MASS SPECTROMETRY OF PERMETHYLATED PEPTIDES CONTAINING TYROSINE AND TRYPTOPHAN*

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1. Introduction

Today, mass spectrometry has become an accomplished tool for the rapid determination of the sequence of amino acids in oligopeptide derivatives [2–4]. A significant advance toward the use of this method has been achieved by the introduction of the technique of *N,O*-permethylation of *N*-acetyl(acyl) peptides [5–9]. The usefulness of this technique is being increasingly appreciated by a number of investigators and several laboratories are at present actively enganged in the application of this technique to peptide sequencing [10–12]. In this communication we report an interesting electron impact fragmentation reaction specific for peptides containing two aromatic amino acid residues (tyrosine and tryptophan) directly linked in a peptide chain.

2. Experimental procedure

For *N*-acetylation, the peptide (ca. 0.5 mg) is dissolved in methanol:acetic anhydride (3:1; 0.2 ml) and allowed to stand at room temp. for 2 hr. The reagents are then removed by evaporation on a water bath.

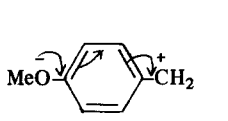
N-, *O*-permethylation is carried out with methyl iodide, using sodium hydride dissolved in dimethylsulfoxide as base [13]. Sodium hydride (5–10 mg, 50% dispersion in oil) is rinsed three times with anhydrous ether and heated under nitrogen at 80° in dimethylsulfoxide (0.3 ml) until hydrogen evolution ceases. This solution is added to the *N*-acetylated peptide (0.2–0.5 mg) at room temp., followed by methyl iodide (0.1 ml). The reaction is continued with stirring for 30 min. The reaction mixture is diluted with water and extracted with chloroform. The chloroform extract of the permethylated peptide is washed with water, dried over anhydrous magnesium sulfate, evaporated to dryness and submitted to mass spectrometric analysis.

Mass spectra are determined with an A.E.I. model MS 9 mass spectrometer operating at 70 eV.

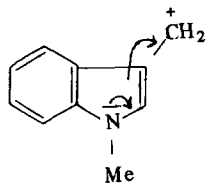
3. Results and discussion

It has been previously noted that the *N*-acetylated and permethylated peptides give rise to mass spectra which consist almost exclusively [14] of "sequence determining peaks" corresponding to acylium ions such as $m_1, m_2, \ldots$, etc., resulting from the cleavage of peptide bonds as shown in (I).

Besides the sequence determining peaks, these mass spectra sometimes exhibit other peaks of significant intensity, particularly at the lower mass region. Some of these non-sequence peaks are indicative of the presence of individual amino acid residues in a peptide. Thus peaks at m/e 121 and 144 may be attributed to ions *a* and *b* arising from the side-chain cleavage of tyrosine and tryptophan residues, respectively.

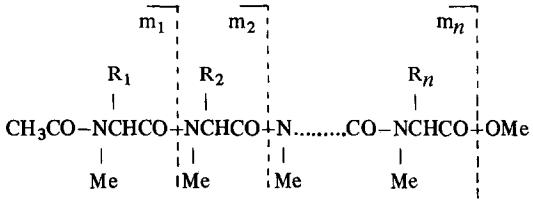


a m/e 121



b m/e 144

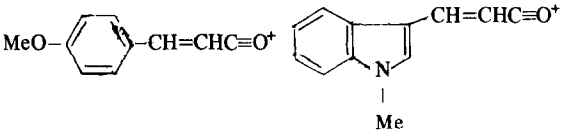
* See [1].



(I)

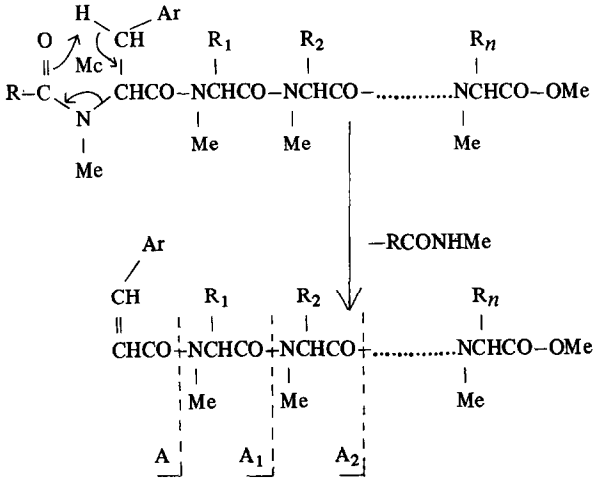
Apart from these peaks due to side-chain cleavage, peptides containing these aromatic amino acids also exhibit a fragmentation reaction (indicated by Mc in II) which involves their N–C bond cleavage along with the transfer of a β-hydrogen (from the benzylic methylene group) and with charge retention on the C-terminal fragment bearing an aromatic conjugated acyl group as shown in (III). Similar fragmentation has also been observed with peptides containing Asp or Asn residue.

A series of peaks corresponding to the peptide bond cleavage (A_1, A_2,... etc) of the C-terminal fragment (III) may thus be observed in the mass spectra. Aromatic conjugated acyl groups (corresponding to cleavage A in III) originating from tyrosine and tryptophan are revealed in the mass spectra by the presence of peaks at m/e 161 (ion *c*) and 184 (ion *d*), respectively. Tyrosine or tryptophan occurring at the C-terminus of a peptide chian may manifest itself by the presence of a peak at m/e 192 (*c* + OMe) or at m/e 215 (*d* + OMe) due to the terminal methoxy group.



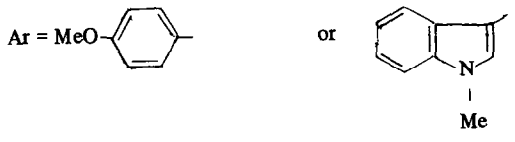
c m/e 161 *d* m/e 184

During our investigation on the sequence determination of Tyr and Trp-containing peptides by mass spectrometry, we have noted that the peak due to ion fragment *c* or *d* corresponding to cleavage A in (III) is invariably accompanied by a peak 30 mass units higher whenever two of these aromatic amino acids are directly linked in a peptide chain. This can be explained as due to a second Mc-type fragmantation as shown in (IV).



(II)

(III)



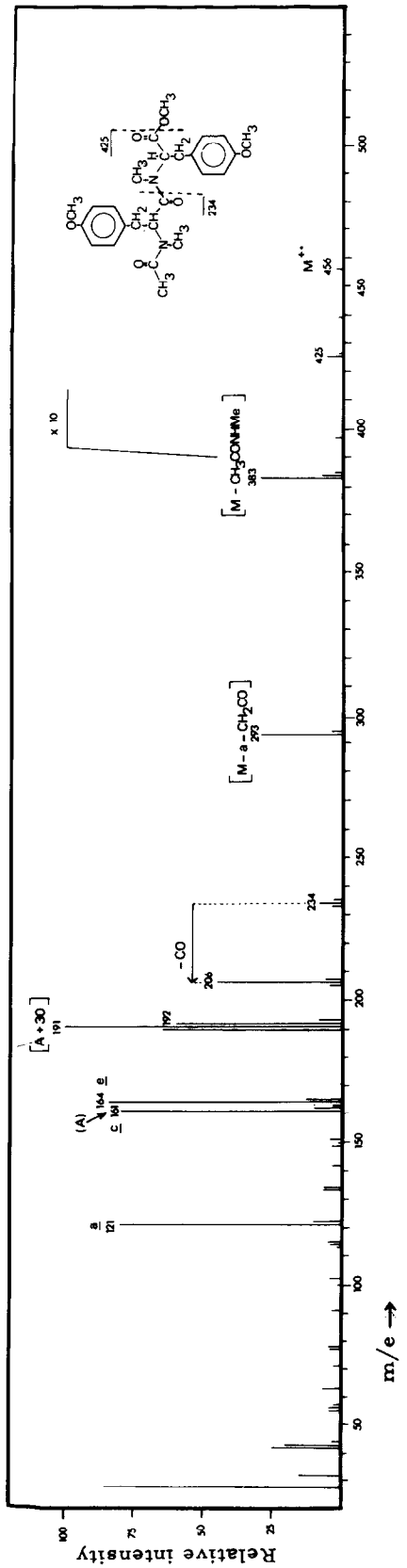


Fig. 1. Mass spectrum of Try–Tyr after *N*-acetylation and permethylation.

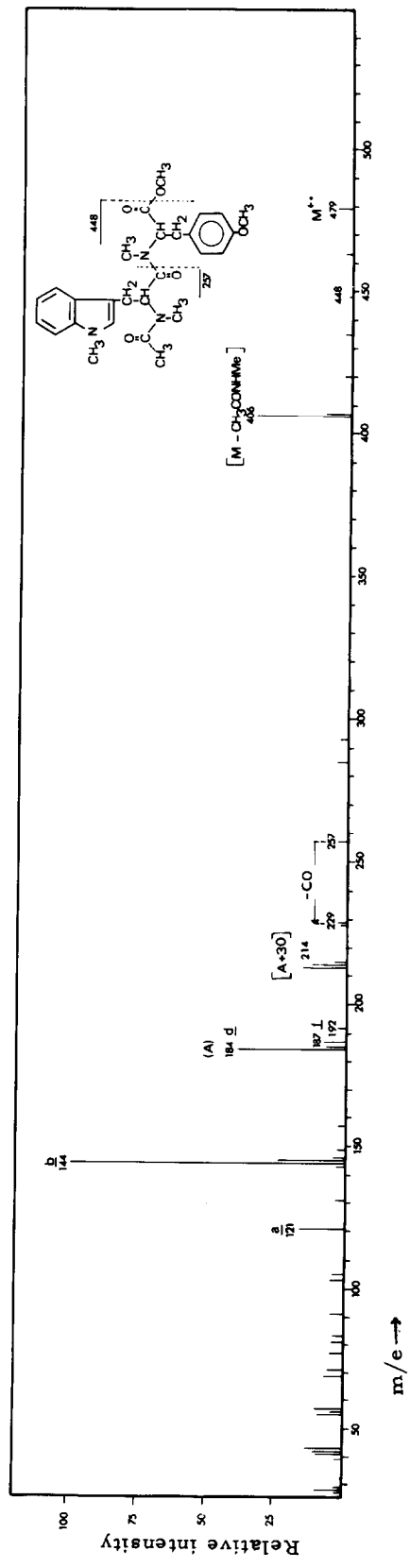
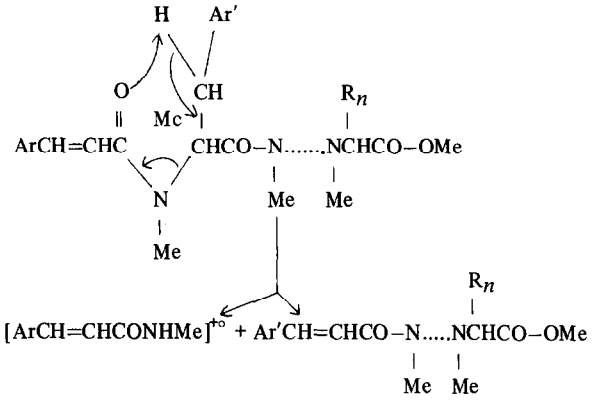
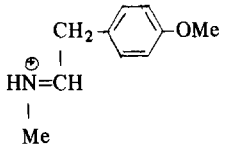


Fig. 2. Mass spectrum of Trp–Tyr after *N*-acetylation and permethylation.

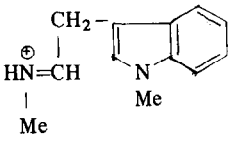


(IV)

Thus, when tyrosine is followed by another tyrosine or tryptophan in the peptide chain, A and A+30 peaks are observed at m/e 161 and 191. Similarly, if a tryptophan residue precedes a tyrosine or tryptophan, corresponding peaks occur at m/e 184 and 214. This is illustrated by the mass spectra of peptides (see fig. 1 and fig. 2) obtained from Tyr–Tyr and Trp–Tyr after *N*-acetylation and permethylation. The A+30 peak in each of these spectra is accompanied by a peak one mass unit lower due to loss of a hydrogen. The peak at m/e 164 in fig. 1 is attributed to ion *e* originating from a tyrosine residue. Similarly, the peak at m/e 187 in fig. 2 is possibly due to ion *f* from a tryptophan residue.



e m/e 164



f m/e 187

Recently, Morris et al. [10] have published the mass spectrum of a peptide (after *N*-acetylation and permethylation) having the sequence Gln–Tyr–Tyr–Thr–Val.... Besides the sequence determining peaks, the spectrum includes an intense peak at m/e 191. Although no explanation was offered, the origin of this peak now becomes evident.

Occurrence of peaks corresponding to ions A and A+30 in the mass spectra of permethylated peptides containing Tyr and Trp can therefore be of diagnostic value for ascertaining the relative position of these amino acids when two or more of these amino acid residues are present in a peptide chain.

Acknowledgements

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