from C18, since C19 is retained. The other intense peak observed in steroidal amides is at m/e 60. It shifts completely to m/e 63 in the spectrum of the trideuterio acetyl analog and so must be the protonated acetamido ion (CH3CO^+NH3). It is
Types of fragmentation of N-acetyl amino asteranes

CH₂CH₂CONH₂

CH₃

M/e 258

OR

Retro Diels–Alder rearrangement

M/e 204

M/e 139
Double Hydrogen Transfer

M/e 60

M/e 56
produced directly from the molecular ion as demonstrated by appropriate metastable ions. The peak at m/e 56 is produced by the elimination of ketene group forming more stable amine type ion, which is further cleaved as a typical amine fragmentation process.

P. Longevialle et al.\textsuperscript{49} have shown that the presence of a hydroxyl group in steroidal amines, amides and imines may influence their fragmentation pattern. This fragmentation is initiated by the rearrangement of the hydroxylic hydrogen on the nitrogen containing groups and form ions characteristic of the hydroxyl site in the molecule. Sometimes this may occur even when the two groups are situated at remote positions. The mechanism is given as below (Chart 2).

C. Marazano and Pierre Longevialle\textsuperscript{51,52} have studied mass spectrometric fragmentation of 2 and 4 amino steroids, and the principal fragment ions have been interpreted. A comparative study with the mass spectra of 3\textsubscript{2} -hydroxy-4\beta amine allows the determination of the initial C-C bond rupture in the mechanism of the formation of the principal fragment ions. The fragmentation pattern of 2 & 3 amino steroids were compared (Chart 3).

Z. Pelah et al.\textsuperscript{53} have given the mass spectral fragmentations of dimethyl amines substituted at C\textsubscript{1}, C\textsubscript{2}, C\textsubscript{3}, C\textsubscript{6}, C\textsubscript{7}, C\textsubscript{11}, C\textsubscript{16} and C\textsubscript{17} in androstane and cholestane skeletons.
(CHART 3)

\[
\begin{align*}
\text{N}^+ & \rightarrow \text{N}^+ \\
\text{M/e 84} & \rightarrow (M - 71)^+ \\
& + \text{M/e 84} \\
\end{align*}
\]

\[
\begin{align*}
\text{N}^+ & \rightarrow \text{N}^+ \\
\text{M/e 110} & \rightarrow \text{M/e 84} \\
\end{align*}
\]
R. Taubianu and co-workers\textsuperscript{54} reported cleavage of dimethylamine in the cholestane skeleton having hydroxyl function at a neighbouring carbon atom as given below (Chart 4). Cleavage of C\textsubscript{5}-C\textsubscript{6} bond with hydrogen rearrangement appears to be a characteristic mode of fragmentation in case of functional group at the C\textsubscript{6} position insteroids.

C.R. Narayanan and Lala\textsuperscript{55} have also observed similar type of fragmentation in cholestan 6\textbeta-yl methyl ethers.

![Diagram of compound]

**PRESENT WORK**

As depicted in the earlier chapter several steroidal amides have been synthesised by Ritter reaction, by incorporating an amide moiety at C\textsubscript{4}, C\textsubscript{5} or C\textsubscript{6} carbon atom in the steroid skeleton with hydroxy functions at vicinal positions. There is not much information in the literature on electron impact mass spectral fragmentations of such types of amides having other functional groups at the neighbouring carbon atoms. Therefore to study the
CHART - 4

M/e 276

M/e 260
influence of hydroxyl and acetoxy functions at \( \beta \) or \( \gamma \) carbon atoms from the hetero atom mass spectral fragmentation study of these compounds was undertaken.

The mass spectra of all the compounds were recorded at 200-250°C by using a direct inlet system in order to prevent thermal decomposition.

The compound 3 \( \beta \)-acyl amino 5\( \alpha \)-cholestan (A) showed prominent molecular ion peak at m/e 429 (14%) followed by usual six membered rearranged (M-59)\(^+\) cleavage of amide at m/e 370 (12%) as a second significant peak. However, the base peak was observed at m/e 60, clearly indicating a loss of protonated amide moiety -NH\(_2\)-C-CH\(_3\) by double hydrogen transfer similar to the pattern reported by C. Djerassi et al.\(^{47}\).

3\( \alpha \), Acylamino, 5\( \alpha \)-cholestan (B) also exhibits the same mode of fragmentation on electron impact giving the same molecular ion peak at m/e 429, m/e 370 (10%) and a base peak at m/e 60. But the intensities of the peaks at both high and low mass region are much higher in the 3 \( \beta \)-acylamino compound than in the 3\( \alpha \) one, thus making it easy to distinguish in two if both the spectra are given. In both the above compounds a loss of amide moiety -NH\(_2\)-C-CH\(_3\) is supported by the presence of the corresponding metastable ions.
In the case of 6\(\beta\) acyl amino 5\(\alpha\) -cholestane (C) also a strong molecular ion peak at m/e 429 (33%) was observed along with the other usual peaks at m/e 370 (30%) and m/e 60 as a base peak. It appears that in the absence of any other functional group this type of cleavage with double hydrogen transfer is the important mode of fragmentation. However, in this compound a peak at m/e 247 was observed which could be ascribed to the cleavage as shown in Scheme (I).

This kind of hydrogen transfer cleavage is analogous to the fragmentation described by R. Taubianu\textsuperscript{54} et al. for dimethoxy amines and Narayanan et al.\textsuperscript{35} for 6\(\beta\)-yl methyl ether at C\(\alpha\) carbon atom.

\[
\begin{align*}
\text{II} \quad & R = H \\
\text{II' } & R = \text{Ac}
\end{align*}
\]

5\(\alpha\) acyl amino 6\(\beta\) hydroxy cholestane (IIb) showed the usual (M-57)\(^+\) peak quite prominent at m/e 386 (31%). The base peak was observed at m/e 371 which could be easily assigned to loss of \(-\text{CH}_2\text{COCH}_3\) followed by a loss of angular methyl radical \(\text{C}_1\) as shown below (Scheme II).

* I am thankful to Dr. B.M. Sawant for the compounds.
The loss of C19 methyl is likely as it gives a more stable allylic hydroxyl radical ion system as a base peak. This mechanism is further supported by the presence of the corresponding metastable ions.

The above compound (IIb) was acylated and the resulting 5α-acyl amino 6β-acetoxy cholestane (IIb') gave on fragmentation (M-59)$^+$ at m/e 428 at relatively reduced intensity (2.4%), whereas (M-60)$^+$ at m/e 427 was less than 0.3% but (M-59-42)$^+$ at m/e 386 was 0.6%. The base peak was at m/e 368, which could be assigned as depicted below (Scheme III).

This implies that there is influence of the adjacent functional groups on the fragmentation pattern. But for the apparent difference, energy of activation was also to play some part. A loss of hydroxyl group in the form of H$_2$O requires more energy of activation and hence it is rather difficult, whereas loss of -OAc as acetic acid is a process of low energy of activation, and hence easier. In both the compounds (IIb & IIb') the obvious tendency is to form the most stable conjugated radical ion as a base peak.

The compound 3β, 6β-dihydroxy 5α-acyl amino cholestane (IIa) showed the familiar (M-59)$^+$ peak as one of the prominent peaks at m/e 402 (54,5%).
The base peak observed was at m/e 43. It should be due to the loss of -CH$_3$C=O$^+$ from the amide, supplemented by the loss of side chain isopropyl group. In cholestane this peak due to the loss of the side chain isopropyl is of low intensity (3-10%). The other prominent peaks observed were at m/e 387 and m/e 369. The mechanism of the cleavage for these peaks is shown in Scheme IV. This mechanism is supported by the presence of appropriate metastable peaks.

However, in the case of $3\beta,5\beta$-diacetoxy 5$\Delta$-acylamino cholestane (IIa') the most intense peak was at m/e 384 (M-59-68-42)$^+$. The other significant peaks were at m/e 486 (M-59)$^+$ (59%), m/e 471 (M-59-15)$^+$ (31%), m/e 426 (M-59-68)$^+$ (45%). The origin of these peaks are depicted in Scheme (V). As would be observed fragmentation of this compound follows that of $6\beta$-acetoxy 5$\Delta$-acyl amino cholestane.

$6\beta$-Acyl amino $3\beta,5\Delta$-dihydroxy cholestane (IVa) did not show a prominent molecular ion as compared to $6\beta$-acyl amino-$5\Delta$-cholestane. It exhibits the usual (M-59)$^+$ peak at m/e 402(14%).
and further dehydration peak at m/e 384 (12%) thus giving probably the same stable diene ion as in Scheme V though the substitution pattern at 3,5 and 6 are different in this compound.

The most prominent peak, however, was observed at m/e 56 which should be assigned to the cleavage of the C₅-C₆ bond. The peaks at m/e 443 (3%) and m/e 425 (3%) indicate elimination of one and two molecules of H₂O respectively. These are supported by the corresponding metastable ion peaks. It may be mentioned here that elimination of water by a hydroxyl group is considered by electron impact when it is 1,3 or 1,4 or more remote whereas when it is 1,2 it is considered as thermal. The peak at m/e 330 (4%) is due to the elimination of ketene (⁻CH₂⁻C=O) from the acyl amino group giving finally the frequently observed m/e 56 ion. All these important fragmentation patterns are summarised in Scheme VI.
SCHEME - IV

SCHEME - V
SCHEME VI
3β-Acetoxy-5α-hydroxy 6β-acyl amino cholestane (IVA') showed the most prominent peak at m/e 43, which could be -CH$_3$C=O$^+$ from acetoxy or amide function. Since this compound also exhibited a prominent peak at m/e 247 (+71%), the possibility of m/e 43 from -NH$_2$-CH=CH$_2$ can also be considered. The other prominent peak at m/e 384 showed elimination of amide and acetic acid (M-59-50)$^+$ giving the 3β-hydroxy 4,6-diene as in the previous cases.

6β-Benzoylamino 3β,5α-dihydroxy cholestane showed the most intense peak at m/e 105 which is obviously a very well known C$_6$H$_5$-C≡O$^+$ moiety similar to that of -CH$_3$C=O$^+$. The peak at m/e 247 (11%) also quite significant was obtained by rupture of the C$_5$-C$_6$-bond as depicted in the earlier schemes. The usual McLafferty rearranged benzamido cleavage at (M-121)$^+$ at m/e 402 was also one of the prominent peaks. In addition to all these peaks a prominent peak at m/e 330 (183) was observed by rupture of ring A as depicted below in
the Scheme VII. A strong peak at m/e 334 (8.2%) probably due to the 3β-hydroxy 4,5-diene ion was observed in this case also as in all other 3β,5α,6β substituted steroids.

Acetylation of the 3β hydroxyl gave the 3β-acetoxy 6β-benzoyl amino, 5α-hydroxy cholestane (IV'). This showed the most prominent peak at m/e 356 which is obviously the more stable conjugated radical ion system. The mode of cleavage leading to this ion is illustrated in Scheme VIII. The 6β-benzamide group probably assists the removal of 2β-H to give the triene m/e 366. However, in this case also, the 3β-hydroxy 4,6 diene ion was 70.4% of the base peak.

4α-Acyl amino 5β,6β-dihydroxy cholestane (XXIII') showed the expected (M-59)+ peak at m/e 402 (8%) with the most prominent peak at m/e 43, which could be mostly the CH3C=O+ peak supplimented by the isopropyl group from the side chain. Other prominent peaks observed were at m/e 384 (38%) due to diene ol ion and m/e 331 (71%). The mode of cleavage is summarised in Scheme (IX).
are cis fused, rupture of ring A is very significant. Hence, fragmentation leading to the ion at m/e 331 appears to be an important mode of cleavage. An analogous fragmentations has been given by S.G. Wyllie\textsuperscript{56} in the case of steroidal diols.

4\(\alpha\)-Acyl amino 5\(\beta\),6\(\beta\) dihydroxy cholestane 6\(\beta\)-acetate (XXIII) also exhibited remarkably similar mode of fragmentation with the most intense peak at m/e 43. The other prominent peaks were at m/e 384 and m/e 331.

CONCLUSION

From the compounds studied it appears that in the absence of any other functional group steroid amides generally give molecular ion peak as one of the prominent peaks. The cleavage with double hydrogen transfer seems to be an important mode of fragmentation. Steroid amides having hydroxy or acetoxy groups at the \(\beta\) or \(\gamma\) carbon atoms from the hetero atom did not show significant molecular ion peak. In a C\(_5\)-hydroxy steroid, whenever the other hydroxyl group was converted to an acetoxy group, the process of elimination of acetic acid and amide first takes place leaving the tertiary hydroxy group at C\(_5\). In all 3,5,6 hydroxy, acetoxy and amide substituted steroids fragmentation leading to a stable conjugated dienol was always found to be a prominent peak, irrespective of the position of the acetoxy or amide group. Subsequent
dehydration and loss of a molecule of water give another stable conjugated triene. The amides at tertiary carbon atoms usually do not give cleavage of C-C bond of a steroidal ring due to their rigid nature. Elimination of $-\text{NH}_2\text{COCH}_3$ by MacLafferty rearrangement is very common. A rupture of $\text{C}_5-\text{C}_6$ bond with hydrogen rearrangement appears to be a general phenomena in the case of $\text{C}_6$-amides, with or without substituent at $\text{C}_3$ or $\text{C}_5$. This mode of cleavage was observed before in the case of $\text{C}_6$-amines and $\text{C}_6$ ethers. It can now be extended to $\text{C}_6$-amides also. In many cases ring A gets cleaved off when a tertiary hydroxyl group is present at $\text{C}_5$. This mode of fragmentation is very prominent if the A/B rings are cis fused. Another mode of fragmentation of secondary amides is the rupture of the $\alpha,\beta$ carbon-carbon-bonds of the steroid ring. In the case of tertiary amides cleavage between the nitrogen and the ring carbon giving rise to the more stable conjugated polycyclic steroid skeleton appears to be preferred.
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Metastable peaks m/e</th>
<th>Calculated for $M^+ = (M)\frac{2}{m}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 β-acylamino 5α-cholestanone (A)</td>
<td>319</td>
<td>319.1</td>
</tr>
<tr>
<td>2</td>
<td>3α-Acylamino 5β-cholestanone (B)</td>
<td>319</td>
<td>319.1</td>
</tr>
<tr>
<td>3</td>
<td>6β-Acylamino 5α-cholestanone (C)</td>
<td>319</td>
<td>319.1</td>
</tr>
<tr>
<td>4</td>
<td>5α-Acylamino 6β-hydroxy-cholestanone (IIb)</td>
<td>335, 357</td>
<td>334.8, 356.7</td>
</tr>
<tr>
<td>5</td>
<td>5α-Acylamino-cholestan-3β,6β-diol (IIa)</td>
<td>352, 372.5</td>
<td>350.5, 372.6</td>
</tr>
<tr>
<td>6</td>
<td>5α-Acylamino-cholestan-3β,6β-diol-diacetate (IIa')</td>
<td>457</td>
<td>456.4</td>
</tr>
<tr>
<td>7</td>
<td>6β-Acylamino-cholestan-3β,5α-diol (IVA)</td>
<td>408, 426</td>
<td>407.9, 425.7</td>
</tr>
<tr>
<td>8</td>
<td>4α-Acylamino-cholestan-5β,6β-diol (XXIII')</td>
<td>367</td>
<td>366.7</td>
</tr>
</tbody>
</table>
FIG. 7. MASS SPECTRUM OF 3\(\beta\)-ACYLAMINO-5\(\beta\)-CHOLESTONE (A)
Fig 6. (B) Mass Spectrum of 3α-Acylamino-5α-Cholesterol
FIG. 10. MASS SPECTRUM OF 5-ACETYLMINO-CHELSTAN 3\%, 6\% -DIOL (II a)
FIG. 12. MASS SPECTRUM OF 3-ACYLAMINO-5-OXYHYDRAZINE
FIG. 16 MASS SPECTRUM OF 3α-HYDROXYCHOLESTAN-3β-5α-D OL (IV1)
FIG 20 B. MASS SPECTRUM OF 4α-ACYLAMINO 5α-6β-DIHYDROXY CHOLESTANE (XXIII)
XXX IR SPECTRUM OF 5β-HYDROXY 4β,5β-EPOXY CHOLESTANE.

XXX NMR SPECTRUM OF 5β-HYDROXY 4β,5β-EPOXY CHOLESTANE.
XXL IR SPECTRUM OF 3,6-ACETOXY 4β,5,6-EPOXY CHOLESTANE.
XXIII IR SPECTRUM OF 4α, ACYLAMINO-5β-HYDROXY-CHOLESTANE-6β-ACETATE.

XXIV NMR SPECTRUM OF 4α, ACYLAMINO-5β-HYDROXY-CHOLESTANE-6β-ACETATE.

(FIG. 20)
XXIV IR SPECTRUM OF 4α, 5β-DIHYDROXY CHOLESTANE - 6β-ACETATE.

XXIV NMR SPECTRUM OF 4α, 5β-DIHYDROXY CHOLESTANE - 6β-ACETATE. (FIG 21)
XXIV. IR SPECTRUM OF CHOLESTANE $4\alpha,5\beta,6\beta$-TRIOL $4\alpha,6\beta$-DIACETATE.

XXIV. NMR SPECTRUM OF CHOLESTANE $4\alpha,5\beta,6\beta$-TRIOL $4\alpha,6\beta$-DIACETATE. (FIG. 22)
XXXIII IR SPECTRUM OF 6β-ACETOXY 4α,5α-EPoxy Cholesterol.

XXXIII NMR SPECTRUM OF 6β-ACETOXY 4α,5α-EPoxy Cholesterol. (FIG 23)
XXX IR SPECTRUM OF 4ß-HYDROXY 5α-CHOLESTANE-6 ONE.

XXX NMR SPECTRUM OF 4ß-HYDROXY 5α-CHOLESTANE-6 ONE. (FIG. 24)
XXXIV IR SPECTRUM OF CHOLESTANE 4β - 3α - 5β TRIOL - 6 - ACETATE.
XXXV IR SPECTRUM OF 5β-CHOLESTANE 4-ONE 6β-ACETATE.

XXXV NMR SPECTRUM OF 5β-CHOLESTANE 4-ONE 6β-ACETATE IN CCl₄. (FIG. 26)
XXXVI IR SPECTRUM OF 4α,5α-EPOXY CHOLESTANE 3β-6β-DIACETATE.

XXXVI NMR SPECTRUM OF 4α,5α-EPOXY CHOLESTANE 3β-6β-DIACETATE. (FIG. 28)
XXXVII IR SPECTRUM OF CHOLESTANE 3β, 4β, 5α, 6β TETROL 3, 6 DIACETATE.

XXXVII NMR SPECTRUM OF CHOLESTANE 3β, 4β, 5α, 6β TETROL 3, 6 DIACETATE.

(FIG. 29)
XXXVIII

IR SPECTRUM OF A MIXTURE OF CHOLESTANE 3β,4α,5β,6β AND 3β,4β,5α,6β TETROL 3,6 DIACETATE.

XXXVIII

NMR SPECTRUM OF A MIXTURE OF CHOLESTANE 3β,4α,5β AND 3β,4β,5α,6β TETROL 3,6 DIACETATE. (FIG. 30)
IR SPECTRUM OF 6β-ACETOXY 3β-CHLORO 46,56-EPoxy CHOLESTANE.
XL. IR SPECTRUM OF 6β-ACETOXY 3β-CHLORO 5β-CHOLESTANE 4 ONE.

XXI. NMR SPECTRUM OF 6β-ACETOXY 3β-CHLORO 5β-CHOLESTANE 4 ONE IN CDCl₃
(FIG. 32)
AXI NMR SPECTRUM OF 6ß-ACETOXY 3ß-CH ORO 5ß-CHOLESTANE IN D6-DZENE.

(FIG. 33)
REFERENCES

1 M. Igarashi

2 W.L.G. Gent

3 S. Searles and M. Tamres

4 S. Searles, M. Tamres and E.R. Lippincott

5 H.S. Gutowsky, R.L. Rutledge, M. Tamres and S. Searles

6 R.A. Nelson and R.S. Jessup

7 N.H. Cromwell and G.V. Hudson

8 C.A. Coulson and W.E. Moffitt

9 B. Ottar

10 R.E. Parker and N.S. Isaacs

11 A. Furst and P.J.A. Plattner

12 D.H.R. Barton, D.A. Lewis and J.F. McGhie

13 H.B. Henbest and R.A.L. Wilson

14 C.W. Shoppe, M.E.H. Howden, R.W. Killick and G.H.R. Summers

15 H.B. Henbest and J. McEntee
16 W.G. Young, R.E. Ireland, T.I. Wrigley, C.W. Shoppee, B.D. Agashe and G.H.R. Summers

17 D.J. Collins

18 S. Greenfield, E. Glotter, D. Lavie and (in part) Y. Kashman

19 A.R. Davies and G.H.R. Summers

20 R. Dehghenghi, A. Philipp and R. Gaudry

21 D. Lavie, Y. Kashman and E. Glotter

22 K. Ponsold

23 H.B. Henbest and T.I. Wrigley

24 H.B. Henbest and T.I. Wrigley

25 J.M. Coxan, M.P. Hartshorn and D.N. Kirk


27 C.R. Narayanan, A.K. Kulkarni, A.B. Landge and M.S. Vadia

28 R.J. Ryan and S. Julia

29 C.R. Narayanan and B.M. Sawant

30 C.R. Narayanan, A.K. Kulkarni, A.B. Landge and M.S. Vadia

31 J.P. Pete and M.L. Viriot-Villaume
32 J.D. Conolly and R. McCrindle

33 D.H. Williams and N.S. Bhacca

34 S.M. Kupchan, D. Lavie, C.V. Deliwala and B.Y.A. Andoh

35 A.T. Rowland and H.R. Nace

36 G. Shutzke and A. Velthen
Liebigs Ann. 703, 159 (1967).

37 D.N. Jones, J.R. Lewis, C.W. Shoppee and G.H.R. Summers

38 L. Ruzicka, M. Furter and G. Thomann

39 P.M. Chakravorty and E.H. Levin

40 G. Drefahl and K. Ponsold

41 V.A. Petrow, C. Rosenheim and W.W. Starling

42 E. Glotter, S. Greenfield and D. Lavie

43 P.J. Daughenbaugh and J.B. Allison

44 C.W. Shoppee, R.J. Bridgwater, D.N. Jones and G.H.R. Summers

45 J.M. Heilbron, V. Shaw and F.S. Spring

46 J.A. Gilpin

47 Z.Pelah, M.A. Kiekzewski, J.M. Wilson, M. Ohashi,
H. Budzikiewicz and Carl Djerassi
48  J.H. Beynon  

49  P. Longevialle, J. Einhorn, J.P. Alazord,  
    X. Lurinchi  

50  W.J. Richter, J.M. Bursey and A.L. Burlingame  
    O.M.S., 5, 1275 (1971).

51  C. Morazano and P. Longevialle  
    O.M.S., 10, 435 (1975).

52  C. Morazano and P. Longevialle  
    O.M.S., 10, 442 (1975).

53  Z. Pelah, D.H. Williams, H. Budzikiewicz and  
    C. Djerassi  

54  H. Audier, Sonia Bory, Marcel Fetizon, P. Longevialle  
    and Raul Taubiana  

55  C.R. Narayanan and A.K. Lala  
    O.M.S., 6, 119 (1972).

56  S.G. Wallie  
    O.M.S., 6, 559 (1972).