MASS SPECTROMETRY OF SOME UNSATURATED STEROLS

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Some features of the fragmentation under electron impact of sterol molecules having one or two double bonds in the nucleus are considered. The question of the identiciation of concrete sterols is discussed on the basis of the ratio of the intensities of the peaks of certain fragmentary ions.

Chromato-mass spectrometry is the most convenient method for studying sterols isolated from living organisms [1]. For the successful interpretation of the mass spectra obtained, a detailed study of the nature of the fragmentation of the sterol molecule according to its structure is necessary. This problem has been solved most completely for sterols having Δ^5-[2], Δ^7- and $\Delta^{5,7}$-[3, 4], and Δ^4-[5] double bonds in the nucleus.

We have obtained the mass spectrum of nine sterols — ergosterol (I), ergosta-7,22-dien-3β-ol (II), cholesta-5,7,22-trien-3β-ol (III), 24-ethylcholesta-5,7-dien-3β-ol (7-dihydrositosterol) (IV), cholesta-5,8(9)-dien-3β-ol (V), cholesta-7,9(11)-dien-3β-ol (VI), cholesta-7,-14-dien-3β-ol (VII), cholesta-8,14-dien-3β-ol (VIII), and cholesta-5,24-dien-3β-ol (desmosterol) (IX) — and have considered some features of the fragmentation of their molecules under the action of electron impact.

A considerable number of the peaks in the mass spectra have a common nature for all the sterols considered, but their intensities depend on the positions of the double bonds in the molecules. Some of the most important peaks for interpretation are those of the ions $(M - R)^+$ and $(M - R - 2\,H)^+$. Their intensities, and also the presence or absence of ions corresponding to the breakdown of the side chains, permit the distribution of the double bonds between the nucleus and the side chain to be established. All the sterols considered in the present investigation had a peak with m/z 271, but only for compounds (II) and (IX) did it correspond to the elimination of the side chain with the transfer of two protons (the ion $(M - R - 2\,H)^+$ and was it the maximum peak in the mass spectrum. Such decomposition is very characteristic for sterols with a Δ^5- or a Δ^7- double bond in the nucleus and an unsaturated side chain [3]. Changes in the side chain have practically no effect on the breakdown of the nucleus of the molecule, and its fragmentation will not be considered further [6].

The peak of the molecular ion is the strongest in the spectrum of a sterol having two double bonds in the nucleus (V, VI, VII, VIII). Exceptions are formed by the $\Delta^{5,7}$-sterols (I, III, and IV) in which the strongest peak in each spectrum is that of the $(M - CH_3 - H_2O)^+$ ion.

The peak of the molecular ion is strongest for a sterol with one double bond in the nucleus (II, X).

The m/z value of the strongest peak permits the sterols under consideration to be divided into three characteristic groups: sterols having one double bond at carbon atom 5 or 7 in the nucleus and one in the side chain, sterols having a $\Delta^{5,7}$- conjugated dienic system of double bonds; and sterols having two double bonds in the nucleus not in the $\Delta^{5,7}$- positions.

The most characteristic is the fragmentation of a sterol with a $\Delta^{5,7}$- double bond system. Only in the spectra of compounds (I), (III), and (V) is a strong peak observed for the ion $(M - 59)^+$, which corresponds to the allyl cleavage of the (3—4) and (1—10) bonds in ring A with the migration of one hydrogen atom to the neutral particle [7].

The strongest peaks for (I), (III), and (IV), in comparison with the other sterols, are those of the $(M - H_2O)^+$ and $(M - R - H_2O)^+$ ions and also a peak with m/z 211 connected with the cleavage of ring D at the (13—17) and (14—15) bonds and the elimination of a molecule of water.

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The nature of all the fragmentations described above is not affected by the presence of double bonds in the side chain [3] which permits the unambiguous identification of a $\Delta^{5,7}$-dienic system from the results of mass spectrometry.

The fragmentation of a sterol having one double bond in the nucleus at carbon atom 5 or 7 is characterized by the following features. A high intensity of the $(M - H_2O)^+$ and $(M - CH_3 - H_2O)^+$ peaks in compound (IX) is probably due to the formation of a conjugated system of double bonds at carbon atom 3 and 5 in the fragmentary ions.

Characteristic for Δ^7-sterols is a high intensity of the peak with m/z 246, which is connected with the elimination of the side chain and part of ring D (cleavage takes place at the (13—17) and (15—16) bonds [8].

The absence of the $(M - R - 2\,H - H_2O)^+$ peak from the spectrum of compound (II), which distinguishes it from the other sterols considered, must also be mentioned.

Features of fragmentation under the action of electron impact readily permit Δ^5- and Δ^7-sterols to be distinguished on the basis of their mass spectra.

The least specific in a comparative study is the breakdown of the cholestadienols (V, VI, VII, and VIII). Sterols (VII) and (VIII), which have a (14—15) double bond are, as was expected, characterized by a low intensity of the peaks of the ions with m/z 229 and 211 (5 and 11% for (VII), and 5 and 9% for (VIII), respectively), which are connected with the breakdown of ring D at the (13—17) and (14—15) bonds. The high intensities of the doublet peaks with m/z 256 and 257 for compounds (VII) and (VIII) are caused by the favorable nature of the allyl splitting out of the CH_3 group at carbon atom 13 after the elimination of the side chain. A strong doublet with m/z 238 and 239 is formed on the loss by the loss of H_2O molecules by the fragmentary ions with m/z 256 and 257.

The features mentioned above make it possible to establish the presence of a (14—15) double bond in a sterol molecule.

In the spectrum of the $\Delta^{7,14}$-sterol the peak of the ion $(M - R)^+$ is very high (91% of the maximum), which distinguishes it from the other cholestadienols (V, VI, VIII), and, in particular, from the $\Delta^{8,14}$-sterol having a mass spectrum practically identical with it. We must also mention the absence from the spectrum of (VII) of the peak of an ion with m/z 301, in contrast to the other dienols considered.

Sterols (V) and (VI) are characterized by lower intensities of the majority of the peaks connected with the fragmentation of the nucleus. In the spectrum of the $\Delta^{7,9(11)}$-sterol a peak with m/z 244, which is characteristic of sterols with a Δ^7-double bond, must be noted. However, it is absent from the spectrum (VII), probably because of the stabilization of ring D by the (14—15) double bond.

EXPERIMENTAL

The mass spectra of the sterols were obtained on a LKB-2091 instrument at 70 eV. The temperature of the ion source was 250°C. The purity of the samples was checked chromatographically.

The ten strongest peaks in the mass spectrum ($m/z \geqslant 200$) are given for all the sterols, their intensities being shown as percentages of the maximum peak.

Ergosterol (I), m/z (%): M^+ 396(72), 378(11), 363(100), 337(24), 271(27), 253(38), 251(18), 227(8), 213(13), 211(15).

Ergost-7,22-dien-3β-ol (II), m/z (%): M^+ 398 (40), 383(17), 300(19), 273(45), 271(100), (100), 255(58), 246(37), 231(21), 229(33), 213(40).

Cholesta-5,7,22-trien-3β-ol (III), m/z (%): M^+ 382(63), 364(20), 349(100), 323(37), 271(46), 255(34), 253(63), 251(95), 237(43), 211(54).

7-Dehydrositosterol (IV), m/z (%): M^+ 412(74), 394(15), 379(100), 353(40), 271(15), 253(20), 227(10), 217(11), 213(13), 211(24).

Cholesta-5,8(9)-dien-3β-ol (V), m/z (%): M^+ 384(100), 369(63), 366(5), 351(23), 271(23) 257(9), 253(9), 239(6), 238(7), 217(5).

Cholesta-7,9(11)-dien-3β-ol (VI), m/z (%): M^+ 384(100), 369(26), 351(12), 271(25), 269(20), 244(14), 229(20), 217(18), 211(13), 204(10).

Cholesta-7,14-dien-3β-ol (VII), m/z (%): M^+ 384(100), 369(65), 351(29), 271(91), 269(18), 257(32), 253(28), 239(13), 238(15), 211(12).

Cholesta-8,14-dien-3β-ol (VIII), m/z (%): M^+ 384(100), 369(87), 351(40), 271(28), 257 (15), 256(12), 255(11), 253(13), 239(16), 238(21).

Desmosterol (IX), m/z (%): M^+ 384(32), 369(32), 351(20), 300(27), 299(22), 271(100), 255(17), 253(22), 231(15), 213(34).

CONCLUSIONS

Some laws of the fragmentation of the sterol molecule have been considered and generalized for, as examples, nine sterols.

Sterols with a $\Delta^{5,7}$-dienic system can be identified by comparing the intensities of the peaks of the M^+ $(M - CH_3 - H_2O)^+$ and $(M - 59)^+$ ions and those of dehydration fragments.

The presence of a Δ^5- or a Δ^7- double bond in the nucleus of a sterol with an unsaturated side chain can be established on the basis of the intensities of the peaks of the M^+, $(M - R - 2\,H)^+$, $(M - R)^+$, and $(M - R - H_2O)^+$ ions.

Differences in the positions of the double bonds in the nucleus of a sterol molecule can be established on the basis of the peaks of ions with m/z 246, 229, 211, 256, 257, 238, 239, 301, and 366.

LITERATURE CITED

1. N. Natale, Lipids, 12, 847 (1977).
2. S. G. Wyllie, B. A. Amos, and L. Tokes, J. Org. Chem., 42, 725 (1977).
3. C. Djerassi, Pure Appl. Chem., 50, 171 (1978).
4. A. Lavanchy, T. Varcony, D. H. Smith, N. A. B. Gray, W. C. White, R. E. Carhart, B. G. Buchanan, and C. Djerassi, Org. Mass Spectrom., 15, 355 (1980).
5. V. I. Zaretskii, N. S. Vul'fson, V. G. Zaikin, and I. B. Papernaya, Khim. Prir. Soedin., 383 (1967).
6. S. G. Wyllie and C. Djerassi, J. Org. Chem., 33, 305 (1968).
7. B. A. Knights, J. Gas Chrom., 5, 273 (1967).
8. C. Djerassi, Pure Appl. Chem., 21, 205 (1970).