Short Communications

Mass-Spectrometric Identification of 1-Dimethylaminonaphthalene-5-sulphonyl-Amino Acids

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The fluorescent reagent DNS* chloride is now widely used for the determination of *N*-terminal residues in proteins and peptides (Gray, 1967). In the present paper the mass-spectrometric characterization of DNS-amino acids is reported as a preliminary step towards the rapid detection of the *N*-terminal residue in proteins. The same technique has been proposed for the identification of 3-phenyl-2-thiohydantoin derivatives of amino acids (Wulfson, Stepanov, Puchkov & Zyakoon, 1963) and DNP-amino acids (Penders, Copier, Heerma, Dijkstra & Arens, 1966). The following DNS-amino acids were obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.): DNS-alanine, bis-DNS-cysteine, DNS-glutamate, DNS-glycine, DNS-isoleucine, DNS-leucine, bis-DNS-lysine, DNS-

methionine, DNS-phenylalanine, DNS-proline, DNS-serine, DNS-threonine, DNS-tryptophan, bis-DNS-tyrosine and DNS-valine. Their purity was checked by thin-layer chromatography (Woods & Wang, 1967); with the exception of DNS-tryptophan, all derivatives gave a single fluorescent spot. Each DNS-amino acid was converted into the corresponding methyl ester to obtain more volatile derivatives better suited for mass-spectrometric studies.

The methyl esters were prepared by treating overnight at room temperature in a sealed glass tube 100 μ l. of a solution of each DNS-amino acid in dry methanol (approx. 1 mg./ml.) with 50 μ l. of dry methanol saturated with HCl. The methanolic solutions were then dried under vacuum. Esterification was quantitative as shown by thin-layer chromatography in the system water-90% formic

* Abbreviation: DNS, 1-dimethylaminonaphthalene-5-sulphonyl.

Fig. 1. Spectrum recorded at 12 eV of a mixture of DNS-amino acid methyl esters. The underlined m/e values correspond to the molecular ions of the derivatives of the following amino acids (in order of decreasing molecular weight): phenylalanine; glutamate; methionine; aspartate; leucine and isoleucine; threonine; valine; proline; serine; alanine; glycine.

acid (200:3, v/v) (Woods & Wang, 1967), where the esters generally had R_F values 30–50% lower than those of the corresponding DNS-amino acids.

Mass spectra were recorded on an A.E.I. MS9 mass-spectrometer. A few drops of an acetone solution of the methyl esters were placed on the direct insertion probe, most of the solvent was warmed off and the probe was then directly introduced into the mass-spectrometer source. The temperature of the source was set at 120° for the mono-DNS derivatives and at 210° for the bis-DNS derivatives. Spectra were recorded with an electron-beam energy of either 70 ev or 12 ev.

With the exception of DNS-arginine and bis-DNS-cysteine, all derivatives tested gave excellent spectra with very intense molecular ion peaks. The fragmentation patterns obtained by 70 ev impact were quite complex: they generally showed a base peak at 170 m/e whereas the molecular ion peaks had a relative intensity ranging from 30 to 70%. Simpler spectra, more useful for analytical purposes, were obtained by using the 12 ev impact, as side fragmentation was largely prevented and the base peak generally corresponded to the molecular ion.

The line spectrum of a mixture of 14 amino acid derivatives run at 12 ev is shown in Fig. 1. DNS-amino acid methyl esters are easily recognized through their nominal molecular weights and the characteristic pattern due to the natural abundance of sulphur isotopes. High-resolution measurements unambiguously proved their identity. The isomeric DNS-leucine methyl ester and DNS-isoleucine

methyl ester could be differentiated from each other by running spectra at 70 ev; each isomer, in fact, gave a fragmentation characteristic of the type of side-chain branching (Biemann, Seibl & Gapp, 1959).

Unfortunately quantitative information cannot be obtained through the direct insertion probe method, owing to differences in volatility of the compounds; perhaps the use of an all-glass heated inlet system might circumvent this difficulty.

By considering the high sensitivity of the above suggested technique, it is predictable that the amount of peptide required for *N*-terminal residue identification is of the order of 10 pmoles.

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