MASS SPECTRA OF SOME 5-METHYL-19-NOR-5β-CHOLEST-9-ENES*

František Tureček[a] and Pavel Kočovský[b]

[a] *The Jaroslav Heyrovský Institute of Physical Chemistry and Electrochemistry, Czechoslovak Academy of Sciences, 121 38 Prague 2 and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

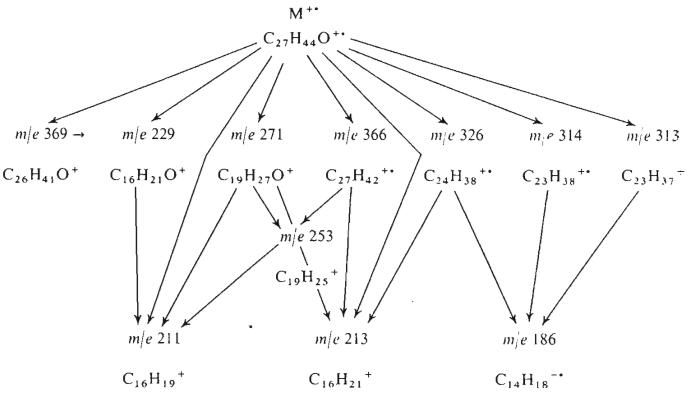
Received January 30th, 1978

The mass spectrometric behavior of isomeric ketones *I*—*V* and alcohols *VI*—*X* of Westphalen type is described. The differences in fragmentation patterns of these compounds are discussed in dependence on the position and the configuration of the substituent.

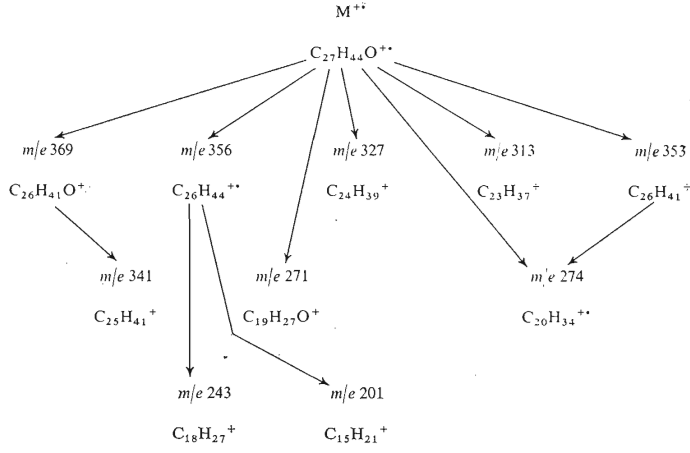
The mass spectrometric behavior of steroid compounds has been the subject of a number of papers in the past. Their results are summarized in several review articles[1,2]. The classical papers by Djerassi and his group[3-5] have explained the effect of the keto group on the fragmentation of steroids with normal cholestane, androstane and pregnane skeletons. Extensive studies of the elimination of water from ionized molecules of steroid alcohols[6-9] have contributed to the elucidation of the mechanism of this reaction and its dependence on structural and stereochemical factors. Therefore it was interesting to investigate the influence of the positions or configurations of the oxygen functions on mass-spectrometric fragmentations of steroids with non-classical skeletons. As suitable models we have used the series of simple steroids of Westphalen type, *i.e.* 5-methyl-19-nor-5β-cholest-9-enes (*A*), prepared earlier (compounds *I*—*X*).

The mass spectra (high mass regions only) of three isomeric ketones *I*, *III* and *V* are shown in Figs 1—3. The results of the measurement of accurate masses of abundant ionic species and the observed metastable transitions are summarized in fragmentation maps (Scheme 1—3). The lower parts of the spectra (*m/e* 20—200) contained the series of homologous ionic species $C_nH_{2n-3}^+$, $C_nH_{2n-5}^+$ and $C_nH_{2n-7}^+$ only. The splitting off of the cholestane side chain (ions $C_{19}H_{27}O^+$, *m/e* 271) and the cleavage of the ring D (ions $C_{16}H_{21}O^+$, *m/e* 229) are common in the majority of ketones with a cholestane skeleton[3]. In addition to these fragmentations, however, fragmentation could be observed in the case of ketones *I*—*V* which had no analogy with the corresponding cholestanones.

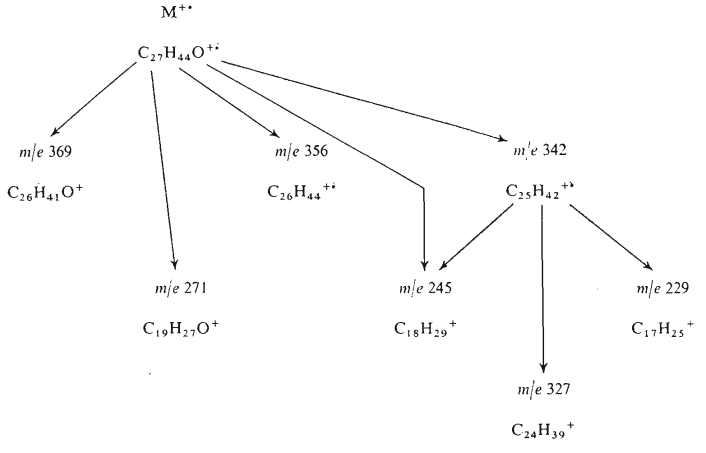
* Part CCXVII in the series On Steroids; Part CCXVI: This Journal *44*, 260 (1979).



SCHEME 1
Partial fragmentation map of 5-methyl-19-nor-5β-cholest-9-en-3-one (*I*)



SCHEME 2
Partial fragmentation map of 5-methyl-19-nor-5β-cholest-9-en-4-one (*III*)



SCHEME 3
Partial fragmentation map of 5-methyl-19-nor-5β-cholest-9-en-6-one (*V*)

Ions $C_{24}H_{38}^{+\cdot}$, *m/e* 326 are formed by the loss of fragment C_3H_6O (Fig. 1). From ionized molecules of 5-methyl-19-nor-5β-cholest-9-en-3-one (*I*). Labelling with deuterium in the positions $C_{(2)}$ and $C_{(4)}$ (compound *II*) has shown that the split off three-carbon fragment contained carbons $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$. The transfer of both hydrogen atoms onto the neutral fragment was not accompanied by the scrambling of hydrogens of

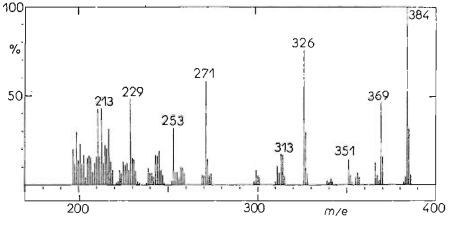


FIG. 1
High Mass Region of the Mass Spectrum of Ketone *I*

the A-ring. The effect of the keto group resembles in this case rather the effect of the 16-keto group of 5α-androstan-16-one[10]. On cleavage of the ring A the ions $C_{23}H_{38}^{+\cdot}$, *m/e* 314 and $C_{23}H_{37}^{+}$, *m/e* 313 are formed (transfer of one hydrogen to the neutral fragment). Labelling with deuterium (compound *II*) again proved the splitting off of the carbons $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$, while the fourth carbon in the neutral fragment comes probably from $C_{(1)}$.

In the spectrum of the isomeric 4-ketone *III* (Fig. 2), the dominant feature is the cleavage of the A-ring. Ions $C_{26}H_{44}^{+\cdot}$, *m/e* 356, $(M—CO)^{+\cdot}$, are further fragmented by losing a methyl (ions $C_{25}H_{41}^{+}$, *m/e* 341), or the cholestane side chain (ions $C_{18}H_{27}^{+}$, *m/e* 243), or by the cleavage of the ring D (ions $C_{15}H_{21}^{+}$, *m/e* 201). An alternative route to ions *m/e* 341, *i.e.* the cleavage of the $C_{(4)}$—$C_{(5)}$ bond, followed by McLafferty rearrangement, can be excluded on the basis of the labelling of the position $C_{(3)}$ with deuterium (compound *IV*). The ions $(M—C_3H_5O)$, *m/e* 327, can be formed either by the loss of the fragment $C_{(2)}$—$C_{(3)}$—$C_{(4)}$ with hydrogen transfer, or by a combined splitting off of the fragment $C_{(3)}$—$C_{(4)}$ and the methyl group from $C_{(5)}$. The formation of homologous ions $(M—C_4H_7O)^{+}$, *m/e* 313, can be represented quite analogously. The extensive cleavage of the ring A, observed in the spectrum of 5-methyl-19--nor-5β-cholest-9-en-4-one (*III*) has no analogy in corresponding 4-cholestanones and 4-androstanones[11] or in 1-cholestanones and 1-androstanones[12] either. The reason for this easy cleavage is evidently the low dissociation energy of the $C_{(4)}$—$C_{(5)}$ bond due to the combined effect of the $C_{(5)}$-methyl group, the double bond in the position 9 and the keto group in the position 4.

The different type of fragmentation of 5-methyl-19-nor-5β-cholest-9-en-6-one (*V*), Fig. 3, can be explained by the activation of the $C_{(5)}$—$C_{(6)}$ bond under the combined effect of the $C_{(6)}$-keto group, the $C_{(5)}$-methyl group and the double bond in the position 9. The main fragmentation reaction of the ionized molecules is the retro-Diels–

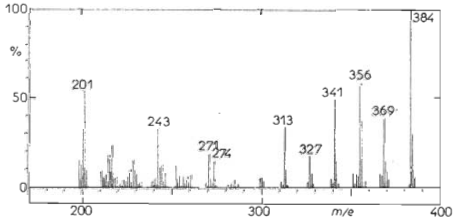


FIG. 2

High Mass Region of the Mass Spectrum of Ketone *III*

–Alder cleavage of the ring B, under formation of the ions $C_{25}H_{42}^{+\bullet}$, *m/e* 342. These are further fragmented by the loss of methyl (ions *m/e* 327), the loss of the side chain (ions $C_{17}H_{25}^{+}$, *m/e* 229) or by the splitting off of the A-ring after migration of the double bond into the ring C (ions $C_{18}H_{29}^{+}$, *m/e* 245). The ions with equal nominal masses but different elemental composition (*m/e* 245, $C_{17}H_{25}O^{+}$ and *m/e* 229, $C_{16}H_{21}O^{+}$) are formed from ionized molecules by cleavage of the ring D.

From a comparison of the mass-spectrometric behavior of ketones of the Westphalen type (*I*, *III*, *V*) with analogous cholestanones[3] with normal skeletons the following can be inferred: *1*) The effect of the double bond in the position 9 and of the $C_{(5)}$-methyl is manifested by a higher abundance of the ions formed by cleavage of the rings A and B, while in the spectra of corresponding cholestanones these processes are substantially less frequent; *2*) the greater extent of the cleavage of the rings A and B is manifested by a deepening of the differences in the spectra of isomeric ketones *I*, *III* and *V*, differing in the position of the keto group.

The effect of the position of the hydroxyl group on the cleavage of 5-methyl-19-nor-5β-cholest-9-ene skeleton of isomeric alcohols *VI*–*X* is less pronounced in comparison with ketones *I*–*V*. The main fragmentations of ionized molecules of alcohols *VI*–*X* are the following: Loss of methyl, loss of water, combined loss of methyl and water, splitting off of the cholestane side chain (ions $C_{19}H_{29}O^{+}$, *m/e* 273) and the cleavage of the D-ring (ions $C_{17}H_{26}O^{+\bullet}$, *m/e* 246 and $C_{16}H_{23}O^{+}$, *m/e* 231). On combination of the loss of water and the loss of the side chain or the cleavage of the ring D the following ions are formed: $(M-C_8H_{17}-H_2O)^{+}$, *m/e* 255, $(M-C_{10}H_{19}-H_2O)^{+}$, *m/e* 229, and $(M-C_{11}H_{23}-H_2O)^{+}$, *m/e* 255. In addition to these fragmentations which are common for the alcohols *VI*–*X*, we also observed in the case of 4β-hydroxy derivative *VIII* the cleavage of the ring A (ions $C_{24}H_{39}^{+}$, *m/e* 327, and $C_{23}H_{37}^{+}$, *m/e* 313) which is analogous to the fragmentation of the 4-oxo derivative *III*. 6β- and 6α-

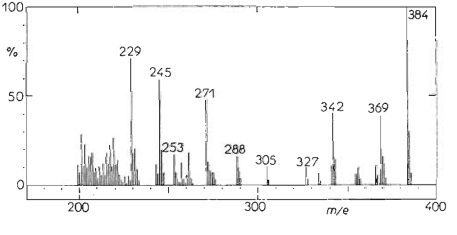


FIG. 3
High Mass Region of the Mass Spectrum of Ketone *V*

-Hydroxy derivatives *IX* and *X* are fragmented, after ionization, by retro-Diels–Alder reaction (ions *m/e* 342) analogously as the corresponding 6-oxo derivative *V*. The abundance of the ions $(M—C_2H_4O)^{+\cdot}$, *m/e* 342, is dependent on the configuration of the hydroxyl.

The effect of the position or of the configuration of the hydroxyl group on the fragmentation of alcohols *VI*–*X* is most strikingly evident from the relative abundance of ions $(M—H_2O)^{+\cdot}$ (Table I). It was shown[13,14] that the loss of water from ionized molecules of cyclic alcohols takes place predominantly as a 1,3- or 1,4-elimination. Under the assumption that in our substances a primary ring cleavage does not take place, the accessibility of the hydrogen atoms in individual positions of the skeleton of alcohols *VI*–*X* can be estimated from Dreiding models (Table II). However, the striking difference in the abundance ratios $[M—H_2O]^{+\cdot}/[M]^{+\cdot}$ of epimeric 3β- and 3α-hydroxy derivatives *VI* and *VII* (Table I) cannot be referred simply to the accessibility of the skeletal hydrogens (Table II). Should we suppose, however, on the basis of analogies[6,15,16] that the hydrogen transfer takes place primarily from the activated allylic position $C_{(1)}$, the effect of the configuration of the

TABLE I

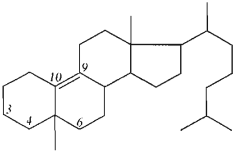
Abundance Ratios $[M—H_2O]^{+\cdot}/[M]^{+\cdot}$ of Alcohols *VI*–*XII* in Their Mass Spectra

Alcohol	*VI*	*VII*	*VIII*	*IX*	*IX* + *X*	*XI*	*XII*
$[M—H_2O]^{+\cdot}/[M]^{+\cdot}$	0·15	0·95	0·32	3·85	2·48	1·32	5·71

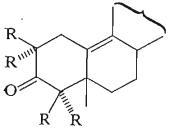
TABLE II

Accessibility of Hydrogens in Compounds *VI*–*X* for the Elimination of Water (according to Dreiding models)

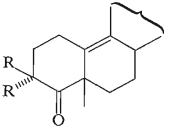
Hydroxyl	3α	3β	4β	6α	6β
Accessible Hydrogens	1α, 6α	1β, 5β-CH_3	1β, 2β, 6α, 6β, 5β-CH_3	3α, 4α, 4β	4α, 4β, 8β, 5β-CH_3



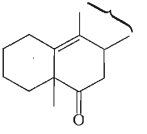
A



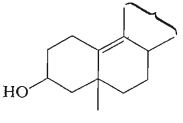
I, R = H
II, R = ^{2}H



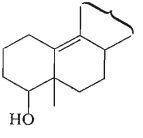
III, R = H
IV, R = ^{2}H



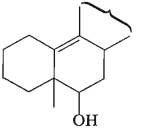
V



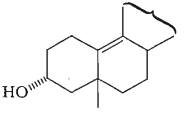
VI



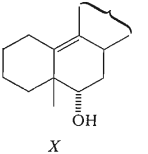
VIII



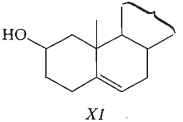
IX



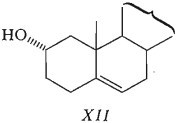
VII



X



XI



XII

hydroxyl can be explained in the following manner: during the ionization of the 3α--hydroxy derivative *VII* a part of excess energy is consumed for the conformational excitation of the A-ring, from the chair conformation A_1 to the boat form A_2 (Fig. 4). The originally equatorial 3α-hydroxy group becomes axial and comes close to the 1α-hydrogen, at a distance permitting the transfer. In contrast to this the originally axial 3β-hydroxy group of compound *VI* becomes equatorial on transition of the A-ring from the chair form A_1 to the boat form A_2, and it is simultaneously removed from the originally close 1β- and 5β-CH_3 hydrogens. The reverse transition of the A-ring to the chair conformation A_1 with an axial 3β-hydroxyl is inevitably impaired by the 1,3-diaxial interaction of the hydroxyl group with the 5β-methyl group. Even though the energy contribution of this 1,3-diaxial interaction is only a few kcal mol^{-1} (ref.[17]), it can contribute to an increase in the activation energy of the loss of water, which requires 1β-H. In order to support this hypothesis we chose as model compounds epimeric 2β- and 2α-hydroxy-5-cholestenes[18] (*XI*, *XII*) which have a classical skeleton with the same relative arrangement of the hydroxyl, methyl and double bonds as both the epimeric alcohols of the Westphalen type, *VI* or *VII*. Table I shows that the dependence of the abundance ratio $[M-H_2O]^{+\cdot}/[M]^{+\cdot}$ on the configuration of the hydroxyl is quite analogous in alcohols *XI* and *XII* as in alcohols *VI* or *VII*, respectively.

The low value of the abundance ratio $[M-H_2O]^{+\cdot}/[M]^{+\cdot}$ in 4β-hydroxy derivative *VIII*, which – of all alcohols investigated – has the highest number of hydrogen atoms accessible for elimination (Table II), can be explained in a similar manner: In order to enable the transfer of the allylic 1β-hydrogen the A-ring must assume the

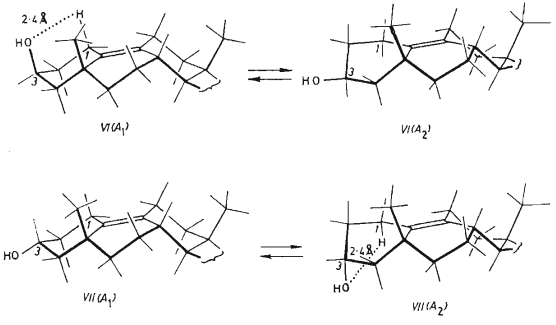


FIG. 4
Conformations of Alcohols *VI* and *VII*

twist-boat conformation A_3 (Fig. 5) that permits the approach of the 4β-hydroxyl to the 1β-hydrogen. During this conformational change, however, the 4β-hydroxyl becomes prone to a synperiplanar interaction with the 5β-methyl. The energy of this interaction can then contribute to the increase in activation energy of the water elimination proceeding by this mechanism. The values of the abundance ratios $[M—H_2O]^{+\cdot}/[M]^{+\cdot}$ of alcohols *VI* and *VIII* in which a hindered access to the activated allylic hydrogens may be assumed are comparable to the same values of steroidal alcohols that do not contain a double bond[2,6], while the values for alcohols *VII* and *IX* with easily accessible allylic hydrogens are higher by almost one order of magnitude (Table I). The high abundance ratio $[M—H_2O]^{+\cdot}/[M]^{+\cdot}$ in the 6β-alcohol *IX* (Table I) is given probably by the accessibility of the activated 8β-hydrogen in conformation B_2 (Table II, Fig. 5). The epimeric 6α-alcohol *X* which does not contain an allylic hydrogen accessible to elimination (Table II) was available only in the form of an unseparable mixture with the 6β-isomer *IX*. The lower abundance ratio $[M—H_2O]^{+\cdot}/[M]^{+\cdot}$ in the spectrum of a mixture of *IX* and *X* (Table I) shows that the process of water loss from ionized molecules is less pronounced in the case of the 6α-alcohol *X*. This is consistent with the higher proportions of the products of the competitive retro-Diels–Alder cleavage of the ring B (ions *m/e* 342) in the spectrum of a mixture of *IX* and *X*, in comparison with the pure 6β-alcohol *IX*.

EXPERIMENTAL

The mass spectra were measured on a JEOL JMS D-100 spectrometer, 75 eV, with a direct inlet system. The temperature of the direct inlet was kept at the lowest values sufficient for the evaporation of the sample (90—100°C) so that thermal dehydration should be avoided. The temperature of the ion source was always 140—150°C. Moreover, the ion source was also tested with cholesterol, as a compound inclining to thermal dehydration[19]. The metastable transitions were measured by the defocusing technique using the accelerating voltage scan. The syntheses of compounds *I*, *III*, *V*, and *VI—X* have been described earlier[20—22]. The specifically labelled

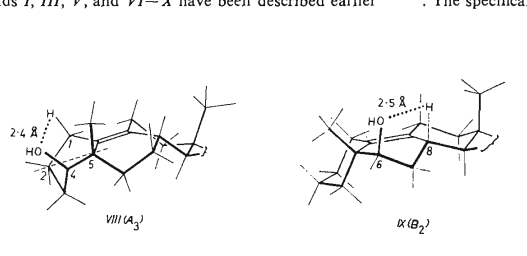


FIG. 5
Conformations of Alcohols *VIII* and *IX*

derivatives *II* and *IV* were prepared from corresponding ketones *I* or *II* by exchange of hydrogen for deuterium (deuterium oxide, tetrahydrofuran, sodium deuteroxide, decyltrimethylammonium bromide, 20°C, 24 h). The content of deuterium in ionized molecules was determined at 13 eV; *II*: 81·2% d_4, 16·7% d_3, 2·1% d_2; *IV*: 90·0% d_2, 8·0% d_1, 2·0% d_0.

REFERENCES

1. Budzikiewicz H., Djerassi C., Williams D. H.: *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II. Holden-Day, San Francisco 1964.
2. Spiteller-Friedmann M., Spiteller G.: Fortschr. Chem. Forsch. *12*, 440 (1969).
3. Budzikiewicz H., Djerassi C.: J. Amer. Chem. Soc. *84*, 1430 (1962).
4. Shapiro R. H., Djerassi C.: J. Amer. Chem. Soc. *86*, 2825 (1964).
5. Beugelmans R., Shapiro R. H., Durham L. J., Williams D. H., Budzikiewicz H., Djerassi C.: J. Amer. Chem. Soc. *86*, 2832 (1964).
6. Karliner J., Budzikiewicz H., Djerassi C.: J. Org. Chem. *31*, 710 (1966).
7. Egger H., Spiteller G.: Monatsh. Chem. *97*, 579 (1966).
8. Fenselau C. C., Robinson C. H.: J. Amer. Chem. Soc. *93*, 3070 (1971).
9. Jovanovic J., Spiteller G.: Tetrahedron *29*, 4017 (1973).
10. Beard C., Wilson J. M., Budzikiewicz H., Djerassi C.: J. Amer. Chem. Soc. *86*, 269 (1964).
11. Gutzwiller J., Djerassi C.: Helv. Chim. Acta *49*, 2108 (1966).
12. Powell H., Williams D. H., Budzikiewicz H., Djerassi C.: J. Amer. Chem. Soc. *86*, 2623 (1964).
13. Kingston D. G. I., Holbroeck B. W., Bursey M. M., Bursey J. T.: Chem. Rev. *75*, 693 (1975).
14. Green M. M., Cook R. J., Schwab J. M., Roy R. B.: J. Amer. Chem. Soc. *92*, 3076 (1970).
15. Wulfson N. S., Zaretskii V. I., Sadovskaya V. L., Zakharychev A. V., Ananchenko A. N., Torgov I. V.: Tetrahedron *23*, 3667 (1967).
16. Tureček F., Vystrčil A., Hanuš V.: Org. Mass Spectrom. *12*, 3 (1977).
17. Allinger N. L., Miller M. A.: J. Amer. Chem. Soc. *83*, 2145 (1961).
18. Černý V., Kasal A., Šorm F.: This Journal *35*, 1235 (1970).
19. Spiteller-Friedmann M., Spiteller G.: Org. Mass Spectrom. *2*, 901 (1969).
20. Coxon J. M., Hoskins P. R., Ridley T. K.: Austr. J. Chem. *30*, 1735 (1977).
21. Kočovský P., Černý V.: This Journal *41*, 2620 (1976).
22. Kočovský P., Černý V., Vašíčková S., Synáčková M.: This Journal *42*, 3339 (1977).

Translated by Ž. Procházka.