THE STEREOSPECIFIC LOSS OF WATER FROM B-HOMOCHOLESTANOLS UNDER ELECTRON IMPACT*

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The mass spectra of six isomeric B-homocholestanols are reported. The spectra differ in relative intensities of ions originating by losses of water, acetic acid and ethylene. The differences are rationalized with the help of labelled compounds and metastable spectra.

The electron-impact-induced loss of water from cyclic alcohols is known to exhibit a remarkable stereospecificity[1-3]. While much effort has been concentrated on elucidation of the water elimination mechanism from the alcohols containing five or six-membered rings, compounds carrying a hydroxyl group on the seven-membered rings have been almost omitted[4]. In a recent paper[5], we noted a rather peculiar loss of water in the mass spectra of the steroid stereoisomers which differred in the configuration of the hydroxyl group located on the seven-membered B-homo ring. Apparently, the behaviour of these compounds has contradicted the stereochemical rules which are known to hold for the electron-impact-induced loss of water from the alcohols with six-membered rings[6,7]. This observation prompted us to investigate the water elimination from the six B-homocholestanols which differ in both the positions and configurations of hydroxyls on the seven-membered B-homo ring.

The mass spectra of the isomers *I*–*VI* (Figs. 1–4, Table I) differ markedly in relative intensities of several ions in the high mass region, namely m/z 460 – $C_{30}H_{52}O_3$, $M^{+\cdot}$, m/z 442 – $C_{30}H_{50}O_2(M—H_2O)^{+\cdot}$, m/z 414 – $C_{28}H_{46}O_2(M-H_2O—C_2H_4)^{+\cdot}$, m/z 400 – $C_{28}H_{48}O$ $(M-CH_3COOH)^{+\cdot}$, m/z 382 – $C_{28}H_{46}$ $(M-H_2O—CH_3CO.OH)^{+\cdot}$, and m/z 354 – $C_{26}H_{42}$. Since the alcohols *I*–*VI* might show different propensity to thermal elimination of water, we checked the extent of this reaction by labelling the hydrogens in the neighbourhood of the hydroxyl group (compound *Ia*–*VIa*). Table II shows that the thermal loss of water is negligible in all cases.

The mass spectrum of the alcohol *I* (Fig. 1) contains abundant ions m/z 414 and 354. The former ions originate from the $(M-H_2O)^{+\cdot}$ ions by loss of ethylene as

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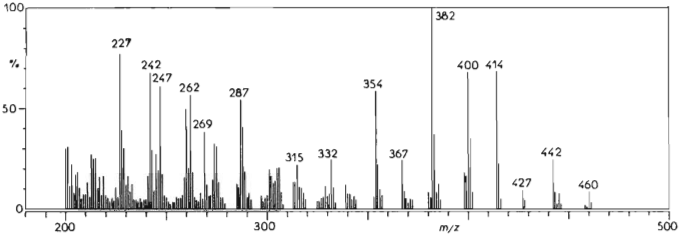


FIG. 1

The Mass Spectrum of *I* (high mass region)

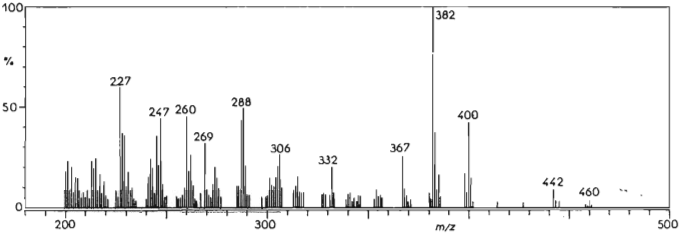


FIG. 2

The Mass Spectrum of *II* (high mass region)

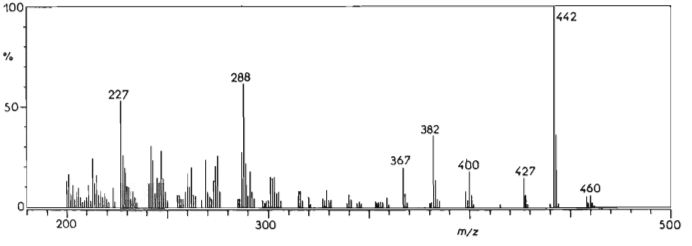


FIG. 3

The Mass Spectrum of *III* (high mass region)

evidenced by the decomposition of the single metastable ion in the first field free region (FFR). Since the mass spectrum of the 7,7-$^2H_2$-derivative *Ia* shows very cleanly ions of the same m/z 414, the expelled ethylene moiety must involve the methylenes from $C_{(7)}$ and $C_{(7a)}$). The ions m/z 354 arise from the ions m/z 414 by loss of acetic acid and from the $(M-CH_3COOH-H_2O)^{+\cdot}$ ions by loss of ethylene. Again, the spectrum of *Ia* shows only a few percent of deuterium left in the ions m/z 354. The facile elimination of ethylene from both the $(M-H_2O)^{+\cdot}$ and $(M-CH_3COOH-H_2O)^{+\cdot}$ ions is not observed in the spectra of the remaining isomers *II* – *VI*. This striking difference can be explained by stereospecific formation

TABLE I

Relative Intensities (%) — Related to the Sum of All Ions Above m/z 200 — of Important Ions and $[M-H_2O]^{+\cdot}/[M]^{+\cdot}$ Abundance Ratios

Compound	m/z						$[M-H_2O]^{+\cdot}/[M]^{+\cdot}$
	460	442	414	400	382	354	
I	0·34	1·01	2·88	2·87	4·21	2·47	3·0
II	0·20	0·52	0·18	2·48	5·87	0·52	2·6
III	0·43	7·71	—	1·36	2·74	—	17·9
IV	1·39	2·80	—	1·27	4·84	—	2·0
V	0·34	2·02	0·16	3·09	3·61	0·64	5·9
VI	0·25	2·27	0·10	2·27	5·83	2·17	9·1

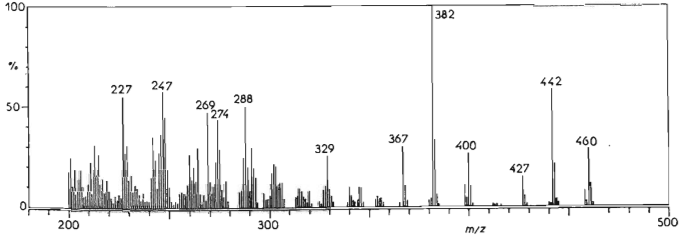


FIG. 4

The Mass Spectrum of *IV* (high mass region)

TABLE II

Deuterium Contents of $(M)^{+\cdot}$, *a* $(M-H_2O)^{+\cdot}$, *b* $(M-CH_3COOH)^{+\cdot}$ and *c* $(M-CH_3COOH-H_2O)^{+\cdot}$ Ions

Compound	Ion	$^2H_4$	$^2H_3$	$^2H_2$	$^2H_1$	$^2H_0$
Ia	$M^{+\cdot}$	—	5	63	20	12
	a	—	5	60	19	16
	b	—	6	57	25	12
	c	—	6	63	18	13
IIIa	$M^{+\cdot}$	48	40	12	—	—
	a	49	41	10	—	—
	b	47	40	13	—	—
	c	47	40	13	—	—
IIIb	$M^{+\cdot}$	—	—	—	82	18
	a	—	—	—	82	18
	b	—	—	—	82	18
	c	—	—	—	78	22
IIIc	$M^{+\cdot}$	—	—	53	39	8
	a	—	—	54	39	7
	b	—	—	52	39	9
	c	—	—	52	39	9
IVa	$M^{+\cdot}$	48	36	12	—	—
	a	45	42	13	—	—
	b	45	41	14	—	—
	c	46	41	13	—	—
Va	$M^{+\cdot}$	—	—	72	28	—
	a	—	—	71	29	—
	b	—	—	70	30	—
	c	—	—	70	30	—
IVb	$M^{+\cdot}$	—	—	—	84	16
	a	—	—	—	82	18
	b	—	—	—	79	21
	c	—	—	—	46	54
IVc	$M^{+\cdot}$	—	—	53	37	10
	a	—	—	54	37	9
	b	—	—	52	38	10
	c	—	—	30	50	20
VIa	$M^{+\cdot}$	—	—	70	28	2
	a	—	—	68	30	2
	b	—	—	70	28	2
	c	—	—	68	30	2

TABLE II
(*Continued*)

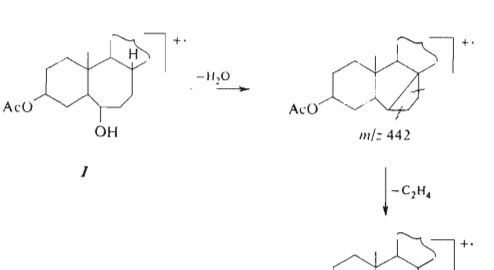
Compound	Ion	$^2H_4$	$^2H_3$	$^2H_2$	$^2H_1$	$^2H_0$
VIb	$M^{+\cdot}$	—	—	45	51	4
	a	—	—	42	43	15
	b	—	—	39	55	6
	c	—	—	23	45	32

TABLE III
Accessibilities of the Skeletal Hydrogens Measured on Relaxed Dreiding Models

Hydroxyl	Accessible hydrogens (minimum distance in nm)
6β	10-CH_3 (0·05), 8β (0·13), 4β (0·08), 7aβ (0·17), 4α (0·10)
6α	4α (0·11), 9α (0·12), 4β (0·10), 7aα (0·17)
7β	10-CH_3 (0·00), 8β (0·17), 4β (0·14)
7α	5α (0·17), 9α (0·13), 14α (0·15)
7aβ	10-CH_3 (0·03), 6β (0·17)
7aα	5α (0·15), 6α (0·17), 9α (0·17), 14α (0·20)

of the $(M-H_2O)^{+\cdot}$ ions in *I*. Inspection of Dreiding models shows that there are several types of hydrogens available to the 6β-hydroxyl group (Table III). The 7aβ- and 8β-hydrogens and the hydrogens from the $C_{(10)}$-methyl group, may approach as close as 0·2 nm to the 6β-hydroxyl by conformational changes of the B-homo ring. The boat form of the A-ring enables the access of the 6β-hydroxyl group to the 4β-hydrogen. Owing to its easy accessibility, the tertiary hydrogen at $C_{(8)}$ is probably most prone to be transferred onto the 6β-hydroxyl and then lost as water. The resulting $(M-H_2O)^{+\cdot}$ ions (Scheme 1) could further lose ethylene by a simple fission of the $C_{(6)}$—$C_{(7)}$ and $C_{(7a)}$—$C_{(8)}$ bonds. In contrast, the 6α-hydroxyl of *II* can reach the 9α- and 7aα-hydrogens *via* the conformational changes of the B-homo ring. If the conformational changes in both rings – A and B – are assumed, the 4α- and 4β-hydrogens can approach the 6α-hydroxyl group. However, none of the mechanisms listed above for the elimination of water from *II* can give rise to the $(M-H_2O)^{+\cdot}$ ions with the structure depicted in Scheme 1. Although the transfer of the 8β-hydrogen is not documented by appropriate labelling and it is tacitly

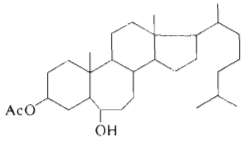
assumed that the $(M-H_2O)^{+\cdot}$ ions do not interconvert prior to further fragmentation, the mechanism outlined in Scheme 1 can account for the striking differences in the spectra of *I* and *II*.



SCHEME 1

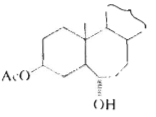
The mass spectra of the alcohols *III* and *IV* differ markedly in relative intensities of the $M^{+\cdot}$, $(M-H_2O)^{+\cdot}$, $(M-CH_3COOH)^{+\cdot}$ and $(M-CH_3COOH-H_2O)^{+\cdot}$ ions (Fig. 3 and 4). Inspection of models shows that number of hydrogens accessible to the 7α- and 7β-hydroxyls is rather limited (Table III). In *IV*, there are three tertiary hydrogens, *i.e.* 5α-, 9α- and 14α-, which can be made accessible to the 7α-hydroxyl in different conformations of the B-ring. The alcohol *III* has only one accessible tertiary hydrogen (8β) in addition to those of $C_{(10)}$-methyl and 4β-. Approaching the last hydrogen neccessitates a conformational excitation of both A and B rings. By contrast, the 7β-alcohol *III* shows more abundant $(M-H_2O)^{+\cdot}$ ions than the 7α-epimer. The extraordinary behaviour of *III* and *IV* may be, *a priori*, due to two factors: *a*) the isomers differ mainly in the rates of formation of the $(M-H_2O)^{+\cdot}$ ions; *b*) they differ mainly in the overall rates of decomposition of the latter ions. The metastable spectra of the $(M-H_2O)^{+\cdot}$ ions generated from both *III* and *IV* show the same decompositions, *i.e.* the loss of acetic acid, the loss of the cholestane side chain (m/z 329) and the cleavage of the D-ring (m/z 303, 302, 301, 288), so that they do not permit any definite conclusion. The spectra of the labelled derivatives *IIIb*, *IIIc*, *IVb* and *IVc* shed more light on the mechanism in question. Table II shows that the 5α-deuterium is preserved in the $(M-H_2O)^{+\cdot}$ and $(M-CH_3COOH)^{+\cdot}$ ions of *IVb* and *IVc*. On the other hand, the $(M-CH_3COOH-H_2O)^{+\cdot}$ ions exhibit a significant loss of the 5α-deuterium. Decompositions of the metastable ions in the

1st FFR revealed that the $(M-CH_3COOH—H_2O)^{+\cdot}$ ions originate from both the $(M-H_2O)^{+\cdot}$ and $(M-CH_3COOH)^{+\cdot}$ ions. Since the elimination of acetic acid from the alicyclic acetates is known to involve only accessible hydrogens from neighbouring positions[8], the loss of the 5α-deuterium in *IVb,c* can be unambiguously ascribed to the elimination of water from the $(M-CH_3COOH)^{+\cdot}$ ions. The change of the mechanism of water elimination is probably caused by activation of the 5α-hydrogen by the double bond created by the loss of acetic acid. The labelled

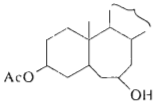


I

Ia: 7α,7β-$^2H_2$

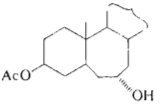


II



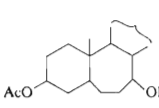
III

IIIa: 6α,6β,7aα,7aβ-$^2H_4$
IIIb: 5α-$^2H_1$
IIIc: 5α,6α-$^2H_2$



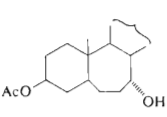
IV

IVa: 6α,6β,7aα,7aβ-$^2H_4$
IVb: 5α-$^2H_1$
IVc: 5α,6α-$^2H_2$



V

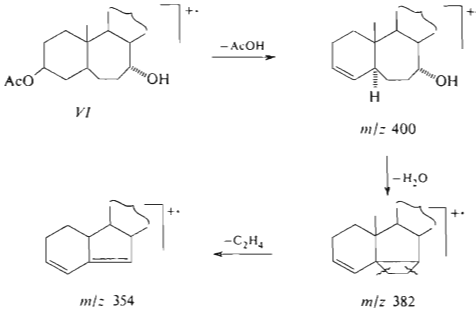
Va: 7α,7β-$^2H_2$



VI

VIa: 7α,7β-$^2H_2$
VIb: 5α,6α-$^2H_2$

7β-alcohols *IIIb* and *IIIc* retained the 5α-deuterium in both the $(M-H_2O)^{+\cdot}$ and $(M-CH_3COOH-H_2O)^{+\cdot}$ ions. This suggests that the configurational arrangement at $C_{(5)}$ and $C_{(7)}$ is preserved prior to the water elimination. In a summary, the differences in the spectra of *III* and *IV* can be interpreted in the following manner. The molecular ions of *III* lose water *via* 1,3-elimination with abstraction of the accessible 8β-hydrogen. The water elimination is sufficiently rapid to compete with the concurrent loss of acetic acid from $M^{+\cdot}$. The loss of water from the $(M-CH_3COOH)^{+\cdot}$ ions proceeds neithter with any apparent change of mechanism nor with any enhanced abundance (Fig. 3). The molecular ion of *IV* loses water *via* the 1,4-elimination, the 9α- and/or 14α-hydrogens taking part in this elimination. Nevertheless, the access of the 7α-hydroxyl to these hydrogens is probably hindered, due to non-bonding interactions of the 7aβ-hydrogen with the $C_{(10)}$-methyl group. Consequently, the greater part of the molecular ions decomposes by loss of acetic acid. However, the latter decomposition opens a new reaction channel for the 1,3-elimination of water from the $(M-CH_3COOH)^{+\cdot}$ ions, by activating the accessible 5α-hydrogen. This results in enhanced abundance of the ions *m/z* 382 in the spectrum of *IV* (Fig. 4). It is noteworthy that the same configurational dependence of the loss of water has been described earlier for more complex 7-hydroxy-B-homocholestane derivatives[5].



SCHEME 2

The mass spectra of the 7a-alcohols *V* and *VI* (Table I) also differ in relative intensities of the $M^{+\cdot}$, $(M-H_2O)^{+\cdot}$, $(M-CH_3COOH)^{+\cdot}$ and $(M-CH_3COOH-H_2O)^{+\cdot}$ ions. Nevertheless, the differences are less pronounced than in the preceding isomers. Analysis of Dreiding models shows that the 7aβ-hydroxyl can reach

to 6β- and $C_{(10)}$-methyl hydrogens. The 7aα-hydroxyl can approach the 5α-, 6α-, 9α- and 14α-hydrogens. The $(M-H_2O)^{+\cdot}$ ions of the labelled derivative *VIb* exhibit only a small decrease of the deuterium content. Since both the 5α- and 6α-positions are labelled in *VIb*, it follows that most of water is eliminated *via* the 1,3-mechanism involving the 9α- and/or 14α-hydrogens. By contrast, the $(M-CH_3COOH-H_2O)^{+\cdot}$ ions show a considerable loss of deuterium. This phenomenon can be interpreted in a similar way as done for *IV*. A part of the $(M-CH_3COOH)^{+\cdot}$ ions is formed with the $C_{(3)}$—$C_{(4)}$ double bond which activates the allylic 5α-hydrogen for the transfer onto the 7aα-hydroxyl group. Of course, only careful labelling of the A-ring would distinguish whether the $C_{(3)}$—$C_{(4)}$ double bond is formed immediately or whether it is located by subsequent rearrangements in the A-ring. The change of the water elimination mechanism from the $(M-CH_3COOH)^{+\cdot}$ ions affects the further fragmentation of the $(M-CH_3COOH-H_2O)^{+\cdot}$ ions. As demonstrated by Table I, the alcohol *VI* shows more abundant ions m/z 354, formed by loss of ethylene from m/z 382, than the isomer *V*. This effect can be rationalized by assuming that the 1,4--elimination of water from the $(M-CH_3COOH)^{+\cdot}$ ions in *VI* creates the four--membered ring which decomposes by elimination of ethylene (Scheme 2). Indeed, the spectrum of the 7,7-$^2H_2$-derivative *VIa* displays a clean loss of $C_2H_2{}^2H_2$ thus confirming that the ethylene eliminated contains the $C_{(6)}$ and $C_{(7)}$ methylenes.

When comparing the mass spectral behaviour of alcohols *I* – *VI*, it can be pointed out that the relations between the relative intensities of the $(M-H_2O)^{+\cdot}$ ions and the number and nature of accessible hydrogens are by no means straighforward. This is illustrated by Tables I and III. The striking example of isomers *III* and *IV* shows that the accessibility of hydrogens, as measured on molecular models, may be only formal. The real accessibility can be governed by conformational properties of the cyclic framework.

EXPERIMENTAL

The mass spectra were recorded on a JEOL JMS D-100 spectrometer at 75 eV. The samples were introduced using a direct inlet at 140—150°C, the ion source was maintained at 140°C. Decompositions of metastable ions in the 1st FFR were recorded by scanning the accelerating voltage. Preparation of *I*, *II*, *III*, *IIIb*, *IIIc*, *IV*, *IVb*, *IVc*. *V*, *VI* and *VIb* was described earlier[9–11]. The labelled derivatives *Ia IIIa*, *IVa*, *Va* and *VIa* were prepared from the corresponding ketones[9] by exchange of the labile hydrogens for deuterium (lithium deuteroxide, deuterium oxide, tetrahydrofuran, triethylbenzylammonium chloride, 100 h at 20°C), followed by acetylation (acetanhydride, pyridine), and hydride reduction[9,10]. The configurational isomers were separated on silica gel plates and identified by R_F values. The deuterium contents were determined by mass spectrometry at 14 eV.

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