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Mass-spectrometric Determination of the Configuration of Steroid Alcohols

N.S.Vul'fson, V.I.Zaretskii, and V.G.Zaikin

A large number of epimeric secondary alcohols of the 3-, 11-, 12-, 14-, 15-, 16-, 17-, 20-, and 24-hydroxy-steroid series, derivatives of androstane, pregnane, or cholestane, and also tertiary alcohols of the oestrane series which are epimeric at $C_{(17)}$ show that, in all cases in which the hydroxyl is situated in a conformationally sufficiently rigid part of the steroid skeleton, mass spectrometry can be used to establish the configuration of the steroid alcohols. The main conclusions can be drawn on the basis that, in most cases of electron impact an axial hydroxyl is split off more readily than is an equatorial hydroxyl, as a consequence of the possibility of a mechanism of 1,3- or 1,4-*cis*-diaxial elimination of the elements of water. A list of 31 references is included.

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1. INTRODUCTION

Mass spectrometry, which is widely employed in solving structural problems of organic chemistry and especially the chemistry of naturally occurring compounds, has hitherto found only limited application to the solution of stereochemical problems. The primary reason is that aliphatic stereoisomers differ little if at all in internal energy. As a consequence the mass spectra of optical enantiomers are usually identical not only to each other, but also to the spectrum of their racemic mixture[1]. The presence of two or more asymmetric carbon atoms is sometimes sufficient to produce differences, though slight, in the mass spectra of the diastereomers. Thus Ryhage and Stenhagen[2] observed slight differences in the mass spectra of dimethyl *meso*- and (−)-3,4-dimethyladipates (I) and methyl 2L, 9L- and 2L, 9D-dimethyltetracosates (Ia)

$$MeOCOCH_2CHMeCHMeCH_2COOMe \quad (I);$$

$$Me[CH_2]_{14}CHMe[CH_2]_6CHMeCOOMe \quad (Ia).$$

As could be expected, alicyclic compounds exhibit sharper differences in the mass spectra of stereoisomers, which are larger the greater the conformational stability of the cyclic system. The difference in internal energy between epimeric molecules may depend significantly on the nature of the substituent, its spatial arrangement, and the character of non-bonded interactions, and this in turn leads to variation in the intensities of the characteristic peaks in the mass spectrum.

Differences in the mass spectra of cyclic epimers are due primarily to variation in the ease of elimination of substituents at the asymmetric atom, a process often accompanied by ejection of a hydrogen atom (detachment of water, hydrogen sulphide, and acetic acid from alcohols, thiols, and acetates respectively). Therefore, although all modes of fragmentation of one epimer on electron impact are usually characteristic also of the other epimer, the intensities of the corresponding peaks ($M - CH_3$, $M - H_2O$, $M - H_2S$, $M - CH_3COOH$, etc.) are different. The extent to which the migrating hydrogen atom or group of atoms is involved in specific rearrangement of the given compound may be as important as the purely energetic factor. In this case the peak intensity of the corresponding rearranged ion usually depends on the distance between the hydrogen-donating and the hydrogen-accepting atoms.

The above discussion can be summarised in the conclusion that the differences in the way in which molecular ions of epimers break down on electron impact are governed ultimately by two factors—an energy factor and a so called "distance factor"[3,4].

In one of the earliest mass-spectrometric investigations of epimeric cyclic alcohols Biemann and Seibl[5] showed, with epimeric secondary alcohols of bicyclo[3,3,0]octan-2-ol, bicyclic terpenes, and steroids as examples, that the peak due to the molecular ion is less intense in the mass spectrum of the epimer having the more "compact" configuration than in the spectrum of the less "compact" epimer.

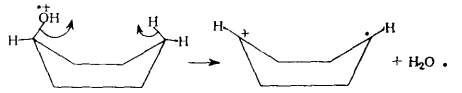
Before passing to a detailed examination of work on the mass spectrometry of steroid alcohols, we must first consider the mechanism of the dehydration of alcohols under conditions of mass spectrometry.

2. MECHANISM OF THE DEHYDRATION OF ALCOHOLS ON ELECTRON IMPACT

Momigny[6], McFadden et al.[7,8], Meyerson and Leitch[9], and also Benz and Biemann[10] showed, by means of deuterated analogues of straight-chain aliphatic alcohols, that in dehydration under the influence of electron impact ~ 90% of hydrogen atoms are lost from position 4: i.e. a mechanism of dehydration involving a six-membered transition state is preferred.

Natalis[11,12] was early able to show that all the deuterium atoms are retained when 2,2,5,5-tetradeuterocyclopentanol undergoes dehydration. It was suggested that 1,3-*cis*-diaxial elimination of water is the most probable process in the case of cycloalkanols. This question has been studied in greater detail by Shannon et al.[13] and by Djerassi et al.[14].

Investigation of 2,2,6,6-tetra- and 1,4,4-tri-deuterocyclohexanols showed[13] that the dehydration of the molecular ion of cyclohexanol (under conditions excluding random migration of hydrogen atoms) is effected by 1,3- and 1,4-elimination in the proportions 0.7 : 1. If the latter process does not involve preliminary opening of the ring, it must be assumed that the molecular ion of cyclohexanol has the boat conformation (we are concerned, of course, with the reactive conformation):

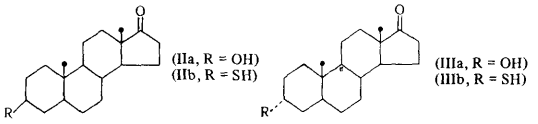


In this case *trans*-cyclohexane-1,4-diol should be more easily dehydrated than its *cis*-isomer on electron impact. In fact, the peak due to the $[M - DOH]^{+}$ ion in the spectrum of *trans*-1,4-dideuterocyclohexane-1,4-diol exceeds the corresponding peak in the spectrum of the *cis*-diol by a factor of 8.

By means of the mass spectra of deuterated cyclohexanols containing deuterium in various positions Djerassi[14] confirmed the suggested 1,3- and 1,4-mechanism for the elimination of water. However, his results indicated that the relative probabilities of the ejection of a hydrogen atom from positions 3 and 4 were 0.5 : 1.

3. 3-HYDROXY-STEROIDS

As remarked above, Biemann and Seibl[5] observed significant differences in the mass spectra of epimeric steroid alcohols. Thus the intensity ratio of the peaks $I_{M-H_2O}/I_{M^+} = 0.11$ for epiandrosterone (IIa) with an equatorial 3β-hydroxy-group, but the ratio is 0.19 for androsterone (IIIa) with a 3α-hydroxyl.

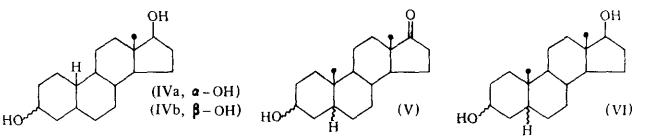


These workers found a similar but still more clearly defined difference in the mass spectra of the acetates of (IIa) and (IIIa), where elimination of a molecule of acetic acid occurs far more readily from the epimer having an axial acetoxy-group.

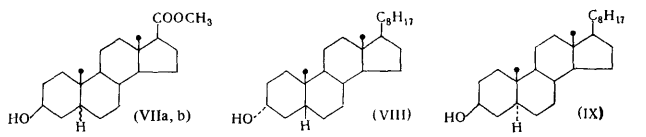
However, the work of Egger and Spiteller[15] casts about on the reliability of Biemann and Seibl's results. The mass spectra of epiandrosterone (IIa) and androsterone (IIIa), obtained by the later workers by direct introduction of the specimen into the ion source at 70°C, were completely identical, showing no difference in the intensity ratio of the peaks due to M^+ and $[M - H_2O]^{+}$ ions. Thermal effects obviously played a quite important part in Biemann and Seibl's experiment, since they used an instrument with a heated metallic admission system. Therefore their mass spectra represented a difference in thermal dehydration rather than in the degree of dehydration on electron impact.

An independent study of the mass spectra of the epimeric androsterones (IIa) and (IIIa) was made by Zaretskii and Zaikin[16]. They also detected no difference between the mass spectra of the epimers while using a system of direct introduction of the test material into the ion source† (at a temperature 20–30 deg below the melting point). Nor were differences evident in the mass spectra of their thio-analogues (IIb) and (IIIb). Thus the mechanism of the elimination of hydrogen sulphide from thiols on electron impact is evidently similar to the mechanism of the elimination of water.

In view of the results obtained by Shannon[13] and by Djerassi[14] we may suppose that the dehydration of androsterone (IIIa)[15,16], containing an axial hydroxyl, takes place mainly by the 1,3-type of elimination. On the other hand, the dehydration of epiandrosterone (IIa) may be accompanied by transition of ring A into the boat form, which evidently increases the probability of elimination of the 3β-hydroxyl, since hydrogen atoms from other rings, situated closest to the hydroxyl-group, may also be involved in the dehydration. The ultimate result is that the ratio I_{M-H_2O}/I_{M^+} is almost the same in the spectra of the 3α- and 3β-epimers. The spectra of 19-norandrostane-3α,17β- and -3β,17β-diols (IVa) and (IVb) provide some support for this view.



The peak due to the $[M - H_2O]^{+}$ ion is considerably larger in the spectrum of the 3α-hydroxy-epimer (IVa) than in that of the 3β-hydroxy-epimer (IVb)[15]. If it is assumed that on electron impact the ring A is in the boat form, conditions are favourable in the β-epimer for 1,4-elimination of water involving the 10β-hydrogen atom (which, incidentally, is absent from androstanes containing a 19-methyl group). In steroids having *cis*-fusion of rings A and B a hydrogen atom may be removed from $C_{(9)}$ (if ring A is also in the boat form). Such a possibility is not available to alcohols having *trans*-fusion of these rings, so that the 5β-alcohols undergo dehydration more easily than do their 5α-epimers. Nor are differences observed in the spectra of 3-hydroxyandrostan-17-ones (V) and of androstane-3,17β-diols (VI), whether of the 5β- or of the 5α-series, these compounds being epimeric at $C_{(3)}$.



However, compounds having the same configuration of the 3-hydroxyl but isomeric with respect to the fusion of rings A and B have significantly different spectra: dehydration under the influence of electron impact takes place more readily in the 5β-series. A more intense $M - H_2O$

† In all the work discussed below the mass spectra were obtained with direct entry of the specimen into the ion source.

peak and a less intense M^+ peak are observed also in the mass spectra of methyl 3β-hydroxy-5β-etianate (VIIa) and coprostan-3α-ol (VIII) in comparison with those of the stereoisomeric methyl 3β-hydroxy-5α-etianate (VIIb) and cholestan-3β-ol (IX) respectively[15].

The problem of elucidating the mechanism of the elimination of water on electron impact with epimeric cholestan-3-ols have been examined by Djerassi et al.[17] In order to exclude the effect of thermal dehydration preceding electron impact, these workers consider the dehydration of the $[M - CH_3]^+$ ion, not that of the molecular ion as is usually done. The mass spectra of the deutero-derivatives of cholestan-3α-ol showed (Table 1) that 73% of hydrogen atoms involved in formation of the ion $[M - CH_3 - H_2O]^+$ came from the 5α-position (ion a) and 7% from $C_{(1)}$ (ion a'). In the case of cholestan-3β-ol formation of the $[M - CH_3 - H_2O]^+$ ion involves the loss of 28% of hydrogen atoms from position 1 and 11% of 5α-hydrogen atoms (Table 2). The remaining hydrogen atoms involved in dehydration of the ion $[M - CH_3]^+$ are eliminated from other rings. If ring A is in the chair form, 1,3-diaxial detachment of hydrogen involving the 5α-hydrogen atom is possible with cholestan-3α-ol. This possibility is clearly absent from the cholestan-3β-ol molecule, where the opening of ring A and random ejection of hydrogen atoms from the other rings constitute the more probable mechanism[17].

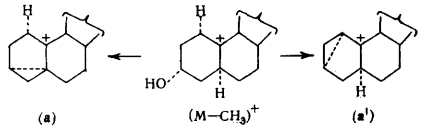
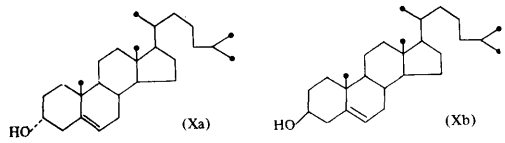


Table 1. Intensity of $[M - CH_3 - HDO]^+$ peak as percentage of $[M - CH_3 - H_2O]^+$ peak in the spectra of labelled cholestan-3β-ols.

2,2,4,4-Tetradeuterated	0
1,1,3β-Trideuterated	7 ± 2
1α-Monodeuterated	4.5 ± 2
5α-Monodeuterated	73 ± 2

It follows from the above discussion that the configuration at $C_{(3)}$ has little effect on the ease of elimination of water on electron impact with epimeric saturated 3-hydroxy-steroids.



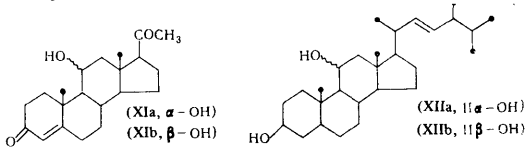
The introduction of a double bond at the 5–6 position of a steroid molecule fixes the conformation of ring A, which changes the situation significantly. In fact the mass spectra of epicholesterol (Xa) and cholesterol (Xb)[16] differ in the relative intensities of the peaks due to $[M - H_2O]^{+\cdot}$ and $M^{+\cdot}$ ions. In the case of (Xa) (axial hydroxyl) the intensity of the peak due to the former ion is 90% of that due to M^+, whereas in the spectrum of (Xb) (equatorial hydroxyl) the corresponding ratio is 50%. Thus, on the assumption that water is removed in this case by a process of 1,3-elimination (involving hydrogen from $C_{(1)}$), the axial 3α-hydroxy-group is more easily removed, owing to the 1,3-*cis*-diaxial arrangement of the groups undergoing elimination.

Table 2. Intensity of $[M - CH_3 - HDO]^+$ peak as percentage of $[M - CH_3 - H_2O]^+$ peak in the spectra of labelled cholestan-3β-ols.

2,2,4,4-Tetradeuterated	0
3α-Monodeuterated	0
5α-Monodeuterated	11
1,1-Dideuterated	28

4. 11-HYDROXY-STEROIDS

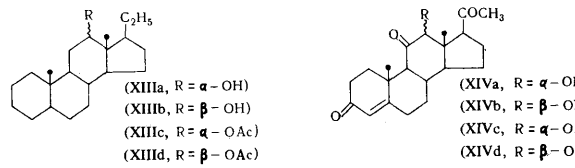
The mass spectra of steroid alcohols containing the hydroxyl in conformationally fixed rings, unlike those of 3-hydroxy-steroids, differ markedly among themselves, primarily in the peak intensity ratio $I_{M - H_2O}/I_{M^+}$. Comparison of the mass spectra of 11α- and 11β-hydroxy-progesterone (XIa) and (XIb) shows[18-20] that the alcohol (XIb), whose molecule contains an axial hydroxyl, readily eliminates a molecule of water on electron impact, whereas the molecular ion of its epimer (XIa), containing an equatorial hydroxyl, is more stable, and is dehydrated to a less extent (the intensity ratios for (XIa) and (XIb) are respectively 0.7 and 5.7).



The mass spectra of ergost-22-ene-3β,11α-diol (XIIa) and of the corresponding 3β,11β-diol (XIIb), which were studied by Fitches[21], also differ in the ratio of the $M - H_2O$ and M^+ peaks: the intensity ratio is considerably smaller in the spectrum of the 11α-epimer (XIIa) (equatorial hydroxy-group) than in that of the 11β-epimer (XIIb) (axial hydroxyl).

5. 12-HYDROXY-STEROIDS

Studies have been made of the mass spectra of pregnan-12α- and 12β-ols (XIIIa and XIIIb), their acetates (XIIIc and XIIId), 12α- and 12β-hydroxypregn-4-ene-3,11,20-triones (XIVa and XIVb), and also the acetates of the latter compounds (XIVc and XIVd)[16]. The pregnan-12-ols (XIIIa) and (XIIIb) are easily dehydrated on electron impact (in both cases the peak due to the $[M - H_2O]^{+\cdot}$ ion considerably exceeds the M^+ peak). However, the $I_{M - H_2O}/I_{M^+}$ ratios in the spectra of the epimers differ markedly. As has been noted with 11-hydroxyprogesterones, an axial hydroxy-group is also eliminated more easily in the series of pregnan-12-ols.



A curious feature of the mass spectra of (XIIIa) and (XIIIb) is the difference in intensity of the peak having $m/e = 257$, corresponding to ejection of an ethyl radical from the $[M - H_2O]^{\cdot+}$ ion: this peak is considerably more intense in the spectrum of (XIIIa). It may be expected that, as a consequence of the *cis*-arrangement of the 12β-hydroxyl and the 17β-ethyl group, the elimination of water from the molecular ion of pregnan-12β-ol will be accompanied, at least partly, by loss of a hydrogen atom from the side-chain; whereas the 17β-ethyl group located *trans* to a 12α-hydroxyl is not involved in dehydration of the molecular ion of pregnan-12α-ol. This situation is probably responsible also for the diminution in the intensity of the elimination of the ethyl group from the $[M - H_2O]^{\cdot+}$ ion in the case of (XIIIb).

The mass spectra of the acetates (XIIIc) and (XIIId) differ little between themselves. Both epimers eliminate a molecule of acetic acid so easily on electron impact that the peak of the molecular ion cannot be recorded. However, breakdown of the $[M - CH_3COOH]^{\cdot+}$ ion, accompanied by ejection of an ethyl radical, occurs rather more readily with the 12α-hydroxy-epimer, as for the free alcohols.

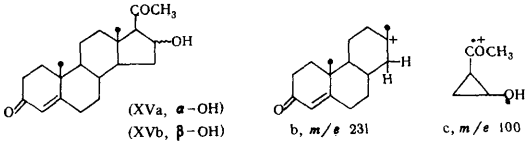
Table 3. Intensity ratios $I_{M - H_2O(CH_3COOH)}/I_{M^+}$ in the mass spectra of (XIIIa)–(XIVd).

Compound	$I_{M - H_2O(AcOH)}/I_{M^+}$	Compound	$I_{M - H_2O(AcOH)}/I_{M^+}$
(XIIIa)	57	(XIVb)	0.6
(XIIIb)	14	(XIVc)	7.3
(XIVa)	0.3	(XIVd)	4.3

The dependence on configuration of the ease of dehydration on electron impact, which has been reported for pregnan-12-ols, is not maintained in the 12-hydroxypregn-4-ene-3,11,20-triones (XIVa and XIVb)[16]. In this case the mass spectrum of (XIVb) (equatorial hydroxyl) exhibits double the $I_{M - H_2O}/I_{M^+}$ ratio shown by (XIVa) (Table 3). This phenomenon is obviously explained by the ease of tautomerisation of α-hydroxy-ketones, on electron impact, to the corresponding 11-hydroxy-12-keto-isomers. This hypothesis is supported indirectly by the mass spectra of the acetates (XIVc) and (XIVd) of (XIVa) and (XIVb), in which high intensity of the peak due to the $[M - CH_3COOH]^{\cdot+}$ ion is characteristic of the 12α-acetoxy-epimer (XIVc) (axial acetoxy-group) (Table 3).

6. 16-HYDROXY-STEROIDS

The mass spectra of 16α- and 16β-hydroxyprogesterones (XVa) and (XVb)[18,19], while indicating that both epimers are easily dehydrated on electron impact, exhibit significant differences. Firstly, the ease of dehydration of the molecular ion of (XVa) (16α-hydroxyl) exceeds that of its β-epimer (XVb) by a factor of 3 (Table 4). This indicates that an α-bond at $C_{(16)}$ is almost axial in character, and a β-bond almost equatorial.



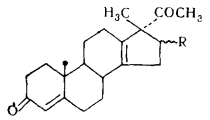
(XVa, α-OH)
(XVb, β-OH)
b, *m/e* 231
c, *m/e* 100

Secondly, the mass spectrum of (XVa) contains an intense peak due to the ion (b) having $m/e = 231$, which is almost completely absent from the spectrum of (XVb). The fragment (b) contains the rings A–C and is formed by rupture of the 13–17 and 14–15 bonds with migration of a hydrogen atom from position 16 to the charged fragment. However, rupture of the same bonds but with the charge localised on the fragment containing the atoms $C_{(15)}$–$C_{(17)}$, $C_{(20)}$, and $C_{(21)}$ yields the ion (c) having $m/e = 100$, whose peak is quite intense in the spectrum of 16α-hydroxyprogesterone (XVa) and is almost completely absent from the spectrum of its 16β-epimer (XVb).

Table 4. Intensity ratio $I_{M - H_2O}/I_{M^+}$ and intensities of peaks due to characteristic ions (% of Σ) in mass spectra of (XVa) and (XVb).

Cpd	I_{M-H_2O}/I_{M^+}	Ion (b) (*m/e* 231)	Ion (c) (*m/e* 100)
XVa	13	4.6	3.2
XVb	4.3	0.65	0.6

The above results were used to determine the configuration at position 16 in the pregnen-16-ol derivatives (XVIa) and (XVIb)[16]. In the mass spectrum of (XVIa) the ratio $I_{M - H_2O}/I_{M^+} = 20$, whereas in the spectrum of (XVIb) this ratio is only 8.7. By analogy with the 16-hydroxyprogesterones (XVa and XVb), therefore, the alcohol (XVIa) should have the α-configuration of the hydroxy-group, and the alcohol (XVIb) the β-configuration.



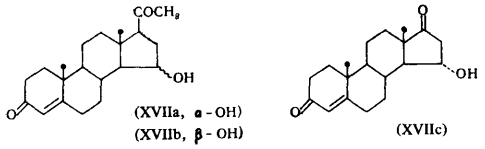
(XVIa, R = α-OH)
(XVIb, R = β-OH)
(XVIc, R = α-OAc)
(XVId, R = β-OAc)

Further confirmation of the configuration at position 16 was obtained by comparing the mass spectra of the corresponding acetates (XVIc) and (XVId). Primarily, the ratio of the peaks due to the ions $[M - CH_3COOH]^{\cdot+}$ and M^+ is different in the spectra of the epimers (120 and 36 respectively). Therefore the acetate (XVIc) must be assigned to the 16α-series, and its epimer (XVId) to the 16β-series. Another striking feature of the spectra of the four compounds (XVI) is the difference in intensity of the peak due to the ion $[M - COCH_3]^+$. The great intensity of this peak in the spectra of the 16α-OR′ epimers (XVIa and XVIc) is evidently due to the *cis*-arrangement of the 17α-acetyl and the 16α-OR′ groups, which facilitates elimination of the 17-acetyl group.

7. 15-HYDROXY-STEROIDS

Studies have been made of the mass spectra of 15α- and 15β-hydroxyprogesterones (XVIIa) and (XVIIb), and also of 15α-hydroxyandrost-4-ene-3,17-dione (XVIIc)[18,19]. The 15β-hydroxy-epimer (XVIIb) had a high $I_{M - H_2O}/I_{M^+}$ ratio (Table 5). The explanation is that the hydroxy-group

is equatorial in character in the (XVIIa) molecule, but axial in (XVIIb).



(XVIIa, α-OH)
(XVIIb, β-OH)
(XVIIc)

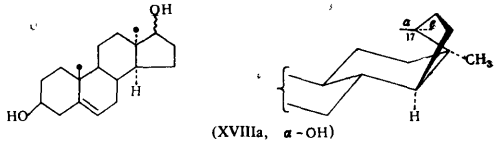
Table 5. Intensity ratio I_{M-H_2O}/I_{M^+} and intensities of peaks due to characteristic ions (% of Σ) in mass spectra of (XVIIa–(XVIIc).

Cpd.	I_{M-H_2O}/I_{M^+}	Ion (b) (*m/e* 231)	Ion (c) (*m/e* 100)
XVIIa	0.21	0.7	0.3
XVIIb	4.9	3.0	1.3
XVIIc	0.07	0.2	—

A similar situation is found in the mass spectrum of (XVIIc), in which the intensity of the peak due to the $[M - H_2O]^{+\cdot}$ ion is only 7% of the intensity of the M^+ peak, which dominates the spectrum. The characteristic difference in the mass spectrum of (XVIIb), as in the case of its 16α-hydroxyl analogue (XVa), is the considerable intensity of the peaks due to the ions (b) and (c), the mechanism of whose formation is similar to that described for (XVa) and (XVb) (Table 5).

8. 17-HYDROXY-STEROIDS

The behaviour of epimeric 17-hydroxy-steroids of the normal series on electron impact has not been studied. However, it is noteworthy that steroids of the 17β-hydroxy-series (oestradiol, 19-nortestosterone)[22], in which the hydroxyl is equatorial in character, eliminate water with difficulty under these conditions.



(XVIIIa, α-OH)
(XVIIIb, β-OH)

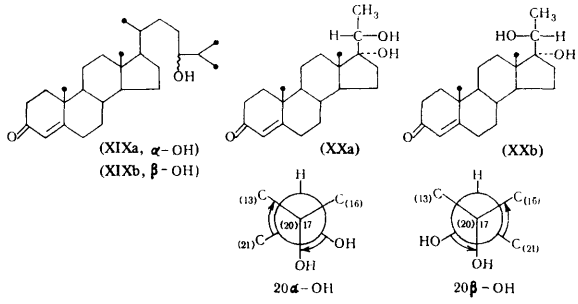
Comparison of the mass spectra of epimeric alcohols of the 13-iso-series—13-isoandrost-5-ene-3β,17α- and -3β,17β-diols (XVIIIa and XVIIIb)[16]—shows that electron impact has different dehydrating effects: the M^+ peak is more intense than the peak due to the $[M - H_2O]^{+\cdot}$ ion in the mass spectrum of (XVIIIa), but the opposite situation is observed in the spectrum of (XVIIIb). This observation is fully consistent with the equatorial character of an α-bond at $C_{(17)}$ in steroids of the 13-iso-series, and the axial character of a β-bond (in relation to ring C—see above scheme). Thus in this case also an axial hydroxyl is more easily eliminated as water than is an equatorial hydroxyl. A similar relation is found in several other fragments formed when molecular ions of compounds (XVIIIa) and (XVIIIb) break down. Thus the ratios

$$I_{M-2H_2O}/I_{M-H_2O} \text{ and } I_{M-H_2O-CH_2-H_2O}/I_{M-H_2O-CH_2}$$
$$I_{m/e\,197}/I_{m/e\,205} \text{ and } I_{m/e\,161}/I_{m/e\,179}.$$

change similarly to I_{M-H_2O}/I_{M^+} on passing from (XVIIIa) to (XVIIIb). In these two cases ions having m/e = 205 and 179 are characteristic of steroids having a 5–6 double bond and contain the ring C together with parts of rings A and B[23–25].

9. 20- AND 24-HYDROXY-STEROIDS

Epimeric aliphatic alcohols containing a single asymmetric centre usually have completely identical mass spectra. This held for 24α- and 24β-hydroxycholest-4-en-3-one (XIXa and XIXb), which contain a hydroxy-group in the aliphatic side-chain. Nevertheless, 17α,20α-dihydroxypregn-4-en-3-ones show significantly higher I_{M-H_2O}/I_{M^+}, I_{M-2H_2O}/I_{M-H_2O}, and also I_{M-CH_3CHOH}/I_{M^+} ratios in the mass spectrum of the 20α-hydroxy-epimer (XXa) than in that of the 20β-epimer (XXb).



(XIXa, α-OH)
(XIXb, β-OH)
(XXa)
(XXb)
20α-OH
20β-OH

Inspection of Dreiding models shows that an intramolecular hydrogen bond is possible between the 20β- and 17α-hydroxyls in (XXb), whereas this possibility is difficult to realise in the case of the 20α-epimer (XXa), owing to steric hindrance caused by the close mutual approach of the 20-methyl and angular 13-methyl groups (see above scheme). Formation of a hydrogen bond is probably the reason why a water molecule and a CH_3CHOH radical are not ejected from the molecular ion of the 20β-hydroxy-epimer (XXb).

10. TERTIARY ALCOHOLS OF THE D-HOMO-SERIES

Study of epimeric tertiary alcohols derived from (±)-17a-alkyl-3-methoxy-D-homo-oestra-1,3,5(10),8-tetraen-17a-ols—(XXIa), (XXIb), (XXIIa), and (XXIIb)—revealed exceptionally large differences between the mass spectra of epimers[26–28]. First of all, the intensity of the peak due to the $[M - H_2O]^+$ ion in the spectra of alcohols having an axial hydroxyl (XXIa and XXIb) far exceeds that of the M^+ peak, whereas the opposite situation is found with

epimers having an equatorial hydroxyl (XXIIa and XXIIb) (Table 6).

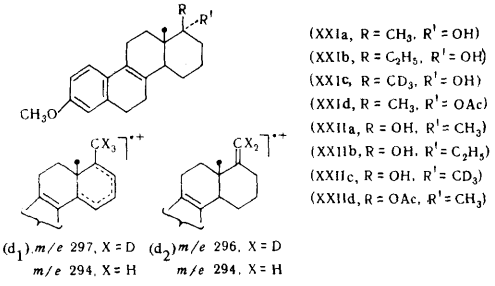


Table 6. Intensity ratios I_{M-H_2O}/I_{M^+} and $I_{M-H_2O-(17a\text{-Alk})}/I_{M-H_2O}$ in mass spectra of (XXIa)–(XXIIb).

Cpd	I_{M-H_2O}/I_{M^+}	$I_{M-H_2O-(17a\text{-Alk})}/I_{M-H_2O}$
(XXIa)	3.2	2.3
(XXIIa)	0.4	0.6
(XXIb)	2.3	3.5
(XXIIb)	0.2	0.6

The mass spectra of the 17a-trideuteromethyl analogues (XXIc and XXIIc) of (XXIa) and (XXIIa) were studied in order to elucidate the mechanism of dehydration on electron impact. Comparison of the mass spectra of (XXIc) and (XXIIc) (Figs. 1 and 2) shows that the readily dehydrated epimer (XXIIc)—and hence also (XXIa) and (XXIb)—form almost exclusively the $[M-H_2O]^{\cdot+}$ ion having m/e = 297 (ion d_1) on dehydration of the molecular ion.

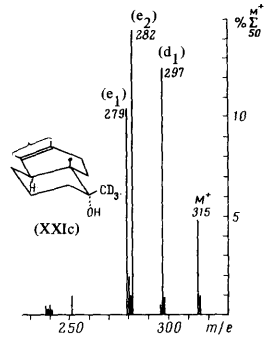


Figure 1.

Since dehydration takes place mainly by a mechanism of 1,3-elimination, the loss of hydrogen in this case takes place most probably from the atom $C_{(16)}$ or $C_{(14)}$. The epimer (XXIIc)—and hence also (XXIIa) and (XXIIb)—which eliminates water with far greater difficulty on electron impact, forms not only the ion d_1 but also the $[M-DOH]^{\cdot+}$ ion having m/e = 296 (ion D_2) with an exocyclic double bond. The formation of the fragment d_1 (not d_2) from the molecular ion of (XXIc) suggests an axial arrangement of the 17a-hydroxyl (since only then can one of the axial hydrogen atoms of the D ring be removed together with the hydroxy-group), and thus provides fresh evidence of the configuration at position 17a in the molecule of this compound. The appearance of twin $M-H_2O$ and $M-DOH$ peaks in the mass spectrum of (XXIIc) indicates an *ax*-alkyl-*eq*-hydroxyl configuration at position 17a.

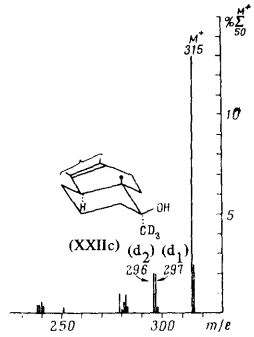
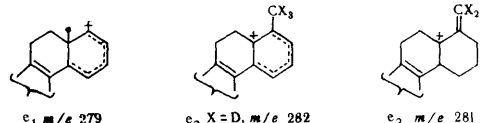
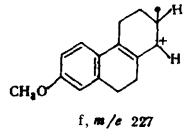


Figure 2.

The mass spectra of these compounds differ also in the relative intensities of the ions $[M-H_2O-17a\text{-Alk}]^+$ (e) and $[M-H_2O]^{\cdot+}$. In the case of epimers having an axial hydroxyl (XXIa and XXIb) the intensity of the former peak far exceeds that of the latter, but the opposite relation is found in epimers having an equatorial hydroxyl (XXIIa and XXIb) (Table 6). Comparison of the mass spectra of the deuterated analogues (XXIc and XXIIc) with those of the unlabelled compounds (XXIa and XXIIa) shows that the peak due to the ion (e) is composite, representing the formation of ions of the types (e_1), (e_2), and (e_3). In the spectra of epimers having an axial hydroxyl this peak represents the ions (e_1) and (e_2). In alcohols having an equatorial hydroxyl, however, removal only of the angular methyl group (ion e_3) is possible after the elimination of water, since the presence of an exocyclic double bond in the ion (d_2) makes loss of the 17a-alkyl group impossible.



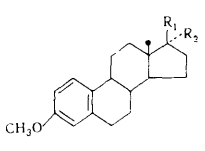
Another characteristic peak in the mass spectra of (XXIa), (XXIb), (XXIIa), and (XXIIb), whose intensity depends on the configuration of the epimer, is that due to the ion (f) having m/e = 227. Its intensity in the spectrum of the epimer with an equatorial hydroxyl is 16% (of $\Sigma_{50}^{M^+}$), whereas in the spectrum of the epimer having an axial hydroxyl it is only 1.2%. The ion (f) is formed directly from the molecular ion by detachment of ring D and migration of the hydrogen atom from the 17a-hydroxyl.



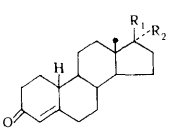
Comparison of the mass spectra of the acetates (XXId) and (XXIId)[30] reveals easier elimination of an axial than of an equatorial acetoxy-group as acetic acid on electron impact, similarly to the behaviour of the free alcohols. The mass spectra of (XXId) and (XXIId) differ also in the intensity of peaks due to ions of type (e), formed by ejection of a methyl group from ions of type (c) $[M - CH_3COOH]^{\cdot+}$. As with the free tertiary alcohols, the peak (e) is composite, the difference in its intensity being due to a difference in the mechanism of the elimination of acetic acid from the molecular ions of the epimers.

11. TERTIARY ALCOHOLS OF THE NORMAL SERIES

In contrast to alcohols of the D-homo-series, tertiary alcohols which are epimeric at $C_{(17)}$—(±)-17-alkyl-3-methoxyoestra-1,3,5(10)-trien-17-ol (XXIIIa,b–XXXVa,b) and (±)-17-alkyl-19-nortestosterone (XXXVIa,b–XXVIIa,b)[29] derivatives—have essentially similar mass spectra[29], differing only in the intensity ratio of the peaks due to $[M - H_2O]^{\cdot+}$ and M^+ ions. The ratio is higher in alcohols having an α-hydroxyl (XXIIIa–XXVa) than in their β-hydroxy-epimers (XXIIIb–XXVb) (Table 7). This result is consistent with general views on the conformation of bonds at the $C_{(17)}$ atom (an α-bond is axial in character, and a β-bond equatorial).



(XXIIIa, R_1 = CH_3, R_2 = OH)
(XXIIIb, R_1 = OH, R_2 = CH_3)
(XXIVa, R_1 = C_2H_5, R_2 = OH)
(XXIVb, R_1 = OH, R_2 = C_2H_5)
(XXVa, R_1 = $CH_2C(=CH_2)CH_3$, R_2 = OH)
(XXVb, R_1 = OH, R_2 = $CH_2(=CH_2)CH_3$)



(XXVIa, R_1 = CH_3, R_2 = OH)
(XXVIb, R_1 = OH, R_2 = CH_3)
(XXVIIa, R_1 = C_2H_5, R_2 = OH)
(XXVIIb, R_1 = OH, R_2 = C_2H_5)

Table 7. Intensity ratio $I_{M - H_2O}/I_{M^+}$ in mass spectra of (XXIIIa)–(XXVb).

Compound	I_{M-H_2O}/I_{M^+}	Compound	I_{M-H_2O}/I_{M^+}
(XXIIIa)	45	(XXIVb)	21
(XXIIIb)	14	(XXVa)	62
(XXIVa)	41	(XXVb)	21

In the 19-nortestosterones (XXVIa,b–XXVIIa,b) the $I_{M - H_2O}/I_{M^+}$ ratios show smaller differences in the spectra of epimers. In this case, however, the 17α-hydroxy-epimer undergoes dehydration more readily on electron impact.

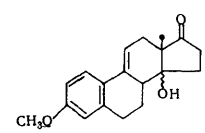
The spectra of epimers of this group differ more markedly in the ratio of the peaks due to the ions $[M - H_2O - 17\text{-Alk}]^+$ (m/e = 255) and $[M - H_2O]^{\cdot+}$ (Table 8). The considerable decrease in this ratio in the spectra of the 17β-hydroxy-epimers (XXVIb and XXVIIb) in comparison with the 17α-epimers (XXVIa and XXVIIa) is obviously due to a difference in the mechanism of the dehydration of their molecular ions. The process probably resembles that described for the D-homo-alcohols: removal of an α-hydroxyl from a molecular ion is accompanied by that of a hydrogen atom from the ring D, with formation of an ion of type (d_1); whereas in the case of the β-hydroxy-epimers an ion of type (d_2) having an exocyclic double bond is also formed. Ejection of the 17-alkyl is impossible in the latter case, and therefore the peak at m/e = 255 becomes less intense.

Table 8. Intensity ratios $I_{M - H_2O}/I_{M^+}$ and $I_{M - H_2O - 17\text{-Alk}}/I_{M - H_2O}$ in mass spectra of (XXVIa)–(XXVIIb).

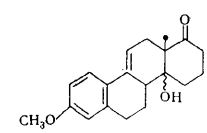
Compound	$\frac{I_{M-H_2O}}{I_{M^+}}$	$\frac{I_{M-H_2O-17\text{-Alk}}}{I_{M-H_2O}}$
(XXVIa)	36	1.8
(XXVIb)	17.4	0.3
(XXVIIa)	21	1.8
(XXVIIb)	15	0.9

12. 14-HYDROXY-STEROIDS

Mass spectrometry has been used also to determine the configuration at positions 8 and 14 in 14-hydroxy-derivatives of 3-methoxyoestra-1,3,5(10),9(11)-tetraen-17-one (XXVIII) and 3-methoxy-D-homo-oestra-1,3,5(10),9-(11)-tetraen-17-one (XXIXa and XXIXb)[31].

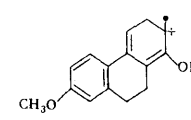


(XXVIII)



(XXIX, a α-OH; b β-OH)

The mass spectra of the hydroxy-ketones (XXIXa) and (XXIXb) differ substantially in the intensity of the peak due to the ion (g) having m/e = 241

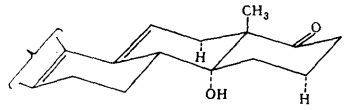


g, m/e 241

formed by cleavage of the bonds 13–17a and 14–15 in the molecular ion. The fivefold increase in this ion on passing from (XXIXa) to (XXIXb) enables us to assign the former to the *trans*-C–D series (14α-hydroxyl), and the latter to the *cis*-C–D series (14β-hydroxyl), by analogy with *cis*- and *trans*-α-decalins, 14α- and 14β-equilenins, 8α- and 8β-oestrones, etc.

Furthermore, the spectra of (XXIXa) and (XXIXb) differ in the intensity ratio of the $[M - H_2O]^+$ and M^+ peaks (1.2 and 0.3 respectively). The 8-deuterated analogues have been used to establish that the 8-hydrogen atom is not involved in dehydration. The other possible 1,2-elimination, involving the 15-hydrogen atom, is obviously also improbable. Ejection of DOH is not observed from the 17,17-dideutero-analogues, which refutes the possibility of 1,4-elimination. The most probable process is therefore the 1,3-elimination of water involving a 12- or 16-hydrogen

atom. This supports the conclusion that the ease of dehydration of the hydroxy-ketone (XXIXa) is associated with the α-configuration of the 14-hydroxyl (*trans*-C–D fusion), when the hydroxyl is located axially with respect to both rings C and D, with the preferred 1,3-*cis*-diaxial orientation of the groups undergoing elimination.



The insignificant dehydration of the molecular ion of the hydroxy-ketone (XXIXb) is evidently due to the equatorial orientation of the 14-hydroxy-group with respect to the ring which donates the hydrogen atom in the elimination of water on electron impact. This condition is feasible only with *cis*-C–D ring fusion (14β-hydroxy-configuration), since in any of the theoretically possible conformations of (XXIXb) the 14-hydroxyl is located equatorially with respect to one of the rings (C or D).

The mass spectrum of the hydroxy-ketone (XXVIII) is very similar to that of (XXIXb), so that *cis*-C–D fusion of the rings and equatorial orientation of the hydroxy-group can be attributed to the former.

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Although sufficient experimental results are not yet available, it can be concluded from the above survey that mass spectrometry can be used to establish configuration in all cases in which the steroid alcohol molecule contains a sufficiently rigid cyclic system, since in most cases electron impact eliminates an axial more readily than an equatorial hydroxyl.

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Institute of the Chemistry of Natural Compounds,
USSR Academy of Sciences,
Moscow