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MASS SPECTROMETRY AS A DIAGNOSTIC TOOL FOR 5-HYDROXYSTEROIDS[a]

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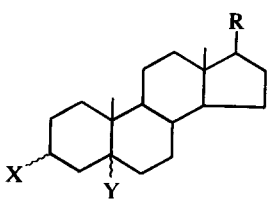
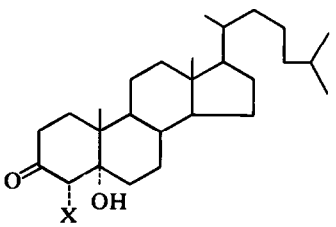
Abstract—The mass spectra of twelve 5-hydroxysteroids (**1–12**), indicate that a common feature is the formation of the ion **a** by loss of the four ring A C atoms 1, 2, 3, and 4, with the H atoms and other substituents attached.

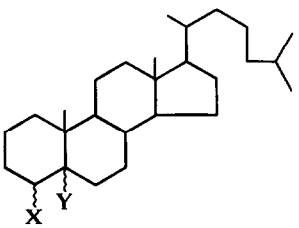
In the course of a study on radical oxidations in the steroid series we were in need of a simple diagnostic method for the determination of the site of the new OH group. We found that mass spectrometry can be a useful and sensitive tool for the determination of 5-hydroxysteroids **1–12**.



1: X = H; Y = β-OH; R = C_8H_{17}
2: X = H; Y = α-OH; R = C_8H_{17}
3: X = H; Y = α-OH; R = OAc
4: X = β-OH; Y = β-OH; R = C_8H_{17}
5: X = β-OH; Y = α-OH; R = C_8H_{17}
6: X = α-OH; Y = β-OH; R = C_8H_{17}
7: X = β-OAc; Y = α-OH; R = OAc



8: X = OH
9: X = H



10: X = α-OAc; Y = α-OH
11: X = Y = β-OH
12: X = Y = α-OH

We examined the mass spectra of twelve 5-hydroxysteroids (**1–12**). The general phenomenon indicated by the mass spectra of the 12 compounds was the formation of the radical-ions **a** by the loss of four ring A C atoms 1, 2, 3 and 4 with the H atoms and other substituents attached. The *m/e* values and abundances of ions **a** are listed in the Table and their formation is visualized in the Scheme. In most cases metastable transitions have been detected, which indicate that ions **a** are formed by primary processes from the molecular ions.

Ions **a** have been detected both in 5α-OH and 5β-OH compounds. However, they are more abundant in the 5β-hydroxy compounds **1** and **4** (rings A and B *cis*-fused) than in the 5α-OH stereoisomers **2** and **5** (Table 1). This difference may be due to the more facile cleavage of the C-4, C-5 bond in the less stable A-B *cis*-fused compounds than in the *trans*-isomers.

It is possible that primary fragmentation is not the only process which leads to the formation of ion **a** from the molecular ion of **7**, which has an acetoxy substituent at C-3. A part of the most abundant *m/e* 278 ion **a** in **7** may be formed by a

Table 1

	m/e	$\frac{[\text{ion a}]}{[M^+]}$	$\%\Sigma_{40}$ ion **a**	($M^+ \xrightarrow{m^*}$ ion **a**)
1	332	0·97	1·4	[b]
2	332	0·57	0·4	[b]
3	278	1·08	2·1	[b]
4	332[a]	10·9	25·1	273·2
5	332	3·8	1·8	[b]
6	332	8·0	4·1	273
7	278[a]	12	9·6	[b]
8	332[a]	1·85	22·0	264
9	332	1·0	1·9	274·2
10	332	2·0	2·3	247
11	332[a]	2·7	7·2	273·2
12	332	2·2	6·1	273·2

[a]Most abundant ion.
[b]No metastable transition detected.

[a]Part of this work was presented at the 41st annual meeting of the Israel Chemical Society, October 1971.

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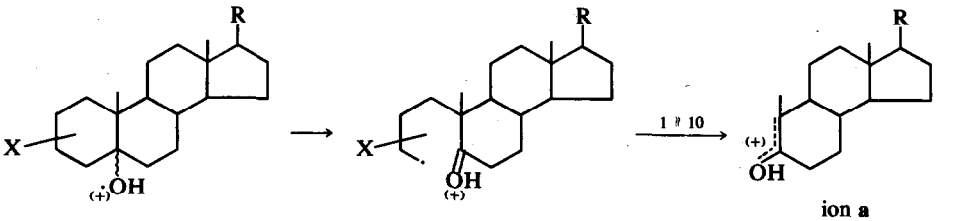
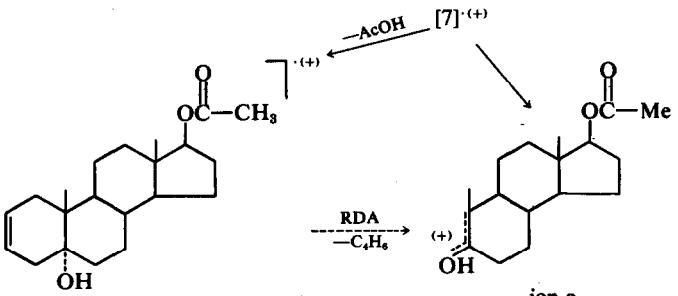


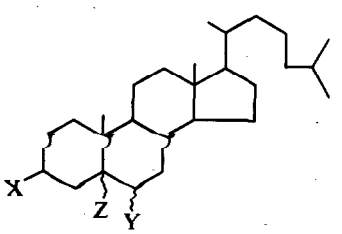
SCHEME



secondary "retro-Diels-Alder" (RDA) fragmentation of the *m/e* 332 elimination product ion $[M-MeCO_2H]^+$. Such fragmentation route has been reported for 3-acetoxysteroids,[1] even if they are not substituted at C-5. No metastable transition has been detected for any of the two possible processes.

Radical ions analogous to ions **a** (corresponding to the loss of carbons 1, 2, 3 and 4) were found in 1960 in the mass spectra of several 3-hydroxysteroids.[2] It appears that these ions are formed by a "retro Diels-Alder" fragmentation after the elimination of H_2O from the molecular ions. This elimination of H_2O seems to occur mainly in the high temperature inlet system, which was employed in 1960 for the introduction of the materials to the ion source. In later works on mass spectrometry of 3-hydroxysterols[3] as well as in our measurements of 3β-cholestanol and 3β-coprostanol, the ions analogous to ions **a** were found to be absent or of very low abundance. Recently, however, it has been shown[4] that 3α-hydroxysteroids in which rings A and B are *cis*-fused give rise to abundant $[M-72]^+$ ions, which result from dehydration followed by loss of C_4H_8 unit from C-1–C-4. It is therefore possible that in compounds **b**, in which the two conditions (*cis*-fusion and 3α-OH) are fulfilled, the $[M-72]^+$ ions are formed in part by two consecutive processes, namely elimination of H_2O followed by the loss of C_4H_8. The other two 3,5-dihydroxysteroids **4** and **5**, however, are believed to give rise to ions **a** only by the primary process directly from the molecular ion, due to the presence of the hydroxyl group at position 5.

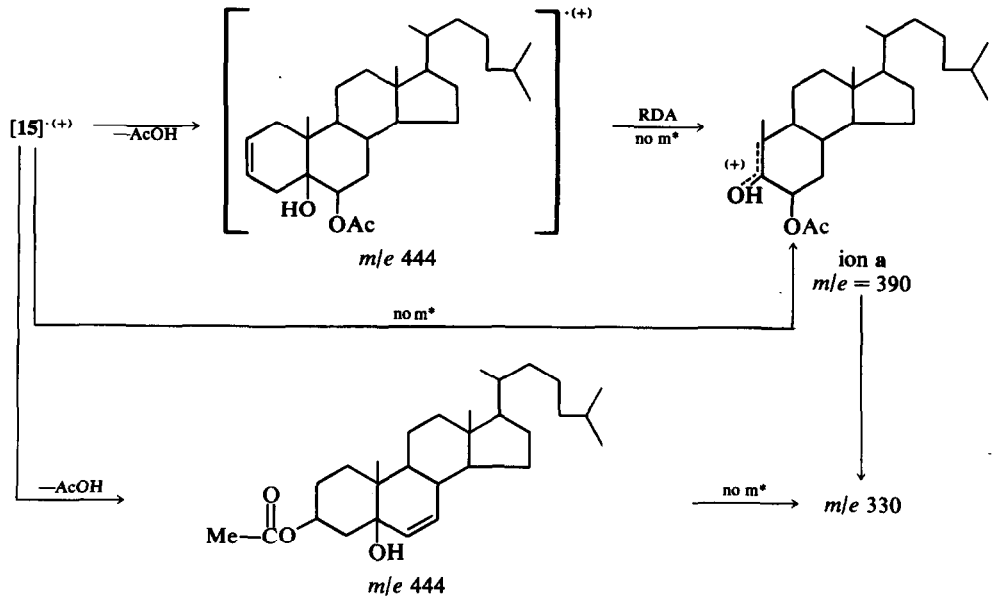
The presence of substituents at C-6 has been found to suppress the formation of ions **a**. Thus 5α,6β-dihydroxycholestane (**13**) and 3β-methoxy-5α,6β-dihydroxycholestane (**14**) do not exhibit ions **a** in their mass spectra. The loss of one and two molecules of water are the most important



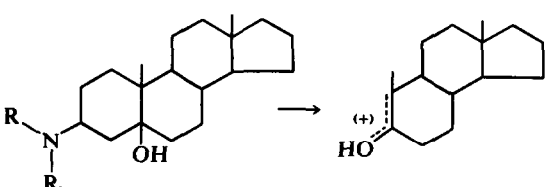
13: X = H; Y = βOH; Z = αOH
14: X = OCH_3; Y = βOH; Z = αOH
15: X = Y = $OCOCH_3$; Z = βOH
16: X = H; Y = αOH; Z = αOH

processes in the case of **13** and **16**. In the mass spectrum of **14** the loss of H_2O gave rise to the most abundant ion (*m/e* 416, $\%\Sigma_{40}$ 10·9), and it was followed by the loss of an additional molecule of water (*m/e* 398, $\%\Sigma_{40}$ 2·2) and of methanol (*m/e* 384, $\%\Sigma_{40}$ 2·9). In the mass spectrum of **15** the *m/e* 390 peak ($\%\Sigma_{40}$ 2·7) corresponds to ion **a**, and the *m/e* 358 ion ($\%\Sigma_{40}$ 8·3) can be formed by the elimination of acetic acid from ion **a**. We cannot, however, exclude the possibility that the *m/e* 358

ion **a** is formed by a secondary "retro-Diels Alder" process as outlined above. No metastable transition has been detected for the formation of *m/e* 390 ion.



Mass spectral data of several 5-hydroxysteroids have been reported or discussed in the literature, but the formation of ions **a** has not been pointed out. Thus a relatively intense peak at *m/e* 220 has been observed for compounds **17** and **18**,[5] but no route was suggested for the formation of the corresponding ions. It seems reasonable that the *m/e* 220 ions may be ions **a**, which are formed as a result of the presence of the hydroxyl group at position **5**.



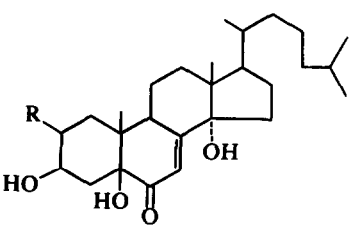
17: R = Me; R_1 = H

18: R = Me; R_1 = CF_3C(=O)

m/e 220
ion a_{17}, 38%
ion a_{18}, 100%

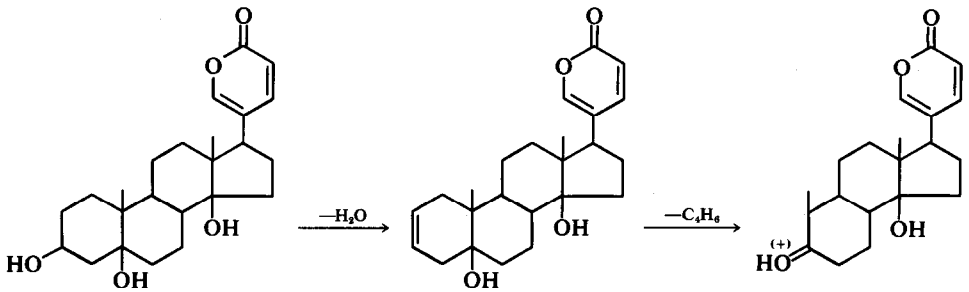
Partial mass spectral data have also been published previously,[6] for isomeric 3,5-dihydroxycholestanes **4**, **5**, and **6**, but the presence of ions **a** has not been mentioned.

In the data reported for 3β,5β,14α-trihydroxy-5β-cholest-7-en-6-one (**19**) no peak at *m/e* 360 which would correspond to ion **a** was listed.[7a] This seems to be in agreement with the previously discussed suppression of formation of ion **a** when a substituent is present at position 6. The *m/e* 360 peak is, however, of relatively high intensity (~ 30%) in the mass spectrum of 2β,3β,5β,14α-trihydroxy-5β-cholest-7-en-6-one (**20**).[7a]



19: R = H
20: R = OH

Recently the formation of analogous ions has been reported in the case of 3,5-dihydroxybufadienolides.[7b] The authors claim that this fragmentation is best rationalized in two steps, by (a) expulsion of H_2O from ring A, followed by (b) retro-Diels-Alder elimination of ring A, rather than as a single-step process as suggested for 5-hydroxysteroids examined by us. However, the elimination of H_2O from 3-hydroxysteroids is known generally not to be followed by a "retro-Diels-Alder" fragmentation (step b). Moreover, the presence of metastable peaks in the mass spectra of the majority of both 5-hydroxy and 3,5-dihydroxysteroids reported here (Table 1) clearly indicates that the formation of ions **a** in these compounds is (at least in part) a single-step process. It should be noted that we did not detect any metastable transitions for the two-step pathway. Similar support for the two steps mechanism in the 5-



hydroxybufadienolides was not provided by the above authors since metastable peaks were not presented.

Attempts have recently been made to develop automatic techniques for the interpretation of mass spectral data with the aid of computer facilities. Monofunctional simple compounds have been shown to be good examples for such an approach, and gross structures as well as more subtle structural details could be deduced by the computer.[8]

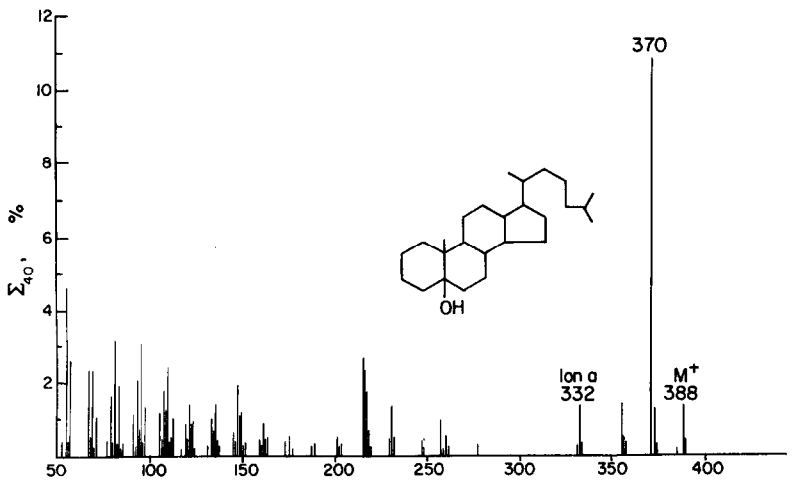


Fig 1. Mass spectrum of 5β-cholestane-5-ol (1).

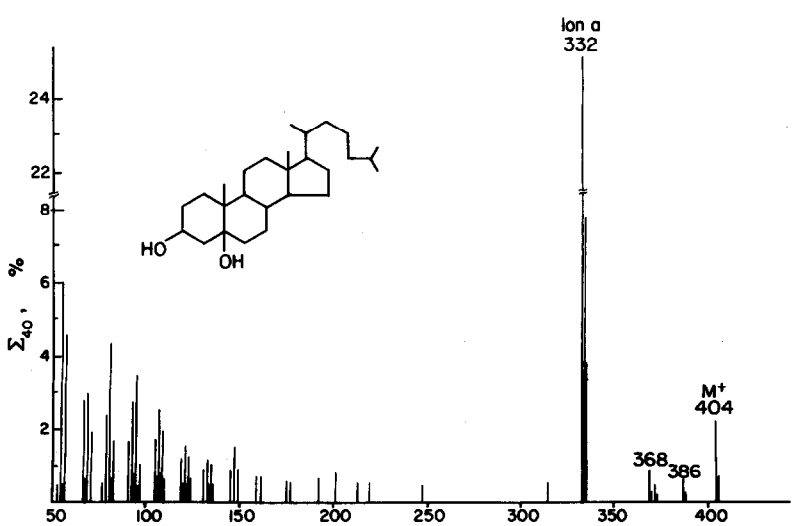


Fig 2. Mass spectrum of 5β-cholestane-3β,5-diol (4).

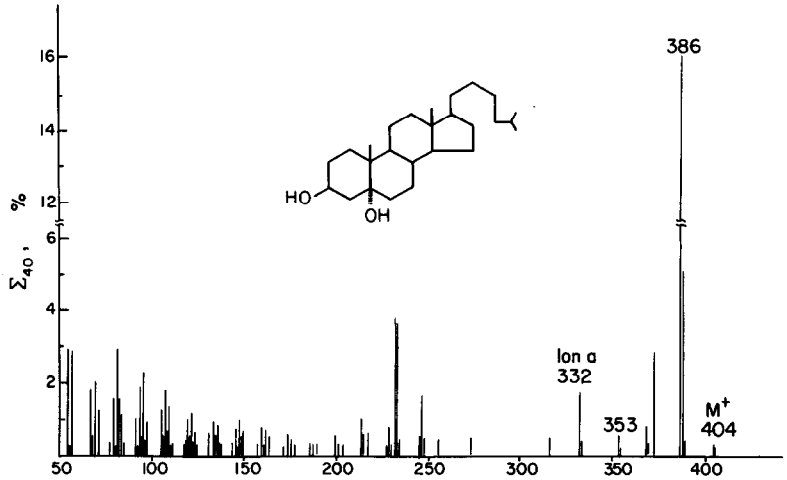


Fig 3. Mass spectrum of 5α-cholestane-3β,5-diol (5).

Fig 4. Mass spectrum of 5β-cholestane-3α,5-diol (6).

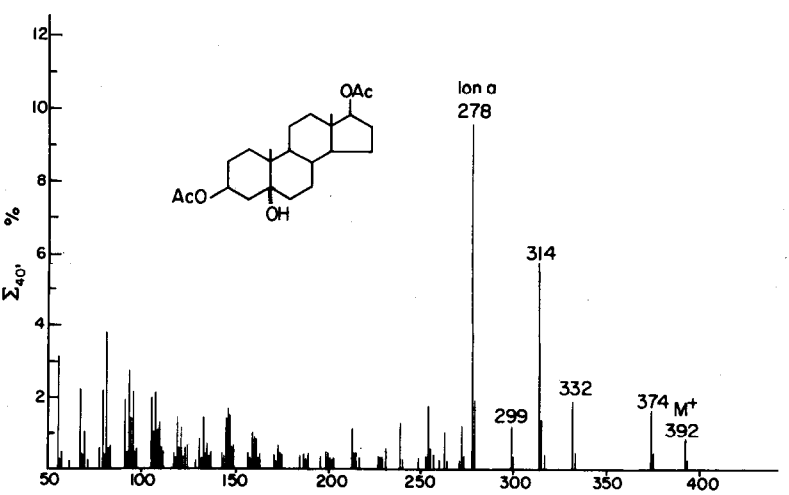


Fig 5. Mass spectrum of 5α-androstane-3β,5,17β-triol-3,17-diacetate (7).

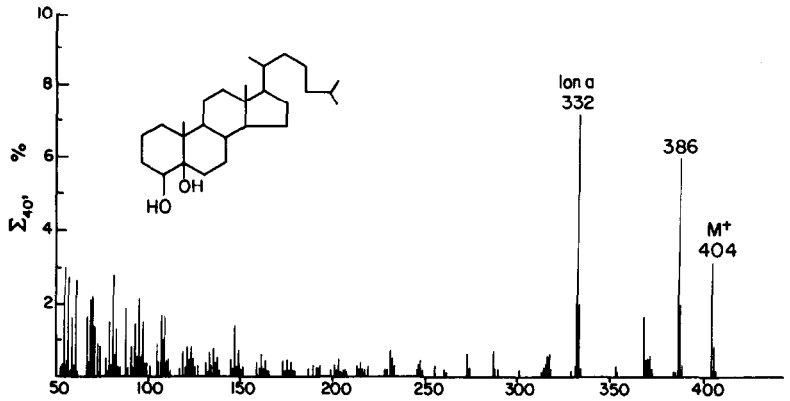


Fig 6. Mass spectrum of 5β-cholestane-4β,5-diol (**11**).

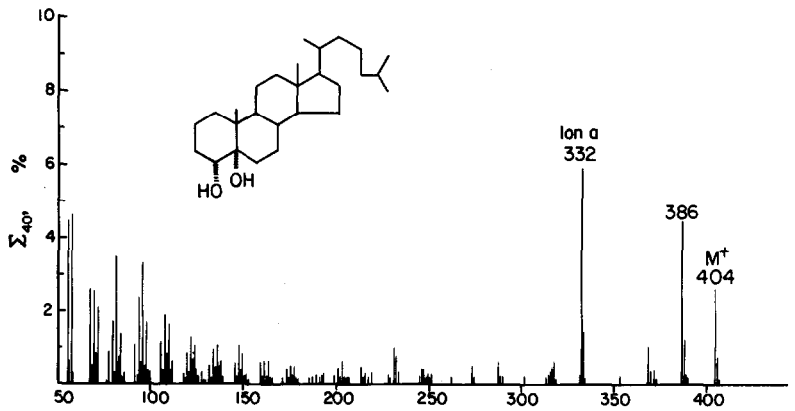


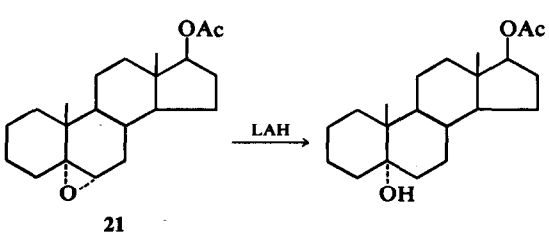
Fig 7. Mass spectrum of 5α-cholestane-4α,5-diol (**12**).

It appears that first steps are being made to extend the potential of this technique to more complicated problems, and in this context a list of common mass spectral features of differently substituted steroids has been compiled.[4a,b] We think that the results of the present work (together with the limitations outlined above), could be added to such a list.

EXPERIMENTAL

Compounds **1**[1b], **2**[11], **4**[12], **5**[1b], **6**[12], **7**[13], **8**[11], **9**[1b], **10**[11], **11**[11], **12**[11] were prepared by procedures reported in the literature. Compounds **4**[8c], **5**[6b], **6**[6b] were kindly supplied by Prof. B. Witkop. Compounds *1*, *2* were kindly supplied by Prof. D. Lavie and Mr. M. Weissenberg from the Department of Chemistry, The Weizmann Institute of Science. Compound **3** was prepared by LAH reduction of *5α-androstane-5,6-epoxy, 17-acetate (21) m.p. 146°*.

Mass spectra were measured with an Atlas CH-4 mass spectrometer fitted with a TO-4 ion source. The ionisation energy was maintained at 70 eV. The samples were introduced through the direct inlet system, and heated (if necessary) until usable mass spectra could be obtained.



21

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We thank Mr. S. Gattegno for measuring the mass spectra.

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