Mass spectrometry of permethylated peptide derivatives ; extension of the technique to peptides containing arginine or methionine*

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For the sequence determination of oligopeptides by mass spectrometry, N-permethylated derivatives have been shown to offer two major advantages : a) volatility is increased by permethylation, thus permitting larger oligopeptide derivatives to be vaporized in the mass spectrometer (Das et al., 1967) ; and b) the mass spectra of permethylated peptide derivatives are relatively simple and require a minimum of interpretation to determine the amino acid sequence (Thomas et al., 1968). The technique for permethylation of peptides using methyl iodide and silver oxide has also been applied by Agarwal et al. (1968), and Vilkas and Lederer (1968) have used a method employing methyl iodide and a methylsulfinyl carbanion as base.

However, it became apparent that the success of these methods is sometimes limited by certain functional groups. Thus, in order to render the technique generally applicable to the determination of peptide sequences, regardless of the amino acid residues present, we have examined several operationally simple methods to modify some of the "troublesome" functional groups, such as free amines, the guanido group of arginine, and the thioether group of methionine.

Free amino groups - The most commonly encountered functional group of potential difficulty is a free amine, present either at the N-terminus or as the ω-function of lysine or ornithine. Methylation would produce a quaternized methyl iodide salt with low volatility which could lead to undesirable

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pyrolytic decomposition in the mass spectrometer. This problem is conveniently solved by prior acetylation of the peptide. For this purpose, we find methanolic acetic anhydride (4:1) to be preferable to the conventional pyridine method ; amino functions are selectively acylated and hydroxyl groups, if present, remain free (and are converted to methoxyl functions by the subsequent methylation process). The N-acetyl derivative is isolated after a few hours at room temperature simply by evaporation of solvent and reagent.

Arginine - A more unusual problem was encountered when the methylation of arginine-containing peptide derivatives was attempted. As an example, methylation of compounds 1 and 2 (after acetylation) gave chloroform-soluble products which had sufficient volatility to be vaporized in the mass spectrometer. The spectra* clearly showed the expected "sequence peaks" (resulting from $CO\text{-}NCH_3$ cleavage) up to but not including the arginine residue, and in neither case could a molecular ion be detected. This poses little problem for peptide derivatives known to have a C-terminal arginine residue (such as are obtained by enzymatic hydrolysis with trypsin) especially if information on the total number and type of residues is available from an amino acid analysis. But if arginine is present at an intermediate or N-terminal position, only a partial structure may be obtained by this method. It is possible that a detailed search of a complete high resolution mass spectrum, together with a knowledge of the structure of the product(s) resulting from the arginine residue after methylation, would give evidence from which the entire sequence of such a peptide derivative could be deduced. However, it is a distinct disadvantage that peaks resulting from the desired $CO\text{-}NCH_3$ cleavage, if they exist at all, are not immediately obvious in the case of arginine-containing peptide derivatives ; thus other solutions were considered which retain all advantages of this permethylation technique.

1 H. Phe. Arg. Trp. Gly. OH

2 H. Phe. Ser. Pro. Phe. Arg. OMe

Two methods have been proposed to modify arginine-containing peptides for mass spectrometric analysis. One is the condensation of the gua-

* All mass spectra were determined using the direct-introduction probe of an A.E.I. model MS9 mass spectrometer.

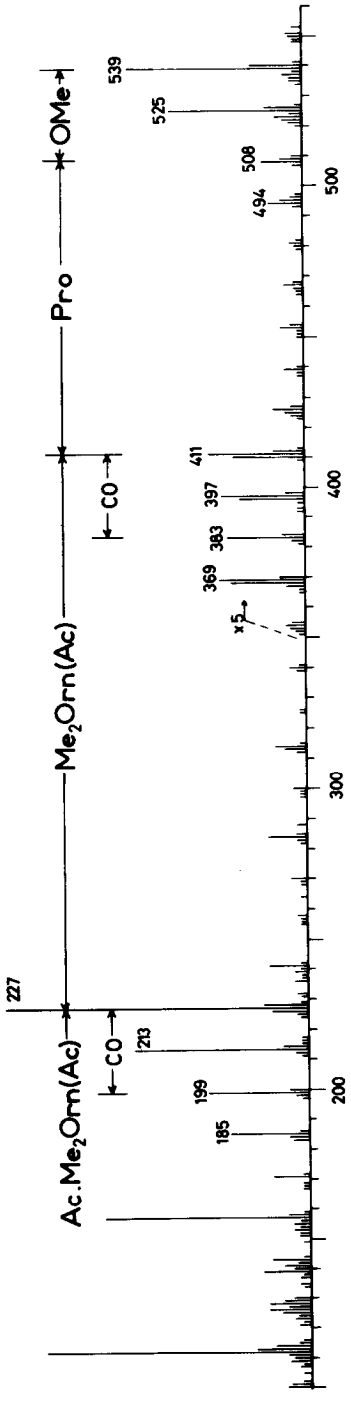


Figure 1 - Mass spectrum of the product obtained after consecutive treatment of H.Arg.Arg.Pro.OBu^t with N_2H_4, Ac_2O, TFA-OH, MeI.

nidine group with β-dicarbonyl compounds (Shemyakin et al., 1967 ; Vetter-Diechtl et al., 1968) ; however, permethylation of the resulting basic pyrimidyl ornithine derivative would give an undesirable quaternized salt. The second method, which involves the conversion of arginine-containing peptides to the corresponding ornithine derivatives by treatment with hydrazine (Shemyakin et al., 1967) seemed particularly suitable for our purposes. With a slight variation of the published procedure (1-10 mg peptide, 0.2 ml hydrazine hydrate/water 1:1, 95°, 1 hour), this reaction was found to give a derivative (readily isolated by evaporation of the reagent) which is relatively free of undesirable side-products. Successive acetylation and permethylation of the resulting ornithine peptide proceed without difficulty, and the mass spectrum of the final product gives prominent peaks for the cleavage at each peptide bond.

An example to illustrate this method is the tripeptide derivative 3, which contains two arginine residues. Consecutive treatment with hydrazine, acetic anhydride, trifluoroacetic acid (to hydrolyse the t-butyl ester), and diazomethane produced the triacetylpeptide methyl ester 4. A mass spectrum obtained at a source/sample temperature of 210° was consistent with this structure (molecular weight 483).

Permethylation of 4 gave a product which could be vaporized in the mass spectrometer at a temperature of 150°. [Such an increase in volatility as a result of permethylation has been shown to be especially important for larger oligopeptide derivatives (Thomas et al., 1968)]. The mass spectrum of this product (Fig. 1) shows, in addition to the expected N-permethylated peptide derivative 5, the presence of a lower homolog (actually a mixture of 6 and 7) which is due to incomplete methylation at the δ-amide of one acetyl ornithine residue. This result has been observed to be characteristic also of lysine, but such homologs will seldom create difficulty in the determination of amino acid sequences. However, if a lower homolog is undesirable for any reason, the fully N-methylated compound, because of its reduced polarity, can be readily separated from incompletely methylated products by thin-layer chromatography (silica gel, $CHCl_3$/MeOH 9:1).

3 H.Arg.Arg.Pro.OBu^t

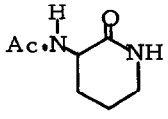
4 Ac.Orn(Ac).Orn(Ac).Pro.OMe

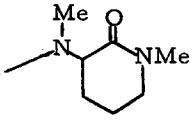
5 Ac. Me_2Orn(Ac). Me_2Orn(Ac). Pro. OMe

6 Ac. MeOrn(Ac). Me_2Orn(Ac). Pro. OMe

7 Ac. Me_2Orn(Ac). MeOrn(Ac). Pro. OMe

Hydrazinolysis of arginine peptide does not always give the free δ-amine of ornithine. An exception occurs with a C-terminal arginine ester (a free acid gives predominantly the normal product) which leads to a lactam rather than an amino ester. For example : a) N,N-diacetyl-arginine methyl ester, after hydrazinolysis, gave a mass spectrum corresponding to the derivative 8 ; and b) the consecutive hydrazinolysis, acetylation and permethylation of the pentapeptide derivative 2, for which no molecular ion could be detected after acetylation and methylation only, led to the mass spectrum of Figure 2 which clearly shows the complete sequence for structure 9, including the molecular ion at m/e 718.

8 

9 Ac. MePhe. Me_2Ser. Pro. MePhe 

Methionine - Permethylation of peptide derivatives containing methionine residues produces a result similar to that encountered with arginine peptides : prominent peaks in the mass spectra delineate the sequence from the N-terminus up to but neither including nor beyond the methionine residue. The relatively greater polarity of these products (tlc), as compared to permethylated peptide derivatives not containing methionine, suggests that the isolated product is a sulfonium iodide. Further evidence is the pyrolytic formation of methyl iodide (m/e 142) observed when the permethylated derivative is vaporized in the mass spectrometer. Agarwal et al. (1968), after a similar methylation procedure, have isolated peptide derivatives containing a desulfurized cyclopropane derivative of the methionine residue. We have found no evidence for such a product ; this apparent discrepancy is undoubtedly due to a difference in the preparation of the silver oxide catalyst used for the methylation reaction (see Thomas et al., 1968).

One solution, which we suggest for the problem of methionine is its desulfurization with Raney nickel. Thus, N-acetylmethionine methyl ester is converted to the corresponding derivative of α-amino butyric acid (Abu) after four hours at reflux with freshly prepared catalyst in ethanol. The α-

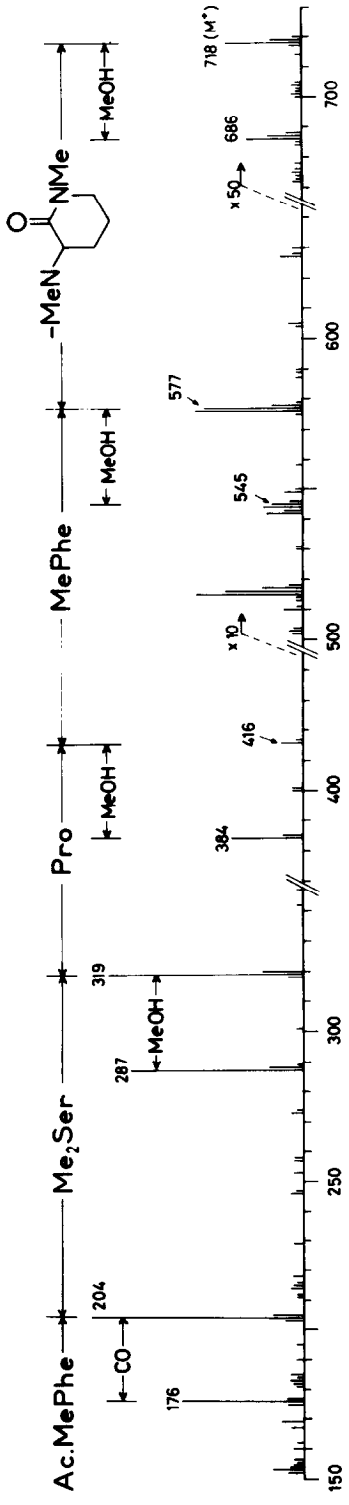


Figure 2 - Mass spectrum of the product obtained after consecutive treatment of H.Phe.Ser.Pro.Phe.Arg.OMe with N_2H_4, Ac_2O, MeI.

amino butyric acid residue may of course be N-methylated without difficulty, and because it differs in mass from the common aliphatic amino acids, the position of this residue in a peptide (and thus of its methionine precursor) may be easily determined.

An example is the conversion of the methionine-containing heptapeptide derivative 10 to the compound 11 by successive treatment with Raney nickel, trifluoroacetic acid, methanolic acetic anhydride, and methyl iodide/silver oxide. The presence of a C-terminal permethylated α-aminobutyramide is indicated by the mass spectrum of this derivative.

10 BOC. Lys(BOC). Phe. pF-Phe. Gly. Leu. Met. NH_2

11 Ac. MeLys(Ac). MePhe. MepF-Phe. MeGly. MeLeu. MeAbu. NMe_2

The same technique could also be used for peptides containing cystine or cysteine, although an unambiguous determination of the sequence position of these residues would be more difficult since their desulfurization gives an alanine residue.

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