DISSOCIATIVE ELECTRON-CAPTURE MASS SPECTROMETRY OF FATTY ACIDS AND THEIR PYRROLIDIDES AND METHYL ESTERS

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The regions of the energies of resonance capture of electrons by the molecules of fatty acids and their pyrrolidides and methyl esters have been established, which has permitted an explanation of the difference between the dissociative electron-capture mass spectra of these compounds and their chemical ionization and fast-atom bombardment negative-ion mass spectra. It has been shown that the dissociative electron-capture mass spectra of a high-energy resonance state characterized by the most far-reaching dissociation permit the unambiguous determination of the positions of multiple bonds in the initial molecules.

In the mass-spectrometric characterization of fatty acids, the greatest attention is devoted to the determination of the positions of multiple bonds. For this, as a rule, the fatty acids are modified at the multiple bonds in such a way that the mass spectrum unambiguously shows their positions in the alkyl chain [1]. Otherwise, high-energy electron impact leads to a migration of the hydrogen atoms in the molecular ion, as a result of which the structural specificity of the mass spectrum is lost. A new approach consists in a technical expedient — the two-stage degradation of the fatty acid molecules. In the first stage, mild ionization takes place, and the anion formed, which retains the structure of the initial molecule to the maximum degree, is subjected in the second stage to collision-induced dissociation. Thus, the chemical ionization by hydroxyl anions of fatty acid methyl ester to the $(M - H)^-$ ion and its subsequent dissociation induced by collision and observable in the kinetic energy spectra of the fragments leads to two types of such ions: $R\bar{C}HCOOCH_3$ and $R\bar{C} = C = O$, where R represents alkyl. The subsequent set of fragments $R\bar{C}{-}C{-}O$ is interrupted at the multiple bond, and in the following set of fragments $R\bar{C}HCOOCH_3$ those which are formed by the cleavage of the bond in the β-position to the multiple bond reckoning from the alkyl end have a higher yield [2].

The successive set of anions $(RCOO - C_nH_{2n+2})^-$ induced by the collison of the $RCOO^-$ ions formed as the result of the bombardment with fast xenon atoms of fatty acids in triethylamine are similarly interrupted at the multiple bond [3]. But if there are more than three multiple bonds, then even such a technically equipped method (FAB MS CAD/MS) proves to be powerless and, in the final account, the problem is solved by chemical modification. For the quantitative analysis of the prostaglandins in low concentrations, use is made of the fact that the dissociative capture of the electrons of a swarm (about 0 eV) by pentafluorobenzyl esters of these compounds leads to the formation of the $RCOO^-$ anion in high yield [4]. No fragments characterizing the structure are detected in this process. However, the methyl esters of fatty acids capture the electrons of a beam of strictly determined energies up to about 8 eV, thanks to which the molecular negative ions acquire an excess of internal energy which is sufficient for the cleavage of carbon bonds [5, 6]. Three-dimensional dissociative electron-capture mass spectra have shown the possibility of distinguishing from one another the methyl esters of hept-2,5- and -3,5-dienoic acids from the set of fragmentary ions $R\bar{C}{-}C{-}O$ and ions with a hydrocarbon composition.

The use of a one-stage scheme for determining the positions of multiple bonds without their modification is also known in electron-impact mass spectrometry for the case of fatty acid pyrrolidides [7]. However, there is no information on electron capture by these derivatives and by the fatty acids themselves. With the aim of a further investigation of fatty acid derivatives by the dissociative electron-capture (DEC) mass-spectrometric method, we have studied the DEC mass spectra of fatty acids and their pyrrolidides and methyl esters.

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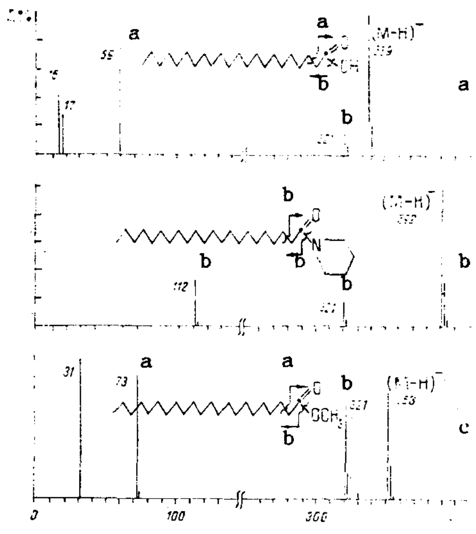


Fig. 1

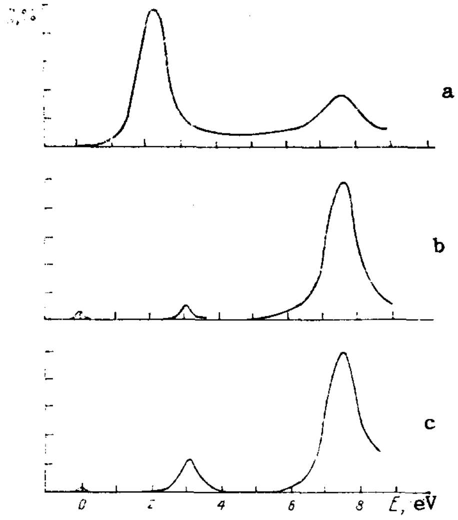


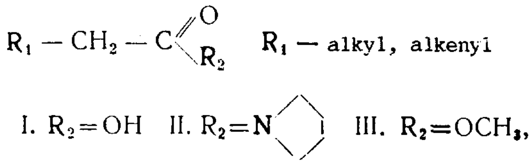
Fig. 2

Fig. 1. Negative ion mass spectra of behenic acid (a), its pyrrolidide (b), and its methyl ester (c). Scanning of the ionizing voltage from −1 to +11 V.

Fig. 2. Effective yield curves of the (M − H)$^-$ ions of behenic acid (a), its pyrrolidide (b), and its methyl ester (c).

In addition, the applicability of the method for determining the positions of multiple bonds has been tested.

The experiments on obtaining the DEC mass spectra of a beam were performed with the fatty acids (I), their pyrrolidides (II), and their methyl esters (III) having alkyl chains of 14, 16, 18, 20, and 22 carbon atoms. Unsaturated fatty acids were represented by linoleic



($C_{18:2}$) and arachidonic($C_{20:4}$) acids and their pyrrolidides and methyl esters. By using a pseudo-wide distribution of electron energy (DEE), comprising 12 eV, the negative ion mass spectra (NIMSs) [8] were recorded, and then for each ion the effective yield curves (EYCs) with real DEE of about 1 eV were obtained. Resonance energies were determined from these curves.

The capture of electrons by the molecules of fatty acids and their pyrrolidides and methyl esters, both saturated and unsaturated, takes place in all the electron-energy regions: the first at about 0 eV, the second at 2-3 eV, and the third at 7-8 eV. As in the case of fatty acid methyl and pentafluorobenzyl esters, the molecular negative ions of the acids themselves and of their pyrrolidides do not reach the detector but break down into fragmentary ions. The number of fragmentary ions is not large, as in the case of electron impact. A typical NIMS of compounds (I-III) using behenic acid ($C_{22:0}$) as an example is given in Fig. 1. As a rule, the (M − H)$^-$ ion is the main one in the NIMS of each of the three types of compound. Figure 2 gives the EYCs of the (M −H)$^-$ ions for compounds (I-III).

The (M − H)$^-$ ions of fatty acids formed at an energy of 2.3 eV have the nature of carboxylate anions $R_1CH_2COO^-$ and are formed with considerable yields. The yields of the (M − H)$^-$ ions at an energy of 7-8 eV are considerably smaller. The carboxylate ion does not dissociate subsequently, which ensures that the (M − H)$^-$ peaks are the highest in the negative-ion spectra of the acids (Fig. 1a). This resonance does not appear for the acid derivatives and the (M − H)$^-$ peak at 3.0 eV has a different nature (the peaks of carboxylate anions do appear in the NIMSs of the methyl esters (Fig. 1c, m/z 339) but this is in the region of energies of 7-8 eV).

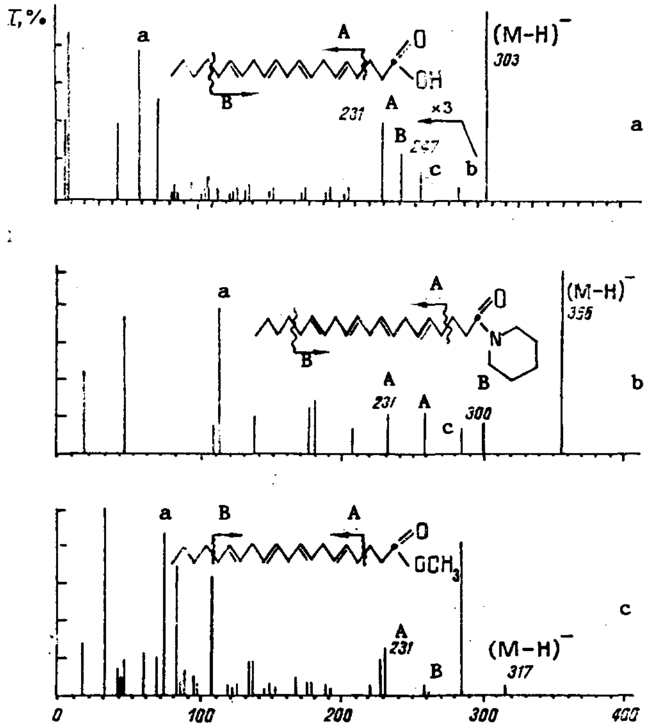
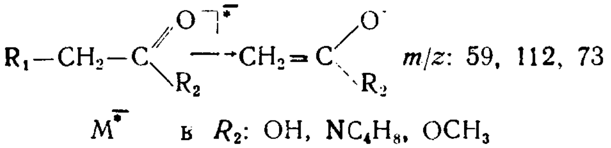


Fig. 3. DEC mass spectra of arachidonic acid (a), its pyrrolidide (b), and its methyl ester (c). Scanning of the ionizing voltage from 7 to 8.5 V.

A critical feature of dissociation is not only the amount of excess internal energy obtained as the result of collisions but also the electronic state of the anion. Thus, the mass spectrum of an acid recorded at an electronic energy of about 0 eV contains only the peak of ion *a* with m/z 59. In the second resonance there is only the peak of $(M - H)^-$ ion, and in the third resonance the peaks of all the ions present in the NIMS appear in the spectrum. The ionic current of O^- and OH^- is recorded over the whole range of energies and may, to a considerable degree, be due to residual gases: O_2, CO_2, H_2O. The peaks of these ions carry little structural information and will not be discussed. Ion b with m/z 321 for behenic acid is formed from $(M - H)^-$ by the loss of R_2H only at 7-8 eV. The metastable peak corresponding to this ion is recorded in the case of the pyrrolidides and methyl esters. In this rearrangement reaction, the migration of hydrogen atoms takes place from the second carbon atom, as has been established with the aid of deuteration in methyl esters [5]. It is reasonable to assume that an analogous mechanism exists for the acids and the pyrrolidides. The formation of this ion in two stages explains its appearance only at high electron energies. If the $(M - H)^-$ ion were formed with a low content of excess energy (as in chemical ionization [10]), then the transition to ion b, $R\bar{C}{=}C{=}O$ takes place only by collision-induced dissociation.

The NIMSs of the pyrrolidide and methyl ester of behenic acid contain ions of the same types as the NIMS of the free acid: $(M - H)^-$, A $(CH_2{=}C(O^-)R_2)$, and also $^-OCH_3$ and $RCOO^-$ for the methyl ester (Fig. 1b, c). A remarkable feature of the dissociation of compounds (I-III) is the simple cleavage of the carbon bond between the second and third atoms with the formation of ion *a* which takes the place at electron energies close to 0 eV.



$$\left[R_1{-}CH_2{-}C(=O)R_2\right]^{\overline{\bullet}} \longrightarrow CH_2{=}C(O^-)R_2 \quad m/z\text{: } 59,\ 112,\ 73$$

$$M^{\overline{\bullet}} \qquad R_2\text{: } OH,\ NC_4H_8,\ OCH_3$$

The considerable inductive effect of the negatively charged oxygen atom in M^- weakens the β-bond to such a degree that the thermal vibrational energy is sufficient for its dissociation. We may note that neither the chemical ionization with ^-OH [3] nor the fast-atom bombardment [4] of fatty acid methyl esters produces the ions *a*, since under these conditions there are no thermal electrons (with energies of about 0 eV) for the formation of M^-, and charge exchange with ^-OH apparently leads to a different state of the M^- ion. The two ions *a* and b characterize the difference between the NIMS shown, which was obtained by resonance capture, and the chemical ionization spectrum [10].

It can be seen from the results given that the modification of fatty acids does not appreciably change the pattern either of the NIMS or of the DEC MS in view of the similarity of the EYCs of the three types of compound studied. Therefore, for the purposes of analysis their relative electron-capture cross-sections have greater value. Among these types of compound, the free acids have the largest cross-section. Consequently, for analysis by the DEC method there is no need whatever for modification if there is no problem of volatility or any other reason.

The NIMSs of the unsaturated compounds (I-III) at the electron energies of the first and second resonances are identical with the spectra of the saturated analogs and contain the peaks of the ions *a* and $(M - H)^-$. At the third resonance the spectra abound in the peaks of fragmentary ions. The NIMSs of the acids and the methyl esters are particularly rich in peaks, as can be seen from the spectra of arachidonic acid and its derivatives (Fig. 3). These peaks are due to ions both of the alkyl moiety, referred to below as the C-type, and of the carboxyl moiety (O-type) of carbon chain of the molecules of the compounds studied. In combination, both types of ions confirm the positions of the double bonds in the chain. For example, fragments of type C formed by the cleavage of the α-bond are shown by wavy lines in the scheme (m/z 177, 137, 97). However, there is a series of their dehydrogenated analogs: m/z 175, 135, 95. The yield of dehydrogenated ions rises with a decrease in the number of carbon atoms, as was to be expected for carbonium anions [9], and their mass numbers erroneously show the presence of one more multiple bond in the direction of the alkyl end.

There is also a series of ions of the C-type formed by the β-cleavage of the 9-10 and 12-13 bonds. Ions of the O-type are not so numerous: m/z 221, 207, 167. When the abundance of fragments, H-migration and the not always considerable yield are taken into account, these ions formed by the cleavage of bonds in the area of unsaturation can be assigned only to the subsidiary ions in the determination of the positions of the multiple bonds. The ions formed by β-cleavage before the first (A) and after the last (B) centers of unsaturation must be regarded as the main ones. These ions are formed in all three types of compounds and give appreciable peaks in the region of high mass numbers (with the exception of the B ion from the methyl esters, which lowers the applicability of these derivatives).

Thus, the number of double bonds determined from the mass of the $(M - H)^-$ ion, and the masses of the A and B ions show the positions of multiple centers of unsaturation. The other centers can be allocated in the light of their natural regularity.

Among the three types of compounds, the pyrrolidides of unsaturated acids have simpler spectra in which bond cleavages are not accompanied by H-migration. For them the ions of the O-type have even masses, which unambiguously shows their structures. Thus, the DEC mass spectrum of the pyrrolidide of linoleic acid, $C_{18:2}$, recorded at an electron energy of 7-8 eV, contains five peaks: $(M - H)^-$ (100%), *a* (53%), b (11%), A(2%), and B(6%). The A and B ions determine the positions of the double bonds, but their low yields and the absence of ions due to the cleavage of the bonds between the centers of unsaturation must be noted.

A study of methyl linoleate also gave the same result — the yields of the A and B ions fell while no ions due to the cleavage of bonds between the centers of unsaturation were observed. In the sepctrum of an acid with the minimum degree of unsaturation, oleic ($C_{18:1}$), there was only one small peak of the B ion, but this was sufficient to localize the double bond on the ninth carbon atom. In another extreme case with the maximum degree of unsaturation — for example, for methyl eicosapentaenoate — the formation of the B ion requires the cleavage of the terminal 19-20 bond, and this process does not take place, but the presence in the spectrum of the peaks of ions A, m/z 229; $(M - H)^-$, m/z 325; and b, m/z 283 unambiguously shows the presence of five double bonds, beginning with the fifth carbon atom.

These results indicate that the presence of the A and B ions in the NIMSs of the compounds studied enables the positions of the double bonds to be established regardless of the degrees of unsaturation.

EXPERIMENTAL

The mass spectra were recorded on a RMU-6D instrument connected to a PDP8/e minicomputer. The scanning of the energy of the electrons was carried out by a twelve-discharge isolated DAC with a minimum step of 3 mV and a fast action of 1 μsec. The signal with reversed SEM with respect to the feed was recorded through a decoupled amplifier with a time constant of 1 μsec.

The substances were introduced directly into the source and into the system heated at 200°C. The cathodic emission current was 3 μA. The energy scale was calibrated with respect to nitrobenzene (0 eV) and to pyridine (5.2 and 8.5 eV) as in [9].

SUMMARY

1. Three regions of the resonance capture of electrons by the molecules of fatty acids and their pyrrolidides and methyl esters have been determined.

2. A high degree of dissociation of the molecular ions of unsaturated fatty acids and their derivatives in the high-energy resonance state has been detected. The mass numbers of the fragmentary ions permit the positions of multiple bonds to be determined unambiguously.

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