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Determination of amino acid sequences in oligopeptides by mass spectrometry. II. The structure of Peptidolipin NA*

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In the first paper of this series (1) the structure of fortuitine, an acyl-nonapeptide methyl ester of molecular weight 1359, was established by mass spectrometry. It was shown that the main fragmentation of the molecule is that of the peptide bond, and this, together with evidence from metastable ions, allows the sequence of amino acids to be determined without ambiguity.

We now show that an analogous cyclic acyl-heptapeptide, peptidolipin NA, is equally accessible to sequential analysis by mass spectrometry.

Based on these results, the next communication will present a method of amino acid sequence determination in oligopeptides by mass spectrometry of N-stearoyl-oligopeptide methyl esters.

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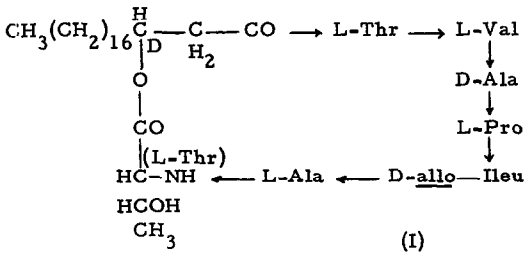
Peptidolipin NA, m. p. 232-233°, $[\alpha]_D$ + 42°, isolated from Nocardia asteroides, is a peptidolipid for which Guinand, Michel and Lederer (1964) (2) have proposed structure (I) corresponding to the molecular formula $C_{50}H_{89}N_7O_{11}$ (mol. weight 963). Mass spectrometry fully confirms this structure, as we shall see in the following.

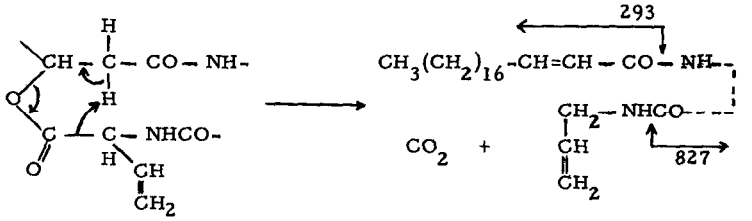


(I)

This compound was introduced into the ion source of an A.E.I. MS9 mass spectrometer using a ceramic direct insertion probe which was admitted through a vacuum lock. The mass spectrum was recorded with the resolving power of the instrument set at about 1500, and then the masses of several peaks in the spectrum were accurately measured with the resolving power set at 10,000.

The low resolution spectrum (Fig. 1) shows a molecular ion at m/e 963 and peaks at M-18 and M-36. A relatively intense peak is observed at m/e 919 due to the loss of 44 and this was first thought to be due to elimination of CO_2 from the lactone group. However, mass measurement showed that it was due to the loss of CH_3CHO apparently from the C-terminal threonine.

The peak at m/e 883 is due to the loss of 2 H_2O and CO_2 from the molecular ion. After the loss of 2 H_2O, the CO_2 can be lost as follows :



The ring is thus open and the molecule is now analogous in structure to that of fortuitine, i.e. a linear N-acyl-oligopeptide (1).

Let us now consider the further fragmentation of the molecule which is basically a sequential cleavage of the peptide bonds leading to the confirmation of the proposed structure (I):

1) The peak at m/e 827 is due to the further loss of 56 from the m/e 883 ion. Mass measurement (see Table) shows this to correspond to the loss of the decarboxylated C-terminal anhydro-threonine together with the hydrogen transferred in the loss of CO_2 described above.

2) The next peak, at m/e 756, is due to the further loss of 71 m.U. and the metastable peak observed at m/e 691.1 shows it to originate directly from the m/e 827 ion ; mass measurement shows that the alanine unit has now been lost.

The m/e 756 ion loses CO to give m/e 728.

3) A small peak at m/e 643 is due to the loss of the allo-isoleucine unit.

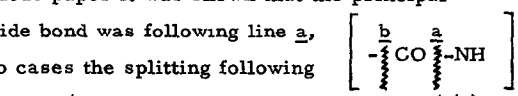
4) The intense peak at m/e 546 is due to the further loss of the proline unit but it should be noted that a metastable peak at m/e 393.5 shows that the isoleucine and proline units can be lost together from the m/e 756 ion to give m/e 546.

5) The m/e 546 ion next loses an alanine unit to give m/e 475. A metastable peak is observed at m/e 413.2.

The m/e 475 ion loses CO to give m/e 447.

6) The peak at m/e 376 is due to the further loss of the valine unit, and this is followed by the loss of CO to give m/e 348.

7) The peak at m/e 293 is due to the loss of the anhydrothreonine unit from m/e 376 leaving the acyl group minus the hydrogen transferred in the original loss of CO_2. The peak at m/e 294 corresponds apparently to the acyl radical having not suffered loss of a hydrogen.

The high intensity of the peaks at m/e 475-28 and 376-28 is noteworthy. In the previous paper it was shown that the principal splitting of the peptide bond was following line a, whereas here in two cases the splitting following  line b is more prominent (see also Heyns and Grützmacher (3)). However, in each case metastable peaks show the initial fragmentation to be via line a.

A sample of methylated peptidolipin NA was next examined in the mass spectrometer ; the low resolution spectrum shows the molecular ion at m/e 991, the di-O-methyl ether having been formed. Mass measurement of the molecular ion confirms the molecular formula of $C_{52}H_{93}N_7O_{11}$. The intense peak at M-58 (not M-44 as for peptidolipin NA itself) was shown to be due to the loss of C_3H_6O thus confirming that a threonine side chain is involved.

All previously mentioned peaks corresponding to the splitting of the peptide bond following line a are also present in the spectrum of the di-O-methyl ether.

The methyl ester of peptidolipic acid, the saponification product of peptidolipin NA (2) was methylated and a low resolution mass spectrum of the product was obtained. This showed a molecular ion at m/e 1037 confirming the expected molecular weight of peptidolipic acid methyl ester tri-O-methyl ether.

The results of the accurate mass measurements are given in the following Table :

m/e	Reference	Measured ratio	Measured mass	Assigned formula	Difference (ppm) from measured mass
Peptidolipin NA:					
963 (M^+)	$C_{24}F_{35}^+$	1.011245	963.6599	$C_{50}H_{89}N_7O_{11}$	+ 2
919	$C_{24}F_{33}^+$	1.005123	919.6345	$C_{48}H_{85}N_7O_{10}$	+ 1
883	$C_{21}F_{33}^+$	1.005351	883.6505	$C_{49}H_{85}N_7O_7$	- 1
827	$C_{23}F_{29}^+$	1.000784	827.6020	$C_{46}H_{79}N_6O_7$	- 1
756	$C_{17}F_{29}^+$	1.002133	756.5640	$C_{43}H_{74}N_5O_6$	0
728	$C_{21}F_{25}^+$	1.002212	728.5681	$C_{42}H_{74}N_5O_5$	+ 1
546	$C_{12}F_{20}N^+$	1.015717	546.4264	$C_{32}H_{56}N_3O_4$	+ 1
475	$C_{12}F_{17}^+$	1.018025	475.3900	$C_{29}H_{51}N_2O_3$	0
348	$C_{10}F_{12}^+$	1.000993	348.3264	$C_{23}H_{42}NO$	0
293	$C_7F_{11}^+$	1.001031	293.2845	$C_{20}H_{37}O$	0
Peptidolipin di-OMe ether:					
991 (M^+)	$C_{24}F_{37}^+$	1.000758	991.6920	$C_{52}H_{93}N_7O_{11}$	+1
933	$C_{25}F_{33}^+$	1.007232	933.6510	$C_{49}H_{87}N_7O_{10}$	0

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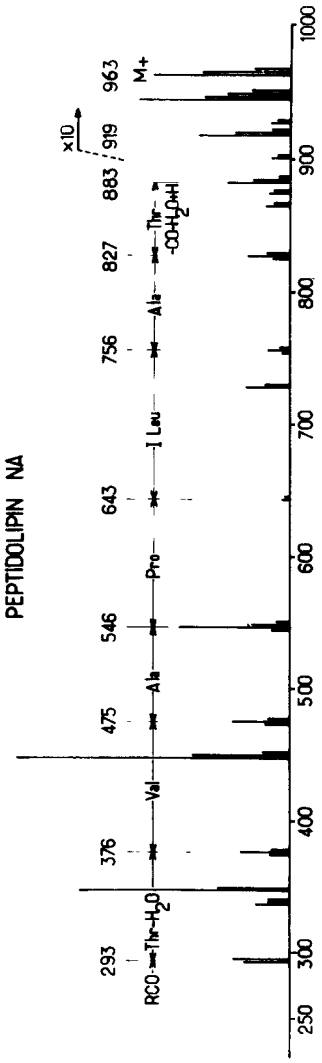

Fig. 1 - Mass spectrum of Peptidolipin NA