MASS SPECTROMETRY OF FLUORESCAMINE DERIVATIVES OF AMINO ACIDS AND PEPTIDES

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Summary: Amino acids and dipeptides may be identified from the mass spectra of their derivatives with 4-phenylspiro-[furan-2(3H),1'-phthalan]-3,3'-dione (fluorescamine) or 2-methoxy-2,4-diphenyl-3(2H)-furanone (MDPF). These compounds may also be used for the determination of the N-terminal amino acids of peptides.

Biological studies are identifying many protein-containing materials, obtainable in only small quantities, for which micro-methods of sequencing are needed. One promising approach utilizes dipeptidylaminopeptidase I (DAPI) (1). The sequence is deduced from the overlapping dipeptides obtained by enzymic digestion of the intact polypeptide and the polypeptide with the N-terminal amino acid removed by a single Edman degradation. The dipeptides have been separated and identified by means of paper and column chromatography (2), by thin layer chromatography of their dansylated derivatives (3), and by mass spectrometry of various volatile derivatives. These derivatives have included pentafluoropropionyl methyl esters (4), N-acetylacetonyl methyl esters (5) and trimethylsilyl derivatives (6).

This report deals with the mass spectrometric identification of dipeptides as derivatives of 4-phenylspiro-[furan-2(3H),1'-phthalan]-3,3'dione (fluorescamine) (I) and of 2-methoxy-2,4-diphenyl-3(2H)-furanone (MDPF) (II).

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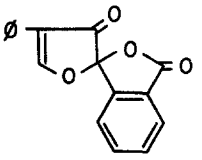

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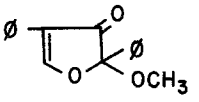


II

Fluorescamine was first recognized in the fluorogenic ninhydrin reaction of Samejima *et al.* (7). Following the elucidation of the structure of the reaction products (8), fluorescamine was synthesized by Weigele (9). The highly fluorescent products formed after reaction of fluorescamine with amino acids, peptides, proteins and primary amines may be used as the basis for their sensitive assay (10,11). Fluorescamine may also be used for the detection of peptides in column effluents (12). The related compound MDPF was also synthesized by Weigele *et al.* (13) and has been used for the fluorescent labeling of proteins.

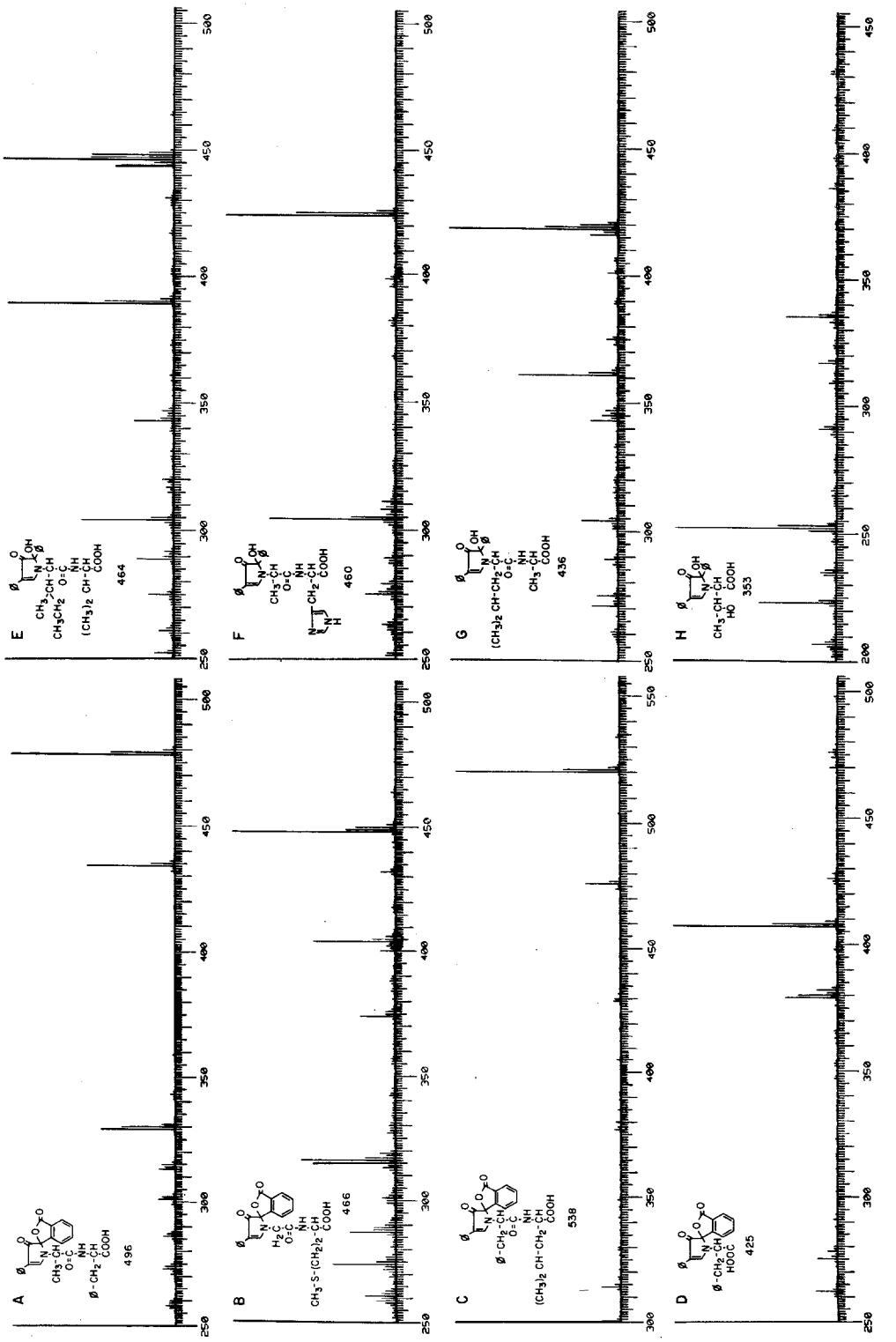
Materials and Methods: Fluorescamine and MDPF were obtained from Hoffmann-La Roche Inc., Nutley, New Jersey.

Amino acids and dipeptides were obtained from Cyclo Chemical, Los Angeles.

Derivatives were routinely prepared by adding 100 µl of a 0.2% solution of fluorescamine in acetone to an equal volume of a 7.2mM solution of the dipeptide in 0.2M pyridine. In the case of MDPF-dipeptides, dimethylbenzylamine-acetate buffer (Beckman Sequanal grade) was used in place of pyridine. The reaction mixture was evaporated to dryness in a vacuum dessicator. A portion of the residue was introduced to the mass spectrometer using a heatable direct insertion probe.

Mass spectra were obtained on a Dupont Instruments Model 21-492B double focusing mass spectrometer in the electron ionization mode. Scans were taken from 200°C to 400°C under control of a probe temperature programer, using an ionizing potential of 70eV. Spectra were processed using a Dupont 21-094B disc based data system.

For the identification of N-terminal amino acids the peptides were treated as described above followed by hydrolysis in 1 ml of 6N HCl at 110° for 24 hours. An ethylacetate extract of the hydrolysis mixture was evaporated to dryness under a nitrogen stream, and a portion of the residue was placed in the direct probe of the mass spectrometer.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Results and Discussion: Figure 1 presents representative spectra, which are the direct output of the data system. For most of the dipeptides examined to date, the base peak appears to correspond to the ketene of the lactonized fluorescamine or MDPF-dipeptide derivative. These spectra are remarkable in their lack of fragmentation. The small amount of fragmentation that does occur is sometimes sufficient to establish the order of the amino acids in the dipeptide. In other cases, it is necessary to hydrolyze the dipeptide derivative as described above and identify the N-terminal amino acid from its mass spectrum (Figures 1D and 1H). Figure 1D is the spectrum of the hydrolysis product of the dipeptide derivative whose spectrum is shown in Figure 1C.

These results suggest that the fluorescamine and MDPF derivatives of peptides and amino acids possess several advantages for the elucidation of peptide sequences by mass spectral examination. Their fluorescence provides a sensitive method to follow their isolation (12). They generally possess sufficient volatility for mass spectral examination. The contribution of the fluorescamine and MDPF residues to the molecular weight moves the characteristic spectral lines to a relatively uncluttered region of the spectrum. The aromaticity of these residues appears to exert a stabilizing effect leading to a surprising absence of fragmentation.

Although some amino acids present special volatility problems, which lead to somewhat more complex spectra than those

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Figure 1. Spectra of fluorescamine and MDPF derivatives. Spectra 1D and 1H are of the hydrolytic products of fluorescamine-phenylalanylleucine and MDPF-threonylalanine, respectively. It is assumed that the fluorescamine derivatives convert to the lactone form on the heated probe.

presented here, these spectra are sufficiently characteristic to permit identification. The nature of these fragmentation pathways and the experimental techniques to provide the most ready identification of these derivatives are being studied.

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