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## Abstract

## Full Text

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# MASS-SPECTROMETRIC STUDY OF METHYLTHIOHYDANTOINS OF AMINO ACIDS

*(Presented by Academician M. M. Shemyakin, January 7, 1964)*

In determining the sequence of amino acids in peptides, Edman' s method ([1]) has become widely used; it makes it possible to cleave amino acids successively, one after another, from the polypeptide chain in the form of the corresponding phenylthiohydantoins (PTH). Recently, Edman' s method has been substantially improved ([2,3]); however, identification of the cleaved PTH derivatives still remains a difficult task. In search of a solution to this problem, we have investigated the applicability of mass spectrometry to the analysis of PTH ([4]).

Further investigations in this direction showed that some PTH derivatives do not give a molecular peak, while other characteristic peaks used for identification have low intensity in the mass spectrum. This is due to the presence of a phenyl group in PTH, which leads to the formation of very intense peaks that are not characteristic of the individual PTH derivatives, for example peaks with $m/e$ $135^+$ ($C_6H_5NCS^+$), 119 ($C_6H_5NCO^+$), etc.

We have established that the methylthiohydantoins (MTH) of amino acids are more convenient for mass-spectrometric identification of amino acids. They sublime without decomposition at a lower temperature (150–170°) than the corresponding PTH derivatives (200–250°) and give more characteristic mass spectra.

Methylthiohydantoins are readily obtained by treating the corresponding amino acids with methyl isothiocyanate, followed by ring closure in an acidic medium. For example: to 2.45 g (15 mmoles) of $\alpha$-phenylalanine in 30 ml of water, with stirring, 1 g (13.7 mmoles) of methyl isothiocyanate is added at 40°, maintaining pH $\sim$ 9.0 by addition of 1 N KOH (autotitrator); after 15 min, conc. HCl is added to pH 1, the mixture is boiled for 10 min, and phenylalanine MTH is obtained, yield 59%, m.p. 136–136.5° (from aq. $CH_3COOH$).

Found, %: C 59.89; H 5.46; N 12.71; S 14.43
$C_{11}H_{12}N_2OS$. Calculated, %: C 59.97; H 5.50; N 12.71; S 14.56

MTH derivatives have characteristic UV absorption spectra with maxima at 235 m$\mu$ ($\varepsilon_{235} \simeq 9500$) and 265 m$\mu$ ($\varepsilon_{265} \simeq 17\,000$); they are less hydrophobic than PTH derivatives, and the differences in solubility among individual MTH derivatives are more pronounced than among PTH derivatives. Using leucylglycine as an example, it has been shown that cleavage of N-terminal amino

acids in the form of MTH proceeds under conditions close to those used in Edman' s method ([1]).

We have studied the mass spectra of the MTH derivatives of 17 amino acids. Their structures are represented by the general formula (A) and by formula (XVII). It should be noted that the MTH derivatives of serine and threonine can undergo dehydration upon heating. Thus, from serine MTH the derivative (XVIII) is formed.

(Figure: Structural formulas (A), XVII, and XVIII: methylthiohydantoin derivatives)

I $R = H$, II $R = CH_3$, III $R = CH(CH_3)_2$, IV $R = CH_2CH(CH_3)_2$, V $R = CH(CH_3)C_2H_5$, VI $R = CH_2C_6H_5$, VII $R = CH_2C_6H_4OH$-$n$, VIII $R =$ (indolyl-3)-methyl, IX $R = CH_2CH_2SCH_3$, X $R = CH_2CONH_2$, XI $R = CH_2COOH$, XII $R = CH_2COOC_2H_5$, XIII $R = CH_2CH_2CONH_2$, XIV $R = CH_2CH_2COOH$, XV $R = CH_2OH$, XVI $R = CH(OH)CH_3$.

The mass spectra of the compounds studied were recorded on an MX-1303 mass spectrometer with a 90° analyzer.

A characteristic feature of the mass spectra of MTH is the presence of a well-defined molecular peak ($M^+$), which in a number of cases (I, II, IX, XVII) has the maximum intensity in the spectrum; this favorably distinguishes the spectra of MTH from the spectra of PTH. Only in the spectra of MTH of tryptophan (VIII) and glutamic acid (XIV) is the magnitude of the molecular peak insignificant.

Fragmentation of the molecular ion, as is seen from consideration of the mass spectra of MTH I–XVII, proceeds in two principal directions: with preservation of the hydantoin ring and with cleavage of bonds of the hydantoin ring. Both the first and the second pathway lead to the formation of characteristic fragments which, together with the molecular peak, make it possible to solve the main analytical problem—the determination of the structure of the amino acid from which a given MTH was obtained (see Table 1).

Fragmentation with preservation of the hydantoin ring proceeds differently, depending on the nature of the radical $R$ that previously belonged to the amino acid. For MTH III, IV, and V, which contain an alkyl chain, elimination of an olefin is characteristic, leading to the formation of a very intense peak corresponding to the "glycine" fragment ($\Phi_1$):

$$[\text{fragment } \Phi_1] \xleftarrow{-CH_3CH=CH_2} [\text{molecular ion } M^+] \xrightarrow{-(CH_3)_2CH} [\text{fragment } \Phi_2]$$
$$[\text{fragment } \Phi_3] + [\text{heterocyclic radical}]$$

This evidently explains the exceptional stability of the molecular peak of MTH of glycine (the intensity of the $M^+$ peak amounts to 30% of the sum of the

peaks). However, in addition to elimination in the form of an olefin, the side chain of the molecular ion of MTH III, IV, and V may be split off in the form of a radical $R\cdot$, with the formation of another "glycine" fragment ($\Phi_2$). On the other hand, upon rupture of the C–C bond between the substituent $R$ and the hydantoin ring, the positive charge may be localized on the side chain, which is split off in the form of fragment ($\Phi_3$), with simultaneous formation of the heterocyclic radical.

In the case of MTH VI, VII, and VIII, in which $R$ contains an aromatic or heterocyclic residue, upon rupture of the C–C bond between the substituent $R$ and the hydantoin ring, the positive charge is localized on fragment ($\Phi_3$) because of its greater stability ([5]).

For MTH IX–XVI, in which $R$ contains a functional group, the formation of "alanine" fragments ($\Phi_4$) and ($\Phi_5$) with $m/e$ 144 and 143 occurs. However, the more preferred direction of fragmentation for these compounds is elimination of HX ($CH_3SH$ for IX, $H_2NCOH$ for X and XIII, $HCOOH$ for XI and XIV, and $HCOOC_2H_5$ for XII), leading to the formation of stable fragments ($\Phi_6$) and

**Table 1**

**Values of $m/e$ (in parentheses) and intensities of the principal peaks in the mass spectra of methylthiohydantoins (intensities are expressed as percentages of the sum of peaks)**

| Amino acid from which MTH was obtained | $[M^+]$ | $[M-2]^+$ | $\Phi_1$ | $\Phi_2$ | $\Phi_3$ | $\Phi_4$ | $\Phi_5$ | $\Phi_6$ | $\Phi_7$ | $\Phi_8$ | $\Phi_9$ | $\Phi_{10}$ | $\Phi_{11}$ | $\Phi_{12}$ | $\Phi_{13}$ | $[M-28]^+$ $\Phi_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I Gly | **30(130)** | 30 | | | | | | | | 5,4 | 0,6 | 9,0 | | | | 8,6(102) |
| II Ala | **35(144)** | 0,8 | 0,8 | | 35 | 0,8 | | | | | 0,7 | 3,7 | 3,7(72) | | **20(44)** | 2,8(116) |
| III Val | 20(172) | **31,7** | | | 5(43) | | | | | 2,9 | 4,0 | 2,5 | | 0,6(72) | 2,3(69) | |
| IV Leu | 8(186) | 4(184) | **11,4** | 2 | 2(57) | 0,2 | 0,4 | | | 2,7 | see $\Phi_3$ | 4,3 | see $\Phi_2$ | 1,2(83) | | |
| V Isoleu | 13(186) | 6(184) | **28,6** | 11 | 6,8(57) | | | | | 2,0 | see $\Phi_3$ | 2,6 | see $\Phi_2$ | | | |
| VI Phe | 8(220) | 9(218) | 0,7 | 0,5 | **16(91)** | 1,4 | 1 | | | 15,9 | | | | 0,2(122) | 2(117) | |
| VII Tyr | 2(236) | 1,6(234) | | | **20(107)** | | | | | 4,2 | 1,2 | 2,0 | | | 2(133) | |

| Amino acid from which MTH was obtained | $[M^+]$ | $[M-2]^+$ | (130) $\Phi_1$ | (129) $\Phi_2$ | $[R]^+$ $\Phi_3$ | (144) $\Phi_4$ | (143) $\Phi_5$ | (142) $\Phi_6$ | (156) $\Phi_7$ | (42) $\Phi_8$ | (57) $\Phi_9$ | (74) $\Phi_{10}$ | $\Phi_{11}$ | $\Phi_{12}$ | $\Phi_{13}$ | $[M-28]^+$ $\Phi_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VIII Trp | 4,3 | see $\Phi_3$ | 6,3 | **19(136)** | 3,6 | 0,4 | 3,8 | | 4,3 | 2,0 | 1 | | | 5(156) | | |
| IX Met | **14(204)** | 10 | 0,7 | 2,0(76) | 6,7 | 5,7 | 1,3 | 6,0 | 2,0 | 1,0 | 6,9 | | | | | |
| X Asp – $NH_2$ | **7(187)** | 2,8 | 2,8 | | 10 | 3,5 | **28** | | 1,5 | 0,5 | 5,0 | see $\Phi_1$ | | | | |
| XI Asp | 3(188) | 5,0 | 1,0 | | 5 | 4,4 | **8,6** | | 7,0 | 2,0 | 10 | | | | | |
| XII Asp – Et ester | **16(216)** | 1,2 | 1,1 | | 2,9 | 2,9 | 16,0 | | 2,5 | 1,2 | 3,2 | | | 0,5(113) | | |
| XIII Glu – $NH_2$ | 3(201) | 1,4 | 0,9 | | 1,3 | 2,2 | **8,0** | 4,7 | 0,5 | | 1,4 | see $\Phi_4$ | **1(101)** | | 1(173) | |
| XIV Glu | 0,6(202) | 1,0 | | | 0,3 | 1,8 | 1,8 | **18** | 4,5 | 0,6 | 6,0 | | | | | |
| XV Ser | | | | | | | **56,0** | | 4,3 | 0,9 | 0,5 | | | 2,6(81) | | |
| XVI Thr | | | | | | | | **44,0** | 1,8 | | 13 | | | | | |
| XVII Pro | **26(170)** | | | 16(70) | | | | | 3,2 | | 3,9 | | | | | 1,3(142) |

**Note.** Bold type denotes the intensity of the maximum peak in the mass spectrum.

($\Phi_7$) with $m/e$ 142 and 156:

(Figure: reaction scheme: formation of $\Phi_6$ ($m/e$ 142) and $\Phi_7$ ($m/e$ 156) by elimination of $-HX$ from the corresponding ions)

Fragmentation with rupture of bonds in the hydantoin ring leads to the forma-

tion both of fragments common to all MTHs ($\Phi_8, \Phi_9$ and $\Phi_{10}$), and of characteristic fragments ($\Phi_{11}, \Phi_{12}$ and $\Phi_{13}$):

(Figure: fragmentation scheme of the hydantoin ring, showing ions $\Phi_8$-$\Phi_{12}$)

Thus, the study of the behavior of MTHs of various amino acids under mass-spectrometric conditions has made it possible to establish the general regularities of fragmentation of the molecular ions of these compounds and to solve the main analytical problem—the determination of the structure of the amino acid from which the given methylthiohydantoin was obtained.

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*Note: Figure translations are in progress. See original paper for figures.*