Tetrahedron Letters No.40, pp. 3015-3022, 1964. Pergamon Press Ltd.
Printed in Great Britain.

MASS SPECTROMETRIC LOCATION OF THE DOUBLE BOND IN STEROID SYSTEMS

N. S. Wulfson, V. I. Zaretskii, V. G. Zaikin*/
G. M. Segal, I. V. Torgov, T. P. Fredkina**/
Institute for Chemistry of Natural Products,
USSR Academy of Sciences, Moscow, USSR

(Received 18 August 1964)

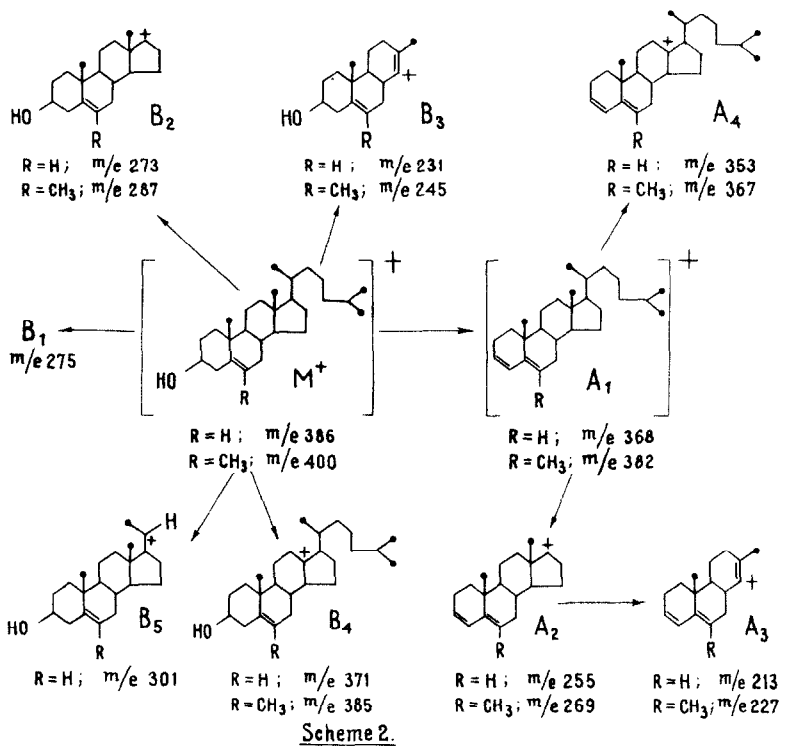
It has previously been shown, that the presence of a double bond in the 12,13-position of a pentacyclic triterpene molecule (I), or in the 2,3-position of a cholestane molecule (2) determines the course of degradation of these compounds induced by electron impact. However, the literature contains no systematic investigation of the mass spectra of unsaturated steroid or triterpenic systems.

Continuing work on the mass spectrometry of steroids (3,4) we made a study of the effect the position of the double bond exerted on the fragmentation of these compounds, using as example allo-cholesterol (Ia), cholesterol (Ib), Δ^4- (IIa) and Δ^5-(IIb) cholesten-3-one, progesterone (IIIa), Δ^5-pregnen-3,20-dione (IIIb) Δ^4- (IVa) and Δ^5- (IVb) androsten-3,17-dione. It was found that under certain conditions the fragmentation of Ia-IVa differs from that of the corresponding Δ^5 isomers, making possible an unambiguous determination of the double bond position in such compounds (see Schemes I and 2 and Fig. I and 2).

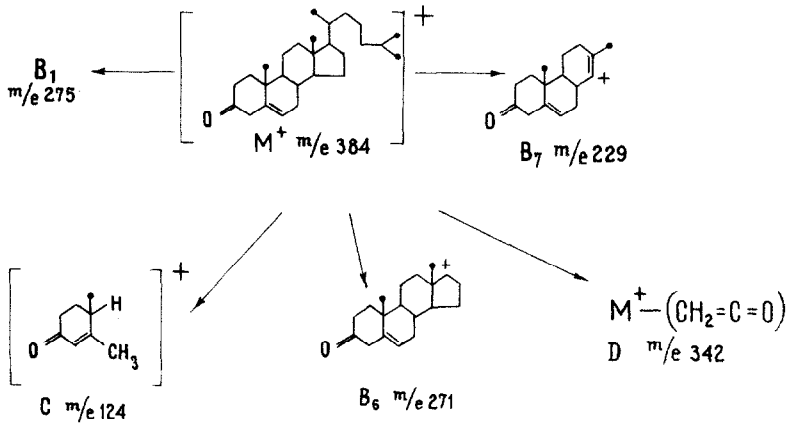
---

*/ Laboratory of Mass Spectrometry of this Institute
**/ Laboratory of Steroid Chemistry of this Institute

Scheme 1.



Scheme 2.



The mass spectra of cholesterol (Ib) and allo-cholesterol (Ia) (200°, glass inlet system) differ considerably from each other. Compound Ib displays an intense molecular peak, whereas its Δ^4 isomer shows no M^+ peak (see Fig. Ia). It therefore follows that fragmentation of Ib can occur in two ways, directly from the M^+ ion (fragments B_I - B_4; Scheme I) and from the fragment A_I with m/e 368 (fragments A_2 - A_4; Scheme I). No B_I - B_4 peaks are present in the spectrum of Ia, the fragmentation of the latter having as its only source the M-18 dehydratation ion. This is also supported by the complete identity of the mass spectra of Ia and of its dehydration product $\Delta^{2,4}$-cholestadiene obtained under identical conditions.

It is to be noted that in the mass spectra of Ia obtained at lower temperatures (100-110°) with direct admission of the sample into the ion source, there is an intense M^+ peak and also peaks due to M^+ fragmentation (m/e 371, 301, 273, 231) and the only basic difference between the Ia and Ib spectra is the presence of a peak with m/e 275 (B_I) in the latter. Hence in such circumstances the use of a hot (200°) glass inlet system is more advantageous than direct admission into the ion source (cf (5)).

The structure of fragments B_2 - B_4 and A_2 - A_4 is confirmed by comparison with the mass spectrum of 6-methylcholesterol (V) for which the corresponding peaks are shifted by 14 m/e units and also by analogs reported in the literature (6). The structure of fragment B_I (m/e 275) has not been established with certainty. However, it may by considered as certain that the fragment contains rings C and D with a side chain (the corresponding peaks in the mass spectra of the ketones

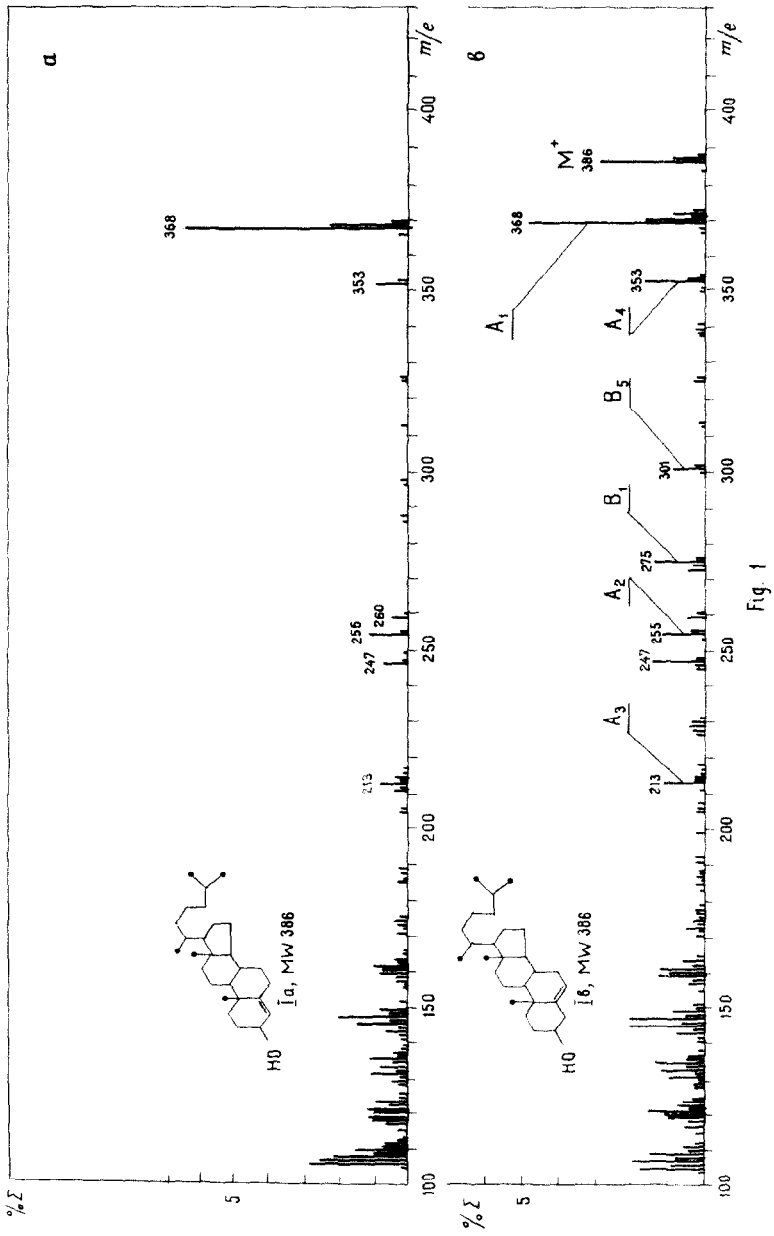

a
b
%Σ
m/e
100
150
200
250
300
350
400
5
368
353
256
247
260
213
HO
Ia, MW 386
Iß, MW 386
M+
386
A1
A2
A3
A4
B1
B5
301
275
255

Fig. 1

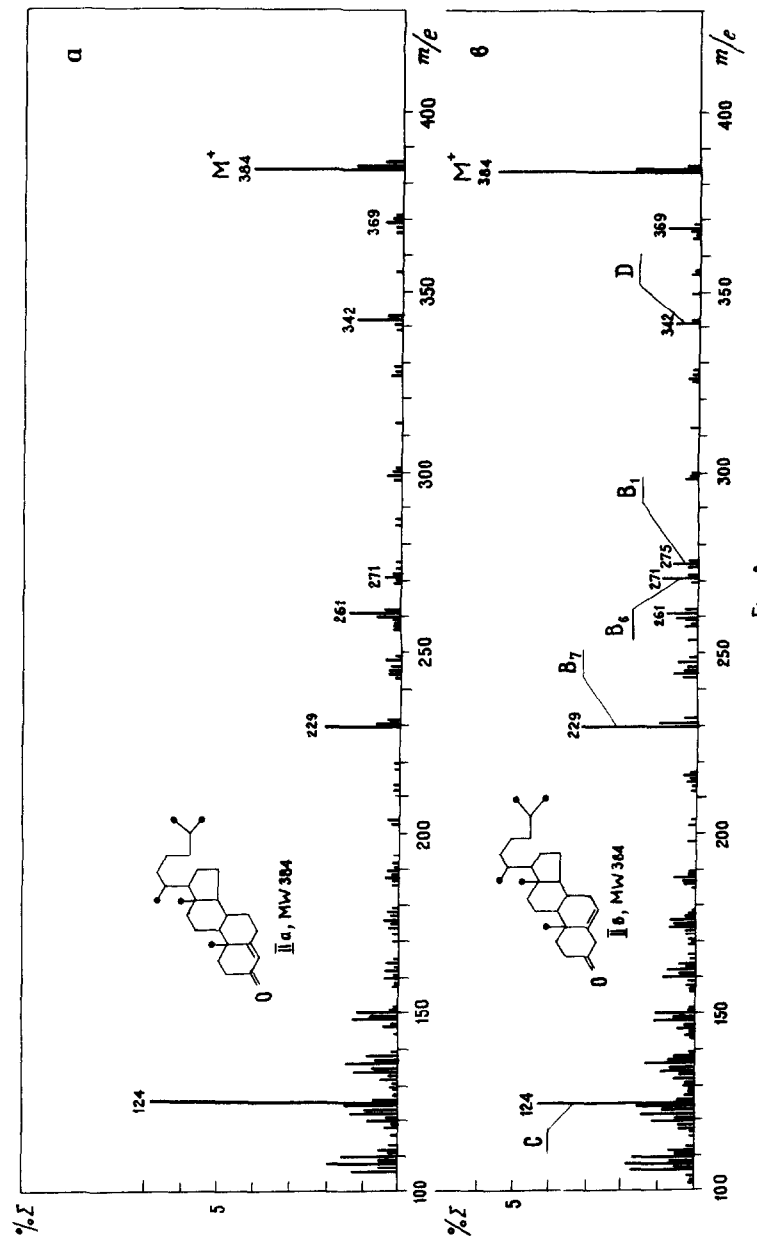

a
б
m/e
%Σ
M+
384
124
229
261
271
342
369
400
350
300
250
200
150
100
5
C
D
B1
B6
B7
275
IIa, MW 384
IIб, MW 384
O

Fig. 2

IIIb and IVb being at m/e 205 and 177). Also, it does not contain ring A, because its m/e value in the case of Δ^5-cholesten-3-one (IIb) is the same as for cholesterol (Ib). The presence of the C_6 atom in the B_I fragment could not be confirmed, because the B_I m/e value remains the same in the mass spectrum of the higher analog V. Perhaps this is due to cleavage of the $C-CH_3$ group from the M^+ ion under the given conditions which seems to be indicated by the unusually intense M^+-15 peak (m/e 385) which predominates in the spectrum of this compound.

The mass spectrum of Δ^5-cholesten-3-one (IIb) obtained with the 200° glass inlet system was found to be completely identical with the spectrum of Δ^4-cholesten-3-one (IIa) which may probably by ascribed to isomerisation of Δ^5-cholesten-3-one (IIb) into its Δ^4 analog (IIa) under the given conditions. A characteristic difference between fragmentations of the ketones IIa and IIb as well as between IIIa and IIIb and IVa and IVb is observed, however, in the spectra obtained at low temperatures (65-90°). The low temperature spectra can be obtained by direct admission of the specimen into the ion source near the ionization chamber. Under such conditions peaks appear in the mass spectra of the ketones IIb, IIIb and IVb at m/e, respectively, 275 (fragment B_I, see Scheme 2 and Fig. 2b), 205 and 177, that are absent from the spectra of the Δ^4 isomers IIa, IIIa and IVa. It might have been expected that also peaks with m/e 124 (fragment B) and M-42 (in the case of the ketone IIb, fragment G with m/e 342) would be present in the spectra of IIb, IIIb and IVb, the formation of these ions being characteristic of Δ^4-3-oxosteroids (7,8). However, it turned out that fragments B and M-42 are formed in the electron impact

induced decomposition of the ketones IIb, IIIb and IVb also when the specimens are let in directly into the ion source (65-90°). This points out to possible partial isomerisation of these compounds into the Δ^4 analogs (IIa, IIIa and IVa) even under such mild conditions. Peaks due to the fragments B_6 and B_7 are present in the spectra of both the IIa and IIb isomers. The mechanism of their formation is apparently similar to that for the fragments B_2 and B_3 (see Schemes I and 2).

Hence the presence of the B_1 peak (m/e 275) in the spectra of compounds Ib, IIb and V or of the peaks of its analogs with m/e 205 and 177, respectively, in the spectra of IIIb and IVb are the most characteristic indicatives of the presence of a Δ^5 double bond in these compounds.

The mass spectra were obtained on the commercial instrument MX-1303. The instrument was furnished with two glass inlet systems, one for temperatures of 200° and ionisation energies of 40 eV, the other for direct inlet into the ionisation chamber at a temperature of 65-110° (the temperature being held constant to ±1°) and ionisation energy of 70 eV.

REFERENCES

(1) C. Djerassi, H. Budzikiewicz, J. Wilson, Tetr. Lett., 263 (1962).

(2) H. Audier, M. Fetizon, W. Vetter, Bull. Soc. Chim., 1971 (1963).

(3) S. N. Ananchenco, V. N. Leonov, V. I. Zaretskii, N. S. Wulfson, I. V. Torgov, Tetrahedron, 20, 1279 (1964).

(4) V. I. Zaretskii, N. S. Wulfson, V. L. Sadovskaja, S. N. Ananchenco, I. V. Torgov, Dokl. Acad. Nauk USSR, 158, N 2 (1964).

(5) M. Chashi, H. Budzikiewicz, J. Wilson, C. Djerassi, J. Levi, J. Gosset, J. Le Men, M.-M Janot, Tetrahedron, 19, 2241 (1963).

(6) C. Beard, J. Wilson, H. Budzikiewicz, C. Djerassi, J. Amer. Chem. Soc., 86, 269 (1964).

(7) R. Shapiro, J. Wilson, C. Djerassi, Steroids, 1, 1 (1963).

(8) L. Petersen, Anal. Chem., 34, 1781 (1962).