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DETERMINATION OF AMINO ACID SEQUENCES IN OLIGOPEPTIDES BY MASS SPECTROMETRY VIII. THE STRUCTURE OF ISARIIN*

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Isariin, a metabolite of Isaria cretacea van Beyma has been shown (1) to contain 1 mole each of glycine, L-alanine, D-leucine and D-β-hydroxydodecanoic acid, and 2 moles of L-valine, linked in a cyclic structure. Partial acid hydrolysis indicated that glycine was attached via an amide bond to the carboxyl function of the hydroxy acid. Mild alkaline hydrolysis afforded isariic acid, an open-chain N-acyl peptide in which L-valine was identified as the C-terminal amino acid. It was concluded that isariin contained the sequence L-valine → D-β-hydroxydodecanoic acid → glycine but the order of the remaining amino acids was not determined.

In a recent series of papers (2,3,4) it has been demonstrated that mass spectrometry can be used to obtain the complete structure of

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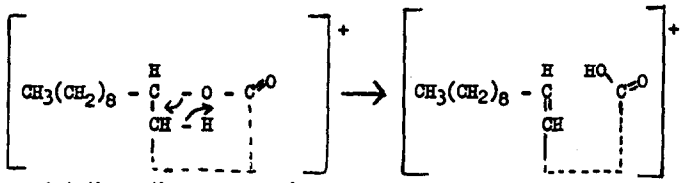
naturally occurring peptidolipids and, based on this work, a procedure was developed for amino acid sequence analysis of oligopeptides as their N-acyl esters (5). We now report the establishment of a complete structure for isariin by this method.

About 150 μgm of isariin was mounted on the end of a ceramic direct insertion probe which was then admitted through a vacuum lock to the ion source of an Associated Electrical Industries MS9 mass spectrometer. The sample was heated by heating the ion chamber with which the probe was in contact, and the mass spectrum was scanned with the resolving power of the instrument set at 1000. The masses of several peaks in the spectrum were then measured very accurately at a resolution of about 15,000.

The low resolution spectrum is shown in Fig. 1. The molecular ion is observed at m/e 637 and the mass measurement confirms the molecular formula $C_{33}H_{59}N_5O_7$. Although the fragmentation pattern as a whole is somewhat complex, peaks are observed which could correspond to simple cleavage of the peptide bonds which has been shown to occur in other natural peptides (2,3). For example, the peak at m/e 522 is due to $C_{28}H_{50}N_4O_5$, i.e. the loss of $C_5H_9NO_2$. This could correspond to the cleavage of the ring and loss of the terminal valine unit. The peak at m/e 451 could then be due to further loss of an alanine unit, and mass measurement shows the m/e 451 peak to be due to $C_{25}H_{45}N_3O_4^+$ which is in agreement with this interpretation. The peak at m/e 337 is due to $C_{19}H_{33}N_2O_3^+$ and this could be explained by the further loss of a leucine unit accompanied by a

hydrogen transfer. However, this peak is probably better explained by transfer of a hydrogen atom with initial ring cleavage followed by simple cleavage of the peptide bond e.g.

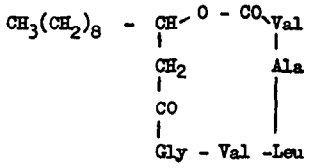


At this point then, the sequence is:

- Leu - Ala - Val -

The peak at $^{m}/_{e}$ 238 due to $C_{14}H_{24}NO_2^+$ represents further loss of a valine unit from $^{m}/_{e}$ 337 and that at $^{m}/_{e}$ 181, $C_{12}H_{21}O^+$, results from loss of the glycine unit from $^{m}/_{e}$ 238.

All these peaks can only be rationalised with the following sequence of amino acids in isariin:



Since the mass spectrum contains several intense peaks due to ring cleavage other than at the lactone group and further cleavages either of or adjacent to the peptide bonds, e.g. $^{m}/_{e}$ 509, 480, 438, 410, a check on this amino acid sequence was made.

Isariin was saponified and a portion of the acid esterified with diazomethane. The remainder was treated with carboxypeptidase A for 18 hours and the chloroform-soluble acidic product (1) treated with diazomethane. The low resolution mass spectra of methyl isariate, m.p. 202-203° ν_{max}^{KBr} 3285, 1747 cm^{-1}, and methyl desvalinoisariate, m.p. 186-188°, υ_{max}^{KBr} 3280, 1748 cm^{-1}, were run in the same way as that of isariin and several accurate mass measurements were again carried out.

The results give excellent confirmation of the amino acid sequence determined from the spectrum of isariin itself.

The low resolution scan of methyl isariate is shown in Fig. 2. The expected molecular ion is observed at $^{m}/_{e}$ 669 and mass measurement confirms the molecular formula of $C_{34}H_{63}N_5O_8$. A relatively intense peak at M-18 is observed, and the fragmentation is then typical of this type of peptide (5). Pairs of peaks 18 mU apart corresponding to M+ and (M-18)+ are observed due to simple cleavage of the peptide bonds. These peaks occur at $^{m}/_{e}$ 539 and 521, 468 and 450, 355 and 337, 256 and 238, then 199 and 181. This gives the amino acid sequence in methyl isariate, as in isariin itself:

$$-CH_2 - CO - Gly - Val - Leu - Ala - Val$$

The results of the accurate mass measurements which confirm this interpretation are given in the table.

The mass spectrum of methyl desvalinoisariate exhibited the expected molecular ion at $^{m}/_{e}$ 570 together with a more intense peak at 571 which mass measurement showed was due to $C_{29}H_{55}N_4O_7$ (molecular ion + H). Pairs of peaks occurred at $^{m}/_{e}$ 468 and 450, 355 and 337, 256 and 238, then 199 and 181 corresponding to those found in methyl isariate and supporting the amino acid sequence given above. The position of alanine was confirmed by hydrazinolysis (6) of desvalinoisariic acid.

Isarlin.

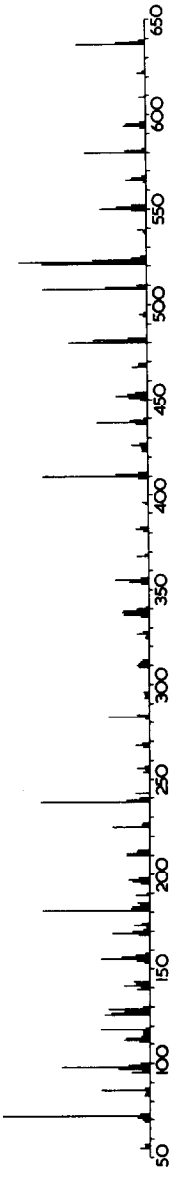


Fig. 1. Mass spectrum of isarlin.

Methyl Isariate.

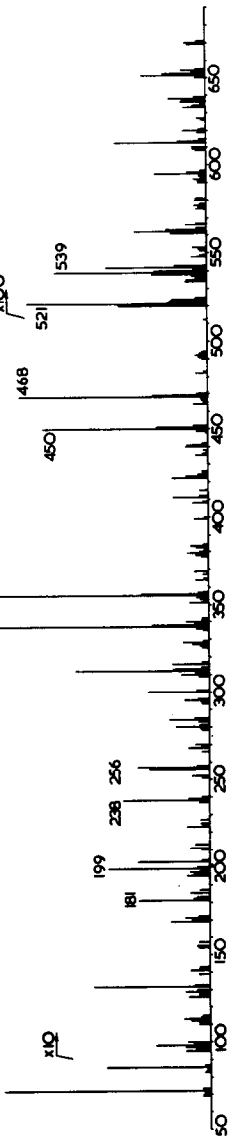


Fig. 2. Mass spectrum of methyl isariate.

Results of Accurate Mass Measurements

Isariin:

m/e	Measured Mass	Assigned Formula	Difference (mmU) from measured mass
637	637.4421	$C_{33}H_{59}N_5O_7$	- 0.7
522	522.3781	$C_{28}H_{50}N_4O_5$	0
451	451.3394	$C_{25}H_{45}N_3O_4$	+ 0.6
438	a) 438.3079	$C_{24}H_{42}N_2O_5$	+ 1.5
	b) 438.2593	$C_{22}H_{36}N_3O_6$	+ 1.1
410	410.2781	$C_{22}H_{38}N_2O_5$	0
337	337.2479	$C_{19}H_{33}N_2O_3$	+ 1.2
238	238.1802	$C_{14}H_{24}NO_2$	+ 0.5
181	181.1591	$C_{12}H_{21}O$	+ 0.1

Methyl Isariate:

669	669.4702	$C_{34}H_{63}N_5O_8$	- 2.5
539	539.3795	$C_{28}H_{51}N_4O_6$	+ 1.4
468	468.3449	$C_{25}H_{46}N_3O_5$	- 1.2
355	355.2605	$C_{19}H_{35}N_2O_4$	- 0.8
256	256.1912	$C_{14}H_{26}NO_3$	+ 0.1
181	181.1593	$C_{12}H_{21}O$	- 0.1

Note: Mass measurement shows the m/e 199 peak to be due to $C_{19}H_{15}N_2O_3^+$. This ion obviously cannot contain the acyl group. The lack of a peak due to m/e 181 ($C_{12}H_{21}O$) + H_2O is not too surprising since in this case, the "anhydro" ion $CH_3(CH_2)_8$ CH=CH-CH=O$^+$ will be afforded additional stabilisation by the presence of the double bond.

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