MASS SPECTROMETRY OF BIOLOGICALLY ACTIVE SUBSTANCES.

II.* MASS SPECTRA OF BARBITURIC ACID DERIVATIVES

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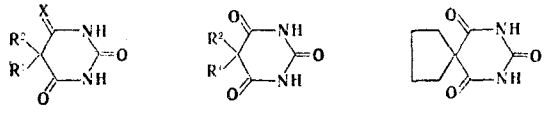
The mass spectra of 2- and 4-iminobarbituric acid derivatives were studied in relation to the mass spectra of their oxygen analogs. It is shown that the pathways of fragmentation of the investigated compounds depend on the type of substituent attached to the C_5 atom, the position of the imino and oxo groups in the ring, and the specific mass-spectral properties. The fragmentation was studied by means of low-voltage mass spectrometry and deuterium labeling.

In a continuation of our mass spectral study of barbituric acid derivatives [1] in the present research we made a comparative study of the mass spectra of I-XI obtained at ionizing-electron energies of 70 and 14 eV with the application of deuterium labeling.



I-VII VIII-X XI

I $R^1=R^2=CH_3$, X=NH; II $R^1=R^2=CH_3$, X=O; III $R^1=R^2=H$, X=NH; IV $R^1=R^2=H$, X=O; V $R^1=R^2=C_2H_5$, X=NH; VI $R^1=R^2=C_2H_5$, X=ND, H=D; VII $R^1=C_2H_5$, $R^2=C_6H_5$, X=NH; VIII $R^1=R^2=C_2H_5$, X=NH; IX $R^1=R^2=C_2H_5$, X=O; X $R^1=C_2H_5$, $R^2=C_6H_5$, X=O

A joint examination of the spectra of I-XI makes it possible to make a qualitative and quantitative estimate of the effect of the functional groups, substituents, and ionization energies on the character of the fragmentation of the barbituric acid ring. The fragmentation of barbituric acid derivatives under the influence of electron impact has not been heretofore studied from this point of view, whereas studies of this sort are extremely valuable for the mass-spectral determination of the structures of products of the metabolism of barbiturates and related medicinals [2-4].

In the barbituric acid series primarily the mass spectra of substances with branched dialkyl substituents attached to the C_5 atom have been studied. In this case the principal fragmentation pathways are associated with splitting out of the alkyl substituents, which is often accompanied by rearrangement processes, whereas the heterocyclic system remains almost uninvolved [1-4].

A comparison of the mass spectra of I-IV with simpler substituents in the 5 position makes it possible to ascertain the peculiarities of the fragmentation of the ring itself at various ionization energies. The principal pathways of fragmentation of barbiturates I-II are represented by Scheme 1 (in the schemes and in the text the numbers next to the letter designations of the fragments correspond to the m/e values).

In the case of the fragmentation of barbituric acid derivatives one must primarily take into account the possibility of tautomeric transformations [1]; these transformations will subsequently be reflected in the fragmentation schemes without explanation in the text. Fragmentation pathways A, C, and A^1, C^1 are common to I and II (Scheme 1); the peaks of the *a*, c, and c' fragments in the spectra at an ionizing energy of 70 eV have approximately identical relative intensities. However, the processes involving elimination of the CH_3,

*See [1] for communication I.

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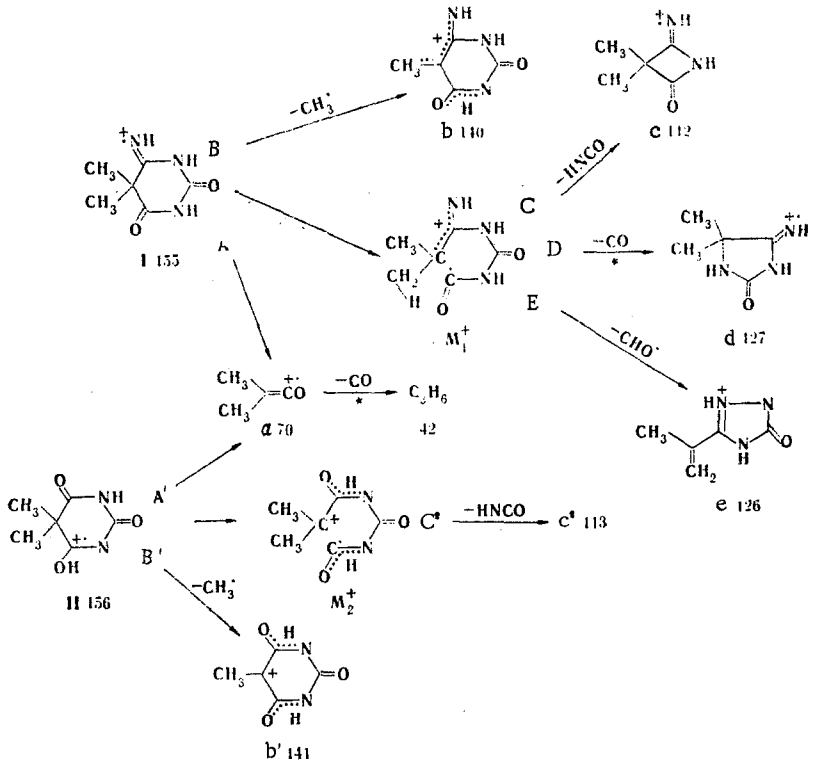
CO, and CHO fragments (pathways B, D, and E) proceed intensively only in the case of I; this is explained by ionization of the molecule at the double bond or at the nitrogen atom of the imino group, subsequent α cleavage (with respect to the position of the charge in the ring), and rearrangement. Stable b, d, and e ions are formed as a result of this. When the ionizing voltage is reduced to 14 eV, the peaks of the d and e fragments retain their high intensities; this confirms the rearrangement character of their formation processes. The peak of the b ion (140) remains the maximum peak in the mass spectrum at the same energy, whereas the intensity of the peak of the analogous b' fragment ion (141) in the spectrum of II increases by a factor of more than two. The stability of the b and b' ions is apparently explained by effective charge stabilization.

Scheme 1



The specific character of the behavior of II under electron impact is due to localization of the charge on the amide bond and the symmetry of the molecule, since the fragmentation of M^+ is realized via pathway A', independently of cleavage of the C_6—C_5 or C_5—C_4 bonds, which are equivalent in this case. This also probably leads to the maximum intensity of the peak of the α fragment (70) in the spectra at high and low ionizing energies. It should be noted that in the case of I an intense peak of a 69 fragment formed as a result of a similar process is also observed along with ion α (70). Peaks of rearranged ions are absent in the spectra of III and IV, and the fragmentation pathways of these compounds therefore virtually coincide.

It may be assumed that methyl groups promote ring opening and the occurrence of rearrangement processes. The fragmentation of I and II through the open forms of the molecular ions (M_1^+ and M_2^+) is confirmed by a comparison of the stabilities of I, II and III, IV molecules with respect to electron impact. The W_M values of I and II are 4.4 (I) and 12.7% (II), while higher values (32.6 and 23.1%, respectively), are observed for analogs III and IV.

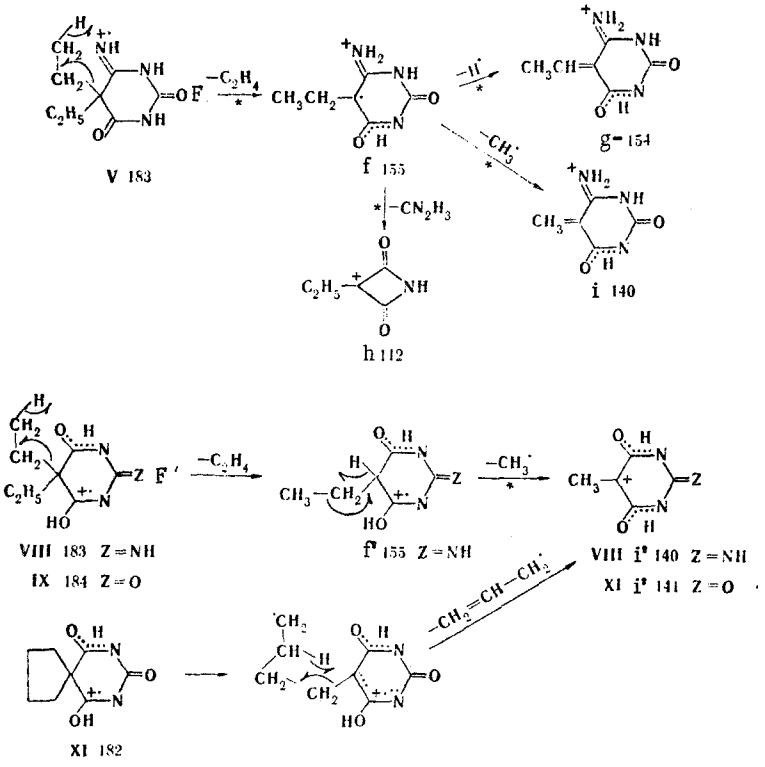
It should be assumed that the formation of open forms M_1^+ and M_2^+ for I and II and their subsequent fragmentation are possible owing to the stabilizing effect of the methyl groups attached to the radical or ion-radical centers.

With this in mind, it seemed of interest to study the peculiarities of the fragmentation of X and XI. First of all, it should be noted that, as compared with I-IV, the intensities of the molecular-ion peaks for structural isomers V and VIII at 70 and 14 eV are extremely

low (~1%). This sort of behavior of derivatives V, VIII, and IX is associated with two principal factors: fragmentation of the diethyl derivatives with detachment of a C_2H_4 fragment, and the specific effect of the imino group, since the corresponding thio analogs of V and VIII have high stabilities with respect to electron impact, and the molecular-ion peaks have considerable intensities [1]. It is interesting to note that the relative intensity of the M^+ ion peak for VIII is higher by a factor of more than two than in the case of isomer V.

One's attention is drawn to the high intensities of the $[M - C_2H_4, - H]^+$ ions in the spectra of V-VII; fragments of this sort are absent in the case of the fragmentation of their oxo analogs IX and X. The features of the fragmentation of isomers V and VIII are presented in Scheme 2.

Scheme 2



It follows from Scheme 2 that the processes involving the elimination of C_2H_4 molecules for V and VIII occur via different mechanisms (pathways F and F'). The subsequent detachment of $\dot{H}$ and $\dot{C}H_3$ radicals from ion f can be considered to be the result of stabilization of the radical center at the C_5 atom to give ions g and i. The mechanisms of fragmentation of derivative V are in agreement with distribution of the deuterium label in the spectrum of analog VI. The f, g, and i ion peaks in this case are shifted three units and the peak of the h fragment is shifted one unit to the high-mass region. In the case of fragmentation of f' ions detachment of a methyl group is possible only via the f' → i' pathway; this is confirmed in the fragmentation of XI (Scheme 2).

The principal process in the fragmentation of this analog is manifested in the intensive elimination from M^+ of an allyl radical and the formation of ion i', which is common to both VIII and XI. The relative intensities of the peaks of the i' fragments in the spectra of derivatives VIII and XI at electron energies of 70 and 14 eV change identically (see Table 1).

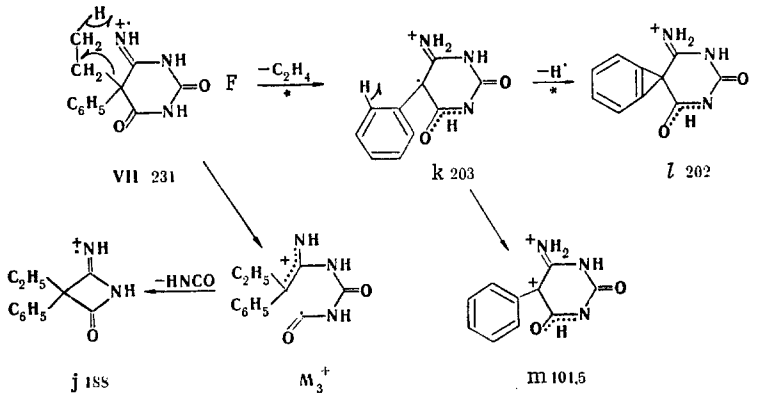
In comparison with the spectra of diethyl derivatives V and VIII, the intensities of the M^+ peak in the spectrum of VII increases sharply. The features of the fragmentation of VII are shown in Scheme 3.

As in the fragmentation of derivatives I and II, the stabilization of M^+ for the compound is realized through open form M_3^+. When substituents such as CH_3 and C_6H_5 are attached to C_5, the structures of ions of the M_1^+, M_2^+, or M_3^+ type are probably extremely stable, and this is reflected in the spectra of VII at 70 at 14 eV. One of the intense $[M - HNCO]^+$ ion peaks (j, 188) at 70 eV becomes the maximum peak in the low-voltage spectrum. At an ionizing-electron energy of 70 eV the principal peak in the spectrum corresponds to the rearranged

TABLE 1. Mass Spectra of I-VIII and XI at 70 (a) and 14 eV (b)

Compound	m/e values (relative intensities of the ion peaks)
I[a]	39 (31), 40 (12), 41 (56), 42 (100), 43 (5), 44 (28), 52 (9), 53 (6), 54 (19), 55 (19), 56 (5), 57 (7), 68 (23), 69 (45), 70 (80), 71 (6), 83 (8), 84 (22), 85 (4), 86 (5), 97 (55), 98 (5), 111 (12), 112 (40), 113 (7), 126 (50)
I[b]	127 (40), 128 (4), 140 (85), 141 (9), 155 (40), 156 (10), 69 (7), 70 (18), 84 (12), 97 (20), 112 (70), 113 (10), 126 (73), 127 (70), 140 (100), 141 (12), 155 (42), 156 (13)
II[a]	39 (16), 40 (6), 41 (14), 42 (61), 43 (8), 79 (8), 70 (100), 71 (4), 85 (4), 98 (8), 113 (26), 141 (7), 156 (44), 157 (3)
II[b]	70 (100), 85 (5), 98 (4), 113 (55), 114 (3), 128 (3), 141 (14), 156 (90), 157 (6)
III[a]	39 (5), 40 (10), 41 (14), 42 (20), 43 (40), 44 (8), 54 (13), 56 (6), 67 (6), 68 (38), 84 (20), 85 (5), 99 (100), 127 (100), 128 (6)
III[b]	84 (15), 99 (15), 127 (100), 128 (6)
IV[a]	41 (6), 42 (100), 43 (10), 44 (6), 69 (7), 70 (3), 85 (15), 100 (3), 128 (47), 128 (3)
IV[b]	42 (33), 85 (27), 100 (6), 128 (100), 129 (5)
V[a]	39 (8), 40 (3), 41 (12), 42 (5), 43 (9), 44 (6), 51 (7), 52 (3), 53 (5), 54 (3), 55 (12), 56 (3), 68 (7), 69 (13), 70 (6), 82 (14), 83 (12), 84 (4), 97 (20), 98 (3), 111 (28), 112 (5), 125 (15), 140 (100), 141 (8), 154 (47), 155 (70), 168 (2), 183 (0,1)
V[b]	140 (40), 154 (21), 155 (100), 156 (8), 155 (100), 168 (3), 183 (0,6)
VI[a]	39 (20), 41 (26), 42 (5), 43 (10), 44 (10), 45 (9), 53 (10), 54 (5), 55 (30), 56 (6), 57 (5), 67 (5), 68 (6), 69 (10), 70 (15), 81 (5), 82 (24), 83 (5), 84 (2), 97 (10), 98 (12), 99 (16), 111 (5), 112 (19), 113 (16), 125 (4), 126 (16), 127 (13), 128 (4), 140 (22), 141 (72), 142 (100), 143 (56), 155 (34), 156 (96), 157 (94), 158 (44), 183 (0,1), 184 (0,15), 185 (0,15), 186 (0,4), 187 (0,2)
VII[a]	39 (10), 41 (4), 42 (5), 43 (9), 44 (5), 50 (4), 51 (16), 63 (8), 64 (3), 65 (8), 66 (3), 76 (5), 77 (22), 78 (7), 89 (12), 90 (5), 91 (15), 92 (3), 102 (5), 103 (33), 104 (10), 105 (3), 115 (20), 116 (8), 117 (36), 118 (14), 119 (3), 128 (4), 129 (3), 130 (10), 131 (27), 132 (15), 143 (6), 144 (3), 145 (7), 146 (12), 147 (6), 158 (6), 159 (30), 160 (10), 161 (3), 173 (50), 174 (7), 187 (3), 188 (58), 189 (10), 202 (100), 203 (82), 204 (12), 216 (13), 217 (3), 230 (7), 231 (32), 232 (4)
VII[b]	173 (7), 188 (100), 189 (12), 202 (72), 203 (92), 216 (8), 217 (7), 230 (4), 231 (38), 232 (6)
VIII[a]	39 (8), 41 (16), 42 (6), 43 (12), 44 (5), 54 (2), 55 (16), 82 (16), 97 (2), 98 (12), 112 (10), 113 (2), 140 (100), 154 (12), 155 (67), 156 (6), 183 (0,3)
VIII[b]	140 (54), 141 (6), 154 (8), 155 (100), 156 (8), 183 (0,2)
XI[a]	39 (8), 40 (7), 41 (8), 58 (4), 54 (3), 55 (2), 65 (2), 66 (2), 67 (8), 68 (4), 83 (4), 95 (3), 96 (3), 98 (24), 101 (4), 141 (100), 142 (7), 154 (3), 167 (1), 182 (18), 183 (3)
XI[b]	141 (100), 142 (6), 154 (2), 167 (2), 182 (32), 183 (3)

Scheme 3



$[M - C_2H_4, - H]^+$ ion (*l*, 202), which retains its high intensity also at 14 eV. The $[M - C_2H_4]$ fragment gives an intense peak of a doubly charged m ion (101.5). Fragments of the j, *l*, and m type are absent in the fragmentation of the corresponding oxo analog X (Scheme 3) [1].

Thus fundamental differences are observed in the behavior of 2- and 4-iminobarbituric acid derivatives and the corresponding barbituric and thiobarbituric analogs under electron impact. The principles found in this research can be used to establish the structures of related compounds or the products of their metabolism in biomedical studies.

EXPERIMENTAL

The mass spectra of X and XI were recorded with an LKB-9000 mass spectrometer at ionizing voltages of 70 and 14 eV, an emission current of 20 μA, and an ion-source temperature of 250°C. The temperature of the admission system was 20-50°C.

Compounds I, III, V, VII, and VIII were synthesized by published methods [5-7], whereas II, IV, and IX-XI were obtained by acid hydrolysis of their 4-imino analogs. The deutero analog of VI was obtained by heating V in deuteromethanol. The purity of the products was monitored by chromatography on Silufol UV-254 plates [chloroform–methanol (10:1)] and elementary analysis. The melting points of the synthesized compounds were in agreement with the literature values.

LITERATURE CITED

1. V. G. Voronin, S. B. Goncharenko, A. I. Ermakov, and Yu. N. Portnov, Khim. Geterotsikl. Soedin., No. 10, 529 (1976).
2. M. Mizugaki, Y. Suzuki, M. Uchugama, and H. Abe, Eisei Kagaku, 17, 241 (1971); Chem. Abstr., 76, 68814 (1972).
3. M. Vore, N. Gerber, and M. T. Bush, Pharmacol., 13, 220 (1971).
4. I. T. Watson and F. C. Folkner, Mass Spectrom., 7, 1227 (1973).
5. M. Conrad, Ann., 340, 310 (1905).
6. M. Conrad and A. Zart, Ann., 340, 326 (1905).
7. E. Fischer and A. Dilthey, Ann., 335, 352 (1904).

MASS SPECTROMETRY OF BIOLOGICALLY ACTIVE SUBSTANCES.

III.* MASS SPECTRA AND PECULIARITIES OF THE FRAGMENTATION OF 4-AMINO-5-PYRAZOLONES

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As compared with alkyl substituents, amino and amido groups in the 4 position of 1-phenyl-2,3-dimethyl-5-pyrazolones substantially reduce the stability of the molecule with respect to electron impact. The rearrangements in the heteroring that are characteristic for alkyl-substituted pyrazolones are absent in the fragmentation of these compounds. The principal fragmentation process in this case is manifested in the primary formation of a $CH_3C{\equiv}NCH_3^+$ ion with cleavage of the ring C=C and N–N bonds. The peculiarities of the mass spectra of 4-aminopyrazolones are explained by ionization of the molecule at the C=C bond and competitive distribution of the charge in the molecular ion between the nitrogen atom of the substituent and the pyrazolone ring. The spectra at low ionizing-electron energies were studied.

The mass-spectrometric fragmentation of the simplest pyrazolone systems has been studied in detail in the case of Δ^2-pyrazolin-5-one derivatives with alkyl substituents in the 4 position [2]. Less study has been devoted to Δ^3-pyrazolin-5-one derivatives in this respect, but compounds with alkyl or acyl substituents also predominate in this series [3, 4].

In the present research we examined the mass spectra, obtained at different ionizing energies (70 and 14 eV), of a number of 1-phenyl-3-methyl-5-pyrazolones (I-VII) with nitro-

*See [1] for communication II.

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