UDC 545.85+541.632

MASS SPECTROMETRY OF STEROID SYSTEMS

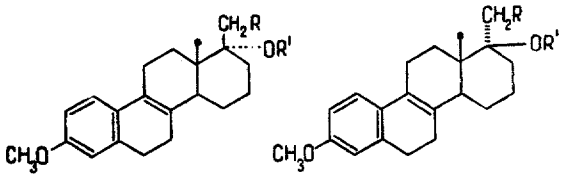
XI. Determination of the Configuration of Steroid Alcohols

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

Khimiya Prirodnykh Soedinenii, Vol. 3, No. 6, pp. 369-382, 1967

The mass spectrometric method, which is widely used to establish the structure of complex natural compounds [1], has still been applied inadequately to the solution of stereochemical problems. There are only occasional papers [2-4] and there is no published information on systematic investigations in this field. With the epimeric tertiary alcohols of D-homestrane and the secondary alcohols of the pregnane series as examples, we have studied the relationship between the configuration and the routes of decomposition of these compounds under the action of electron impact.

Tertiary alcohols of the D-estrane series. Previously [5, 6], by using the method of fragmentation mass spectrometry, we showed that of the two pairs of epimeric alcohols Ia, Ib, IIa, IIb, which are derivatives of d*l*-3-methoxy-17a-alkyl-D-homo-1, 3, 5(10), 8-estratetraen-17α-ol, the first have the e-alkyl-a-OH configuration at C-17 and the second the a-alkyl-e-OH configuration. It was also observed [6] that the epimers IIa, IIb, which readily undergo dehydrogenation under the action of $POCl_3$ in pyridine at 20° C, have a smaller tendency to form the fragments M^+-18 under the conditions of mass spectrometry than the epimers Ia, Ib, which do not undergo dehydrogenation at all by the action of $POCl_3$ under the conditions given.



Ia R = R' = H
Ib R = CH_3, R' = H
Ic R = H, R' = $COCH_3$

IIa R = R' = H
IIb R = CH_3, R' = H
IIc R = H, R' = $COCH_3$

This permitted the assumption that the mechanisms of chemical dehydration and of the splitting out of the elements of water under the action of electron impact are different. To elucidate this question, we have studied the mass spectra of the 17a-trideuteromethyl analogs of the epimers Ia and IIa [Ia-CD_3 and IIa-CD_3]. On comparing their mass spectra (Fig. 1a, 1b) it can be seen that the epimer Ia-CD_3, which is readily dehydrated under the action of the electron impact, forms fragment a_1 (M^+-H_2O, m/e 297) with an endocyclic double bond almost exclusively, while the epimer IIa-CD_3, which dehydrates with greater difficulty under these conditions, gives fragments with both endocyclic (a_1, m/e 297) and exocyclic double bonds (a_2, M^+-DOH, m/e 296) (Scheme 1 and Table 1). The formation from the molecular ion of Ia-CD_3 of the fragment a_1 (and not a_2) shows the axial location of the 17a-OH group in the latter (only in this case is it possible for one of the axial hydrogen atoms of ring D to leave the OH group) and, thus, this is a new proof of the configuration of the 17a center in the molecule of this compound. On the other hand, the presence in the spectrum of the epimer IIa-CD_3 of peaks of the fragments a_1 (M^+-H_2O, m/e 297) and a_2 (M^+-DOH, m/e 296) with approximately equal intensities shows the a-alkyl-e-OH configuration of the 17a center in this compound.

The results obtained have enabled us to propose a new approach to the determination of the configuration of steroids and, possibly, other cyclic tertiary alcohols by fragmentation mass spectrometry. Thus, the presence in the mass spectrum of the trideuteromethyl analog of a cyclic alcohol of a doublet of dehydration peaks of comparatively low intensity (corresponding to the elimination of H_2O and DOH and the formation of endo- and exocyclic double bonds) is an indication of the a-alkyl-e-OH configuration. Likewise, the presence in the mass spectrum of such a compound of an intense M^+-H_2O peak and an extremely small M^+-DOH peak indicates the e-alkyl-a-OH configuration.

A comparison of the mass spectra of Ia-CD_3 and IIa-CD_3 (cf. Fig. 1a, 1b) with the spectra of the unlabeled epimers Ia and IIa (Fig. 2a, 2b) also enables us to show that the M-H_2O-CH_3 peak in the spectra of the latter is composite, and its appearance is connected with the formation of fragments of types b_1, b_2, and b_3 (cf. Scheme 1). The influence of the configuration of epimeric alcohols on the intensity of the characteristic peaks in the mass spectra of these compounds obtained in the instrument with a metallic inlet system that we have described [5] can be seen particularly clearly on comparing their spectra taken with the use of a system for the introduction of a sample directly into the ion source (Figs. 2a, 2b, 3a, 3b, and Table 2).

As has been mentioned previously [5, 6], one of the characteristic peaks, the intensity of which depends on the configuration of the epimer, is that with m/e 227 (fragment c, Scheme 1). To elucidate the mechanism of the formation of fragment c (in particular, the influence of the configuration of the 17a center on this process), we recorded the

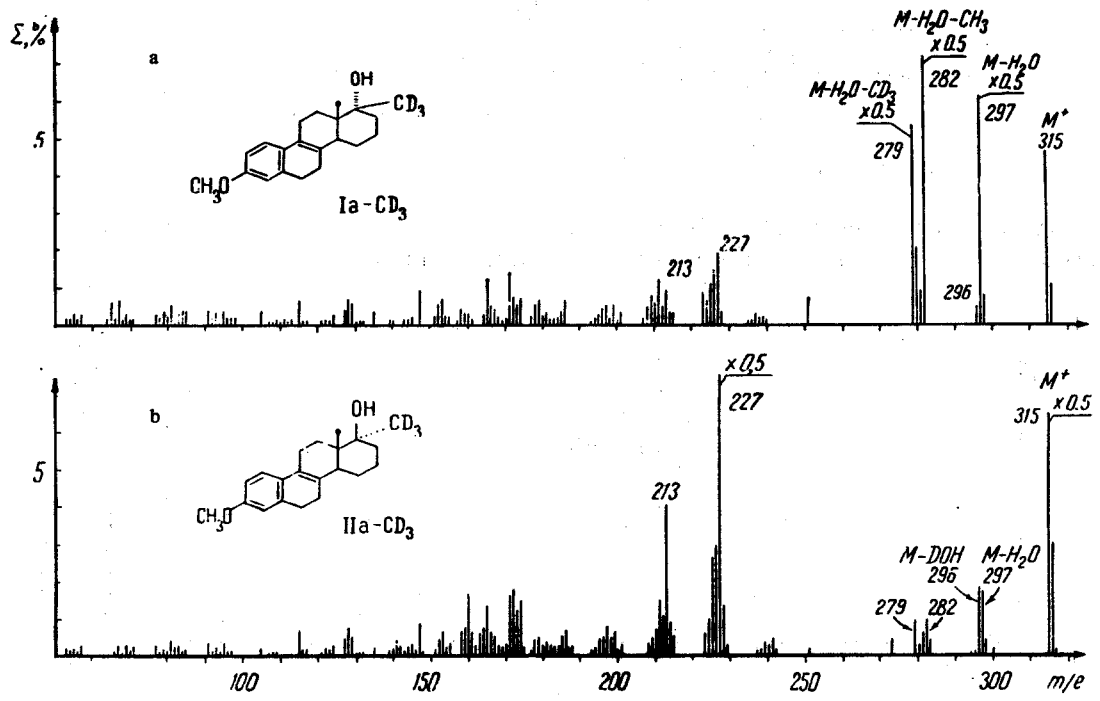
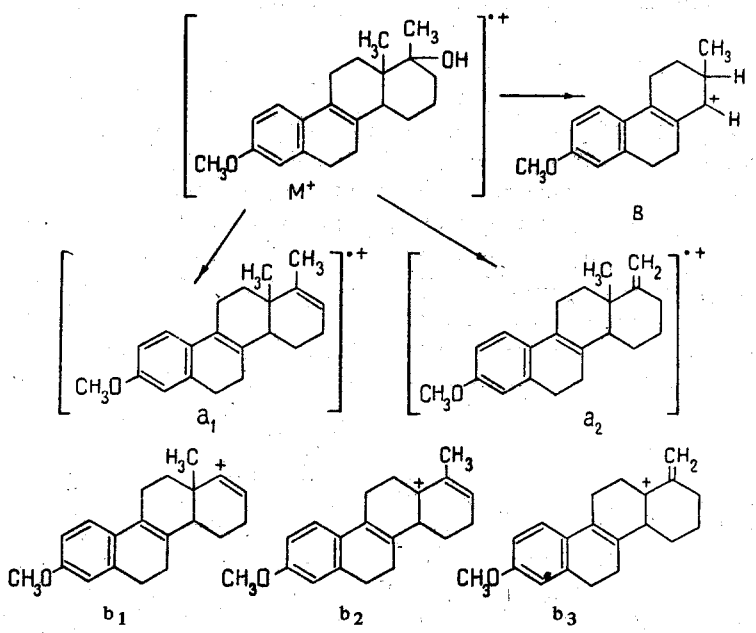


Fig. 1

mass spectrum of the O-deutero analog of the epimer IIa [IIa-OD]. The mass spectrum of this compound clearly shows the shift in the peak of fragment c by one unit (to m/e 228). This shows that the H atom of the hydroxy group takes part in the formation of the rearranged fragment c and, consequently, the difference in the intensity of this peak with m/e 227 in the epimers is connected with its configuration (the H atom from an equatorial OH group migrates more readily than from an axial group).

Scheme 1



Acetates of tertiary steroid alcohols. A comparison of the mass spectra of the acetates Ic and IIc shows that, as in the case of the free steroid alcohols, the elimination of an axial $OCOCH_3$ group under electron impact takes place more

readily than that of an equatorial group. In actual fact, the spectrum of the acetate Ic contains a small peak of the molecular ion and a very intense peak of the ion M^+-CH_3COOH (m/e 294), while for the epimer IIc, the M^+ peak is only three times smaller than the peak with m/e 294 (the ion M^+-CH_3COOH) (Table 3 and Fig. 4a, 4b).

Table 1

Compound	$\frac{M-H_2O}{M^+}$	$\frac{M\text{-}DOH}{M^+}$	$\frac{M-H_2O-CH_3}{M^+}$	$\frac{M-H_2O-CD_3}{M^+}$	$\frac{M-DOH-CH_3}{M^+}$
(Ia) — CD_3	2.6	0.1	3.1	2.3	0.2
(IIa) — CH_3	0.14	0.14	0.08	0.08	0,05

The mass spectra of Ic and IIc also differ with respect to the intensities of the peaks with m/e 279, owing to the detachment of a CH_3 radical from the M^+-CH_3COOH ion. As was mentioned above for the tertiary alcohols, the peak of

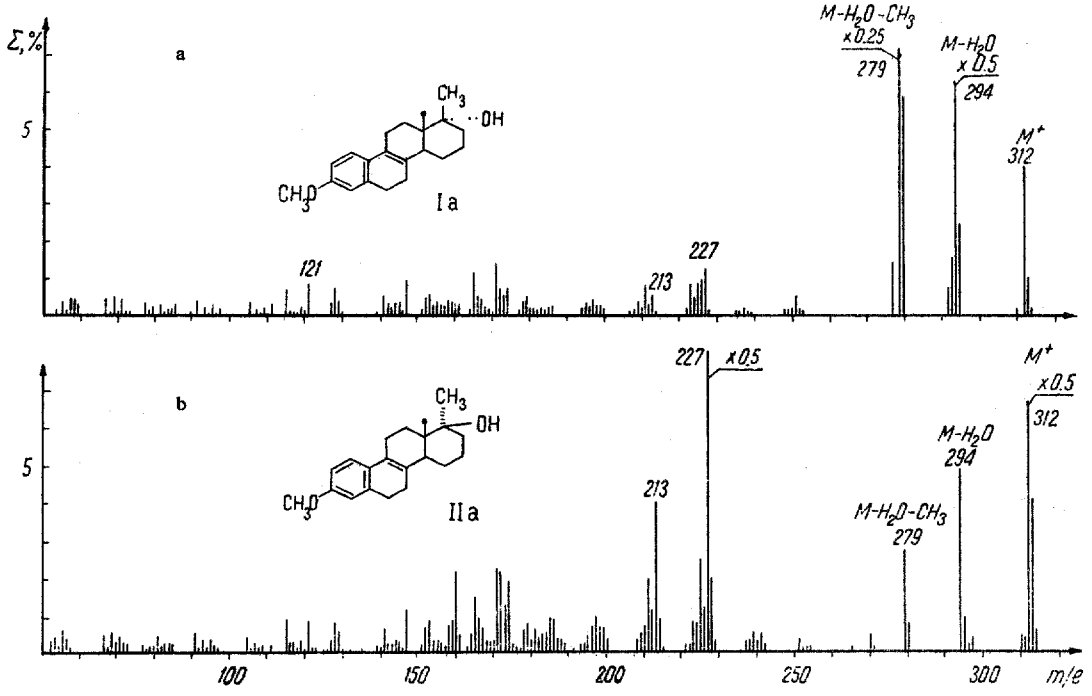


Fig. 2.

the ion M^+-H_2O-CH_3 is complex and corresponds to the elimination of both the angular and the 17a CH_3 groups. The difference in the intensities of the peaks of the ions M^+-CH_3COOH-CH_3 (m/e 279) for the epimers Ic and IIc shows that this peak is also complex, and the mechanisms of the elimination of a molecule of CH_3COOH by the molecules of the ions of these compounds differ. The high intensity of the peak with m/e 279 (M^+-CH_3COOH-CH_3) in the spectrum of the epimer Ic (a-$OCOCH_3$) shows that in this case the elimination of the elements of acetic acid leads to the formation of the ion a_1 with an endocyclic double bond, while for the acetate IIc (e-$OCOCH_3$ group) the appearance of the ion a_2 with an exocyclic double bond, in addition to the ion a_1, is characteristic. In actual fact, in the formation of the fragment with the exocyclic double bond a_2, the splitting out of the 17a CH_3 group is impossible, so that the intensity of the peak of the M^+-CH_3COOH-CH_3 (m/e 279) decreases (cf. Table 3 and Fig. 4a, 4b).

Table 2

Compound	M^+	M^+-18	m/e 279		m/e 227	$\frac{M^+-18}{M^+}$	$\frac{m/e\ 279}{M^+}$
			$M^+-18-15$	$M^+-18-29$			
Ia	4.0	12.6	28.8	—	1.2	3.2	7.2
IIa	13.4	4.8	2.8	—	16	0.4	0.2
Ib	3.2	7.3	—	26	2.6	2.3	8.2
IIb	13	2.3	—	1.4	16	0.2	0.1

Secondary steroid alcohols. A determination of the configuration of secondary cyclic alcohols and their acetates by means of mass spectroscopy was first reported by Biemann and Seibl [2]. However, the mass spectra of the epimers were obtained by these American workers under fairly severe experimental conditions (with evaporation of the sample in a hot metallic inlet system).

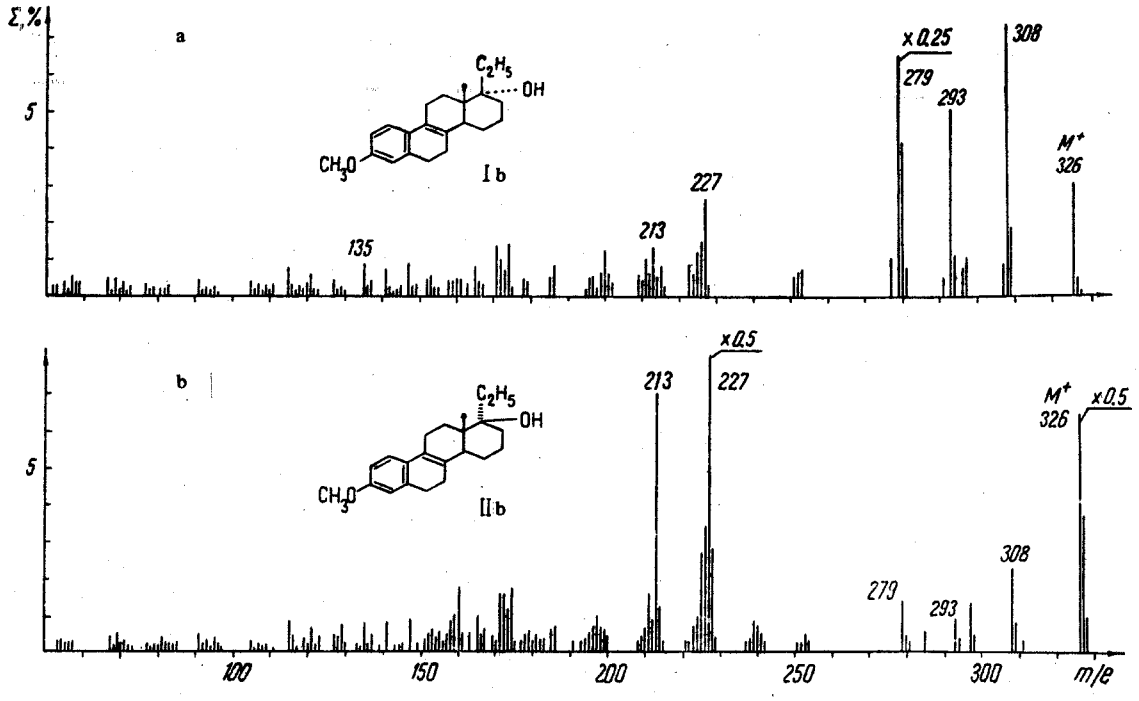


Fig. 3

By using the system of introducing the sample directly into the ion source close to the ionization chamber, we obtained the mass spectra of the epimers III–X under milder conditions (145–165° C), i.e., at a considerably lower temperature than the melting point of the compounds studied. The mass spectra of the α-hydroxysteroids containing an OH group in rings C and D obtained under these conditions differ considerably from the spectra of the corresponding

Table 3

Compound	M+ m/e 354	M+−AcOH m/e 294	$\frac{M^+-AcOH}{M^+}$	M+−AcOH−CH_3 m/e 279
Ic	0.17	10.7	63	11.9
IIc	2.8	8.5	3	5

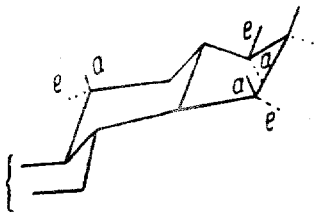
β-hydroxy epimers mainly by the ratio of the intensities of the peaks M-H_2O and M^+(cf. Table 4). This enables us, in a series of similar monohydroxysteroids, to determine the configuration of the corresponding alcohol on the basis of its mass spectrum even when the second epimer is lacking.

Table 4

Position of the OH group	M+	M−H_2O	$\frac{M-H_2O}{M^+}$	m/e 231	m/e 100
11α (III)	3.2	2.1	0.66	—	—
11β (IV)	0.9	5.1	5.7	—	—
16α (V)	0.2	2.6	13	4.6	3.2
16β (VI)	0.75	3.2	4.3	0.65	0.6
15α (VII)	7.6	1.6	0.21	0.7	0.3
15β (VIII)	1.0	4.9	4.9	3.0	1.3
15α (X)	7.6	0.5	0.07	—	—

11α-(III) and 11β-hydroxyprogesterones (IV). On comparing the mass spectra of the compounds, it can be seen that, like the tertiary alcohols of the D-homo series, 11β-hydroxyprogesterone (IV) with the axial OH group readily eliminates

a molecule of water under electron impact (Fig. 5, Table 4), while the molecular ion of the 11α-epimer (III), in which the OH group is equatorial, is more stable under these conditions and undergoes dehydration to a smaller extent.



16α-(V) and 16β-hydroxyprogesterones (VI)*. In spite of the fact that both epimeric 16-hydroxyprogesterones undergo dehydration fairly readily under electron impact there are considerable differences in their mass spectra (Fig. 6a, 6b). In the first place, the degree of dehydration of the molecular ion of 16α-hydroxyprogesterone (V) is greater than

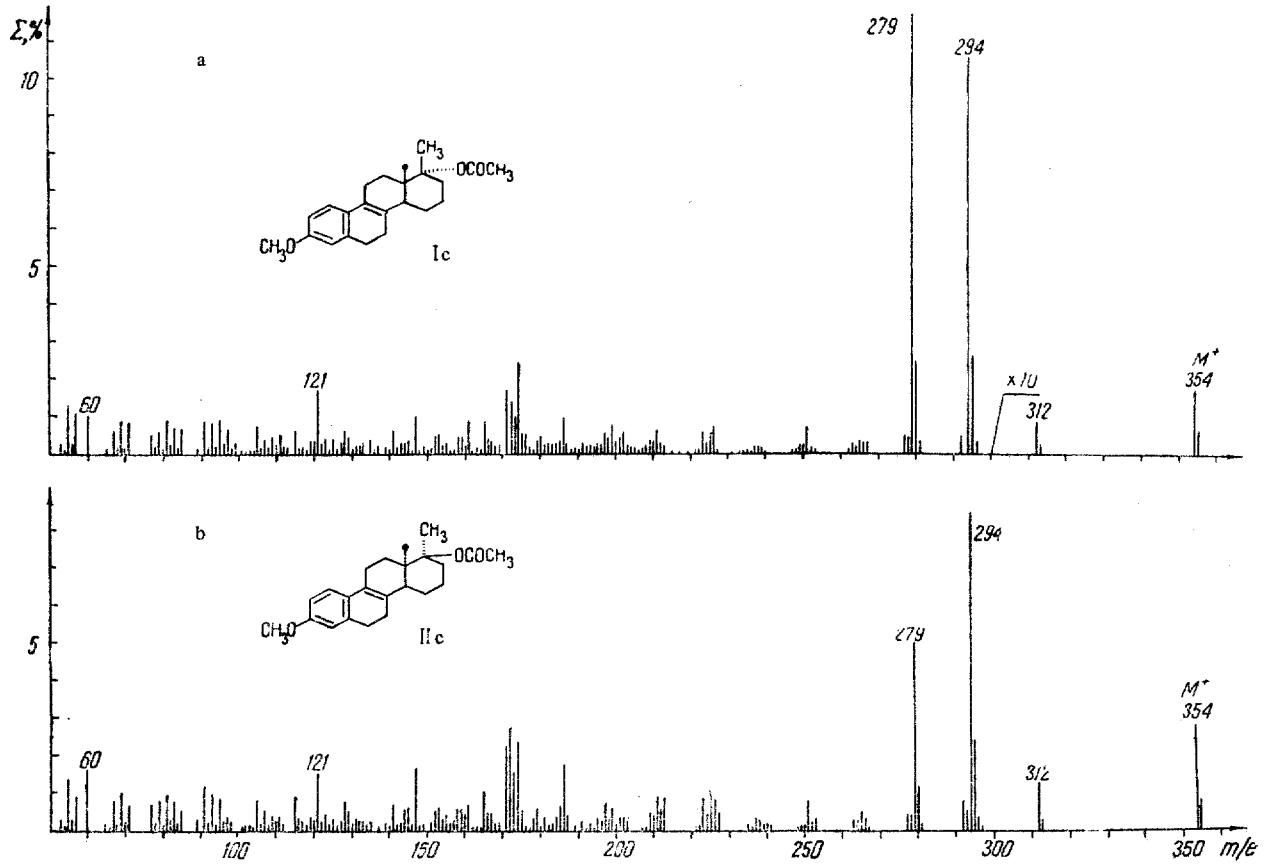
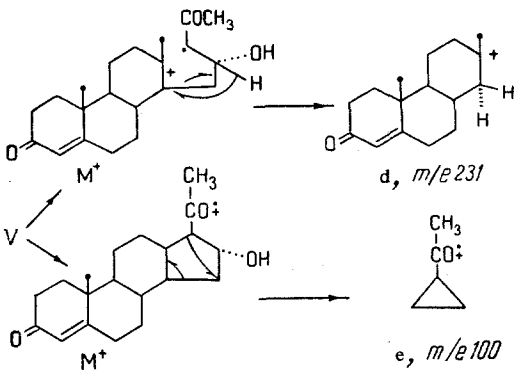


Fig. 4

that of its β-epimer. This enables us to conclude that the α-bond at C-16 is similar in nature to an axial bond and the β-bond to an equatorial bond. In the second place, a characteristic feature of the mass spectrum of V is an intense peak with m/e 231 (the ion d) which is practically absent in the case of VI. The fragment d contains the rings A, B, and C [which is confirmed by the shift in the mass number of the corresponding peak by 16 units (to m/e 247) in the spectrum of 11α, 16α-dihydroxyprogesterone (IX)] and is formed as the result of the rupture of the 13–17 and 14–15 bonds and the migration of a hydrogen atom to the charged fragment (Scheme 2). The cleavage of the same bonds, accompanied by the localization of the charge on the fragment containing the atoms C-15–C-17, C-20, and C-21, leads to the appearance of an ion with m/e 100 (e). The peak of this ion is of considerable magnitude in the case of 16α-hydroxyprogesterone (V) and is practically absent from the spectrum of 16β-hydroxyprogesterone (VI). The structure of ion e is shown by the mass spectrum of the 16α-OD derivative, in which the mass number of the corresponding peak has been shifted by a unit (to m/e 101), and also by the fact that in the spectrum of 15-hydroxyprogesterones m/e for this peak is unchanged. It might be assumed that the hydrogen atom from the 16-OH group takes part in the formation of the ion d (m/e 231), as was mentioned above for the tertiary alcohols. However, in the mass spectrum of the 16α-OD analog (V)

Scheme 2



the peak with m/e 231 is shifted to the peak with m/e 232 to the extent of only 24%. The process of formation of the ion d apparently includes the migration of a hydrogen atom from C-16, while the greater intensity of the peak with m/e 231 in the case of V as compared with its β-analog may be connected with the fact that a 16β-hydrogen atom migrates more readily than a 16α-H atom.

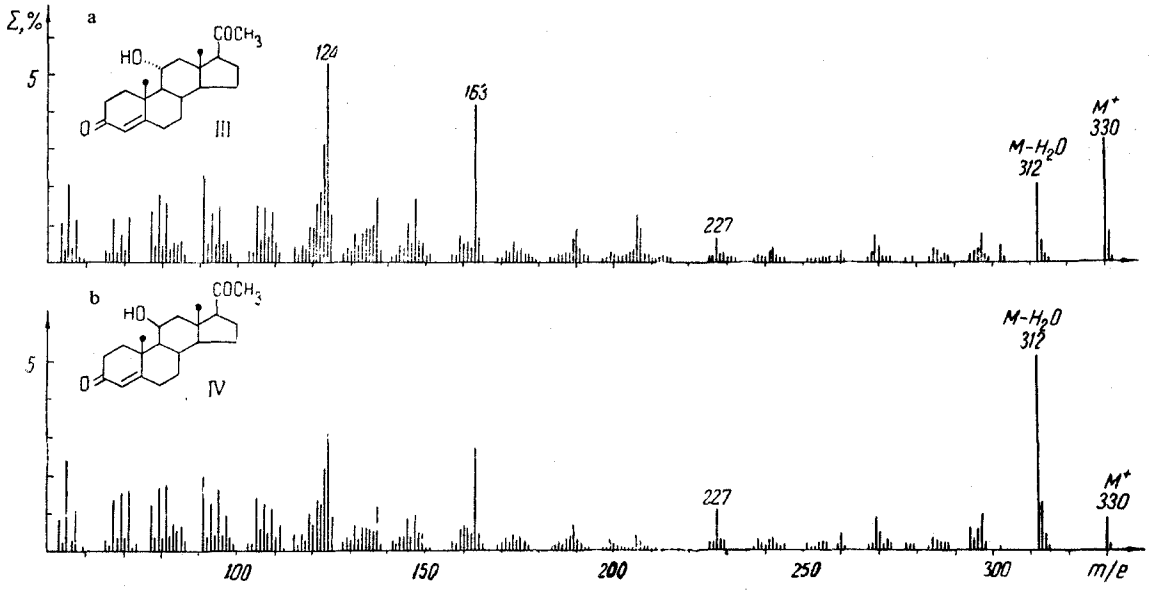


Fig. 5

15α- (VII) and 15β-hydroxyprogesterone (VIII). In contrast to the isomeric 16-hydroxyprogesterones V and VI, in whose mass spectra a large value of the ratio $M-H_2O/M^+$ (cf. Table 4) is characteristic for the α-isomer, in the case of their 15α- and 15β-analogs the opposite relationship is found (Fig. 7a, 7b). This is due to the fact that in the molecule of VII the OH group has an equatorial character, while in the β-isomer (VIII) it is axial. A similar pattern is found in the mass spectrum of 15α-hydroxy-4-androstene-3,17-dione (X) (cf. Table 4). The characteristic difference of the mass spectrum of 15β-hydroxyprogesterone (VIII), just as in the case of its 16α-hydroxy analog (V) is the considerable intensity of the peaks of fragments d (m/e 231) and e (m/e 100), the mechanism of the formation of which is apparently similar to that described above for V. In the mass spectrum of 15α-hydroxyprogesterone (VII) and 15α-hydroxy-4-androstenedione (X), the intensity of the peaks with m/e 231 and 100 is very small (cf. Table 4 and Fig. 7a).

We had no samples of 17α-hydroxysteroids. However, it must be mentioned that the steroids of the 17β-hydroxy series (estradiol, 19-nortestosterone), in whose molecules the OH groups have an equatorial character, undergo dehydration with difficulty under electron impact.

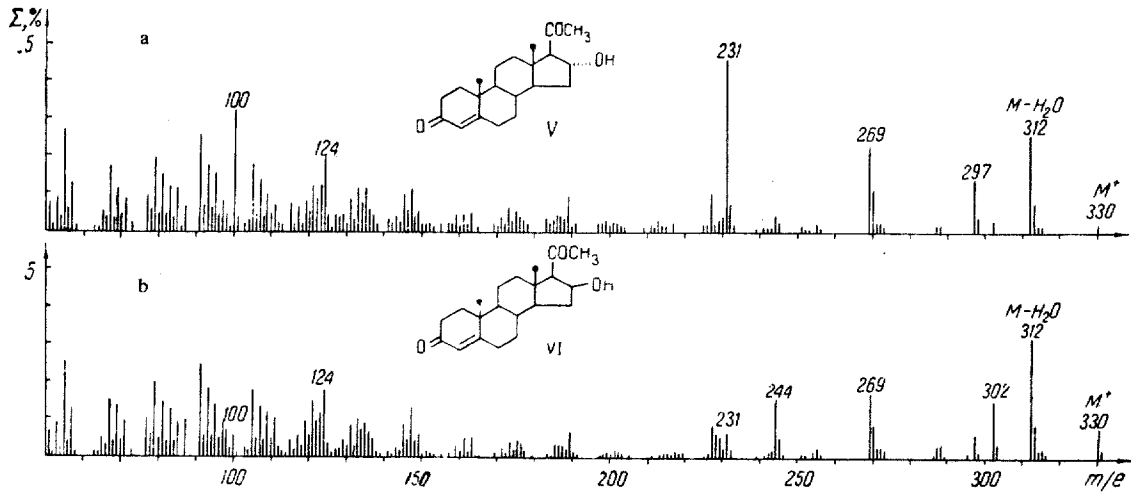


Fig. 6.

Experimental

The mass spectra were taken on a commercial MKh-1303 instrument fitted with a glass system for the introduction of the sample directly into the ion source close to the ionization chamber and with stabilization of the inlet temperature (±1° C) with an ionization energy of 70 eV at temperatures of 100–120° C (for compounds Ia–Ic, IIa–IIc) and 145–165° C (for compounds III–X).

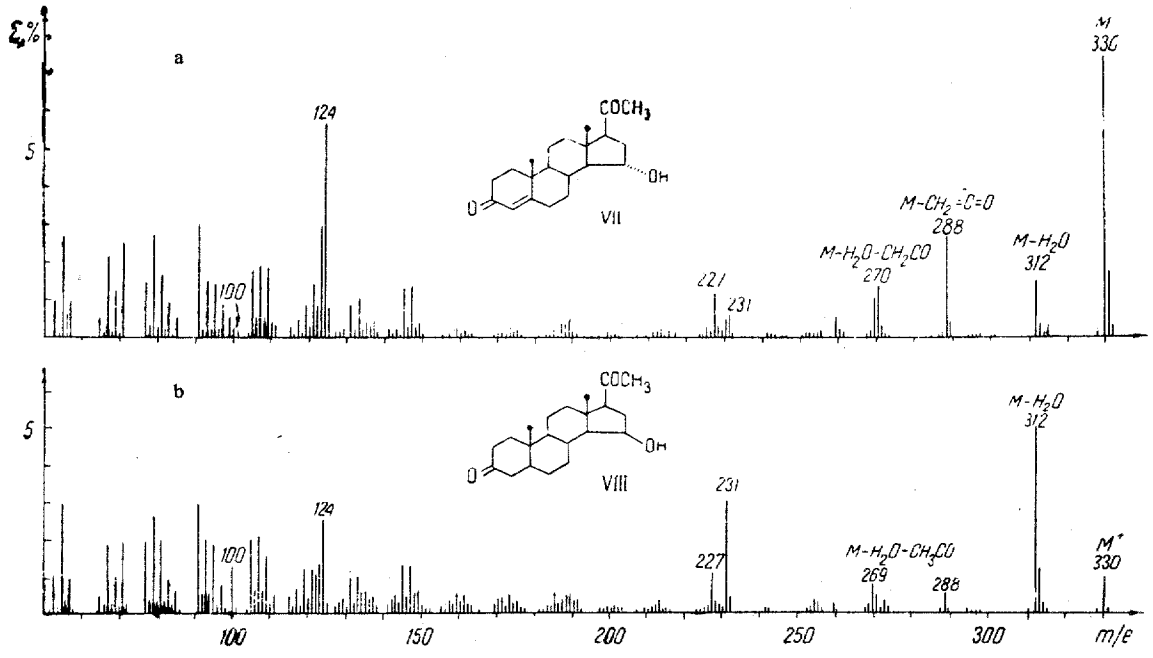


Fig. 7.

Compound IIa-OD was obtained by keeping a solution of IIa in C_2H_5OD at room temperature for 3 hr, after which this solution, containing a mixture of IIa and IIa-OD was used directly for obtaining the mass spectrum.

Compounds Ia–Ic, IIa–IIc, Ia-CD_3, and IIa-CD_3 were kindly given to us by V. N. Leonov, 11β-hydroxyprogesterone (IV), 16β-hydroxyprogesterone (VI), and 11α, 16α-dihydroxyprogesterone (IX) by L. M. Kogan, 15α-hydroxyprogesterone by Professor B. Camerino (Italy), 15β-hydroxyprogesterone by Dr. P. Diassi (USA), and 16α-hydroxyprogesterone by Dr. O. Hanč (Czechoslovakia), to whom we express our deep gratitude.

Summary

1. The mass spectra of epimeric tertiary alcohols of the D-homoestrane series and the acetates and 17a-trideuteromethyl analogs corresponding to them have been studied. A new approach to the determination of the configuration of tertiary steroid alcohols has been proposed in which the trideuteromethyl analogs of the epimeric alcohols are subjected to mass-spectrometric analysis. The presence in the mass spectrum of an epimer of a doublet of peaks M-H_2O and M-DOH indicates the a-alkyl-e-OH configuration, while the presence in the spectrum of an intense M-H_2O peak shows the presence of an axial OH group.

2. The mass spectra of the epimeric secondary alcohols of the pregnane series taken with the use of a system of introducing the sample into the ion source has been investigated. It has been shown that the mass spectra of alcohols containing an a-OH group in rings C and D differ markedly from the spectra of the e-OH epimers with respect to the ratio of the intensities of the peaks M-H_2O and M^+.

REFERENCES

1. H. Budzikiewicz, C. Djerassi, and D. H. Williams, Structure Elucidation of Natural Products by Mass Spectrometry, Holden-Day, Inc., San Francisco, 1, 2, 1964.

2. K. Biemann and J. Seibl, J. Am. Chem. Soc., 81, 3149, 1959.

3. H. Budzikiewicz and C. Djerassi, J. Am. Chem. Soc., 84, 1430, 1962.

4. C. Djerassi, J. Wilson, H. Budzikiewicz, and J. Chamberlin, J. Am. Chem. Soc., 84, 4544, 1962.

5. N. S. Vul'fson, I. V. Torgov, V. I. Zaretskii, V. N. Leonov, S. N. Ananchenko, and V. G. Zaikin, Izv. AN SSSR, ser. khim., 184, 1964.

6. S. N. Anachenko, V. N. Leonov, V. I. Zaretskii, N. S. Wulfson, and I. V. Torgov, Tetrah., 20, 1279, 1964.

7. K. Biemann, Mass Spectrometry, McGraw-Hill Book Co., Inc., New York, 147, 1962.

8. V. I. Zaretskii, N. S. Wulfson, V. G. Zaikin, L. M. Kogan, N. E. Voishvillo, and I. V. Torgov, Tetrah., 22, 1399, 1966.

25 July 1966

Institute of the Chemistry of Natural Compounds, AS USSR