Tetrahedron, 1966, Vol. 22, pp. 1885 to 1892. Pergamon Press Ltd. Printed in Northern Ireland

MASS SPECTROMETRY OF STEROID SYSTEMS—VI*

CIS-TRANS ISOMERISM IN THE ESTRANE AND D-HOMOESTRONE SERIES

N. S. WULFSON, V. I. ZARETSKII, V. L. SADOVSKAYA†, S. N. ANANCHENKO,
V. M. RZHEZNIKOV and I. V. TORGOV‡
Institute for Chemistry of Natural Products, USSR Academy of Sciences,
Moscow, USSR

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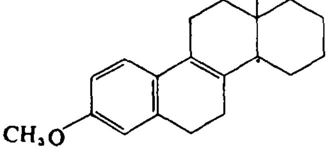
Abstract—The fragmentation behaviour of *cis*- and *trans*-isomers of $\Delta^{8(9)}$-dehydro-D-homoestrone, estradiol and the 19-nortestosterone series upon electron bombardment has been investigated. The intensity differences between the characteristic fragment peaks provides a basis for *cis*- or *trans*-configurational assignments to these compounds.

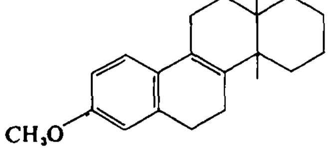
EARLIER[1,2] using as examples, 14α- and 14β-D-homoequilenin methyl ethers, 8β- and 8α-D-homoestrone methyl ethers and A/B-*cis*- and *trans*-sclareoloxides, it was possible by means of mass spectrometry to determinate the mode of ring fusion in the polycyclic systems. Continuing work in this sphere, the mass spectra of isomers of $\Delta^{8(9)}$-dehydro-D-homoestrone, estradiol, 19-nortestosterone and the Δ^{4}-androsten-3,17-dione series have been investigated.

I. C/D-cis- *and* trans-*Isomers of* $\Delta^{8(9)}$-*dehydro-*D*-homoestrone series*

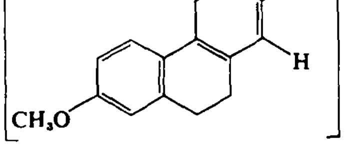
A comparison of the mass spectra of known $\Delta^{8(9)}$-dehydro-D-homoestrone methyl ether (I; obtained by hydrogenation of $\Delta^{8,14}$-*bis*-dehydro-D-homoestrone methyl ether over 10%-Pd on $CaCO_3$) and of its isomer (II) with unknown configuration (prepared by hydrogenation of $\Delta^{8,14}$-*bis*-dehydro-D-homoestrone methyl ether under pressure in the presence of rhenium heptasulfide) show that they differ from each other by the mode of fusion of rings C and D, the former (I) belongs to the 14α- and



I



II



a, *m/e* 226

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* For paper V see V. I. Zaretskii, N. S. Wulfson, V. G. Zaikin, Leonid M. Kogan, N. E. Voishvillo and I. V. Torgov, *Tetrahedron* **22**, 1399 (1966).

† Laboratory of Mass Spectrometry of this Institute.

‡ Laboratory of Steroid Chemistry of this Institute.

[1] V. I. Zaretskii, N. S. Wulfson, V. L. Sadovskaya, S. N. Ananchenko and I. V. Torgov, *Dokl. Akad. Nauk SSSR* **158**, 385 (1964).

[2] N. S. Wulfson, V. I. Zaretskii, V. L. Sadovskaya, A. V. Semenovsky, W. A. Smit and V. F. Kucherov, *Tetrahedron* **22**, 603 (1966).

the latter (II) to the 14β-series. The intensity of the peak of the tricyclic fragment (*a*, *m*/*e* 226), formed by cleavage of the bonds at the C and D ring junction (as well as in case of 14α- and 14β-D-homoequilenin[1]) is seven times as large in the spectrum of II as in that of I (Table 1 and Fig. 1a,b). The configuration at C-14 in the molecules of I and II is also confirmed by a strong increase in intensity of the M-2H (*m*/*e* 294) peak by passing from *cis*(II)- to *trans*-isomer (I) (M–2H peak is almost five times as large in spectrum of I as in that of II, cf.[1]).

Table 1. Abundance of characteristic peaks (% of M^+) in the mass spectra of 14α(I)- and 14β-$\Delta^{8(9)}$-D-homoestrone methyl ether (II)

m/*e*	I	II	II:I
294	42·0	9·0	0·2
281	74·0	45·0	0·6
253	32·0	6·0	0·2
226	6·5	46·0	7·0
225	20·0	45·0	2·0

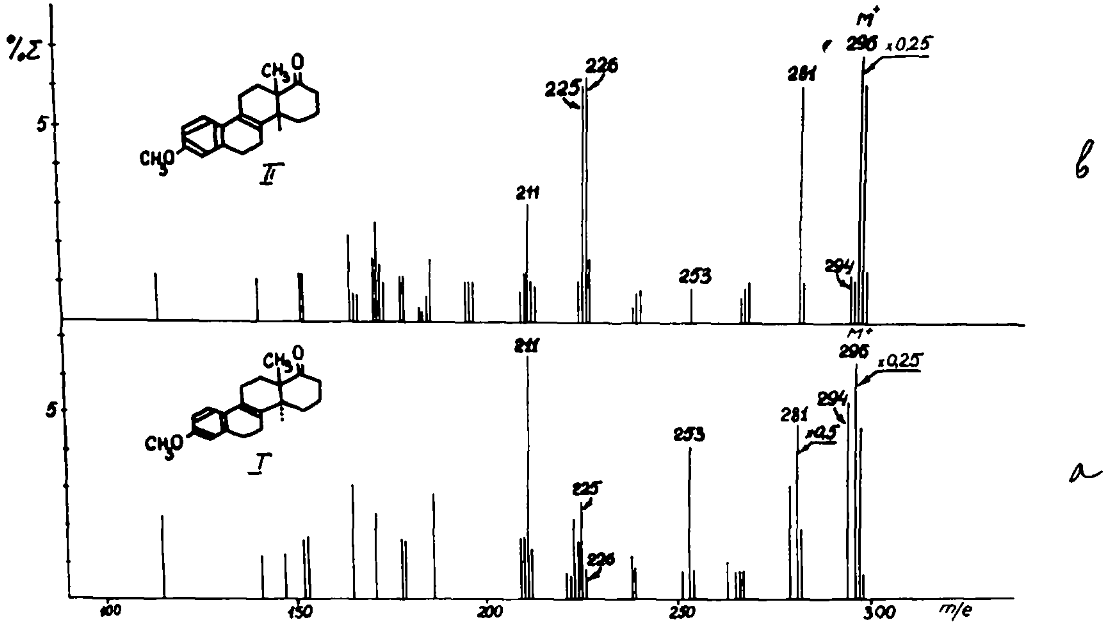

Fig. 1. Mass spectra of:
(a) $\Delta^{8(9)}$-dehydro-D-homoestrone methyl ether (I)
(b) 14β-$\Delta^{8(9)}$-Dehydro-homoestrone methyl ether (II)

II. *B*/*C*-cis- *and* trans-*Isomers of estradiol series*

The mass spectrum of estradiol methyl ether (III) was taken by Djerassi[3] on an instrument provided with an all-glass inlet system heated to 200°. But a comparison

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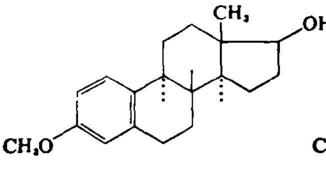
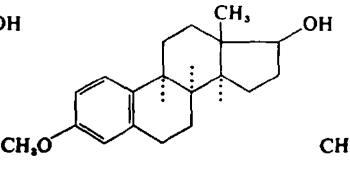
[3] C. Djerassi, J. M. Wilson, H. Budzikiewicz and J. W. Chamberlin, *J. Amer. Chem. Soc.* **84**, 4544 (1962).

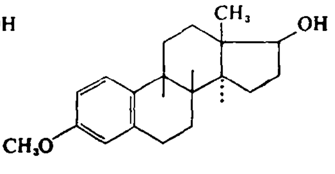
of the mass spectrum of III with that of its 8α-(IV) and 9β-isomers (V) has not been made. We have studied the mass spectra of these B/C-*cis*- and *trans*-isomeric estradiol methyl ethers taken on a spectrometer with glass inlet system (200°) and under more gentle conditions (100°)—by using a direct inlet system. The comparison of the mass spectra showed that the *m/e* 160 (ion *b*) and 186 (ion *c*) peak intensities increase on passing from the compounds III (8β,9α-configuration) and V (8β,9α-configuration) to their 8α,9α-isomer (IV). This must be due to a great thermodynamic lability of the 8α-isomer (IV) in the estradiol series, whereas the stability of the 9β-isomer (V) resembles that of estradiol methyl ether with the natural configuration (III). The analogous dependence of peak *b* intensity from the mode of fusion of rings B and C has been noted earlier in the estrone series.[1,3]



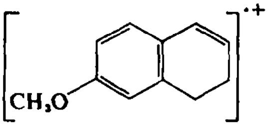
III



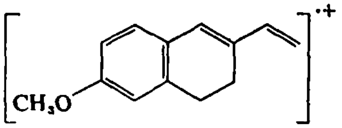
IV



V



b, *m/e* 160



c, *m/e* 186

The absence of the M–H_2O (*m/e* 268) peak in the mass spectra of III and V, taken by using a direct inlet system, is also due to a greater stability of isomers III and V as compared with their 8α-isomer (IV). The mass spectrum of III (200°, hot inlet system), reveals a M-18 peak. Its intensity, however, as in case of the 8α-isomer (IV) is low. The small degree of dehydration of molecular ions of III and IV even under these conditions are due to an equatorial 17-OH-group.[4]

The mass spectra of III and V are also distinguished from that of IV by the presence in the first two of a M–2H peak which is more prominent in the mass spectrum of the 9β-isomer (V) (21% from molecular ion intensity, Table 2). It is to be noted that in

TABLE 2. ABUNDANCE OF CHARACTERISTIC PEAKS (% OF M^+) IN THE MASS SPECTRA OF ESTRADIOL METHYL ETHER (III) AND ITS 8α(IV)- AND 9β-ISOMER (V)

m/e	III	IV	V	IV:III	V:III
284	2·5	—	21·0	—	8·5
268	—	3·6	—	—	—
186	19·3	46·0	24·5	2·4	1·3
160	15·3	50·0	22·5	3·2	1·5

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[4] V. I. Zaretskii, N. S. Wulfson, V. G. Zaikin, S. N. Ananchenko, V. N. Leonov and I. V. Torgov, *Tetrahedron* **21**, 2469 (1965).

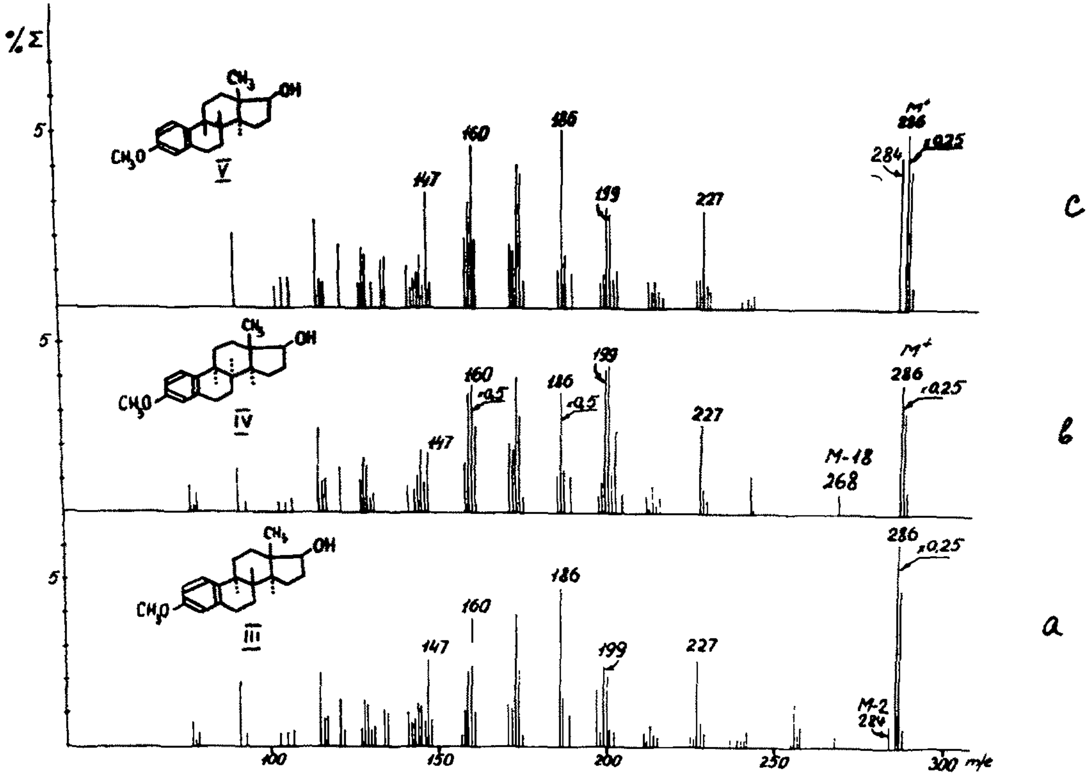
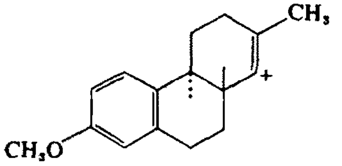


FIG. 2. Mass spectra of:
(a) Estradiol methyl ether (III)
(b) 8α-Estradiol methyl ether (IV)
(c) 9β-Estradiol methyl ether (V)

the mass spectra of III and especially V there are both tricyclic ion *d* (*m/e* 227) and *d*-2H (*m/e* 225) peaks, the latter being absent in spectra of the 8α-isomer. The formation mechanism of ions M–2H and *d*-2H is not clear, but one may suppose, that formation of these fragments in case of 9β-estradiol methyl ether (V) is connected with the favourable arrangement of both eliminating hydrogen atoms in the molecule of this compound.

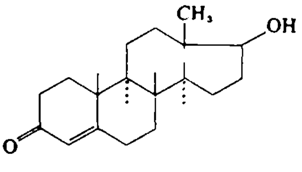


d, m/e 227

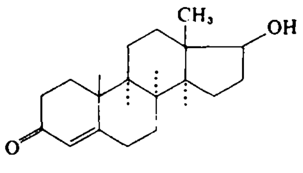
III. Δ^4-3-*Oxo*-19-*norsteroids*

The mass spectra of 19-nortestosterone (VIa), 8α(VIIa)- and 9β-19-nortestosterone (VIIIa), their D-homo-analogs (VIb, VIIb, VIIIb) and Δ^4-androsten-3,17-dione (IXa) and its 9β-isomer (IXb) as well as the *cis-trans*-isomers of $\Delta^{8(9)}$-dehydro-D-homoestrone and the estradiol series differ strongly by the characteristic fragment peak intensities.

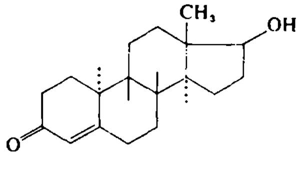
The difference in configuration between isomers VIa, b, VIIa, b, VIIIa, b and IXa, b have a pronounced influence on the value of the *m/e* 110 (ion *e*) peak. This ion is formed as a result of the cleavage of 6–7 and 9–10 bonds and migration of two hydrogen atoms to the charge fragment. As reported before,[5,6] the similar fragmentation upon electron impact is peculiar to Δ^4-3-oxo-steroids and therefore the corresponding fragment *e* structure must resemble that of homologous ions at *m/e* 124 in the case of Δ^4-cholesten-3-one, progesterone, Δ^4-androsten-3,17-dione[5] and a number of Δ^4-androsten-3-one derivatives.[6] In the spectra of testosterones with the natural configuration (VIa, b) and their 9β-isomers (VIIIa, b) the ion *e* peak intensity is considerably greater than that of 8α-testosterones (VIIa, b) (Figs. 3 and 4, Table 3.)



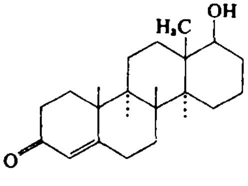
VIa



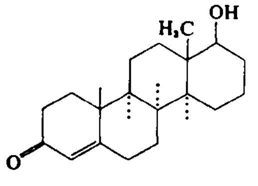
VIIa



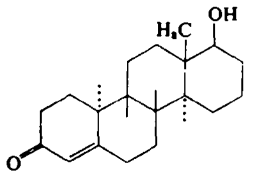
VIIIa



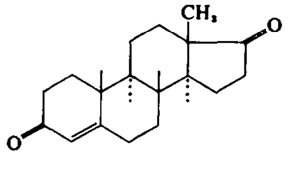
VIb



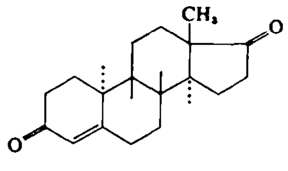
VIIb



VIIIb



IXa



IXb

TABLE 3. ABUNDANCE OF CHARACTERISTIC PEAKS (% OF M^+) IN THE MASS SPECTRA OF 19-NORTESTOSTERONES (VIa, VIIa, VIIIa) AND THEIR D-HOMO-ANALOGS (VIb, VIIb, VIIIb)

m/e	VIa	VIb	VIIa	VIIb	VIIIa	VIIIb	VIIa:VIa	VIIb:VIb	VIIIa:VIa	VIIIb:VIb
256	14·8	—	14·0	—	16·0	—	0·9	—	1·1	—
270	—	22·4	—	22·0	—	24·3	—	1·0	—	1·1
215	18·0	13·0	30·4	21·0	21·4	19·0	1·7	1·7	1·2	1·5
165	5·5	—	9·3	—	61·0	—	1·7	—	11·0	—
179	—	18·0	—	19·8	—	250·0	—	1·1	—	14·0
147	16·4	—	18·6	—	60·9	—	1·1	—	4·2	—
161	—	33·0	—	36·5	—	119·0	—	1·1	—	3·6
110	63·0	138·0	28·0	114·0	350·0	530·0	0·5	0·8	5·6	3·8

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[5] N. S. Wulfson, V. I. Zaretskii, V. G. Zaikin, G. M. Segal, I. V. Torgov and T. P. Fradkina, *Tetrahedron Letters* 3015 (1964).

[6] R. Shapiro and C. Djerassi, *J. Amer. Chem. Soc.* **86**, 2825 (1964).

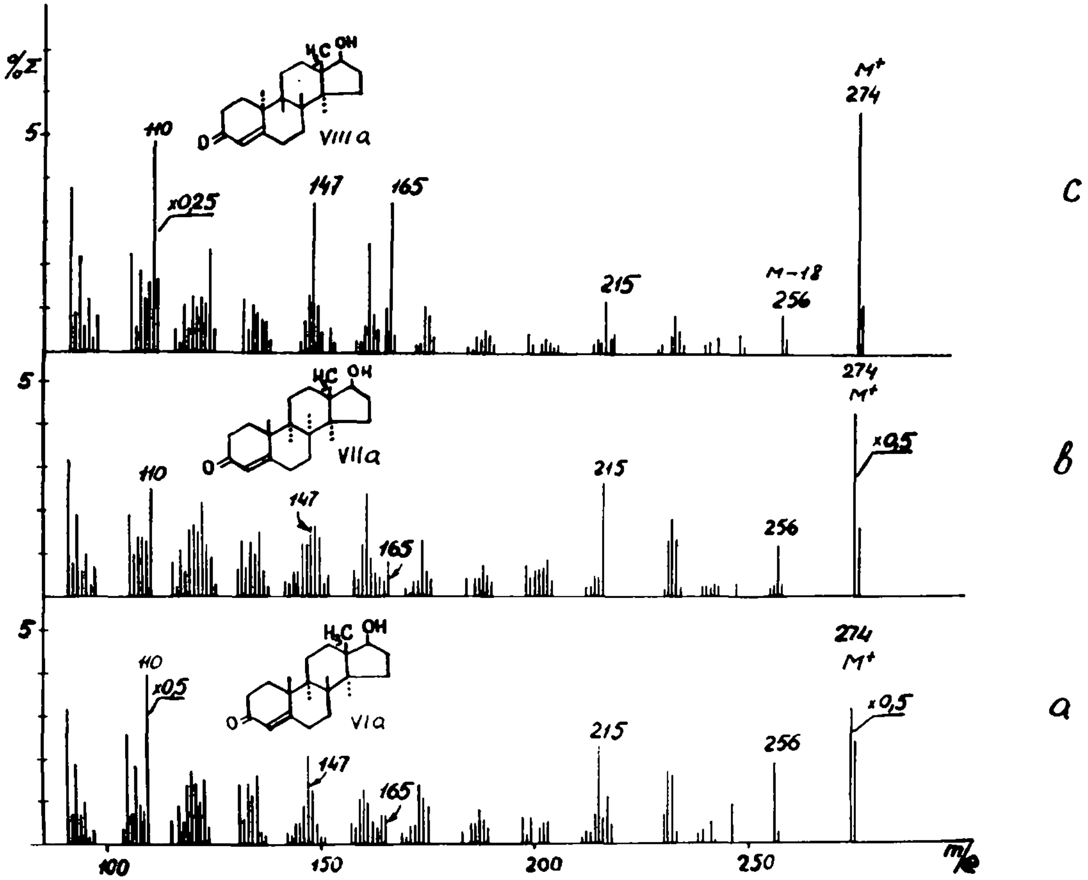
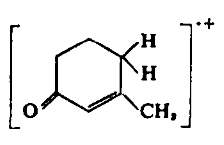
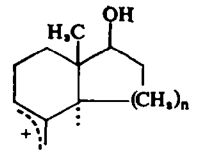


FIG. 3. Mass spectra of:
(a) 19-Nor-testosterone (VIIa)
(b) 8α-19-Nor-testosterone (VIa)
(c) 9β-19-Nor-testosterone (VIIIa)

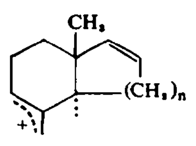
It testifies that the difference in *m/e* 110 peak intensity mainly depends on the distance between the departing hydrogen atoms and the receptor C-atom, and not on energy difference between isomers (in the latter case the *m/e* 110 peak must be greater in spectra of 8α-isomers owing to the presence in VIIa, b of a *cis*-structure as compared to testosterone with the natural configuration). In the spectra of 9β-isomers (VIIIa, b) the ion *e* peak intensity is greater than that of compounds VIa, b and VIIa, b. This may be connected with the favourable arrangement of migrating hydrogen atoms in the molecule of the 9β-isomer.



e, *m/e* 110



f_1 $n = 1$ *m/e* 165
f_2 $n = 2$ *m/e* 179



g_1 $n = 1$ *m/e* 147
g_2 $n = 2$ *m/e* 161

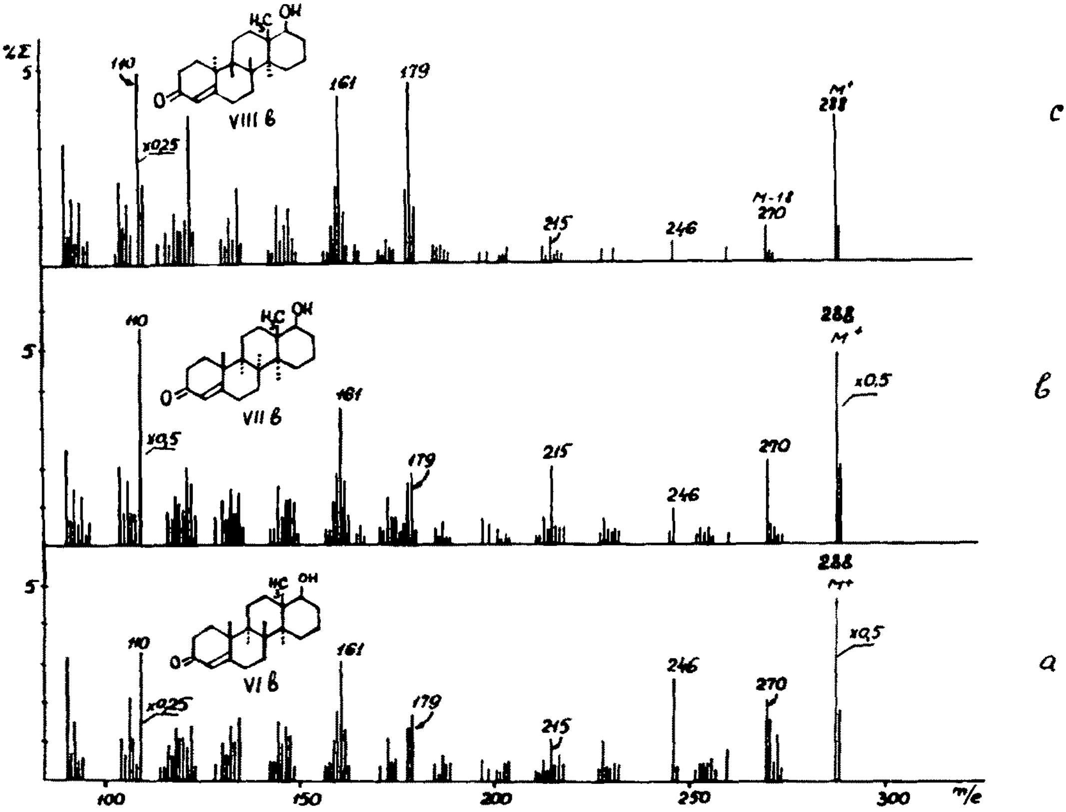


FIG. 4. Mass spectra of:
(a) 19-Nor-D-homotestosterone (VIb)
(b) 8α-19-Nor-D-homotestosterone (VIIb)
(c) 9β-19-Nor-D-homotestosterone (VIIIb)

Stereochemical differences in the structure of isomeric 19-nortestosterones (VIa, VIIa, VIIIa) and their D-homoanalogs (VIb, VIIb, VIIIb) have also a great effect on the abundance of fragments f_1 and f_2 (m/e 165 and 179, Figs. 3 and 4) and g_1 and g_2 (m/e 147 and 161), formed by cleavage of the bonds at the B and C ring junction, ions g_1 and g_2 being formed by the dehydration of f_1 and f_2, respectively. The structure of ions f_1, f_2, g_1 and g_2 is confirmed by the fact that m/e of the corresponding ions (f_2 and g_2) is displaced by a value of homologous difference (14 mass units) in the spectra of D-homo-19-nortestosterones, and by literature data.[6] Thus the comparison of mass spectra of isomeric testosterones (VIa, VIIa, VIIIa) and their D-homo-analogs (VIb, VIIb, VIIIb) shows that these peaks intensities are maximum in the case of 8α(VIIa, b)- and 9β-isomers (VIIIa, b) owing to more intensive decomposition of molecular ions, with a *cis*-system of B and C ring junction, which is more labile than a *trans*-system (Table 3).

The structure of 8α-19-nortestosterone (VIIa) and its D-homo-analog (VIIb) was established earlier.[7,8] The configuration at C-10 in the molecule of 9β-isomers

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[7] K. K. Koshoev, S. N. Ananchenko and I. V. Torgov, *Chim. Prirod. Soed.* 180, No. 3 (1965).

[8] V. M. Rzheznikov, S. N. Ananchenko and I. V. Torgov, *Chim. Prirod. Soed.* 90, No. 2 (1965).

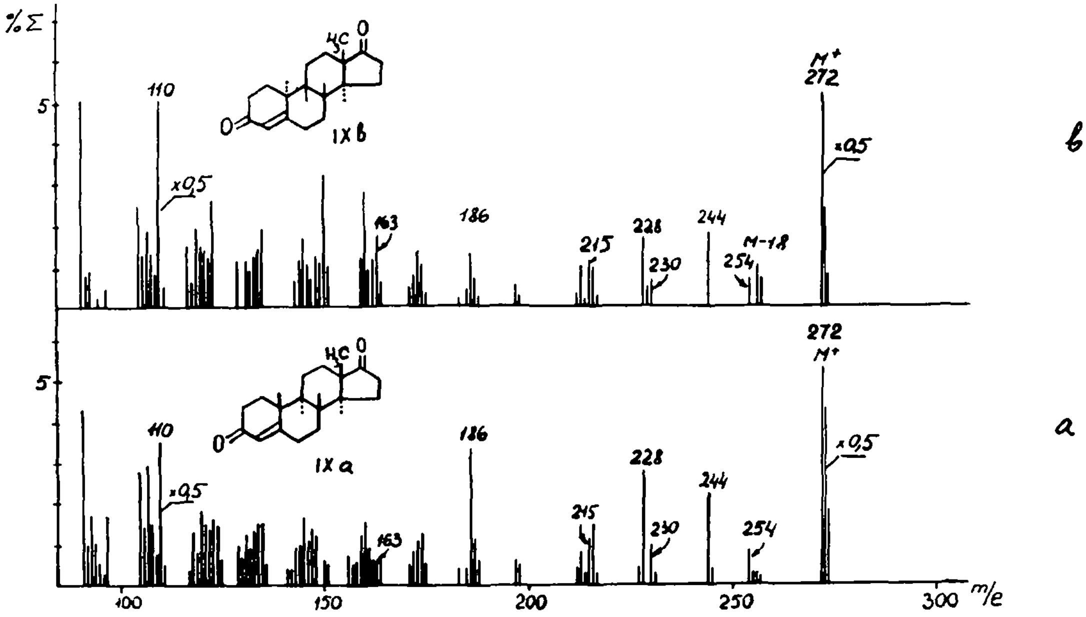

FIG. 5. Mass spectra of:
(a) Δ^4-Androsten-3,17-dione (IXa)
(b) 9β-Δ^4-Androsten-3,17-dione (IXb)

(VIIIa, b) is proved by identity of the mass spectra of VIIIa with that of 8β,9β,10α-19-nortestosterone.[9]*

The above mentioned regularity in the intensity ratio of peaks at m/e 110 (ion e) is observed also in mass spectra of Δ^4-androsten-3,17-dione (IXa) and its 9β-isomer (IXb) (Figs. 5a, b).

EXPERIMENTAL

Mass spectra were taken on the commercial mass spectrometer MX-1303 by using a direct inlet system, at a temp of 130–140° (the temp being held constant to ±1°) and electron energy 70 eV.

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* We express our deep gratitude to Prof. L. Velluz for this substance.

[9] L. Velluz, G. Nominé, R. Bucourt, A. Pierdet and J. Tessier, *C.R. Acad. Sci. Paris* **252**, 3903 (1961).