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THE APPLICATION OF PYROLYSIS METHYLATION AND ESTERIFICATION TO THE MASS SPECTROMETRIC SEQUENCING OF SMALL PEPTIDES

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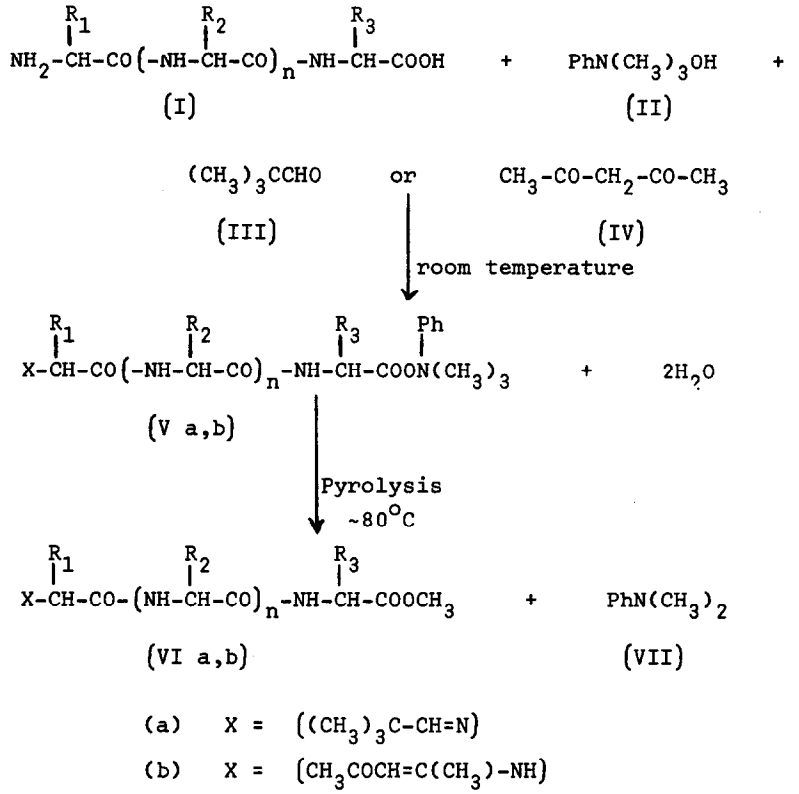
Established methods for the mass spectrometric sequence analysis of peptides require time-consuming chemical processing and manipulations to convert the peptides into suitable volatile derivatives[1,2]. We now describe a simple procedure for the amino acid sequencing of small peptides (2 - 8 amino acid residues), which is based on the quantitative pyrolitic conversion of a peptide trimethylanilinium salt to its corresponding methyl ester in the solid probe of a mass spectrometer. Since derivatization can be achieved in the instrument, the new pyrolysis esterification procedure offers advantages in convenience, speed, sensitivity and reliability over previously described mass spectrometric sequencing methods. The derivatization of a peptide (I) for mass spectrometry involves treatment with excess trimethylanilinium hydroxide (II) and either pivaldehyde (III) or acetylacetone (IV) in the solid probe tip of a mass spectrometer. The intermediate salt (V a,b) is quantitatively converted into the volatile methyl ester derivative (VI a,b) in the mass spectrometer, when the solid probe is heated to ~80°C. The progress of the pyrolysis esterification can be conveniently followed on an oscilloscope display by monitoring the mass spectrum of the liberated dimethylaniline (VII).



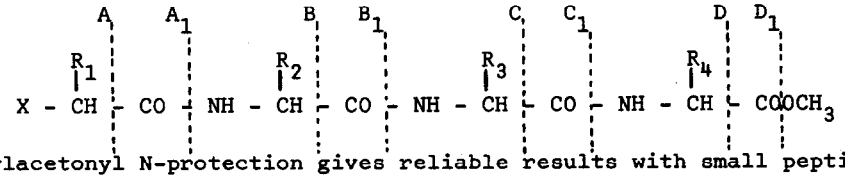
(a) X = $((CH_3)_3C-CH=N)$

(b) X = $(CH_3COCH=C(CH_3)-NH)$

Under the pyrolysis conditions used, tyrosine residues are O-methylated, but other functional groups such as $-CONH_2$, -OH and the indole residue present in the protein amino acids remain unmethylated.

A typical sequencing experiment involves treatment of the peptide (0.05 - 0.2 mg, 0.4 μM) in the probe tip (EAI Quad 300) with trimethylanilinium hydroxide reagent (2 μℓ, 0.2M in methanol, "Methelute", Pierce Chemical, Ill.) pivaldehyde (0.4 μℓ) or acetylacetone (0.4 μℓ) in the presence of molecular sieve (1 stick, 3A, Matheson, N.J.). After 5 min. (neopentylidene derivatives) or 3 hrs. (acetylacetonyls) the molecular sieve is withdrawn with a pair of tweezers, and the probe is inserted into the mass spectrometer (EAI, Quad 300). After removal of the excess solvent and reagents, the tip is heated to ~80°C till a steady evolution of dimethylaniline (VII) is observed on the oscilloscope display (mass ion peaks m/e 121 and 120). After the pyrolysis conversion is complete (5-10 min.) the probe temperature is increased gradually till the pep-

tide spectrum appears on the oscilloscope display. On the basis of the mass spectra of some 60 model peptides, we find that the mass spectra of (VI a) and (VI b) peptide esters obtained by the pyrolysis procedure are essentially identical to the spectra obtained previously, by the conventional esterification and condensation procedure[3]. The interpretation of (VI a) and (VI b) mass spectra is facilitated by the presence of prominent ions corresponding to the "A" fragment and these afford an unambiguous starting point for the search for the other "sequence ions" (B, C, D and B_1, C_1, D_1) (Fig. 1 and 2).



The acetylacetonyl N-protection gives reliable results with small peptides regardless of the amino acids present. Pivaldehyde does not condense with N-terminal proline or hydroxy proline peptides but sequencing proceeds normally, however the neopentylidene method fails altogether with arginine-containing peptides.

A number of acetyl-, formyl-, benzoyl and pivaloyl peptides have also been sequenced by pyrolysis esterification. The results have been in good agreement with those in the literature obtained by other methods[4,5].

Finally, some preliminary work with some other tetra-substituted ammonium bases suggests that pyrolysis conversion may offer an alternative route to permethylated peptides[6,7].

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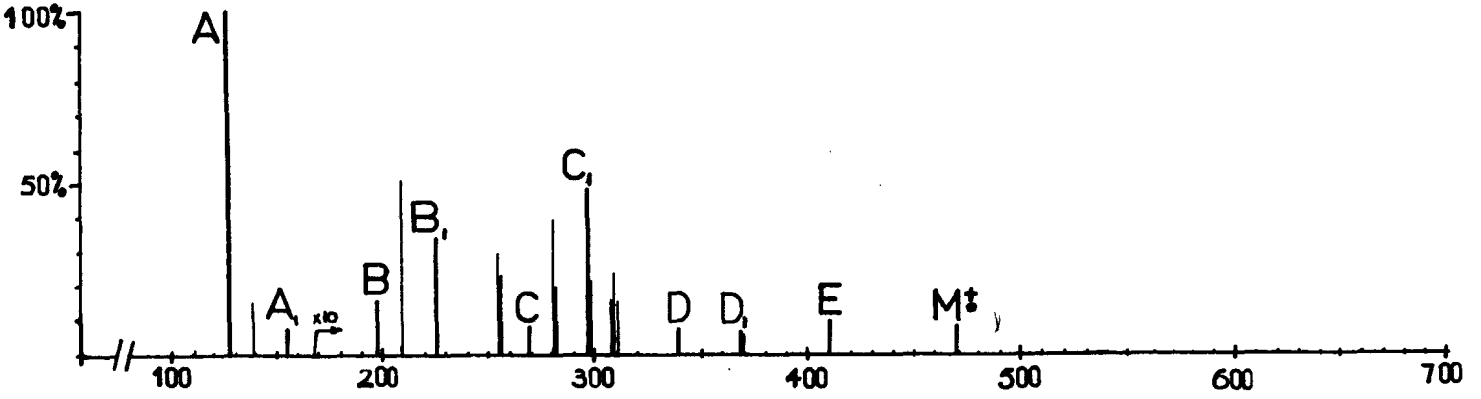

FIG. 1. $(ALA)_5$ AS ACETYLACETONYL DERIVATIVE (VI b)

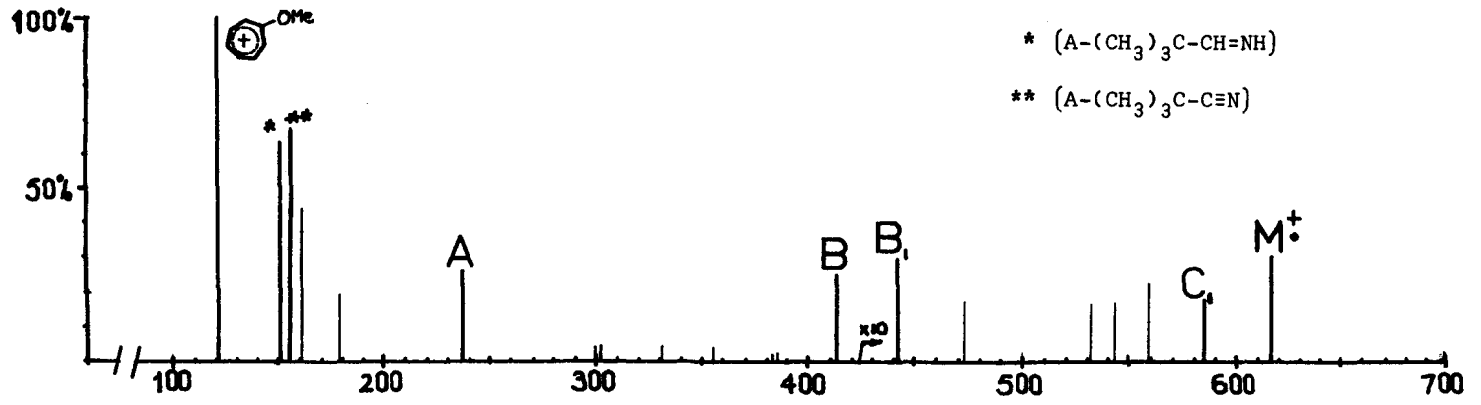

FIG. 2. LYS-TYR-GLU AS NEOPENTYLIDENE DERIVATIVE (VI a)