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MASS SPECTROMETRY OF STEROID SYSTEMS

XII. Determination of the Position of the Double Bond in Some Steroid Systems by the Method of Fragmentation Mass Spectrometry

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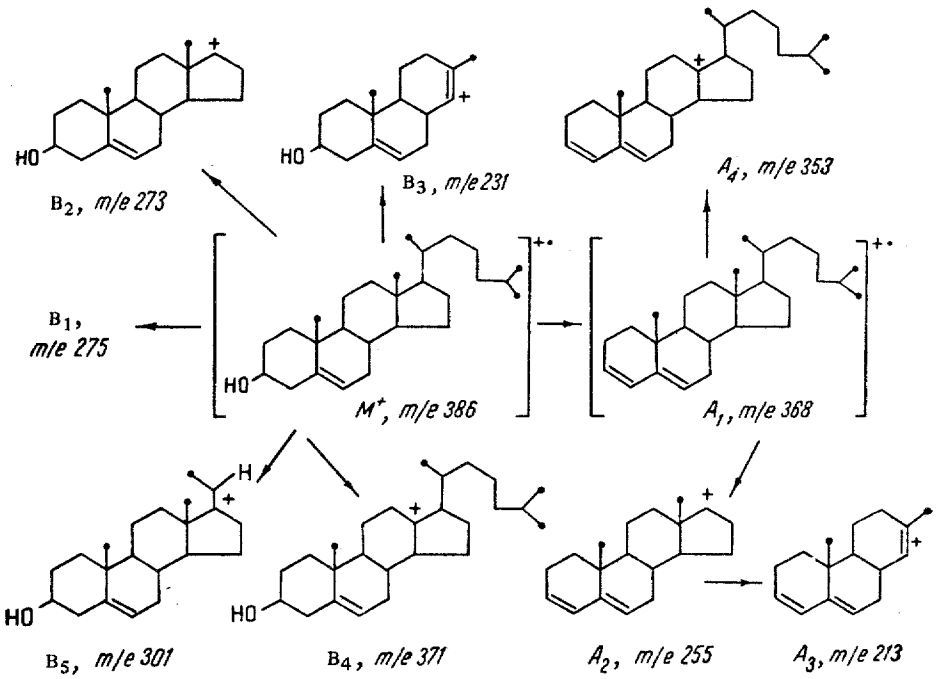
The method of fragmentation mass spectrometry has been used repeatedly to determine the position of the double bond in a number of pentacyclic triterpenes and steroids [1-3]; neverthless, no systematic investigations in this field have been carried out.

In a search for a general method of determining the position of a double bond in steroid systems with Δ^4 and Δ^5 isomers of the cholestane, androstane, and pregnane series as examples, we have studied the influence of the position of the double bond on the decomposition routes of these compounds under electron impact. It has been found that under definite conditions the nature of the decomposition of Δ^4 isomers differs from the fragmentation of the corresponding Δ^5 isomers, which enables the position of the double bond in compounds of this type to be determined unambiguously.

Allocholesterol (Ia) and cholesterol (Ib). The mass spectra of cholesterol (Ib) and allochosterol (Ia), obtained on an instrument with a heated glass inlet system at 200° C, differ considerably. The spectrum of Ib has an intense peak of

Scheme 1



the molecular ion M^+, while in the spectrum of its Δ^4-isomer (Ia), the peak with the highest mass number corresponds to the dehydration ion (A_1, scheme 1). In view of this, the decomposition of Ib takes place by two routes – directly from the ion M^+ (fragments B_1–B_5, cf. scheme 1), and from the dehydration ion A_1 with m/e 368 (A_2–A_4); meanwhile in the spectrum of Ia the peaks of the fragments B_1–B_5 are absent and the fragmentation of this compound begins only from the ion A_1. This is also confirmed by the complete identity of the mass spectra of Ia and the product of its chemical dehydration (2, 4-cholestadiene), taken under identical conditions.

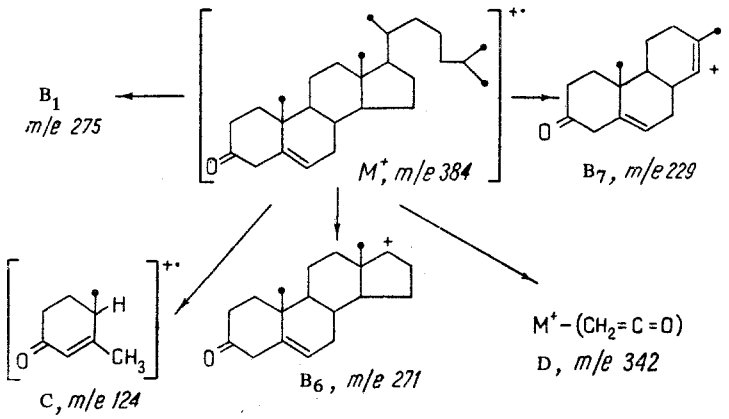
It might be expected that at a lower experimental temperature (with the system of introducing the sample directly into the ion source close to the ionization chamber), the peak of the ion M^+ should be recorded in the mass spectrum of Ia. Consequently, it was of interest to determine whether the mass spectra of Ia and Ib taken under these conditions differed. In actual fact, in this case the peak of M^+, and also peaks of the fragments B_2–B_5 formed from it (m/e 371, 301, 273, 231) appeared in the spectrum of Ia. The only marked difference between the mass spectra of Ia and Ib that

remained was the presence in the spectrum of Ib of a peak with m/e 275 (B_1) (Fig. 1a, 1b). Thus, for compounds similar to Ia and Ib the use of a hot (200° C) glass inlet system has a certain advantage over the direct introduction of the sample into the source (cf. [4]).

Δ^4- and Δ^5-3-ketosteroids. The mass spectra of cholest-4-en-3-one (IIa) and cholest-5-en-3-one (IIb), taken at 200° C with a glass inlet system, proved to be absolutely identical which apparently shows the isomerization of cholest-5-en-3-one (IIb) into its Δ^4 analog (IIa) under these conditions.

However, the spectra of these compounds taken at a lower temperature (65–90° C) with direct introduction of the sample into the source showed characteristic differences (Fig. 2a, 2b). Under these conditions the mass spectra of the ketone IIb and also of 5-pregnene-3, 20-dione (IIIb) and androst-5-ene-3, 17-dione (IVb) have peaks with m/e 275 (fragment B_1, scheme 2, Fig. 2b), 205 (Fig. 3a) and 177, respectively (Fig. 3b) which do not appear in the spectrum of the Δ^4 isomers: IIa, progesterone (IIIa), and androsten-4-ene-3, 17-dione (IVa) (cf. respectively, Figs. 2a and 3b). It might be expected that in the spectra of ketones IIb, IIIb, and IVb peaks with m/e 124 (ion C) and M-42 [ion D, m/e 342 for IIb] would be absent, since the formation of these ions is characteristic for Δ^4-3-ketosteroids [5, 6]. However, it was found that ions C and D are formed, although at a lower intensity, in the decomposition of ketones IIb, IIIb, and

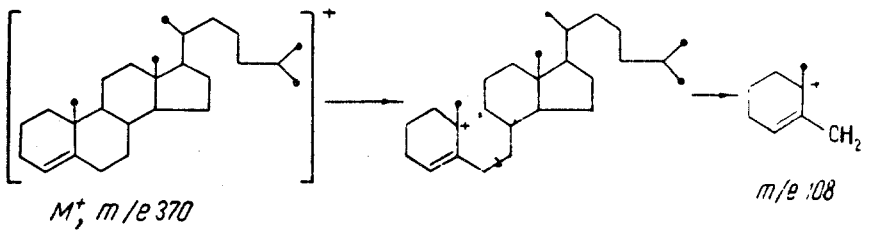
Scheme 2



IVb under electron impact and when the sample is introduced directly into the ion source (65–90° C). This apparently shows the partial isomerization of these compounds into their Δ^4 analogs IIa, IIIa, and IV even under mild experimental conditions. Peaks of the fragments B_6 and B_7 are present in the spectra of both isomers IIa and IIb. The mechanism of their formation is probably similar to the mechanism of formation of fragments B_2 and B_3 (cf. schemes 1 and 2).

Cholest-4-ene (Va) and cholest-5-ene (Vb). The features of the decomposition of Δ^5-dehydrosteroids shown above are also observed in the mass spectra of cholest-5-ene (Vb). As in the examples illustrated previously, the presence of a peak with m/e 275 (ion B_1) is characteristic for the Δ^5 isomer (Vb). No less characteristic is the presence in the mass spectrum of Δ^4 isomer (Va) of an intense peak with m/e 108 (scheme 3). This fragment is formed by the simple cleavage of the two allyl bonds at the 9, 10 and 6, 7 positions. In the case of Vb, this process is blocked by the double bond in the 5, 6 position. The peaks of the fragments with m/e 355, 257, and 215 are observed in the spectra of both isomers and, probably, the mechanism of their formation is similar to that of the formation of the fragments B_2, B_3, and B_4 (cf. scheme 1 and Fig. 4a, 4b).

Scheme 3



Structure of the main fragments. The structure of the main ions formed in the decomposition of the molecular ions of compounds I–V, with cholesterol (Ib) and cholest-5-en-3-one (IIb) as examples (cf. schemes 1 and 2), is confirmed by the following facts: the fragments A_2, B_2, and B_6 arise through the loss of the side chain by ions A_1 and M^+, respectively. The ions A_2, B_2, and B_6 contain ring A, since the mass numbers of the ions B_2 and B_6 differ by 2 units and the mass number of fragment B_2 is 18 units greater than m/e for fragment A_2 (the latter is formed from the dehydration ion A_1). In the case of Ib, the transition $A_1 \rightarrow A_2$ is confirmed by a metastable peak with m/e 176.5 (calc. 176.5). The ions A_2, B_2, and B_6 also contain ring B which is shown by the shift in the peaks of these fragments by 14 units in the spectrum of 6-methylcholest-4-en-3-one (VI) (Fig. 5a).

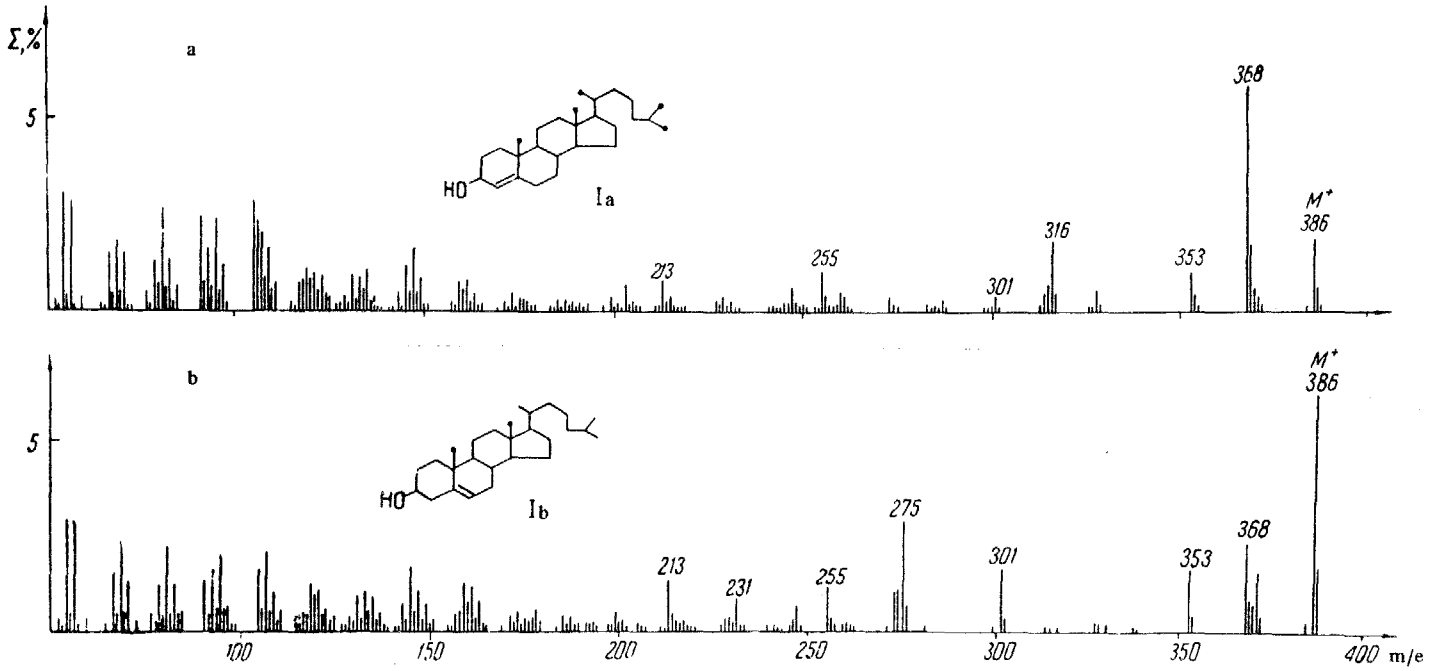


Fig. 1. Mass spectra (direct introduction into the source). a) Allocholesterol (Ia); b) cholesterol (Ib).

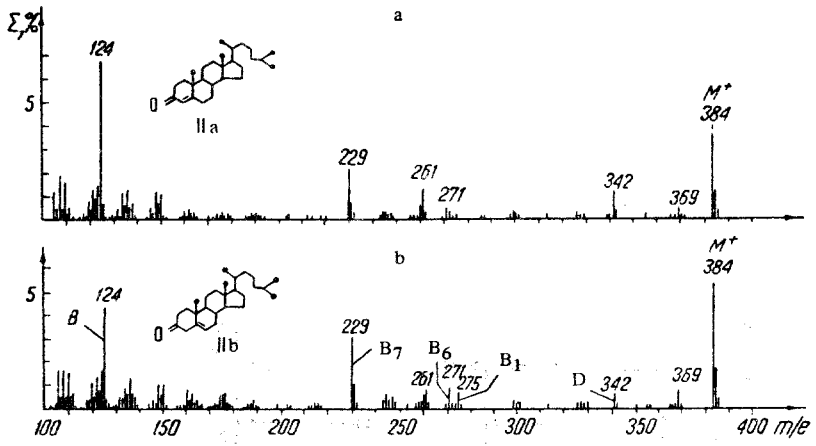


Fig. 2. Mass spectra. a) Cholest-4-en-3-one (IIa); b) cholest-5-en-one (IIb).

Fragments A_3, B_3, and B_7 contain rings A, B, and C: The mass number of the peak of fragment B_3 in the spectrum of Ib (m/e 231) is shifted by 2 units in the spectrum of IIb (m/e 229), and the presence of ring B is confirmed by the mass spectrum of VI, where the corresponding peaks are shifted by 14 mass units. The structure of fragment B_1, the formation of which is characteristic for steroids with a Δ^5 double bond (see above), could not be established. However, it may be regarded as demonstrated that it contains rings C and D with a side chain (table). This is confirmed by the mass spectra of the ketones IIIb and IVb (cf. table and Fig. 3a, 3c), the mass spectra of β-sitosterol (VII) (Fig. 5b) and of stigmasterol (VIII) (Fig. 5c), and also by the mass spectrum of 16α, 17α-epoxypregnenolone (IX) (Fig. 6a), in which peaks of the ion B_1 have m/e 205, 177, 303, 301, and 219, respectively. Fragment B_1 does not contain ring A. The peak of this ion remains unchanged both in the case of cholest-5-en-3-one (IIIb), cholesterol (Ib), 4, 4-dimethylcholesterol (X) (Fig. 5d), and 4, 4-dimethylcholest-5-en-3-one (XI) and in the case of 17β-hydroxyandrost-5-en-3-one (XII) (Fig. 6b) and androst-5-ene-3β-17β-diol (XIII) (Fig. 6b), having m/e 275 and 179, respectively.

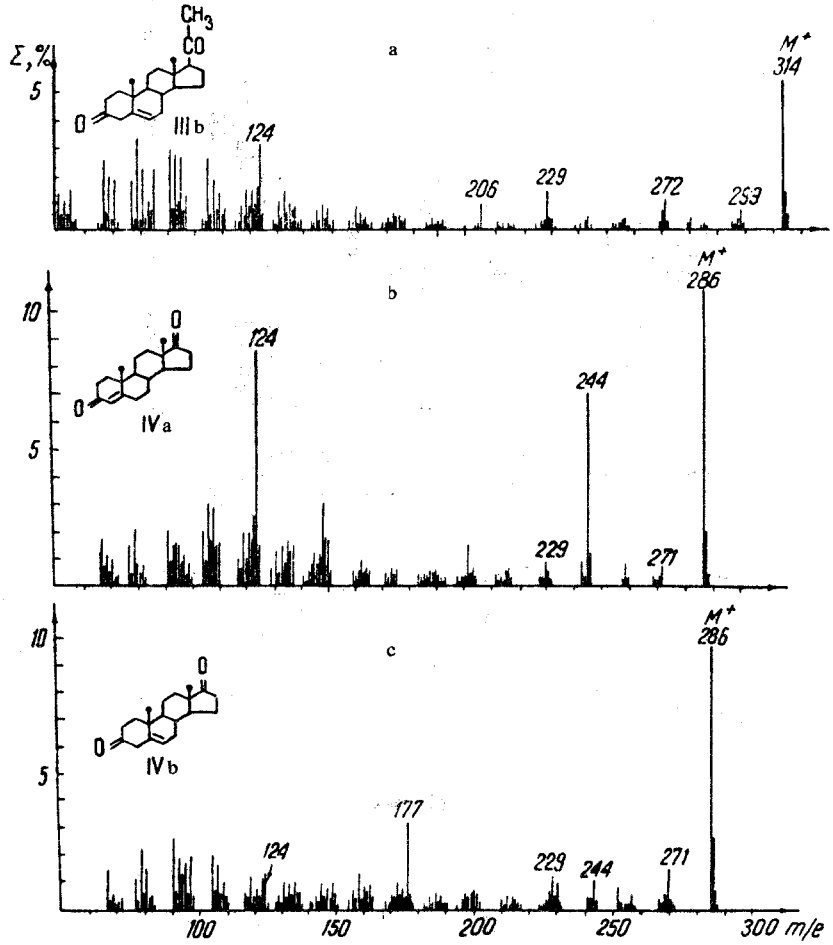


Fig. 3. Mass spectra. a) 5-Pregnene-3, 20-dione (IIIb); b) androst-4-ene-3, 17-dione (IVa); c) androst-5-ene-3, 17-dione (IVb).

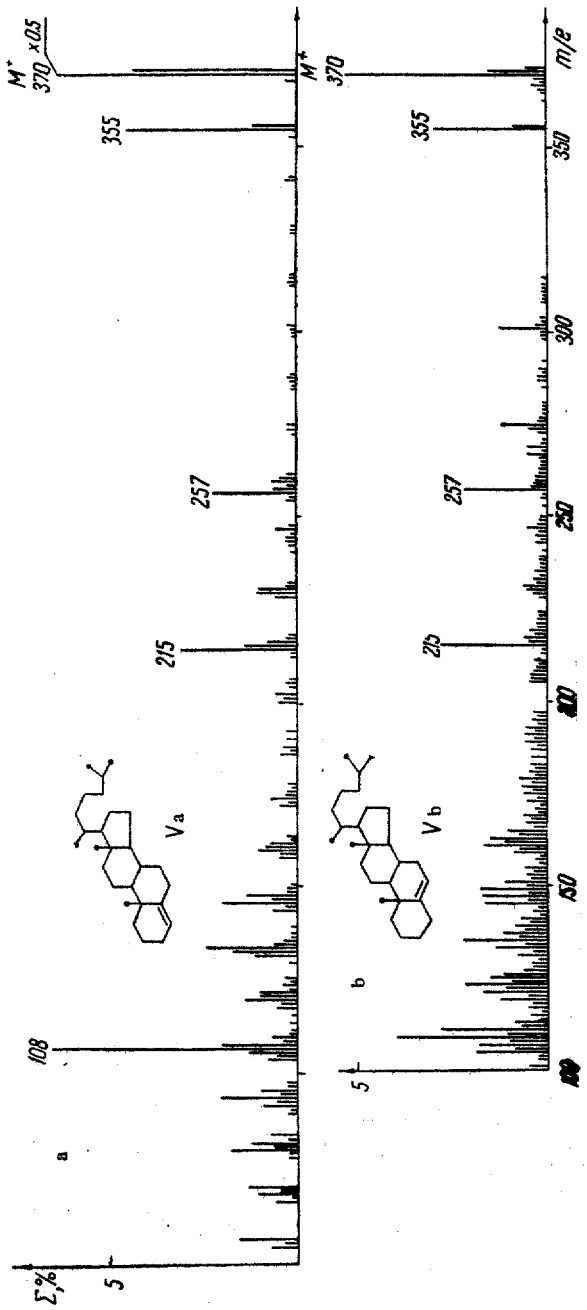


Fig. 4. Mass spectra. a) Cholest-4-ene (Va); b) cholest-5-ene (Vb).

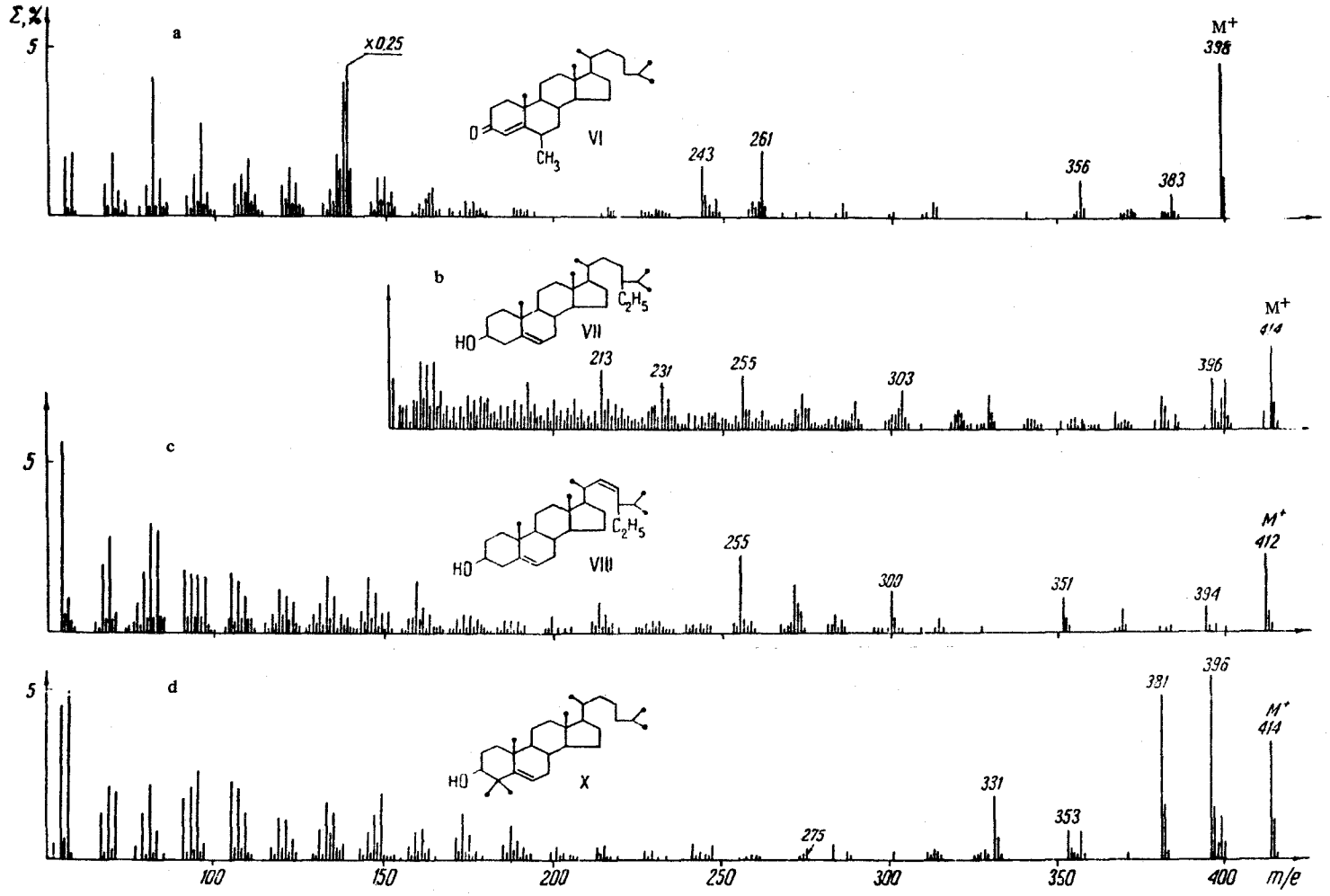


Fig. 5. Mass spectra. a) 6-Methylcholest-4-en-3-one (VI); b) β-sitosterol (VII); c)stigmasterol (VIII); d) 4, 4-dimethylcholesterol (X).

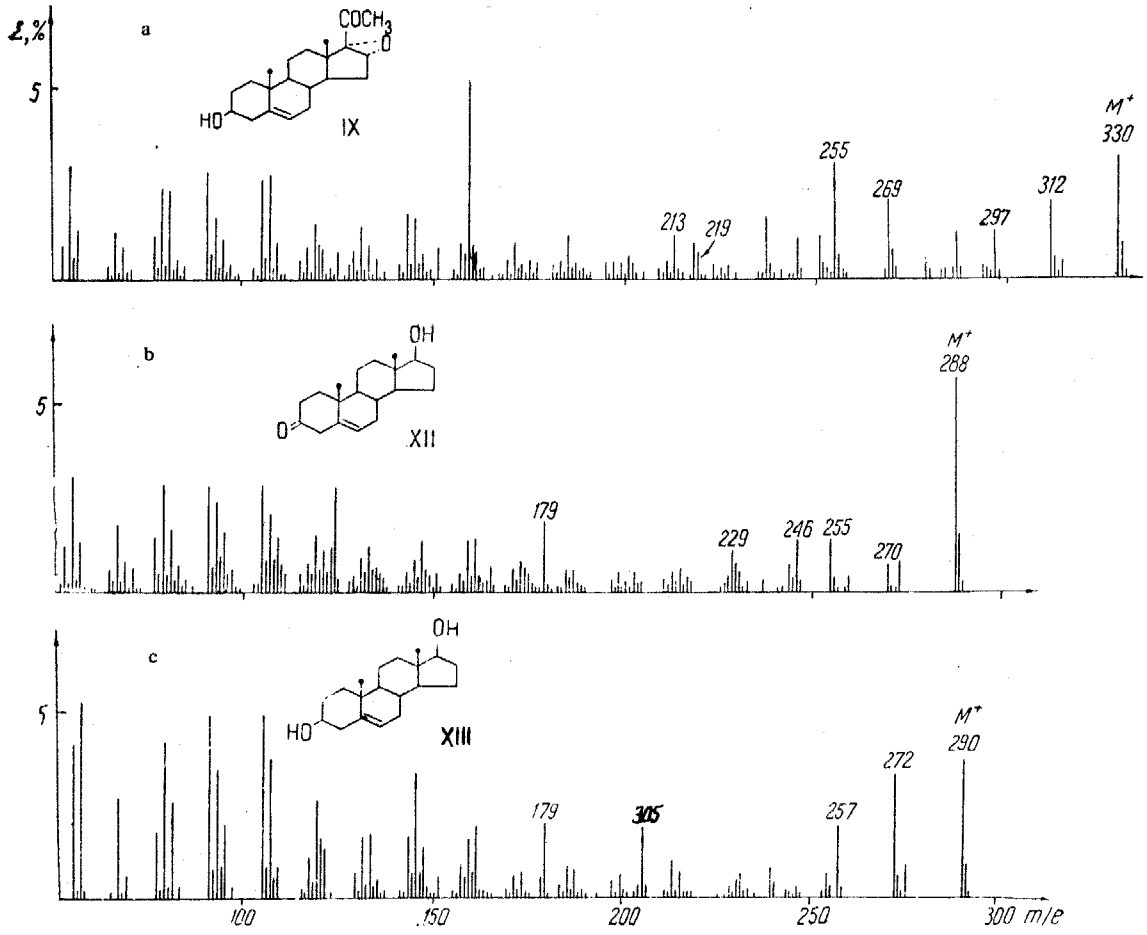
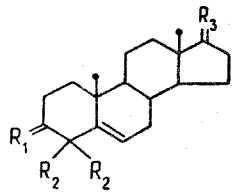


Fig. 6. Mass spectra. a) 16α,17α-epoxypregnenolone (IX); b) 17β-hydroxyandrost-5-en-3-one (XII); c) androst-5-ene-3β, 17β-diol (XIII).

It may be assumed that fragment B_1 is formed from the ion M^+ as a result of the cleavage of bonds 1–10, 5–10, and 7–8. For a definitive solution of the question of the structure and mechanism of the formation of the ion B_1, the mass spectra of the corresponding deutero analogs containing deuterium in positions 7 and 18 must be obtained. Work in this direction is proceeding.



The mass spectra were obtained on a MKh-1303 instrument with a glass admission system at 200° C and an ionization energy of 40 eV, and in the same instrument with a system of admitting the sample directly to the ion source close to the ionization chamber at 50–100° C (temperature stability ±1°) at an ionization energy of 70 eV.

Compound	R_1	R_2	R_3	m/e of fragment B_1 – Δ^5 decomposition
Ib	OH, H	H	C_8H_{17}, H	275
IIb	O	H	C_8H_{17}, H	275
IIIb	O	H	CH_3CO,H	305
IVb	O	H	O	177
Vb	H, H	H	C_8H_{17}, H	275
VII	OH, H	H	$C_{10}H_{21}$, H	303
VIII	OH, H	H	$C_{10}H_{19}$, H	301
X	OH, H	CH_3	C_8H_{17}, H	275
XI	O	CH_3	C_8H_{17}, H	275
XII	O	H	OH, H	179
XIII	OH, H	H	OH, H	179
IX 16α,17α-epoxy-5-pregnenolone				219

Compounds I-VIII and X-XIII were kindly given to us by G. M. Segal and compound IX by Leonid M. Kogan.

Summary

The fragmentation under electron impact of Δ^4 and Δ^5 isomers of the cholestane, androstane, and pregnane series have been studied. It has been found that there are marked differences in the routes of decomposition of steroid systems containing double bonds in positions 4 and 5. This permits compounds to be assigned to the Δ^4 or Δ^5 series on the basis of mass-spectrometric data.

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