N-METHYLATION OF N-ACYL OLIGOPEPTIDES*

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Received September 19, 1967

Summary. - N-acyl oligopeptide methyl esters such as (II), (III) and (IV) can be easily methylated to yield the permethylated derivatives, such as (V). These are more volatile than the non methylated compounds and can be used with advantage for the mass spectrometric determination of amino acid sequence.

In recent years mass spectrometry has found promising applications in the determination of the sequence of amino acid residues in N-acyloligopeptide esters (see the recent reviews of Ovchinnikov et al., 1967, Prox & Weygand, 1967 and of Lederer & Das, 1967). However, the successful exploitation of this technique is limited by the poor volatility of higher oligopeptides.

In a previous communication (Van Heijenoort et al., 1967) it was mentioned that an important factor concerning the low volatility of peptide derivatives might be hydrogen bonding due to the presence of -CO-NH- groups. As an argument in favour of this hypothesis, it was shown that the tetrapeptide methyl ester H-Ile-Pro-Sar-MeVal-OMe containing no peptide hydrogen (i.e., absence of -CO-NH-) gave a mass spectrum exhibiting a molecular ion peak even without acylation of the terminal amino group. Also, the satisfactory volatility of the naturally occurring nonapeptide derivative fortuitine (Vilkas et al., 1963) (I), which gave a perfectly interpretable mass spectrum (Barber et al., 1965), could be explained by the presence of three tertiary amide bonds (two -MeLeu- and one -Pro-).

* Part X in the series "Determination of amino acid sequences in oligopeptides by mass spectrometry" ; part IX, Van Heijenoort et al., 1967.

$CH_3(CH_2)_n$-CO-Val-MeLeu-Val-Val-MeLeu-Thr(Ac)-Thr(Ac)-Ala-Pro-OMe

(I)

n = 18,20 M^+ 1359 (for n = 20)

These observations suggested that, if a procedure leading to permethylation of the -CO-NH- groupings of oligopeptide derivatives could be found, the resulting modified peptide might be more volatile and particularly suitable for the determination of the amino acid sequence by mass spectrometry. In this communication we describe such a method of complete methylation of peptide derivatives and the mass spectrometric results obtained with the methylated products.

The N-methylation procedure consists in treating an N-acyl peptide methyl ester in dimethylformamide with an excess of methyl iodide in the presence of silver oxide (as described by Kuhn et al., 1957, for carbohydrates). The following three peptide derivatives* have been used :

II. Dipeptide : Ac-Leu-Gly-OMe (M^+ 244)

III. Tetrapeptide : Ac-Ala-Phe-Ile-Gly-OMe (M^+ 462)

IV. Heptapeptide : Ac-Gly-Phe-Phe-Tyr(Me)-Thr-Pro-Lys(Ac)-OMe (m.w. 970)

$\downarrow$ MeI/Ag_2O

V. Ac-MeGly-MePhe-MePhe-MeTyr(Me)-MeThr(Me)-Pro-MeLys(Me,Ac)-OMe (M^+ 1082)

peaks m/e	43	114	275	436	627	756	
						↓ - 32 (MeOH)	
						724	

A typical procedure for the methylation of a peptide derivative is as follows : the N-acyl heptapeptide methyl ester (IV)** (0.024 g) was dissolved in anhydrous dimethylformamide (5 ml) and to the solution silver oxide (1.5 g) and methyl iodide (2.5 ml) were added. The suspension was stirred continuously for 3 days at room temperature. The mixture was diluted with dimethylformamide (4 ml) and then filtered off. To the filtrate chloroform (40 ml) was added, whereupon a complex separated, which was removed by washing with 5% KCN solution. The chloroform layer was washed repeatedly with water, dried over anhydrous sodium sulphate, and evaporated in vacuo. The solid permethylated heptapeptide (V ; 0.026 g) was then submitted to mass spectrometry (the compounds were introduced into the ion-source of an A.E.I. MS9 mass spectrometer using a direct insertion probe).

* For the nomenclature of peptide derivatives see Biochemistry, 5, 2485 (1966).

** The free N-acyl oligopeptides can be used just as well.

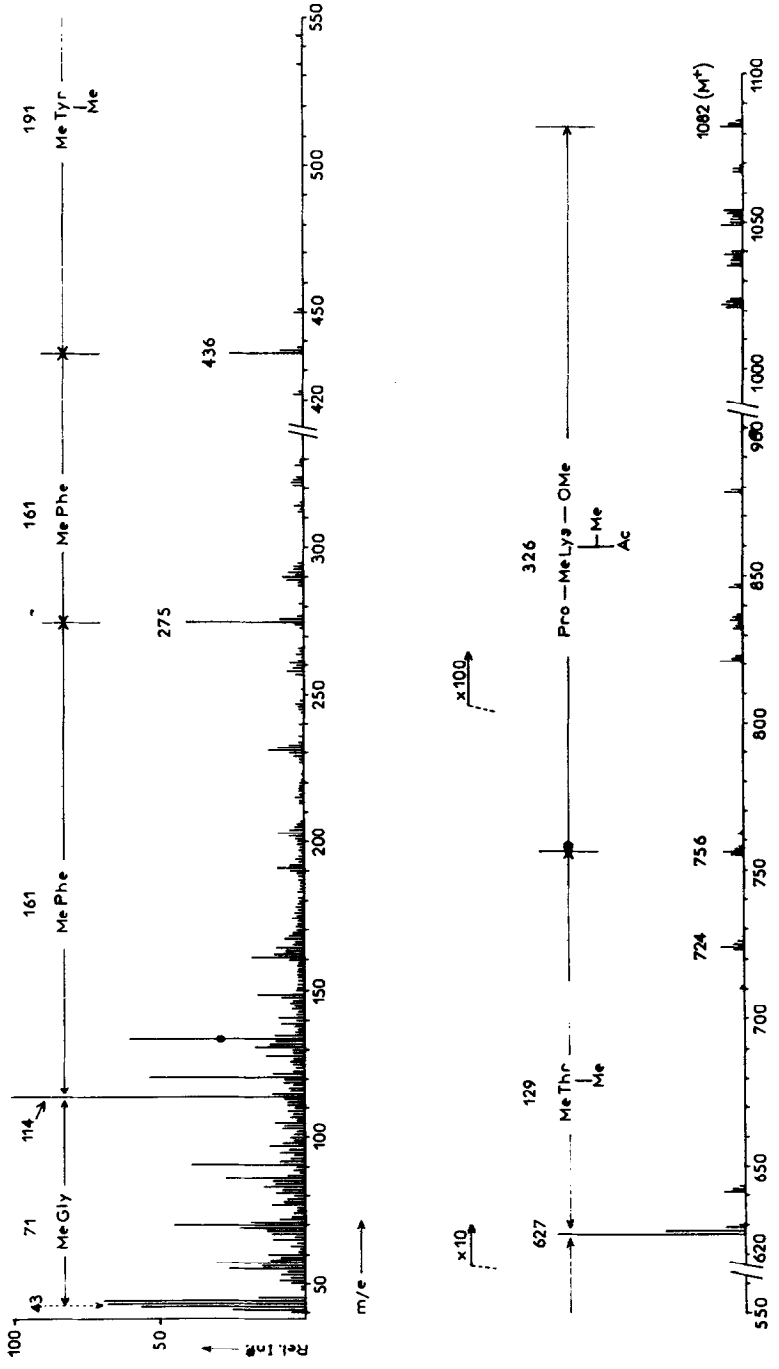

Figure 1 - Mass spectrum of the methylated heptapeptide derivative (V).

Discussion of mass spectra : The mass spectra of the di-(II) and the tetrapeptide (III) showed the respective molecular ion peaks at m/e 244 and 462 as also the peaks arising by the cleavage of the peptide bonds. After methylation, the mass spectra of the di- and tetrapeptide derivatives exhibited an increase of 28 (2 x 14) and 56 (4 x 14) mass units, respectively, in the molecular weight. This appropriate shift of molecular weight to a higher mass number, as well as the expected fragmentation pattern, indicated the complete N-methylation of the peptides. Moreover, the volatility of the N-methylated peptides was considerably increased since the spectra could be obtained at much lower temperature in the ion source of the mass spectrometer than before methylation.

The heptapeptide derivative (IV) was not volatile enough to give a mass spectrum but after complete N-methylation, (V) gave a satisfactory mass spectrum which exhibited a molecular ion peak at m/e 1082. This molecular weight indicates that the seven -CONH- groups as well as the threonine hydroxyl have been methylated by the methyl iodide-silver oxide treatment. The mass spectral fragmentation pattern (Figure 1) is also consistent with the amino acid sequence of this synthetic heptapeptide derivative (V) and shows that the reaction -CONH- $\longrightarrow$ -CO-NMe- proceeds quantitatively and gives a completely methylated product.

The N-methylation procedure described above seems thus to be a significant advance for sequence determination of oligopeptide derivatives. In the case of peptides such as fortuitine (I), for instance, which already contain some N-methyl amino acids, it is preferable to methylate with CD_3I so as to be able to distinguish the natural N-methyl groups from the newly introduced ones (Das, Géro, Lederer, unpublished results).

The hitherto unknown permethylated peptide derivatives have now become easily accessible ; a study of their chemical and biological properties may be rewarding.

The above method also allows the preparation of monomethyl derivatives of primary amines by the route $R\text{-}NH_2 \longrightarrow RNHAc \longrightarrow \underset{\underset{Me}{|}}{RN\text{-}Ac} \longrightarrow \underset{\underset{Me}{|}}{R\text{-}NH}$.

Acknowledgments : We wish to thank Dr. J. Van Heijenoort (Orsay) for a sample of the dipeptide (II), Dr. J.S. Morley (I.C.I., England) for a sample of the tetrapeptide (III) and Dr. F.H. Carpenter (Berkeley) for a sample of the heptapeptide (IV).

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