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MASS SPECTROMETRIC DETERMINATION OF THE AMINO (HYDROXY) ACID SEQUENCE IN PEPTIDES AND DEPSIPEPTIDES

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The rapid determination of primary structure is still a major problem in protein and peptide chemistry. At present one can easily determine the number, structure and configuration of the amino acid constituents of proteins and peptides. Degradation of the protein molecule into various size fragments and separation of the latter also presents no special difficulties. However, determination of the amino acid sequence in the peptide fragments remains a very difficult and cumbersome task, especially if it be borne in mind that the work must often be carried out on submicro amount of substance.

In the present paper it is intended to show the potentialities of mass spectrometry in the solution of this problem.

Systematic analysis of the mass spectra of various linear

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the N-acetyl derivatives of the more involved, linear tetra- and octadepsipeptides the mass spectra of whose tert.-butyl esters are shown in Table 1 (Comps. 4 and 5) and in Fig. 3. At first a tert.-butoxy group is eliminated from the molecular ion and this is followed by consecutive elimination of amino and hydroxy acid residues. It should be pointed out that in some cases we observed all stages of the amino (hydroxy) acid type of fragmentation in linear depsipeptides with free amino and hydroxy groups (Comps. 6 - 8 in Table 1).

The picture becomes highly complicated in the case of cyclopeptides and cyclodepsipeptides built up of different acid residues, since in that case primary rupture may occur at different amide or ester bonds leading to the formation of linear ion-radicals with different sequences of the acid residues. However, here too one can clearly trace the fragmentation in accordance with the above rule and often detect all possible variants of the amino (hydroxy) acid type of fragmentation. For instance, in the mass spectrum of enniatin A (Comp. 9 in Table 1 and Fig. 4) the two possible paths due to the primary rupture of either an amide or an ester bond can be observed, while the spectrum of the synthetic analog of sporidesmolide (Comp. 10 in Table 1) displays all three routes. Even in the case of the 36 membered cyclododecadepsipeptide (Comp. 11 in Table 1) the alternant paths in this type of fragmentation can be clearly discerned.

It must be remembered, however, the amino (hydroxy) acid type of fragmentation is not the only type that appears in the mass spectra of these classes of compounds, but that it occurs

TABLE 1

N	Compound	M^+	The sequence of residues eliminated (m/e of fragments observed)
1	Ac(Ala)$_6$OMe	500	→MeO(469)→Ala(398)→Ala(327)→Ala(256)→Ala(185)→Ala(114)
2	C_9H_{19}COLeu(Ala)$_3$OMe	512	→MeO(481)→Ala(410)→Ala(339)→Ala(268)
3	(Ala)$_6$	426	→Ala(355)→Ala(284)→Ala(213)→Ala(142)→Ala(71)
4	Ac(Val-Lac-Val-HyV)-OBu^t	486	→Bu^{t}O(413)→HyV(313)→Val(214)→Lac(142)
5	Ac(Val-Lac-Val-HyV)$_2$OBu^t	856	→Bu^{t}O(783)→HyV(683)→Val(584)→Lac(512)→Val(413)→ →HyV(313)→Val(214)→Lac(142)
6	H(Val-Lac-Val-HyV)OBu^t	444	→Bu^{t}O(371)→HyV(271)→Val(172)→Lac(100)
7	H(Leu-Lac-Val-HyV)OBu^t	458	→Bu^{t}O(385)→HyV(285)→Val(186)→Lac(114)
8	H(HyV-MeVal)$_2$OBu^t	500	→Bu^{t}O(427)→MeVal(314)→HyV(214)→MeVal(*/)
9	(MeVal-HyV)$_3$	639	MeVal(526)→HyV(426)→MeVal(313)→HyV(213)→MeVal(100) HyV(539)→MeVal(426)→HyV(326)→MeVal(213)→HyV(113)
10	(MeIle-Val-HyV)$_2$	652	MeIle(525)→Val(426)→HyV(326)→MeIle(199)→Val(100) Val(553)→HyV(453)→MeIle(326)→Val(227)→HyV(127) HyV(552)→MeIle(425)→Val(326)→HyV(226)→MeIle(99)
11	(Val-Lac)$_6$	1026	Val(927)→Lac(855)→Val(756)→Lac(684)→Val(585)→Lac(513)→ →Val(414)→Lac(342)→Val(243)→Lac(171)→Val(72) Lac(954)→Val(855)→Lac(783)→Val(684)→Lac(612)→Val(513)→ →Lac(441)→Val(342)→Lac(270)→Val(171)→Lac(99)

*/ Fragment HOCH(CHMe$_2$)C=O$^+$ is so unstable that one may judge of its formation only by the appearance of its further fragmentation products with m/e 84, 83 and 69.

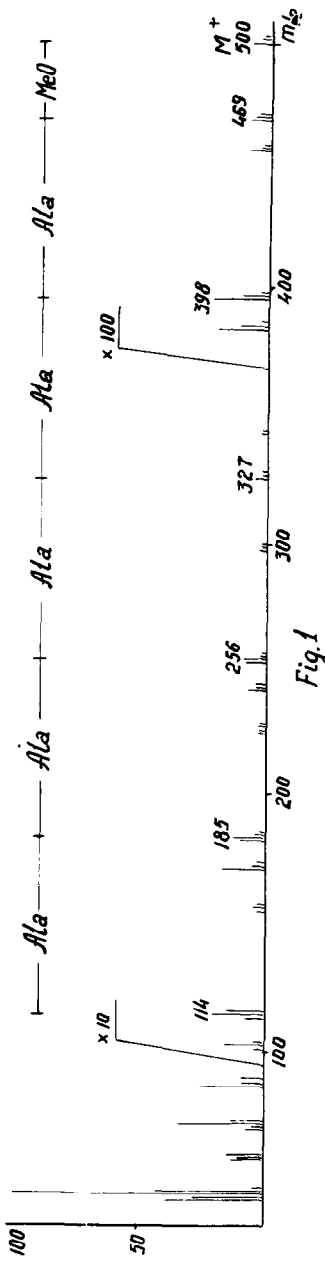
Ala — Ala — Ala — Ala — Ala — MeO
M+ 500
m/e
469
398
x 100
327
256
185
114
x 10
100
200
300
400
100
50

Fig. 1

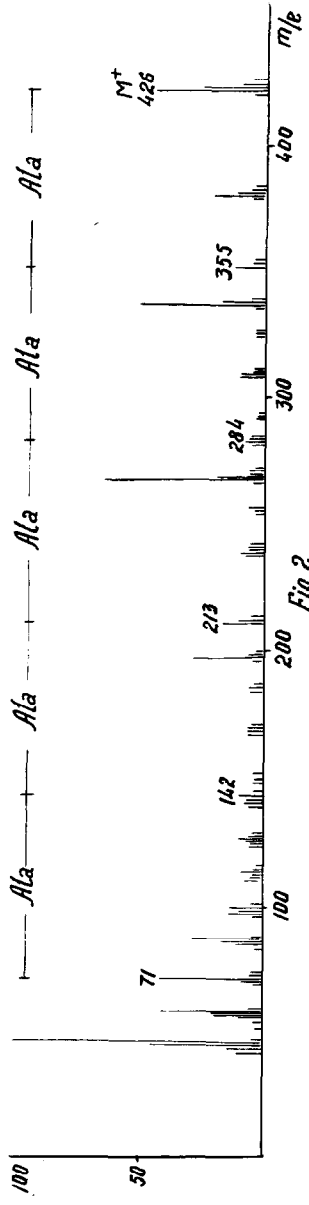
Ala — Ala — Ala — Ala — Ala
M+ 426
m/e
355
284
213
142
71
100
200
300
400
100
50

Fig. 2

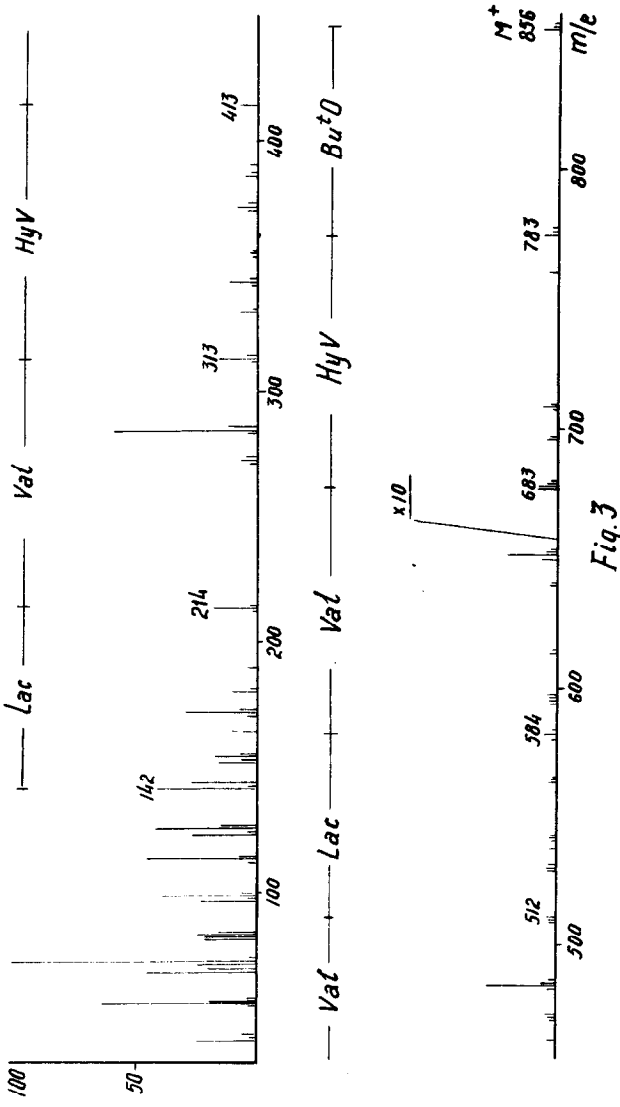
Lac
Val
HyV
Val
Lac
Val
HyV
ButO
100
50
142
214
313
413
100
200
300
400
x10
512
584
683
783
856
M+
500
600
700
800
m/e

Fig. 3

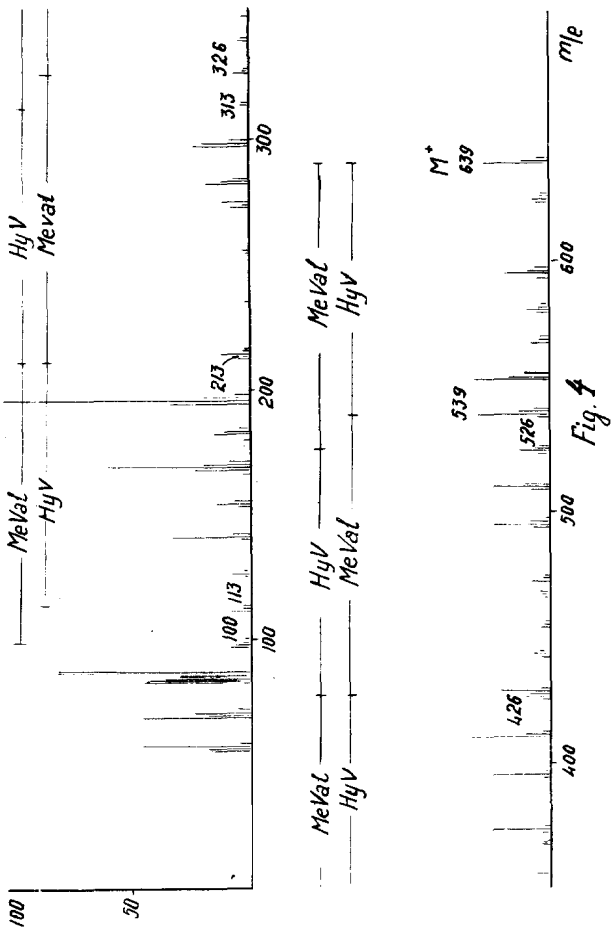
MeVal
HyV
HyV
MeVal
100
50
100
113
213
200
313
326
300
MeVal
HyV
HyV
MeVal
M+
639
539
526
426
500
600
400
m/e

Fig. 4

and cyclic peptides and depsipeptides (injected directly into the ion source) has led us to discovery of the following rule. If, the primary act or one of the subsequent fragmentation stages of molecular ion disintegration affords a linear ion or ion-radical wherein the positive charge is localized on the C-terminus and N(O)-terminus is protected (for instance by acylation, or by the presence of an unpaired electron) then further rupture of the amide (ester) bonds leads to consecutive elimination of the amino (hydroxy) acid residues from the C-terminus with transfer of the positive charge along the chain /see (1)/. This type of fragmentation, which we have termed the amino (hydroxy) acid type, thus permits rapid determination of the sequence of the acid residues in peptides and depsipeptides (2).

In order to increase the volatility of linear peptides and depsipeptides they are feasibly acylated (this simultaneously affords protection to N-terminus) and esterified (the most convenient to use are the methyl, and particularly the tert.-butyl esters) (3).

We shall illustrate the above-said by a simple example, viz. the monotonically constructed methyl N-acetylpentaalanyl-alaninate (Comp. 1 in Table 1 and Fig. 1), whose molecular ion consecutively eliminates alanine residues. For another example see Comp. 2 in Table 1.

Similarly, in the case of the monotonic cyclohexaalanyl (Comp. 3 in Table 1 and Fig. 2) the linear ion-radical resulting from rupture of the amide bond consecutively eliminates five alanine residues.

A completely analogous situation obtains in the case of

along with the COX, morpholinic and acylaminoketenic types we have described earlier (4). It may also turn out that the amino (hydroxy) acid type of fragmentation may not be the predominant one. However, it is analysis of just this type that gives the most complete, reliable and rapid information of the sequence of amino (hydroxy) acid resedues in the chain or ring, particularly when the number and structure of these residues are known.

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