DISCUSSION
A. Enol Lactones

During the period 1947-48, Sorm and coworkers\textsuperscript{44,45} reported an interesting sequence of reactions for the preparation of B-norcholesteryl acetate (C). This involved the reaction of the seco-acid, 3\(\beta\)-acetoxo-5-keto-5,6-secocholestan-6-oic acid (XCVIII) with benzoyl chloride in pyridine. The neutral product of the reaction of (XCVIII) and benzoyl chloride-pyridine was thought to be an enol lactone, 3\(\beta\)-acetoxo-6-oxa-B-homocholest-4-en-7-one (XCIX), which on heating beyond its melting point suffered decarboxylation to give (C) in almost quantitative yield.

\[
\begin{align*}
\text{AcO} & \quad \text{COOH} \\
\longrightarrow & \quad \text{PhCOCl/Py} \\
\text{AcO} & \quad \text{COOH} \\
\longrightarrow & \quad \Delta \\
\text{AcO} & \quad \text{AcO}
\end{align*}
\]
Dauben and Fonken\textsuperscript{46} reinvestigated the above reaction and supported the enol lactone structure (XCIX). Subsequently, based upon the n.m.r. spectrum of the product, Dauben et al.\textsuperscript{47} revised the structure and proposed the isomeric $\beta$-$\lambda$actone structure (CIII) for the neutral product.

\begin{center}
\includegraphics[scale=0.5]{diagram.png}
\end{center}

(CIII)

With the realization of this correction, there appeared a number of papers on the chemistry of the $\beta$-$\lambda$actone, 3$\beta$-acetoxy-5$\beta$-hydroxy-B-norcholestan-6-oic acid 5,6-lactone (CIII)\textsuperscript{54,170-173}, and the "enol lactone" (XCIX) remained completely ignored.

It was further shown that the seco-acid, 5-keto-5,6-secocholestan-6-oic acid (CVII) with benzoyl chloride and pyridine gave B-norcholest-5-ene (CIX) and none of the $\beta$-lactone (CVIII) or the isomeric enol lactone (CXIII) was obtained from this reaction\textsuperscript{55}. 
The present work describes the preparation of the enol lactones, 3β-acetoxy-6-oxa-B-homocholest-4-en-7-one (XCIX), 6-oxa-B-homocholest-4-en-7-one (CXIII) and 6-oxa-B-homocholesta-2,4-dien-7-one (CCXC) from the seco-acids, 3β-acetoxy-5-keto-5,6-secocholestan-6-oic acid (XCXII), 5-keto-5,6-secocholestan-6-oic acid (CVII) and 5-keto-5,6-secocholesta-3-en-6-oic acid (CCLXXXVIII), respectively. Preparation of 4a-oxa-A-homocholest-5-en-4-one (CCXCI) was also attempted from the seco-acid, 5-keto-4,5-secocholestan-4-oic acid (CXX). Attempts have been made to rationalise the difference between the seco-acids (XCXII), (CVII) and (CCLXXXVIII) on one hand and (CXX) on the
other, towards acetic anhydride-fused sodium acetate/acetyl chloride and also for the difference between (XCVIII) and (CVII) towards benzoyl chloride-pyridine.

\[ \text{3β-Acetoxy-6-oxa-3-homocholest-4-en-7-one (XCIX)} \]

There are two conceivable ways to obtain the enol lactone (XCIX). One involves the Baeyer-Villiger oxidation of \( \text{3β-acetoxycholest-4-en-6-one (CLXVII)} \) and the other consists in effecting lactonization of the seco-acid (XCVIII) with acetic anhydride-fused sodium acetate under reflux.

\[ \text{(CLXVII)} \quad \text{(XCIX)} \quad \text{(XCVIII)} \]

An examination of the Baeyer-Villiger oxidation of (CLXVII) using perbenzoic acid as the oxidant and p-toluene-sulphonic acid monohydrate as the catalyst revealed that the reaction leads to several products and therefore this reaction was not considered as a good synthetic route to (XCIX).
As an alternative method, the seco-acid (XCVIII) was heated under reflux with acetic anhydride and fused sodium acetate or acetyl chloride. The reaction mixture after usual work up and column chromatography provided two products, a solid, m.p. 76° and a glassy noncrystallizable material. Both the entities were found to be homogeneous by t.l.c. in different solvent systems.

![Chemical structures](attachment:chemical_structures.png)

Characterization of the compound, m.p. 76° as B-norcholesta-3,5-diene (CCXCI)

The compound, m.p. 76° analysed correctly for C_{26}H_{42} \((M^+ 354, C_{26}H_{42})\) and gave red colour with tetranitromethane.
Its n.m.r. spectrum gave a broad signal spread between δ 5.37-6.37 integrating for 3 protons which are ascribable to C3-H, C4-H and C6-H (vinyllic protons). Methyl signals (5 methyl groups) were observed at δ 0.92, 0.86, 0.82 and 0.7. A mixed m.p. determination with an authentic sample of (CCXCI) did not show any depression.

Characterization of the glassy material as 3β-acetoxy-6-oxa-B-homocholest-4"en-7-one (XCIX)

The glassy compound analysed for C29H46O4 (M+ 458). Its i.r. spectrum showed peaks at 1770s (C=O-C=0, enol lactone carbonyl), 1750s (CH3COO), 1670m (C=C-0-) and 1240s cm⁻¹ (acetate). The n.m.r. spectrum gave signals at δ 5.2-5.6 integrating for 2 protons ascribable to AcO-C3-H and C4-H, (vinyllic proton), 2.4d like (C7a-H2), 2.03s (3 protons, CH3COO), 1.13s (3 protons, C10-CH3), 0.7s (3 protons, C13-CH3), 1.0, 0.92 and 0.83 (other methyl protons). It is pertinent to mention that the signal for C3-H appeared at a lower field; usually AcO-C3-H signals appear in the region δ 4.7-5.0175. The observed downfield shift of C3-H signal could be due to its (C3-H) being allylic to C4-C5 double bond. As anticipated the u.v. spectrum of (XCIX) was devoid of any characteristic maxima in the region 220-360 nm. The mass spectrum exhibited peaks at m/e 458 (M+), m/e 416 (M+-CH2=C=0), m/e 398 (M+-CH3COOH), m/e 370 (m/e 398-CO) and lower mass peaks. The loss of a molecule of
ketene from the molecular ion is suggestive of the presence of an allylic acetate function as in (XCIX)$^{176}$. The detailed interpretation of the mass spectrum is given in the latter portion of the thesis.

The chemical evidence in support of the enol lactone structure (XCIX) was obtained by methanolation of (XCIX). When (XCIX) was treated with either NaHCO$_3$ in methanol or sodium methoxide in methanol, it gave methyl 5-keto-5,6-secocholest-3-en-6-oate (CCLXXXIX)$^{14,170}$.

\[
\begin{align*}
\text{(XCIX)} & \xrightarrow{\text{NaHCO$_3$/MeOH}} \text{(CCLXXXIX)} \\
\end{align*}
\]

The formation of (CCLXXXIX) from (XCIX) amply supports the enol lactone structure (XCIX) and its (CCLXXXIX) formation can be rationalised according to Scheme 34.
The methoxide ion attacks the carbonyl carbon of the lactone moiety with subsequent acyl-oxygen bond cleavage. The acetate function likewise is hydrolysed during the course of the reaction to give the probable intermediate, methyl 3β-hydroxy-5-keto-5,6-secocholestan-6-oate (CI) which being a typical β-ketol suffers base-catalysed dehydration to give (CCLXXXIX).

The identity of (CCLXXXIX) (M⁺ 430, C₂₅H₄₆O₃) was established by its spectral properties; λₑₓₙ 230 nm (log ε 4.04);
$\delta_{\text{max}}$ 3040 w (C=C-H), 1742 s (COOCH$_3$), 1685 s cm$^{-1}$ (C=C=C=O); 
6.7 m (C3-H, $\beta$- to carbonyl group), 5.78 d ($J = 11$ Hz, C4-H),
3.57 s (COOCH$_3$), 2.3 d like (CH$_2$COOCH$_3$), 1.1 s (C10-CH$_3$), 0.68 s 
(C13-CH$_3$), 0.82 and 0.9 (other methyl protons) and by direct 
comparison with an authentic sample prepared according to 
Scheme 35^170.

Scheme - 35

By analogy with the observation of Ourisson and Rull^38
(LXXXVII) $\rightarrow$ (LXXXVIII), it was expected that (CCXC) should
also be formed as one of the products of the reaction of the seco-acid (XCVIII) with acetic anhydride-sodium acetate.

\[
\begin{align*}
\text{OBz} & \quad \text{Ac}_2\text{O}-\text{NaOAc} \\
\text{(LXXXVII)} & \quad \text{(LXXXVIII)}
\end{align*}
\]

On the other hand (XCII) under similar lactonization conditions gave the corresponding enol lactone (XCIII) where no loss of acetic acid occurred.

\[
\begin{align*}
\text{(XCII)} & \quad \text{(XCIII)}
\end{align*}
\]
In our experiments, if (CCXC) was formed, it escaped isolation. It appears that the final products of such reactions depend upon the conditions of reactions.

6-Oxa-B-homocholest-4-en-7-one (CXIII)

As in the case of the enol lactone (XCIX), two approaches can be considered for the preparation of 6-oxa-B-homocholest-4-en-7-one (CXIII). Baeyer-Villiger oxidation of cholest-4-en-6-one (CXLI) is likely to give (CXIII) as one of the products. The other possibility involves the reaction of the seco-acid, 5-keto-5,6-secocholestan-6-oic acid (CVII) with acetic anhydride-sodium acetate.

![Chemical structures](image)

Reaction of perbenzoic acid with cholest-4-en-6-one (CXLI) in a routine manner led to the formation of a complex mixture of products; the isolation and characterization of the individual components proved to be highly discouraging.
Alternatively, the seco-acid (CVII) was subjected to lactonization with refluxing acetic anhydride-fused sodium acetate or acetyl chloride in the manner described for (XCVIII) \( \rightarrow \) (XCIX). After usual work up procedure and column chromatography, two crystalline compounds, m.p.s. 68° and 94° were obtained. Purity of the individual compounds was established by repeated crystallization and t.l.c. in different solvent systems.

Characterization of the compound, m.p. 68° as B-norcholest-5-ene (CIX)

The compound, m.p. 68° analysed correctly for \( C_{26}H_{44}(M^+356) \) and gave positive tetranitromethane test. Its i.r. spectrum gave peaks at 3030w (C=H) and 1625w cm\(^{-1}\) (C=C). The n.m.r. spectrum gave a signal at \( 5.28 \) integrating for 1 proton and was assigned to a vinylic proton at C6. Methyl signals were
observed at δ 1.0s (3 protons, C10-CH$_3$), 0.68s (3 protons, C13-CH$_3$), 0.93 and 0.82 (other methyl groups; in all five methyl groups).

Characterization of the compound, m.p. 94° as 6-oxa-B-homocholest-4-ene-7-one (CXXII)

The compound, m.p. 94° analysed correctly for C$_{27}$H$_{44}$O$_2$ (M$^+$ 400). The i.r. spectrum of (CXXII) gave peaks at 1772s (C=C-0-C=0, enol lactone carbonyl) and 1668s cm$^{-1}$ (C=C-O-). The n.m.r. spectrum gave a signal at δ 5.53t integrating for 1 proton and has been assigned to a vinylic proton at C4. Other signals were observed at δ 2.4 mc (4 protons, C3-H$_2$-allylic to C4-C5 double bond and C7a-H$_2$), 1.0s (3 protons, C10-CH$_3$), 0.73s (3 protons, C13-CH$_3$), 0.93 and 0.83 (other methyl protons).

The u.v. spectrum was featureless in the region 220-360 nm. The mass spectrum gave peaks in the high mass region at m/e 400 (M$^+$, C$_{27}$H$_{44}$O$_2$), m/e 385 (M$^+$-CH$_3$), m/e 372 (M$^+$-28) and lower mass peaks; the detailed interpretation of the mass spectrum is given in the latter part of the thesis.

As expected methanolysis of the enol lactone (CXXII) with either sodium bicarbonate in methanol or sodium methoxide in methanol readily furnished methyl 5-keto-5,6-secocholestan-6-oate (CX)$^{56,177}$ (Scheme 36). The formation of (CX) from (CXXII) supported the enol lactone structure for the compound, m.p. 94°.
The methyl ester (CX) was identified by its spectral properties; 
$\nu_{\text{max}}$ 1742s (COOCH$_3$) and 1708s cm$^{-1}$ (C=O); $\delta$ 3.56s (3 protons, 
COOCH$_3$), 2.2m (4 protons, CO-CH$_2$- and CH$_2$-COOCH$_3$), 1.0s (3 
protons, C10-CH$_3$), 0.88s (3 protons, C13-CH$_3$), 0.90 and 0.83 
(other methyl protons); M$^+$ 432 (C$_{28}$H$_{48}$O$_3$) and also by direct 
comparison with an authentic sample of (CX) prepared according 
to Scheme 37.
6-Oxa-3-homocholesta-2,4-dien-7-one (CGXC)

6-Oxa-3-homocholesta-2,4-dien-7-one (CGXC) was likely to be one of the products of the reaction of the seco-acid (XCVIII) with acetic anhydride and fused sodium acetate. As mentioned earlier, we failed to isolate (CGXC) from this reaction. As an alternative, the seco-acid, 5-keto-5,6-secocholest-3-en-6-oic acid (CCLXXXVIII) was heated with acetic anhydride-sodium acetate or acetyl chloride. After usual work up of the reaction mixture, followed by column chromatography, two compounds, m.p. 76°C and a noncrystallizable oil, were obtained. Both the compounds were found to be homogeneous by t.l.c. in different solvent systems. The compound, m.p. 76°C was identified as B-norcholesta-3,5-diene (CCXCIIO) by comparison with an authentic sample.
Characterization of the oily product as 6-oxa-B-homocholesta-2,4-dien-7-one (CCXC)

This product analysed correctly for C_{27}H_{42}O_{2} (M^+ 398). Its i.r. spectrum gave peaks at 3040w (C=C-H), 1766s (C=C=O-C=O; dienol lactone carbonyl), 1665m (C=C-O) and 1650m cm^{-1} (C=C). The n.m.r. spectrum of (CCXC) gave broad multiplet spread in the region $\delta$ 5.4-6.1 integrating for 3 protons which are ascribable to C2-H, C3-H and C4-H (all vinylic protons). Another broad multiplet centred at $\delta$ 2.4 integrating for 4 protons can be assigned to C1-H$_2$ and C7a-H$_2$ (C=C-CH$_2$-allylic methylene and CO-CH$_2$-C-methylene to a carbonyl group). The methyl signals appeared at $\delta$ 1.05s (3 protons, C10-CH$_3$), 0.70s (3 protons, C13-CH$_3$), 0.91 and 0.81 (other methyl protons). The u.v. spectrum showed absorption maxima at 270 nm (log $\epsilon$ 4.01) thus supporting the presence of a homoannular diene chromophore in the molecule. These spectral data support the assigned structure (CCXC) to the oily product.
Chemical evidence in support of the structure (CCXC) was obtained by methanolysis which gave methyl 5-keto-5,6-secocholest-2-en-6-oate (CCXCV) (Scheme 38).

Scheme 38

\[ \text{NaHCO}_3 - \text{MeOH} \]

\[ \begin{array}{c}
\text{C}_8\text{H}_{17} \\
\text{\includegraphics[width=0.5\textwidth]{image.png}} \\
\text{COOCH}_3
\end{array} \]

The methyl ester (CCXCV) was characterised by its elemental analysis, spectral properties and isomerization to the known compound, methyl 5-keto-5,6-secocholest-3-en-6-oate (CCCLXXXIX). It (CCXCV) analysed correctly for C_{28}H_{46}O_3 (M^+ 430). Its i.r. spectrum gave bands at 3035w (C=C-H), 1740s (COOCH_3), 1708s (C=O) and 1636w cm^{-1} (C=C). The i.r. spectrum thus showed the absence of an \( \alpha,\beta \)-unsaturated carbonyl chromophore which was further supported by a featureless u.v. spectrum in the region 220-360 nm. The n.m.r. spectrum gave a multiplet centred at \( \delta \) 5.6 integrating for 2 protons which are ascribable to C2-H and C3-H (vinylic protons). A sharp singlet at \( \delta \) 3.6
integrating for 3 protons was assigned to COOCH₃. A broad multiplet centred at δ 2.35 integrating for 6 protons was assigned to C₁-H₂, C₄-H₂ and C₇-H₂; the former two being allylic to C₂-C₃ double bond whereas the latter one being adjacent to an ester carbonyl group. The methyl signals were observed at δ 1.0s (3 protons, C₁₀-CH₃), 0.68s (3 protons, C₁₃-CH₃), 0.9 and 0.82 (other methyl protons).

The methyl ester (CCXCV) when subjected to acid-catalysed isomerization of 109 followed by treatment with diazomethane gave the known compound (CCLXXXIX).

![Chemical Structures]

**Reaction of 5-keto-4,5-secocholestan-4-oic acid (CXX) with acetic anhydride-fused sodium acetate**

In an attempted preparation of 4a-oxa-A-homocholestan-5-en-4-one (CCXCI), the seco-acid, 5-keto-4,5-secocholestan-4-oic acid (CXX)₁⁷₈ was heated severally with acetic anhydride
and fused sodium acetate. In each instance, A-norcholestene (CCXCVII) was obtained as the only isolable product together with the starting acid.

A-Norcholestene (CCXCVII), m.p. 78° was identified by its elemental analysis, spectral properties and comparison with an authentic sample. It analysed correctly for C_{26}H_{44} (M^+ 356) and gave positive tetranitromethane test. Its i.r. spectrum was similar to that of B-norcholestene (CIX) and gave weak bands at 3035w (C=C-H) and 1630w cm^{-1}. The n.m.r. signals were observed at \delta 5.3t (1 proton, C3-vinylic proton), 1.0s (3 protons, C10-CH_{3}), 0.7s (3 protons, C13-CH_{3}), 0.92 and 0.82 (other methyl signals).

With acetic anhydride-acetyl chloride of 40

In continuation of our efforts to obtain the enol lactone (CCXCI) from the seco-acid (CXX), the latter was heated
under reflux with acetyl chloride-acetic anhydride. After usual work up of the reaction mixture and column chromatography, two compounds, m.p. 78° and 96° were obtained together with the unreacted starting acid.

The compound, m.p. 78° was identified as (CCXCVII) in the manner described earlier.
Characterization of the compound, m.p. 96° as 5-acetoxy-4, 5-secocholest-5-en-4-oic acid (CCXCVIII)

The compound, m.p. 96° analysed for \( \text{C}_{29}\text{H}_{48}\text{O}_4 \) (\( \text{M}^+ 460 \)) and not for \( \text{C}_{27}\text{H}_{44}\text{O}_2 \) as required by the enol lactone (CCXCI). The composition \( \text{C}_{29}\text{H}_{48}\text{O}_4 \) agrees with the enol acetate (CCXCVIII) as well as with the mixed anhydride (CCC). Both the compounds could be formed from the seco-acid (CXX) under these reaction conditions. The spectral data, however, supports the enol acetate structure (CCXCVIII) and not the isomeric mixed anhydride structure (CCC). The i.r. spectrum of the compound gave a broad peak between 3400-3200 (\( \text{COOH} \)), 3030w (\( \text{C=C-H} \)), 1770s (\( \text{C=C-O} \text{COOCH}_3 \)), enol acetate carbonyl), 1705s (\( \text{COOH} \)), and 1660m cm\(^{-1} \) (\( \text{C=O} \)). These i.r. values are compatible with (CCXCVIII) but do not support the structures (CCC) and (CCXCI). The n.m.r. spectrum gave a singlet at \( \delta 8.6 \) integrating for 1 proton which disappeared on addition of D\(_2\)O and was assigned to \( \text{COOH} \). An unresolved multiplet centred at \( \delta 5.4 \) integrating for 1 proton was ascribable to a vinylic proton at C6 as in (CCXCVIII). A broad signal centred at 2.3 integrating for 2 protons was assigned to a methylene group adjacent to \( \text{COOH} \) (\( \text{CH}_2\text{-COOH} \)) and a sharp singlet at \( \delta 2.1 \) was assigned to an acetate methyl group (\( \text{CH}_3\text{COO} \)). Methyl signals were observed at 0.98s (3 protons, \( \text{C}_{10}\text{-CH}_3 \)), 0.68s (3 protons, \( \text{C}_{13}\text{-CH}_3 \)), 0.96 and 0.8 (other methyl groups).
The presence of a carboxylic group in (CCXCVIII) was further confirmed by its reaction with diazomethane which readily afforded the methyl ester, methyl 5-acetoxy-4,5-secocholest-5-en-4-oate (CCXCIX). This methyl ester analysed correctly for $C_{30}H_{50}O_4$ and its i.r. spectrum gave bands at 1770s (C=O=OCH$_3$), 1740s (COOCH$_3$) and 1660m cm$^{-1}$ (C=C=O).

The n.m.r. spectrum of (CCXCIX) gave no signal for COOH; signals were observed at $\delta$ 5.5t (1 proton, $\text{C}_6$-$\text{H}$, vinylic proton), 3.6s (3 protons, COOCH$_3$), 2.25br (2 protons, $\text{CH}_2$COOCH$_3$), 2.08s (3 protons, $\text{CH}_3$COO), 1.0s (3 protons, C10-$\text{CH}_3$), 0.7s (3 protons, C13-$\text{CH}_3$), 0.94 and 0.82 (other methyl protons).

As expected both the compounds (CCXCVIII) and (CCXCIX) gave the seco-acid (CXX) when subjected to alkaline hydrolysis.

These observations unambiguously support the enol acetate structure (CCXCVIII) for the compound, m.p. 96°.
It is pertinent to mention that the ring A seco-acid (CXX) behaved differently towards sodium acetate-acetic anhydride, and acetic anhydride-acetyl chloride as compared to the ring B seco-acids (XCVIII), (CVII) and (CCLXXXVIII) in the sense that the latter three afforded the corresponding enol lactones whereas the former (CXX) failed to give analogous product (CCXCI). This difference in the behaviour of (CXX) towards acetic anhydride-acetyl chloride can be rationalised on the basis of steric factors. It is reasonable to assume that the carboxylic bearing side chain in (CXX) being less bulky and less crowded than the one in either (XCVIII), (CVII) or (CCLXXXVIII) is more flexible and did not attain close proximity to the enol OH group necessary for lactonization. There is no doubt that the enolic form of (CXX) is obtained under the reaction conditions, since without this the enol acetate (CCXCVIII) would not have been formed. The carboxylic bearing side chain must have attained considerable distance from the enol group as to allow intermolecular reaction to occur at the cost of the expected intramolecular lactonization.

On the other hand the carboxylic bearing side chain in (CVII), as an illustration, being far too bulky is likely to remain less mobile (free rotation about C9-C10 bond being restricted) and as a result forced into close proximity to the enolic OH group to give the product of intramolecular reaction
An examination of the Dreiding models of the secos
acids (CXX) and (CVII) in their enolic forms clearly showed
that in the former the carboxylic bearing side chain is in
maximum staggered conformation when extended away from the
ring B.

On the other hand, in the case of (CVII), due to
restricted rotation about C9-C10 bond and keeping the carboxylic
side chain in staggered conformation, the enolic OH (C5-OH)
and the carboxylic groups come close enough for intramolecular
reaction to become possible.

In order to test the validity of the hypothesis that
the reaction is dependent upon steric factor and not upon
reagents used, (CVII) was heated with acetyl chloride-acetic
anhydride. In this case also the same enol lactone (CXIII)
was obtained. This therefore, implied that the reaction is dependent on the steric factors and not on the reagents used. Similarly, the other seco-acids (XCVIII) and (CCLXXXVIII) with acetyl chloride and acetic anhydride gave the same results as obtained with acetic anhydride-sodium acetate.

It is worth mentioning that both acetic anhydride-fused sodium acetate, and acetyl chloride-acetic anhydride gave similar products as illustrated by the following examples, (LXIV) $\rightarrow$ (CCCI)$_4$ and (LXXXV) $\rightarrow$ (LXXXVI)$_3$.

\[
\begin{align*}
\text{(LXIV)} & \quad \xrightarrow{\text{AcCl-AC}_2O} \quad \text{(CCCI)} \\
\text{(LXXXV)} & \quad \xrightarrow{\text{Ac}_2O-\text{NaOAc}} \quad \text{(LXXXVI)}
\end{align*}
\]
The above examples are in sharp contrast to the present observation, i.e. whereas (LXIV) and (LXXXV) behaved in an expected manner, the seco-acid (CXX) did not give the analogous product (CCXCI). This difference between (LXIV) and (CXX) could be due to the larger carboxylic bearing side chain in (CXX) and also in part, to the greater stability of a six-membered lactone as (CCCI) than the seven-membered lactone as (CCXCI).

B. Saturated \( \varepsilon \) -Lactones

Several papers dealing with peracid oxidation of steroidal ketones have appeared recently. These included saturated ketones with carbonyl function located at different positions and \( \alpha,\beta \)-unsaturated ketones, especially pertaining to rings A and B. A wide variety of products, specially from \( \alpha,\beta \)-unsaturated ketones have been obtained from these reactions; the nature and composition of the products depended largely upon the peracid used, its concentration, catalyst and reaction period.

Previous work from our laboratory described the perbenzoic acid oxidation of 3\( \beta \)-acetoxycholest-5-en-7-one (LXXVIII), cholest-5-en-7-one (LXXXIII)\(^{36} \), 3\( \beta \)-halo-5\( \alpha \)-cholestan-6-ones (XXXI-XXXIII), 3\( \alpha \),5-cyclo-5\( \alpha \)-cholestan-6-one (XXX)\(^{16} \), cholesta-3,5-dien-7-one (LXXV)\(^{35} \), 6\( \beta \)-bromocholest-4-en-3-one (LXVIII)\(^{33} \),
cholesta-2,4-dien-6-one (CLXXVII) and cholest-4-ene-3,6-dione (LXXII)\textsuperscript{34}. 

(LXXVII) \begin{align*}
R, C_{17}H_{35}\\
\text{X, Cl} \\
\text{X, Br} \\
\text{X, I}
\end{align*}

(LXXVIII) R, OAc

(LXXXIII) R, H

(XXXI) X, Cl

(XXXI) X, Br

(XXXI) X, I

(LXXV)

(LXVIII)

(CLXXVII)

(LXXXII)
In continuation of the above work we undertook the study of peracid oxidation of methyl 5-keto-5,6-secocholestan-6-oate (CX), methyl 5-keto-4,5-secocholestan-4-oate (CCLXXXVII) and methyl 5-keto-5,6-secocholest-3-en-6-oate (CCLXXXIX). The selection of these compounds as substrates was largely governed by their accessibility and the expectation of some interesting results.

Methyl 5-keto-5,6-secocholestan-6-oate (CX)\textsuperscript{56,177}, m.p. 103\degree (M\textsuperscript{+} 432, C\textsubscript{28}H\textsubscript{4}O\textsubscript{3}) was treated with a chloroform solution of perbenzoic acid (2.5 mole equivalent) and a few crystals of p-toluenesulphonic acid monohydrate as catalyst for 96 hours at room temperature. The progress of the reaction was monitored by t.l.c. of the reaction mixture from time to time. It was evident that the reaction proceeded at a very
slow rate. At the end of the reaction period (96 hours), the reaction mixture was worked up in the usual manner and the crude product thus obtained was subjected to column chromatography and this provided methyl 5α-oxa-5-keto-5,6-seco-A-homocholestan-6-oate (CCCI), 5α-oxa-5-keto-5,6-seco-A-homocholestan-6-oic acid (CCCIII) (a product of partial hydrolysis of CCCI) and the seco-acid (CVII), a product of hydrolysis of the methyl ester (CX) and the unreacted methyl ester (CX).

\[
\begin{align*}
\text{(CX)} & \xrightarrow{\text{PBA}} \text{A}\quad \text{B} \\
\text{A} & \quad \text{B} \\
\text{C} & \quad \text{D}
\end{align*}
\]

Both the oxidation products (CCCI) and (CCCIII), labelled as A and B, respectively, were obtained as viscous oil and defied all attempts to crystallization. Chromatographically pure products were obtained by repeated column chromatography and were found to be homogeneous by t.l.c. in different solvent systems. The yields of (CCCI) and (CCCIII) were moderate.
Characterization of the oily product 'A' as methyl 5a-oxa-5-keto-5,6-seco-A-homocholestan-6-oate (CCCII)

The compound 'A' analysed correctly for \( C_{28}H_{48}O_4 \) (M⁺ 448). From the molecular composition it was evident that only one oxygen atom has been introduced during the course of the reaction and this leads to two obvious possibilities, (CCCII) and (CCCIV).

\[
\text{(CCCII)} \quad \text{(CCCIV)}
\]

A distinction between the two was obtained by spectral properties. The i.r. spectrum gave peaks at 1740s (COOCH₃) and 1720s cm⁻¹ (ε-lactone carbonyl)¹⁶ which incidentally supported both the structures (CCCII) and (CCCIV) equally well. The n.m.r. spectrum was much more revealing and it gave signals at \( \delta \) 3.57s (3 protons), 2.3br (4 protons), 1.28s (3 protons), 0.67s (3 protons), 0.88, 0.66 and 0.80 (other methyl protons). The singlet at \( \delta \) 3.57 can be assigned to an ester methyl group (COOCH₃) and the broad signal centred at \( \delta \) 2.3 are ascribable to C₄–H₂ and C₇–H₂.
(methylenes \( \sim \) to carbonyl groups as in CCCII). The singlet
at \( \delta 1.28 \) can be assigned to \( \text{C}10-\text{CH}_3 \). This downfield shift of
\( \text{C}10-\text{CH}_3 \) signal clearly indicated that the grouping \( \text{O}^-\text{C}-\text{CH}_3 \)
is present in the compound, as in (CCCII). On the other hand
the isomeric structure (CCCIV) would have given a signal in
the region \( \delta 3.5-4 \) for \( \text{C}4-\text{H}_2 \), i.e. for the grouping \( \text{CH}_2^-\text{O}-\text{CO}^- \).
Further, the \( \text{C}10-\text{CH}_3 \) signal in (CCCIV) is not likely to appear
so downfield. The n.m.r. spectrum thus supported the struc­
ture (CCCII). The mass spectrum of 'A' (M\(^+\) 448) gave the
base peak at m/e 127 (C\(_7\)H\(_{11}\)O\(_2\)); other important peaks in the
high mass region were observed at m/e 433 (M-CH\(_3\)), m/e 420
(M-CO), and m/e 417 (M-OCH\(_3\)). The peak at m/e 433 was fairly
strong. The base peak at m/e 127 strongly supported the
structure (CCCII); the formation of which (m/e 127) has been
rationalized according to the mechanism given below.

There is apparently no rationale for the formation of this
fragment ion m/e 127 from the alternate structure (CCCIV).
Characterization of 'B' as 5a-oxa-5-keto-5,6-seco-A-homocholestan-6-oic acid (CCCIII)

The compound 'B' analysed correctly for C_{27}H_{46}O_{4} (M^+ 434). From the composition it was apparent that this compound was not a methyl ester but it was clearly indicated to be a product of oxidation-cum-partial hydrolysis. Most obviously the methyl ester group was hydrolysed either during the course of the reaction or during the repeated chromatographic purification. The composition C_{27}H_{46}O_{4} agrees with two possible structures (CCCIII) and (CCCV).

The i.r. spectrum of 'B' gave broad peak between 3200-3450 (COOH), 1720s (ε-lactone carbonyl) and 1705s cm^{-1} (COOH); these values support both the structures (CCCIII) and (CCCV). The n.m.r. spectrum displayed a singlet at δ 9.2 (1 proton, exchangeable with deuterium, COOH), 2.2br (4 protons, C4-H\textsubscript{2} and C7-H\textsubscript{2}), 1.29s (3 protons, C10-CH\textsubscript{3}), 0.68s (3 protons,
C13-CH₃, 0.88, 0.85 and 0.8 (other methyl groups). Thus the n.m.r. spectrum supported the structure (CCCIII). This was further substantiated by the fact that (CCCIII) when treated with diazomethane gave (CCCII).

As was expected, the mass spectrum of (CCCIII), like that of (CCCII), gave the base peak at m/e 127 (C₂₇H₅₁O₂).

From these observations it is abundantly clear that the products obtained are (CCCII) and (CCCIII). The formation of (CCCII) further strengthens the view that in Baeyer-Villiger oxidation, a more highly alkylated carbon migrates preferentially. It is possible that the isomeric lactone (CCCIV) might have been also formed but in amounts which escaped isolation by routine methods.

**Perbenzoic acid oxidation of methyl 5-keto-4,5-secocholestan-4-oate (CCLXXXVII)**

Methyl 5-keto-4,5-secocholestan-4-oate (CCLXXXVII)

(M⁺ 432, C₂₈H₄₈O₃, prepared according to known procedure and
purified by column chromatography, t.l.c. homogeneous) was treated with perbenzoic acid (2.5 mole equivalent) and p-toluenesulphonic acid monohydrate as catalyst for 96 hours at room temperature. After usual work up of the reaction mixture, followed by repeated column chromatography, there were obtained 3 compounds along with the starting methyl ester and the seco-acid (CXX).

\[
\begin{align*}
\text{(CCLXXXVII)} & \quad \xrightarrow{\text{PBA}} \quad \text{CH}_3\text{OOC} \\
\text{(CCCVI)} & \quad + \quad \text{CH}_3\text{OOC} \\
\text{'D'} & \quad \text{HOOC} \\
\text{(CCCVII)} & \quad + \quad \text{(CXX)} \\
\text{'E'} & \quad \text{(CCCVIII)} \\
\end{align*}
\]
Characterization of the compound (D) as methyl 5-oxa-6-keto-4,5-seco-B-homocholestan-4-oate (CCCVI)

The compound labelled as 'D', analysed correctly for C_{26}H_{48}O_{4} (M^+ 448). The composition suggested that only one oxygen has been incorporated during the reaction and that the product can be formulated either as (CCCVI) or (CCCVIII). The i.r. spectrum gave bands at 1740s (COOCH_{3}) and 1720s cm^{-1} (δ-lactone carbonyl) and these values supported both the structures (CCCVI) and (CCCVIII). The n.m.r. spectrum gave signals at δ 3.6s (3 protons, COOCH_{3}), 2.28br (4 protons, \text{CH}_3-\text{O}-\text{CO}-\text{CH}_2 and -\text{O}-\text{CO}-\text{CH}_2), 1.28s (3 protons, \text{C10}-\text{CH}_3), 0.7s (3 protons, \text{C13}-\text{CH}_3), 0.96 and 0.84 (other methyl signals). These data, in conjunction with the data for (CCCVII) and (CCCVIII), support the structure (CCCVI) for the compound 'D'.

The mass spectrum of (CCCVI) as was anticipated, gave a very prominent peak at m/e 347(C_{23}H_{39}O_{2}) which further supported the assigned structure (CCCVI). The formation of the fragment ion m/e 347 can be conveniently shown according to the following mechanism.
Characterization of 'E' as 5-oxa-6-keto-4,5-seco-B-
homocholestan-4-0ic acid (CCCVII)

The compound, labelled as 'E' analysed correctly for
\( \text{C}_{27}\text{H}_{46}\text{O}_4 \) and from the composition this appeared to be the product
of partial hydrolysis of either (CCCVI) or (CCCVIII). Its
i.r. spectrum gave a broad peak between 3250-3430 (\( \text{COOH} \)),
1722s (\( \epsilon \)-lactone carbonyl) and 1708s cm\(^{-1} \) (\( \text{COOH} \)). The n.m.r.
spectrum gave a singlet at \( \delta \) 10.1 (1 proton, disappeared on
addition of \( \text{D}_2\text{O} \), \( \text{COOH} \)), 2.3br (4 protons, \( \text{HOOC-CH}_2- \) and
\( -\text{O-CHO-CH}_2- \)), 1.29s (3 protons, \( \text{C}\text{I0-CH}_3 \)), 0.7s (3 protons,
\( \text{C}\text{I3-CH}_3 \)), 0.94 and 0.82 (other methyl signals). A direct
relationship between (CCCVI) and (CCCVII) was readily estab­
lished by the observation that the latter on methylation
(diazomethane) gave the former.
Characterization of 'F' as methyl 6-oxa-5-keto-4,5-seco-
B-homocholestan-4-oate (CCCVIII)

The compound 'F' analysed correctly for C_{25}H_{48}O_{4}
(M^+ 448) and from the molecular composition it was obvious
that 'F' is an isomer of (CCCVI). The i.r. spectrum of 'F'
gave bands at 1742s (COOCH₃) and 1720s cm⁻¹ (α-lactone
carbonyl). The n.m.r. spectrum gave signals at δ 3.84m
(2 protons, -CO-O-CH₂-), 3.5s (3 protons, COOCH₃), 2.26br
(2 protons, CH₃-O-CO-CH₂-), 1.06s (3 protons, C₁₀-CH₃), 0.68s
(3 protons, C₁₃-CH₃), 0.94 and 0.82 (other methyl protons).

A comparison of n.m.r. data for (CCCVI) and (CCCVIII)
proved extremely helpful in the characterization of these
isomers. The signal at δ 3.34 (in CCCVIII) clearly showed
that a methylene group is next to an oxygen atom, i.e. -CO-O-CH₂
grouping is present. Further, the C₁₀-CH₃ signal, as expected,
appeared at relatively high field (δ 1.06) in (CCCVIII).

A comparison of the mass spectra of the seco-acid (CXX)
and (CCCVIII) was also made. In the mass spectrum of the seco-
acid (CXX) a very prominent peak appeared at m/e 332 (C_{23}H_{40}O).
The fragment ion m/e 332 has been shown to occur according to
the following mechanism involving McLafferty rearrangement.
(Details of mass spectral fragmentations of seco acids are
given in another chapter of the thesis).
It was thought that a similar fragmentation will dominate in the mass spectrum of the methyl ester $\epsilon$-lactone (CCCVIII). However, only a weak peak appeared at m/e 348 thus showing that the introduction of an oxygen atom enlarging ring B has affected the required distance between the carbonyl oxygen and C2-H ($\gamma$-hydrogen) for McLafferty rearrangement to be effectively operative.

A parallel observation was made during the examination of the mass spectra of (CCXIX) and (CCXXV)\(^{109}\). The mass spectrum of
(CCXIX) gave the base peak at m/e 110 \((C_7H_{10}O)\) involving McLafferty rearrangement as shown below.

![Diagram](image)

\((\text{CCXIX'})\) \(m/e 110 \,(C_7H_{10}O)\)

On the other hand, the lactam (CCXXVI) with enlarged ring A failed to give analogous ion peak at m/e 141.

![Diagram](image)

\((\text{CCXXVI'})\) \(m/e 141\)

An examination of the Dreiding models of (CCCVIII), (CXX), (CCXIX) and (CCXXVI) clearly showed that the ring enlargement in the case of (CCCVIII) or (CCXXVI) could be the main reason for preventing McLafferty rearrangement to occur.
The distance between the carbonyl oxygen and a γ-hydrogen was extended even when a free rotation about C10-C1 or C9-C10 was fully allowed as might be expected under electron-impact.

From the above discussions it is obvious that the methyl ester (CCLXXXVII), in contrast to (CX), gave both the expected isomers (CCCVI) and (CCCVIII) by the migration of C10 and C6, respectively.

Reaction of methyl 5-keto-5,6-secocholest-3-en-6-oate (CCLXXXIX) with perbenzoic acid

Chromatographically pure methyl 5-keto-5,6-secocholest-3-en-6-oate (CCLXXXIX) was prepared for the present study following literature procedure. It (CCLXXXIX) was treated with perbenzoic acid (2.5 mole equivalent) and p-toluene-sulphonic acid monohydrate as catalyst (4 days). After usual work up of the reaction mixture and column chromatography there were obtained 2 compounds, labelled as 'G' and 'H' together with the starting methyl ester and the seco-acid (CCLXXXVIII). The purity of the products 'G' and 'H' was ascertained by t.l.c. in different solvent systems.
Characterization of 'G' as methyl 3,4-epoxy-5-keto-5, 6-secocholestan-6-oate (CCCIX).

The compound 'G' obtained as a noncrystallizable oil analysed correctly for C_{28}H_{46}O_4, thus showing the introduction of only one oxygen atom during the course of the reaction. Introduction of one oxygen atom leads to several possibilities, such as (CCCIX), (CCCXI) and (CCCXII).

The i.r. spectrum gave peaks at 1740s (COOCH_3), 1710s (C=O) and 870m cm^{-1} (epoxide). From these values, it was obvious
that the compound 'G' is devoid of an \( \alpha,\beta \)-unsaturated carbonyl chromophore as in (CCCXI) or an enol lactone moiety as in (CCCXII). The u.v. spectrum was featureless in the region 220-360 nm thus supporting the absence of an \( \alpha,\beta \)-unsaturated carbonyl chromophore in the molecule. The n.m.r. spectrum gave a singlet at \( \delta 3.6 \) integrating for 3 protons which can be easily assigned to COOCH\(_3\). Two signals, one at \( \delta 3.36 \) (d like, \( J = 5 \) Hz) integrating for 1 proton and another at \( \delta 3.2 \) (unresolved multiplet) integrating for 1 proton were also observed. There was no signal for vinylic protons.

The assignments of these signals (\( \delta 3.36 \) and 3.2) for C4-H and C3-H, respectively, can be made with an epoxy group attached to C3-C4 centres. A broad signal centred at \( \delta 2.3 \) integrating for 2 protons can be assigned to C7-H\(_2\)(-CH\(_2\)-COOCH\(_3\)); other signals were observed at 1.1s (3 protons, C10-CH\(_3\)), 0.75s (3 protons, C13-CH\(_3\)), 0.96 and 0.84 (other methyl groups).

From the shape of the signal at \( \delta 3.36 \) it was obvious that there is a proton at epoxy group bearing carbon adjacent to a carbonyl group, i.e., the grouping \[\text{C}-\overset{3}{\text{H}}\overset{4}{\text{O}}-\text{C}=\text{O}\] is present and that this proton is interacting with another vicinal proton at C3. The \( J \) value (5 Hz) is suggestive of the fact that this interaction is between an axial and an equatorial proton.
The signal at δ 3.2 (unresolved multiplet) which has been assigned to C3-H on the basis of magnitude of splitting has half band width of 6 Hz which implied that this proton is equatorial. The presence of C3-H as equatorial (β-oriented) clearly indicated that C4-H is axial (β-oriented). This then leads to the conclusion that the epoxide ring is α-oriented. The formation of an α-epoxide, such as (CCCIX) is understandable since the peracid will attack the C3-C4 double bond from the less hindered rear side of the substrate (CCLXXXIX). This assumption finds further support from the following observation.

Generally an axial proton resonates at a higher field than an equatorial one but in this case, since axial C4-H is also adjacent to a carbonyl group, the signal appears at a lower field (δ 3.36).
These arguments by and large exclude the structures (CCCXI) and (CCCXII) for the compound 'G' but support the structure (CCCIX).

Characterization of 'H' as methyl 5,6-sec-3,4<eps>epoxy-5-keto-5a-oxa-A-homocholestan-6-oate (CCCX)

The compound 'H' analysed correctly for $\text{C}_{28}\text{H}_{46}\text{O}_5$ and its i.r. spectrum gave peaks at 1740s ($\text{COOCH}_3$), 1718s ($\epsilon$-lactone carbonyl) and 870m cm$^{-1}$ (epoxy group). The u.v. spectrum was featureless in the region 220-360 nm. The presence of 2 additional oxygen atoms with respect to the substrate leads to two obvious possibilities (CCCX) and (CCCX-a).

The n.m.r. spectrum gave signals at $\delta$ 3.58s (3 protons, $\text{COOCH}_3$), 3.3m (2 protons, C3-H and C4-H), 2.3br (2 protons, $\text{CH}_2\text{COOCH}_3$), 1.3s (3 protons, C10-CH$_3$), 0.7s (3 protons, C13-CH$_3$),
0.92 and 0.81 (other methyl protons). These values are compatible with the structure (CCCX). The C4-H signal in (CCCX-a) is expected to appear in the region δ 4.5-4.7. Further the appearance of a singlet at δ 1.3 (3 protons) clearly supported the structure (CCCX). The epoxy group at C3-C4 centres has been tentatively assigned α-orientation by analogy with (CCCIX). From the elemental composition and spectral data, the compound 'H' has been characterized as methyl 5,6-seco-3Δ4-α-epoxy-5-keto-5a-oxa-A-homocholestan-6-oate (CCCX).

C. Reaction of the seco-acids, 5-keto-5,6-secocholest-3-en-6-oic acid (CCLXXXVIII) and 5-keto-4,5-secocholestan-4-oic acid (CXX) with benzoyl chloride-pyridine: Attempted preparation of the β-lactones, 5β-hydroxy-B-norcholestan-3-en-6-oic acid 5,6-lactone (CCCXIII) and 5β-hydroxy-A-norcholestan-4-oic acid 4,5-lactone (CCCXIV).

As mentioned earlier, the seco-acid (XCVIII) with benzoyl chloride-pyridine gave the β-lactone, 3β-acetoxy-B-norcholestan-5β-hydroxy-6-oic acid 5,6-lactone (CIII). On the other hand (CVII) under similar conditions of reactions afforded B-norcholestene (CIX).
It was further shown that the conversion (CVII) $\rightarrow$ (CIX) does not involve the $\beta$-lactone (CVIII)\textsuperscript{56} as an intermediate as this was found to remain unchanged under the conditions of reaction.

In view of these observations, it was considered interesting to study the reaction of the structurally similar seco-acids (CCLXXXVIII) and (CXX) with benzoyl chloride and pyridine. It was hoped to explore these reactions for the preparation of the $\beta$-lactones (CCXIII) and (CCXIV).
Reaction of the seco-acid (CCLXXXVIII) with benzoyl chloride-pyridine

The seco-acid (CCLXXXVIII) was treated with benzoyl chloride and pyridine for variable periods of 48-96 hours at room temperature of 170. Usual work up of the reaction mixture followed by column chromatography gave invariably a single characterizable compound, m.p. 76° in fairly good yields.

\[
\begin{align*}
\text{(CCCXIII)} & \quad \text{(CCLXXXVIII)} \quad \text{(CCXCIII)} \\
\end{align*}
\]

\[
\begin{align*}
\text{(CCXC)}
\end{align*}
\]

The compound, m.p. 76° was identified as B-norcholest-3,5-diene (CCXCIII) by direct comparison with an authentic sample in terms of m.p., mixed m.p., t.l.c. and spectra.
Careful screening of the various fractions obtained during chromatographic separation, as well as the crude reaction mixture did not reveal the presence of the desired β-lactone (CCCXIII). The i.r. spectra of the various fractions or of the crude reaction mixture did not show a peak at about 1820 cm\(^{-1}\) (β-lactone carbonyl). There was, however, some indication of the presence of the dienol lactone (CCXC) as revealed by a peak at about 1765 cm\(^{-1}\) (enol lactone carbonyl). Some of the starting acid was also recovered.

Reaction of the seco-acid (CXX) with benzoyl chloride-pyridine

The seco-acid (CXX) with benzoyl chloride and pyridine afforded A-nor-cholestene (CCXCVII) as the only isolable and characterizable product. The i.r. spectra of the various chromatographic fractions failed to reveal the presence of the β-lactone (CCCXIV); instead we obtained some indication of the presence of the enol benzoate (CCCXVII). However, (CCCXVII) was there only in very small amounts and was not pursued any further. The formation of the enol lactone (CCXCI), however, can not be completely ruled out.
Thus these reactions proved to be unsuccessful for the preparation of the \( \beta \)-lactones (CCCXIII) and (CCCXIV); though it may be claimed that these offer alternative methods for the preparation of B-norcholestadiene (CCXCI) and A-norcholest-3-ene (CCXCII). These observations along with the previous ones\(^{55,170}\) pose problems of some theoretical interest. It can be said that the seco-acid (XCVIII) behaved differently from the seco-acids (CVII), (CCLXXXVIII) and (CXX) towards benzoyl chloride-pyridine. Another point of interest is to have some idea about the intermediates involved in the reactions (CVII) \( \rightarrow \) (CIX), (CCLXXXVIII) \( \rightarrow \) (CCXII) and (CXX) \( \rightarrow \) (CCXCII).
In an attempt to rationalise the difference in the behaviour of the seco-acids (XCVIII) and (CVII) towards benzoyl chloride-pyridine, it can be suggested that a 3\(^\circ\)-acetoxy function in (XCVIII) plays an important role in directing the course of the reaction. From the proposed mechanism for the conversion (XCVIII) \(\rightarrow\) (CIII)\(^{54}\) it is obvious that the enolic form of (XCVIII) is not involved. If enolic form of (XCVIII) was involved then one would expect the formation of the enol lactone (XCIX) by an intramolecular process or an enol benzoate (CCCXV) by an intermolecular process. This however, was not found to be the case.
The suppression of the enolic form of (XCVIII) is understandable. The introduction of a C4-C5 double bond in the enolic form is likely to distort the normal chair conformation of ring A to a quasi chair form with increased 1,4-flagpole interaction between Cl0-substituents and 3β-acetoxy function. On the other hand, the seco-acid (CVII) without a 3β-acetoxy group can be enolized to a greater extent than (XCVIII), hence could lead to a different reaction path. An approximate estimation of the keto-enol components of the seco-acid methyl esters (CCCXVI) and (CI) was obtained by n.m.r. spectroscopy. The routine spectra were run in CDCl₃ (100 MHz), followed by addition of a few drops of pyridine to enolize the C5-keto function. (Enolization of the methyl ester carbonyl function was not expected to offer severe competition). Determination of the spectra in the presence of pyridine revealed the emergence of a new but very weak signal at δ 2.3 which disappeared on addition of D₂O. This new signal was thus assigned to enolic OH. On the other hand, similar experiments with the methyl ester (CI) showed the development of a new signal at δ 3.3 (disappeared on addition of D₂O), of much greater intensity than the one obtained in the case of (CCCXVI). The methyl signals of the ester group in both the esters (CCCXVI) and (CI) remained unaltered. (It must be pointed out that due to the added pyridine it was not possible to ascertain the vinylic proton signals in the enolic forms of the methyl esters.
Further, it was ensured that the new signals were not due to contaminant in the added pyridine by determining the n.m.r. spectrum of pyridine in CDCl₃. From these experiments it was possible to get an approximate estimate of the relative enolization tendencies of (CCCXVI) and (CI).

Thus it appears that the seco-acid (CVII) reacted with benzoyl chloride-pyridine to give initially the enol lactone (CXIII) and this under reaction conditions suffered decarboxylation to give B-norcholestene (CIX). This was substantiated by showing that the enol lactone (CXIII) readily changed to (CIX) when treated with benzoyl chloride-pyridine. The intermediacy of the enol lactone (CXIII) is supported in the conversion (CVII) → (CIX). Similar arguments can be advanced for the conversions (CCLXXXVIII) → (CCXCIII) via the dienol lactone intermediate (CCXC) and for (CXX) → (CCXCVII) via the enol lactone (CCXCI).

However, the mechanism of the conversion (CXIII) → (CIX) in benzoyl chloride-pyridine remains obscure.
Azasteroids

The Beckmann rearrangement and Schmidt reaction of steroidal ketoximes and ketones, respectively, are the two facile and widely employed methods for the preparation of azasteroids. An excellent review entitled "Synthesis of azasteroids using Beckmann rearrangement and Schmidt reaction" has appeared recently.

Several papers dealing with the preparation of azasteroids have appeared from these laboratories and these included azasteroids from 3α-halocholestan-6-ones (XXXI-XXXIII), 3α,5-cyclo-5α-cholestan-6-one (XXX), 3β-acetoxycholest-5-en-7-one (LXXVII), 3β-acetoxycholest-4-en-6-one (CLXVII), cholest-4-en-6-one (CXLII), cholesta-3,5-dien-7-one (LXXV), cholesta-4,6-dien-3-one (CLXXXIV), cholesta-2,4-dien-6-one (CLXXVII) and 4-bromocholest-4-en-3-one (CLXXXIV).

\[ (XXXI) X, \text{Cl} \quad (XXXII) X, \text{Br} \quad (XXXIII) X, \text{I} \]
The present work is an extension of the above and employed hitherto unexplored and yet easily accessible steroidal ketones, such as, methyl 5-keto-4,5-secocholestan-4-oate (CCLXXXVII), 5-keto-4,5-secocholestan-4-oic acid (CXX), methyl 5-keto-5,6-secocholestan-6-oate (CX) and 5-keto-5,6-secocholestan-6-oic acid (CVII).
Schmidt reaction of methyl 5-keto-4,5-secocholestan-4-oate (CCLXXXVII)

Schmidt reaction of (CCLXXXVII) using sodium azide and polyphosphoric acid or sodium azide and sulphuric acid in benzene gave, after usual work up and column chromatography, two crystalline products, m.pts. 155° and 135°. The homogeneity of the products was established by repeated crystallization and t.l.c. in different solvent systems.
Characterization of the compound, m.p. 155° as methyl 5-aza-6-keto-4,5-seco-B-homocholestan-4-oate (CCXCVI)

The compound, m.p. 155° analysed correctly for C_{28}H_{49}O_{3}N and its i.r. spectrum gave peaks at 3310w, 3230m, 3080m (NH), 1745s (COOCH₃) and 1652s cm⁻¹ (N-C=O). These values clearly indicated that the product is a lactam which can be formulated either as (CCXCVI) or (CCCXIX). Formulation of the compound, m.p. 155° as (CCXCVI), rather than its isomer, methyl 5-keto-6-aza-4,5-seco-B-homocholestan-4-oate (CCCXIX) was based upon n.m.r. values of the compound. The n.m.r. spectrum gave a singlet at δ 5.92 integrating for 1 proton and this signal disappeared on addition of D₂O (CO-N-H). Another singlet at δ 3.68 which integrated for 3 protons was assigned to COOCH₃. A broad signal centred at δ 2.3 integrating for 4 protons was assigned to CH₂COOCH₃ and N-CO-CH₂ as in the structure (CCXCVI). The C₁₀-CH₃ signal appeared at δ 1.35. The appearance of this signal at a lower field strongly supported the presence of CH₃-C-N moiety in the molecule. The other methyl signals were observed at 0.7, 0.82 and 0.96. As mentioned above, the signal
at $^6$ 5.92 disappeared on addition of D$_2$O but other parts of
the spectrum remained unchanged which showed the absence of
any vicinal protons about N-H. In the isomeric structure
(CCCXIX), one would have expected a broad signal for NH
(CONH-CH$_2$-) and a multiplet in the region $^6$ 3-3.5 for N-CH$_2$-CH$_2$.
Further, the C10-methyl signal would have appeared at a rela­
tively high field at about 1.0. These spectral data, therefore,
unambiguously support the structure (CCXCVI) for the compound,
m.p. 155$^\circ$.

Characterization of the compound, m.p. 135$^\circ$ as 5-aza-6-keto-4,
5-seco-5-homocholestan-4-oic acid (CCCXVIII)

The compound, m.p. 135$^\circ$ analysed correctly for C$_{27}$H$_{47}$O$_3$N
and from the composition it was suspected that it could be the
product of hydrolysis of (CCXCVI) (Me-0-CO-R $\rightarrow$ HOOC-R). The
i.r. spectrum gave a broad peak in the region 3250-3400 (COOH)
with merging peaks at 3230m, 3100m (NH), 1705s (COOH) and
1655s cm$^{-1}$ (CONH). The n.m.r. spectrum gave a broad signal
centred at $^6$ 6.8 integrating for two protons (COOH and CONH -
rapidly changing protons between themselves). This broad peak
disappeared on addition of D$_2$O. A broad peak centred at $^6$ 2.35
integrating for 4 protons was assigned to HOOC-CH$_2$- and NCOCH$_2$-;
other signals were observed at $^6$ 1.34s (3 protons; C10-CH$_3$),
0.68s (3 protons, C13-CH$_3$), 0.82 and 0.94 (other methyl signals).
Using the preceding arguments, this compound, m.p. 135° can be formulated as (CCCXVIII). Further confirmation to this effect was obtained when (CCCXVIII) was readily converted to (CCXCVI) by diazomethane.

![Chemical structure](image)

**Oximation of 5-keto-4,5-secocholestan-4-oic acid (CXX)**

The seco-acid (CXX) with hydroxylamine hydrochloride and potassium hydroxide in refluxing ethanol afforded the corresponding oxime (CCCXXX), m.p. 163°. Its homogeneity was established by repeated crystallizations, column chromatography and t.l.c. in different solvent systems ($C_{27}H_{47}O_3N$); ν max 3300br (COOH and N-OH), 1700s (COOH) and 1650w cm$^{-1}$ (C=N-O-); δ 7.6br (2 protons, exchangeable with deuterium, COOH and C=N-OH; 1.06s (3 protons, C10-CH$_3$), 0.68s (3 protons, C13-CH$_3$), 0.9 and 0.8 (other methyl protons).
Beckmann rearrangement of the oxime (CCCXX)

The oxime (CCCXX) on Beckmann rearrangement using thionyl chloride followed by alkali treatment gave a single lactam, 5-aza-6-keto-4,5-seco-D-homocholestan-4-oic acid (CCCXVIII), m.p. and mixed m.p. 135°. This on treatment with diazomethane gave (CCXCVI), m.p. and mixed m.p. 155°.
Oximation of methyl 5-keto-4,5-secocholestan-4-oate (CCLXXXVII)

The compound (CCLXXXVII) on oximation with hydroxylamine hydrochloride and sodium acetate in boiling ethanol furnished the oxime (CCCXXI), m.p. 87°. This was also shown to be a single entity by repeated crystallization, column chromatography and t.l.c. in different solvent systems, \( \text{C}_{28} \text{H}_{49} \text{O}_{3} \text{N} \); \( \nu_{\text{max}} \) 3400s (N-OH), 1743s (C=O), and 1655w cm\(^{-1}\) (C=N-0); \( \delta \) 7.2br (1 proton exchangeable with deuterium, C=N-OH); 3.58s (3 protons, COOCH\(_3\)), 1.02s (3 protons, C10-CH\(_3\)), 0.68s (3 protons, C13-CH\(_3\)), 0.91 and 0.8 (other methyl protons). The same oxime (CCCXXI) was also obtained when (CCCXX) was treated with diazomethane (m.p. and mixed m.p. 87°).

Beckmann rearrangement of the oxime (CCCXXI)

Beckmann rearrangement of the oxime (CCCXXI) with thionyl chloride followed by alkali treatment gave the lactams (CCXCVI) (major) and (CCCXVIII), m.p. and mixed m.p.s. 155° and 135°, respectively.

\[ \text{CH}_3\text{COO} \rightarrow \begin{array}{c} \text{NH}_2\text{OH} \\ \text{CH}_3\text{COO} \end{array} \]

\[ \text{CCXXXVII} \rightarrow \text{CCCXXI} \]

\[ \text{CH}_2\text{N}_2 \rightarrow \begin{array}{c} \text{HOOC} \\ \text{HOOC} \end{array} \]

\[ \text{CCCXX} \]

\[ \downarrow \]

\[ \text{CCXCVI} + \text{CCCXVIII} \]
The exclusive formation of the lactams (CCXCVI) and (CCCXVIII) from the oxime (CCCXXI) or of (CCCXVIII) from (CCCXX) clearly showed that the OH group in these oximes, (CCXCXI) and (CCCXX), is away from the more substituted carbon (C10) and this could be rationalised in terms of steric factor. Generally the ring A ketoximes having no bulky $\alpha$-substituents are obtained as a mixture of two isomeric oximes and these naturally lead to two isomeric lactams when subjected to Beckmann rearrangement. On the other hand, C6-ketoximes (ring B ketoximes) exist in only one form in which the OH group (of N-OH system) is away from the more substituted C5- and thus give only one lactam in each case.

Interestingly, attempted Schmidt reaction of methyl 5-keto-5,6-secocholest-6-oate (CX) did not give the corresponding lactam, such as (CCCXXII). Similarly, the oximes (CCCXXIII) and (CCCXXIV) could not be obtained from the respective ketonic compounds (CX) and (CVII).

\[ \text{(CCCXXIII) } R, \text{ CH}_3 \quad \text{(CX) } R, \text{ CH}_3 \]
\[ \text{(CCCXXIV) } R, \text{ H} \quad \text{(CVII) } R, \text{ H} \]

The failure of (CX) to undergo Schmidt reaction or oximation once again supported the view that C5-keto function in (CX) is much more crowded than the C5-keto function in (CCLXXXVII).
Mass Spectral Studies on Steroidal Compounds

A. Rings B and A Seco-5-Keto Compounds in the Cholestane Series

The mass spectra of several structurally related rings B and A seco-5-keto compounds, 5-keto-5,7-seco-6-norcholstan-7-oic acid (LXXXIV), methyl 5-keto-5,7-seco-6-norcholstan-7-oate (LXXXIV a), 5-keto-5,6-secocholestan-6-oic acid (CVII), its methyl ester (CX), 3β-acetoxy-5-keto-5,6-secocholestan-6-oic acid (XCVIII), 5-keto-5,7-seco-6-norcholest-3-en-7-oic acid (LXXXII), methyl 5-keto-5,6-secocholest-3-en-6-oate (CCLXXXIX) and 5-keto-4,5-secocholestan-4-oic acid (CXX) have been examined.
The fragmentation pathways suggested are supported by accurate mass measurements of the salient fragment ions and in some cases by appropriate metastable peaks. In the absence of mass spectra of appropriate deuterated analogues of the compounds under study, these mechanisms should be considered with some reservations.

The mass spectrum of (LXXXIV) (Fig. 1) gave molecular ion peak at m/e 404 \((\text{C}_{26}\text{H}_{44}\text{O}_3)\) followed by other significant peaks at m/e 386 \((\text{M}-\text{H}_2\text{O})\), m/e 376 \((\text{M}-\text{CO})\), m/e 371 \((\text{m/e 386}-\text{CH}_3)\), m/e 360 \((\text{M}-\text{CO}_2)\), m/e 358 \((\text{m/e 386}-\text{CO})\), m/e 343, m/e 319, m/e 275, m/e 273, m/e 264, m/e 247, m/e 245, m/e 233, m/e 231, m/e 163, m/e 161, m/e 135, m/e 133, m/e 121, m/e 119, m/e 112 (base peak) and lower mass peaks.

The formation of some of the salient fragment ions has been proposed to arise according to schemes given below.

\textbf{m/e 386}

The fragment ion m/e 386 represents the loss of a molecule of water from the molecular ion and its composition, \(\text{C}_{26}\text{H}_{42}\text{O}_2\), supports this assumption. The loss of a water molecule can be conveniently shown by involving \(\text{OH}\) portion of the \(\text{COOH}\) group and an appropriate hydrogen, preferably from relative position 4 from \(\text{COOH}^{122}\); although ketones as such are also known to give a molecule of water under electron-impact\(^{122}\).
Figure 1 Mass Spectrum of (LXXXIV)
There are only two positions (C11) and (C15), available from where hydrogen can participate in 1,4-elimination of water molecule. As an illustration, C11-H has been shown to participate in this elimination and this preference will become clear while considering the genesis of other fragment ions.

Scheme - 39a

\[
\text{Scheme - 39a}
\]

\[
\text{LXXXIV'}
\]

\[
\text{m/e 376 (M-CO: C}_{25}\text{H}_{44}\text{O}_{2})
\]

The loss of CO from ketones is of common occurrence and in this case it can be shown to occur according to the following scheme.
The fragment ion m/e 371 can be shown to arise by the loss of a methyl group from the ion m/e 386.

m/e 360 (M-CO₂; \( \text{C}_{25}\text{H}_{44}\text{O} \))

The composition of this fragment ion suggests that a molecule of CO₂ is eliminated from the molecular ion and this can be shown according to Scheme 39c.
The fragment ion m/e 358 obviously results by the loss of a molecule of carbon monoxide from the fragment ion m/e 386. This is supported by a pronounced metastable peak at m/e 332.
\[ m/e \ 275 \ (C_{19}H_{31}O) \text{ and } m/e \ 247 \ (C_{18}H_{31}) \]

The fragment ion \( m/e \ 275 \) arises from the ion \( m/e \ 386 \) as indicated by a prominent metastable peak at \( m/e \ 195.9 \). The fragment ion \( m/e \ 247 \), in turn arises from the ion \( m/e \ 275 \) as supported by a metastable peak at \( m/e \ 221.8 \). Both these ions are shown to arise according to Scheme 39e.

Scheme - 39e

\[ \text{m/e 386} \quad \text{m/e 275} \quad \text{m/e 247} \]

\[ (C_{19}H_{31}O) \quad (C_{18}H_{31}) \]

\[ m/e \ 112 \ (C_{7}H_{12}O) \]

The fragment ion peak at \( m/e \ 112 \) constitutes the base peak of the spectrum, which apparently results from McLafferty rearrangement involving C5-keto function and an appropriate \( \gamma \)-hydrogen either from C8 or C11 or from both. In the absence of appropriate deuterated analogue of (LXXXIV), the relative contribution of C8 and C11 hydrogens could not be ascertained. The genesis of this fragment ion can be shown according to Scheme 39f.
In order to get an idea about the relative distance between C5-keto group and a $\gamma$-hydrogen at C8 or C11, the Dreiding model of (LXXXIV) was examined. At the ground state the distance between the oxygen of the C5-keto function and C8 and C11 hydrogens was much more than the distance required for McLafferty rearrangement to be effective, i.e. more than 1.8Å. However, free rotation about C9-C10 brought both C8 and C11 hydrogens inside 1.8Å from the oxygen at C5-keto function.

The mass spectrum of the methyl ester (LXXXIV-a)(Fig. 2) gave molecular ion peak at m/e 418 ($C_{27}H_{46}O_{3}$) along with other peaks at m/e 400 ($M-H_2O$), m/e 390 ($M-CO$), m/e 387 ($M-OCH_3$), m/e 396 ($M-CH_3OH$), m/e 374 ($M-CO_2$), m/e 371 ($m/e 386-CH_3$), m/e 359 ($m/e 387-CO$), m/e 358 ($m/e 386-CO$), m/e 343 ($m/e 358-CH_3$), m/e 333($M-C_6H_{13}$), m/e 331, m/e 315, m/e 309, m/e 307,
m/e 287, m/e 275 \((C_{19}H_{31}O)\), m/e 247 \((C_{18}H_{31})\), m/e 245, m/e 231, m/e 219, m/e 217, m/e 193, m/e 166, m/e 163, m/e 161, m/e 151, m/e 149, m/e 147, m/e 135, m/e 133, m/e 121, m/e 119, m/e 112 (base peak, \(C_7H_{12}O\)) and lower mass peaks. The mass spectrum of \((LXXXIV-a)\) can easily be related to that of \((LXXXIV)\). A few variations have also been noted and a brief comment on them will not be out of place. Some of the fragment ions have been suggested to arise according to schemes given below. Fragmentations already described in scheme 39 have been avoided unless required to bring out some points of difference between \((LXXXIV)\) and \((LXXXIV-a)\).

m/e 387 and m/e 386 \((C_{26}H_{42}O_2)\)

The fragment ion m/e 387 results by the loss of methoxyl group from the molecular ion; further loss of CO will give the ion m/e 359.

Scheme - 40a
The formation of the fragment ion m/e 386 can be shown to arise by the loss of a molecule of methanol from the molecular ion which is analogous to the loss of a molecule of water from the molecular ion of (LXXXIV).

Scheme - 40b

(LXXXIV-a")

m/e 386

It should be pointed out that the loss of methyl alcohol from (LXXXIV-a) in relation to the loss of water from (LXXXIV) is a slower process as is evident from the peak ratio of m/e 386 and molecular ions. In the methyl ester (LXXXIV-a) the ratio between molecular ion and ion peak m/e 386 is 1:2 whereas in the case of the seco-acid (LXXXIV) it is 1:16.

m/e 374

Apparently the ion m/e 374 (analogous to ion m/e 360 from (LXXXIV) occurs by the loss of CO₂ from the molecular ion, and such a loss, though not commonly encountered, can be shown according to Scheme 40c.
The fragment ion m/e 307 with the composition $C_{20}H_{35}O_2$ indicated the loss of ring A along with C10-CH$_3$ group. This has been shown according to Scheme 40d.
The formation of the ion m/e 307 shows that the loss of methyl alcohol is a slower process; no analogous peak at m/e 293 was observed in the spectrum of (LXXXIV).

\[ \text{m/e 275 (C}_{19}\text{H}_{31}0) \]

The ion m/e 275 in this case can arise, in principle, from the ion m/e 386 \((m^* \text{ 195.9})\) as well as from the ion m/e 307 by the loss of methyl alcohol.

Scheme - 40e

Fragment ions m/e 343, m/e 333 and m/e 247 can be shown to arise by the loss of methyl group from the ion m/e 358, by the loss of \(\text{C}_{6}\text{H}_{13}\) (mass unit 85) from the molecular ion and by the loss of CO from m/e 275, respectively.
The fragment ion peak at m/e 112 \((C_7H_{12}O)\) constitutes the base peak of the spectrum as in the case of (LXXXIV) and its formation need not be repeated here.

The mass spectrum of (CVII)(Fig. 3) gave molecular ion peak at m/e 418 \((C_{27}H_{46}O_3)\) followed by other peaks at m/e 400 \((M-H_2O)\), m/e 390 \((M-CO)\), m/e 385 \((m/e 400-CH_3)\), m/e 374 \((M-CO_2)\), m/e 372 \((M-CO + H_2O)\), m/e 359 \((M-CH_2COOH, C_{25}H_{43}O)\), m/e 344, m/e 341, m/e 333, m/e 318, m/e 315, m/e 314, m/e 306, m/e 305, m/e 291, m/e 289, m/e 287, m/e 277, m/e 273, m/e 265, m/e 261, m/e 247, m/e 231, m/e 217, m/e 193, m/e 166, m/e 153, m/e 135, m/e 133, m/e 121, m/e 119, m/e 112 (base peak, \(C_7H_{12}O\)) and lower mass peaks. The mass spectrum of (CVII) can be easily related to that of (LXXXIV). However, some points of difference have also been observed and these have been commented upon.

\(m/e 400\) \((M-H_2O)\), m/e 372, m/e 289, m/e 261 and m/e 247

\(m/e 400\)

The formation of this fragment ion can be shown by 1,4-elimination (cf Scheme 39a) and the ion m/e 372 can be shown to be derived from it. Alternatively, 1,5-elimination of water from the molecular ion will give the ion m/e 400 and this can conveniently lead to the ions m/e 372, m/e 289, m/e 261, and m/e 247.
Figure 3 Mass Spectrum of (CVII)

$C_{27}H_{46}O_3$

$M^+ 418$
Scheme 41a

1,5-Elimination →

(CVII')

\[ m/e 400 \]

\[ m/e 372 \]

\[ m/e 289 \]

\[ m/e 261 \]

\[ m/e 247 \]
m/e 359 (C_{25}H_{33}O)

This fragment ion is compatible with the loss of CH_2COOH from the molecular ion; there is no rationale for such a loss from (LXXXIV) and (LXXXIV-a).

Scheme - 41b

m/e 333, m/e 318 and m/e 305

The fragment ion, m/e 333 represents the loss of C_6H_{13} (mass unit 85) from the molecular ion. Further loss of a methyl group will account for the ion m/e 318. The ion m/e 305 can be shown to arise by the loss of the side chain (C_8H_{17}) from the molecular ion.
The fragment ion peak at m/e 112 constitutes the base peak of the spectrum.

The mass spectrum of the methyl ester (CX) (Fig. 4) gave molecular ion peak at m/e 432 \((\text{C}_{26}\text{H}_{48}\text{O}_{3})\) along with other peaks at m/e 414 \((\text{M-}\text{H}_{2}\text{O})\), m/e 401 \((\text{M-}\text{OCH}_{3})\), m/e 400 \((\text{M-}\text{CH}_{3}\text{OH})\), m/e 399 \((\text{m/e 414-CH}_{3})\), m/e 338 \((\text{M-}\text{CO}_{2})\), m/e 372 \((\text{M-}\text{CH}_{3}\text{OH + CO})\), m/e 359 \((\text{M-}\text{CH}_{2}\text{COOH}_{3})\), m/e 355, m/e 321, m/e 319 \((\text{M-}\text{C}_{3}\text{H}_{17})\), m/e 289, m/e 261, m/e 247, m/e 231, m/e 217, m/e 207, m/e 189, m/e 167, m/e 163, m/e 161, m/e 149, m/e 147, m/e 145, m/e 135, m/e 133, m/e 121, m/e 119, m/e 112 (base peak) and lower mass peaks. The spectrum of (CX) can be related to (CVII) and (LXXXIV-a). In this case also the loss of methyl alcohol seems to be a slower process than the analogous loss of water from (CVII) and this is reflected by the ratio of molecular ions and m/e 400 peaks. Further a peak at m/e 321 in (CX) finds no analogous peak at m/e 307 in (CVII). Excepting for these variations the spectra of (CX), (CVII) and (LXXXIV-a) are very similar.

**Scheme - 42a**
The mass spectrum of (XCVIII) (Fig. 5) gave no molecular ion peak and the highest mass peak was observed at m/e 416 ($C_{27}H_{44}O_3$). The other peaks were observed at m/e 411 (m/e 416-CH$_3$), m/e 398 (m/e 416-H$_2$O), m/e 370 (m/e 398-CO), m/e 375 (m/e 416-CH$_2$COOH), m/e 331 (m/e 416-C$_6$H$_{13}$), m/e 305, m/e 273, m/e 247, m/e 193, m/e 179, m/e 177, m/e 175, m/e 166, m/e 135, m/e 133, m/e 121, m/e 119, m/e 110 (base peak, $C_7H_{10}O$), m/e 68 ($C_4H_4O$) and lower mass peaks.

m/e 416 (M-CH$_3$COOH, $C_{27}H_{44}O_3$)

The fragment ion m/e 416 results by the loss of a molecule of acetic acid from the molecular ion; acetates usually eliminate acetic acid by 1,2-elimination process. There are two possibilities in this case, i.e., it may involve a C2-hydrogen or C4-hydrogen together with C3-acetate group; in the latter case a more stabilized system would result.

Scheme - 43a

-ACOH

(XCVIII')

m/e 416
Figure 5  Mass Spectrum of (XCVIII)
The other peaks can be rationalized by comparison with the spectrum of (CVII). The base peak appeared at m/e 110 (C\textsubscript{7}H\textsubscript{10}O) which is analogous to m/e 112 in the spectra of the preceding compounds.

\textbf{m/e 110 (C\textsubscript{7}H\textsubscript{10}O)} \hspace{1cm} \textbf{Scheme - 43b}

\[ \text{Scheme - 43b} \]

\[ \begin{array}{c}
\text{m/e 416} \\
\text{m/e 110} \\
\text{m/e 68 (C\textsubscript{4}H\textsubscript{4}O)}
\end{array} \]

This fragment ion can be shown to arise from the ion m/e 110 according to Scheme 43c.

\textbf{Scheme - 43c}

\[ \begin{array}{c}
\text{m/e 110} \\
\text{m/e 68}
\end{array} \]
The mass spectrum of (LXXXII) (Fig. 6) gave molecular ion peak at m/e 402 \((C_{26}H_{42}O_3)\) along with other peaks at m/e 334 \((M-H_2O)\), m/e 369 \((m/e 334-CH_3)\), m/e 356 \((m/e 334-CO)\), m/e 317 \((M-C_6H_{13})\), m/e 299 \((m/e 317-H_2O)\), m/e 282, m/e 289 \((m/e 317-CO)\), m/e 275, m/e 247, m/e 233, m/e 231, m/e 207, m/e 205, m/e 179, m/e 177, m/e 163, m/e 161, m/e 149, m/e 135, m/e 133, m/e 123, m/e 121, m/e 119, m/e 110 (base peak), m/e 68 and lower mass peaks.

The mass spectrum of (CCLXXXIX) (Fig. 7) gave molecular ion peak at m/e 430 \((C_{28}H_{46}O_3)\) followed by other peaks at m/e 415 \((M-CH_3)\), m/e 412 \((M-H_2O)\), m/e 399 \((M-OCH_3)\), m/e 398 \((M-CH_3OH)\), m/e 383 \((m/e 398-CH_3)\), m/e 370 \((M-CH_3OH+CO)\), m/e 357 \((M-CH_2COOCH_3)\), m/e 321, m/e 320, m/e 319, m/e 305, m/e 289, m/e 261, m/e 247, m/e 207, m/e 205, m/e 180, m/e 175, m/e 167, m/e 165, m/e 163, m/e 161, m/e 149, m/e 147, m/e 135, m/e 133, m/e 121, m/e 119, m/e 110 (base peak, \(C_7H_{10}O\)), m/e 68 \((C_4H_4O)\) and lower mass peaks.

The spectra of the compounds (LXXXII) and (CLXXXIX) can be correlated with each other and in turn these can be related to (LXXXIV).

The mass spectrum of the seco-acid (CXX) (Fig. 8) gave molecular ion peak at m/e 418 \((C_{27}H_{46}O_3)\) along with other ion peaks at m/e 403 \((M-CH_3)\), m/e 400 \((M-H_2O)\), m/e 385 \((m/e 400-CH_3/m/e 403-H_2O)\), m/e 332 \((C_{23}H_{40}O)\), m/e 317 \((m/e 332-CH_3)\),
Figure 6. Mass Spectrum of (LXXXII)

C_{26}H_{42}O_3 (M+402)

C_{8}H_{7}

Relative Intensity

m/e
Figure 8 Mass Spectrum of (CXX)
m/e 314 (m/e 332-H_{2}O), m/e 305 (M-C_{8}H_{17}) and lower mass peaks. The isomeric acids (CXX) and (CVII) show considerable difference and some of the pertinent ones have been discussed.

m/e 332 (C_{23}H_{40}O)

The peak at m/e 332 is very intense and its composition as determined by accurate mass measurement was found to be (C_{23}H_{40}O). From the composition it appears that the carboxylic bearing side chain is lost through McLafferty rearrangement as shown in Scheme 44a.

Scheme - 44a

Interestingly the loss of CO and CH_{2}COOH directly from the molecular ion was not observed in this case in contrast to the isomeric acid (CVII).
It is pertinent to mention that the carboxylic or ester carbonyl in (CVII), (XCIII), (CX), (CCLXXXIX) or (CXX) does not seem to take part in the McLafferty rearrangement as is evident from the absence of a significant peak at m/e 60 or m/e 74.

From the above discussion it can be concluded that the mass spectra of (LXXXIV), (LXXXIV-a), (CVII) and (CX) are conspicuous by an intense peak at m/e 112 \((C_7H_{12}O)\) and those of (XCIII) (highest mass peak at \(M-\text{CH}_3\text{COOH}\)), (LXXXII), and (CCLXXXIX) by a prominent peak at m/e 110 \((C_7H_{10}O)\) which apparently results from McLafferty rearrangement involving C5-keto function and an appropriate \(\gamma\)-hydrogen at C8 and C11. This rearrangement leading to aforementioned ions could be of diagnostic value in the characterization of such compounds.

The presence of different substituents at C8 does not seem to change the fragmentation pattern, as is illustrated by the following example.
B. Ring B Enol Lactones

The mass spectra of 6-oxa-B-homocholest-4-en-7-one (CXIII) and 3β-acetoxy-6-oxa-B-homocholest-4-en-7-one (XCIX) have been examined in detail and comparison has been made with the mass spectrum of 6-oxa-B-homocholestan-7-one (XXVIII). The mass spectrum of (XXVIII) has already been described in some length earlier.

The mass spectrum of (CXIII) (Fig. 9) gave molecular ion peak at m/e 400 (C₂₇H₄₄O₂) with other peaks at m/e 385 (M-CH₃), m/e 372 (M-CO and M-C₂H₄), m/e 357 (m/e 372-CH₃), m/e 354 (m/e 372-H₂O), m/e 343 (m/e 385-42), m/e 339 (m/e 354-CH₃), m/e 330 (m/e 372-42), m/e 329, m/e 289, m/e 237, m/e 275, m/e 259, m/e 247, m/e 245, m/e 241, m/e 217, m/e 215, m/e 112, m/e 111 and lower mass peaks. The spectrum was conspicuous by the absence of ion peaks at m/e 382 (M-H₂O) and m/e 318 (base peak of XXVIII).

The formation of some of the fragment ions has been rationalised in Schemes given below.
Figure 9 Mass Spectrum of (CXIII)
The fragment ion peak at m/e 372 is very intense and accurate mass measurement showed that this is composed of two entities of the compositions $C_{26}H_{44}O$ (M-CO) and $C_{25}H_{40}O_2$ (M-CH$_2$=CH$_2$, 70%).

Scheme - 45a

(CXIII')
The analogous fragment ion from (XXVIII) readily lost ring A (Scheme 29) to provide the ion m/e 318 (base peak). As there is no rationale for such a break down in this case, no ion at m/e 318 was observed.

\[ \text{M}-\text{C}_2\text{H}_4(\text{C}_{25}\text{H}_{40}\text{O}_2) \]

The loss of a molecule of ethylene from the molecular ion can be shown to occur according to Scheme 45b.

**Scheme - 45b**

![Diagram of Scheme 45b](image)

\[ \text{m/e 372 } (\text{C}_{25}\text{H}_{40}\text{O}_2) \]
m/e 330 \( (C_{23}H_{38}O) \) and m/e 329

The fragment ion m/e 330 has been shown to occur by the loss of a molecule of ketene from the ion m/e 372. This is supported by a metastable peak at m/e 292.7.

Scheme - 45c

\[
\begin{align*}
\text{m/e 372} & \quad (C_{25}H_{40}O) \\
\text{m/e 330} & \quad (C_{23}H_{38}O) \\
\text{m/e 329} &
\end{align*}
\]

m/e 289 and m/e 247  
Scheme - 45d

\[
\begin{align*}
(C_{\text{XIII}}) & \\
\text{m/e 289} &
\end{align*}
\]
m/e 287, 259 and 241

These fragment ions represent the loss of the side chain (C\textsubscript{8}H\textsubscript{17}) from the molecular ion, the ion m/e 372 and the ion m/e 354, respectively.

m/e 112 (C\textsubscript{7}H\textsubscript{12}O) and m/e 111 (C\textsubscript{7}H\textsubscript{11}O)

Both the ion peaks are fairly intense and their genesis can be shown according to Scheme 45e.

Scheme - 45e

(CXIII*) m/e 112

m/e 111
The mass spectrum of (XCIX) (Fig. 10) gave molecular ion peak at m/e 458 (C_{29}H_{46}O_{4}) with other peaks at m/e 416 (M-CH_2=CH=O), m/e 415, m/e 414, m/e 399 (M-CH_3COO), m/e 398 (M-CH_3COOH), m/e 384 (m/e 399-CH_3), m/e 383 (m/e 398-CH_3), m/e 370 (m/e 398-CO), m/e 355 (m/e 370-CH_3), m/e 354, m/e 352, m/e 289, m/e 285, m/e 247, m/e 110, m/e 109 and lower mass peaks. The formation of some of the salient fragment ions can be shown to arise according to schemes given below.

m/e 416 (M-CH_2=CH=O)

The formation of this fragment ion m/e 416 (C_{27}H_{44}O_{3}) can be shown in two different ways - a molecule of ketene can be ejected either from the acetate function or from the ε-lactone moiety or from both.

Scheme - 46a
Figure 10  Mass Spectrum of (XCIX)
m/e 399 (M-CH$_3$COO)  

m/e 110 (C$_{17}$H$_{10}$O) and m/e 109

These two fragment ions can be shown as arising from the ion m/e 398.
C. **Saturated \( \epsilon \)-Lactones**

The mass spectra of three isomeric \( \epsilon \)-lactones, methyl 5a-oxa-5-keto-5,6-seco-A-homocholestan-6-oate (CCCII), methyl 5-oxa-6-keto-4,5-seco-B-homocholestan-4-oate (CCCVI) and methyl 6-oxa-5-keto-4,5-seco-B-homocholestan-4-oate (CCCVIII) have also been examined.

![Chemical structures](image)

The mass spectrum of (CCCII) (Fig. 11) gave molecular ion peak at m/e 448 \((\text{C}_{28}\text{H}_{48}\text{O}_{4})\) along with other peaks at m/e 433 \((\text{M-CH}_3)\), m/e 430 \((\text{M-H}_2\text{O})\), m/e 420 \((\text{M-CO})\), m/e 417 \((\text{M-CH}_3\text{O})\), m/e 416 \((\text{M-CH}_3\text{OH})\), m/e 402 \((\text{m/e 417-CH}_3)\), m/e 401 \((\text{m/e 416-CH}_3)\), m/e 363 \((\text{M-C}_6\text{H}_{13})\), m/e 289, m/e 247, m/e 128 \((\text{C}_7\text{H}_{12}\text{O}_2)\), m/e 127 (base peak, \(\text{C}_7\text{H}_{11}\text{O}_2\)), m/e 126 and lower mass peaks. The formation of some of the fragment ions, which throw light on the structure, has been considered in the schemes below.
Figure 11: Mass Spectrum of (CCCII)

Molecular formula: C_{28}H_{48}O_{4}

Mass values:
- 127 (C_{7}H_{10}O_{2})
- 128 (C_{7}H_{12}O_{2})
- 448 (M^+448)
- 433 (M-H_2O)
- 430 (M-H_2O)
- 417 (M-OCH_3)
- 410 (M-OCH_3)

Relative Intensity:
- 126
- 121
- 107
- 95
- 81
- 57
- 55
- 440
- 460
m/e 433

The fragment ion m/e 433 can be best represented by the loss of a methyl group from C10 which is next to oxygen of the lactone moiety.

**Scheme - 47a**

[Diagram showing the reaction involving m/e 433]

m/e 128 (C_{7}H_{12}O_{2})

The ion peak at m/e 128 is very intense and its genesis can be rationalised according to Scheme 47b.

**Scheme - 47b**

[Diagram showing the reaction involving m/e 128]
m/e 127 (C_{7}H_{11}O_{2}) and m/e 126

The ion peak at m/e 127 constitutes the base peak of the spectrum and seems to be of considerable diagnostic value. The formation of this ion can be shown by cleavage of C_{10}-C_{9} bond.

Scheme - 47c

\[
\text{(CCCII')} \quad \text{m/e 127} \quad \text{m/e 126}
\]

The formation of the other peaks, such as, m/e 420, m/e 417, m/e 416, m/e 289, and m/e 247 has not been discussed since the mechanisms involved in similar situations have already been discussed earlier.

The mass spectrum of (CCCVI)(Fig. 12) gave molecular ion peak at m/e 448 (C_{25}H_{48}O_{4}) with other peaks at m/e 433 (M-CH_{3}), m/e 430 (M-H_{2}O), m/e 420 (M-CO), m/e 417 (M-OCH_{3}), m/e 416 (M-CH_{3}OH), m/e 402 (m/e 417-CH_{3}), m/e 401 (m/e 416-CH_{3}), m/e 363 (M-C_{6}H_{13}), m/e 347 (base peak, C_{23}H_{39}O_{2}), m/e 335 (M-C_{8}H_{17}), m/e 332 (m/e 347-CH_{3}) and lower mass peaks. The
isomeric lactones (CCCI) and (CCCVI) can easily be distinguished by mass spectrometry. The formation of the ion m/e 347 is of considerable diagnostic value and its genesis can be rationalised according to Scheme 48a.

Scheme 48a

The mass spectrum of (CCCVII) (Fig. 13) gave molecular ion peak at m/e 448 (C$_{28}$H$_{48}$O$_{4}$) along with other peaks at m/e 433 (M-CH$_3$), m/e 430 (M-H$_2$O), m/e 420 (M-CO), m/e 417 (M-OCH$_3$), m/e 416 (M-CH$_3$OH), m/e 402 (m/e 417-CH$_3$), m/e 401 (m/e 416-CH$_3$), m/e 363 (M-35), m/e 348, m/e 335 (M-C$_8$H$_{17}$), m/e 333 (m/e 348-CH$_3$) and lower mass peaks. A distinction between closely related isomeric lactones (CCCVI) and (CCCVIII) was thus made possible by mass spectrometry. As was expected, the loss of methyl group in this case (CCCVIII) was not as pronounced as in the case of (CCCVI) or (CCCI). Further there is no rationale for the loss of carboxylate bearing side chain and therefore no peak at m/e 347 was observed.
The fragment ion m/e 348 apparently resulted by McLafferty rearrangement involving the carbonyl oxygen of the lactone moiety and C2-hydrogen.

Scheme - 49a

From the foregoing observations it has become clear that mass spectrometry offers an excellent means of differentiating between these closely related compounds.