QUANTITATIVE AMINO ACID ANALYSIS BY MASS SPECTROMETRY

K. Biemann and W. Vetter

Department of Chemistry, Massachusetts Institute of Technology
Cambridge, Mass.

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The methods commonly used for amino acid analysis employ an unspecific colorimetric assay of the individual components after a very careful separation by chromatography. This latter step makes such a procedure very time-consuming and even with the elegant, almost fully automatic method of Spackman, Stein and Moore (1958) only one sample per day can be analyzed. Since a much faster but still accurate method would be advantageous for studies of peptide structures, amino acid metabolism, etc., we have investigated the possibility of mass spectrometric analysis of mixtures of amino acids, which was expected to be speedy and accurate because prior separation into the components would not be necessary.

The spectra of ethyl esters of amino acids exhibit relatively few, but very intense peaks which are strictly related to the structure of the individual amino acid (Biemann, Seibl and Gapp, 1959); and even the spectra of isomers, like the esters of leucine and isoleucine, are sufficiently different because of the branching of the side chains to permit quantitative discrimination. Thus, the mass spectrometric quantitative analysis of such mixtures should be possible in much the same way as the hydrocarbon analysis which is now widely used in the petroleum industry (for a review see Dibeler, 1956).

However, the extension of this principle to the analysis of free amino acids is complicated by the necessity to esterify quantitatively (or at least reproducibly) the very small amount of the mixture commonly available for such a determination, to convert it to the free esters and to introduce these into

the mass spectrometer without fractionation in any one of these steps.

We have now achieved this in the following way: The mixture (about one milligram) is refluxed with 2 ml. anhydrous ethanolic hydrochloric acid (5-10%) for three hours, the solvent evaporated in a vacuum desiccator and the residue dissolved (or suspended) in 2 ml. dichloromethane. The hydrochlorides are converted to the free esters by passing anhydrous ammonia through the solution for a few seconds; the solution is then filtered from the ammonium chloride into a small, long-necked bulb type distilling tube and most of the solvent is distilled off. The tube is then connected to the mass spectrometer, cooled to -60°, and the remaining dichloromethane is pumped off through the oil diffusion pump of the inlet system. The mixture of esters is then introduced into the inlet system of the mass spectrometer* by rapidly heating the tube to 160°, and the spectrum is scanned from mass 25 to 200.

Calibration spectra of each individual amino acid have been obtained in the same manner, using an authentic sample. The relative intensities of the peaks used for the measurement of the individual amino acids (its mass number is given in column 2, Fig. 1) were determined from synthetic mixtures of known composition containing aspartic acid or phenylalanine as internal standard. The calculation of the results is facilitated by the fact that many of the amino esters exhibit a strong peak with little or no interference from others and thus can be subtracted one after the other from the spectrum. The few remaining ones are obtained by one or two successive approximations or by the use of simultaneous equations.

Table I gives the results of some preliminary determinations on synthetic mixtures of amino acids, presented as the relative molar ratios since these, rather than the absolute amount, are of interest in most cases, eliminating the necessity of quantitative transfer of the sample during the preparation. However, this could be done without major changes, if essential. The values found deviate 1-5% from the calculated ones; as can be seen from Table I, the

* A CEC 21-103C mass spectrometer, equipped with a heated inlet system, operated at 140°, was used in these experiments.

TABLE I

Amino Acid	Mass Number[a]	Mixture A Calcd.	Mixture A Found[h]	Mixture B Calcd.	Mixture B Found[i]	Mixture C Calcd.	Mixture C Found[j]	Mixture D Calcd.	Mixture D Found[j]
gly	30[b]	2.64[g]	2.63	2.16	2.09	-	-	-	-
ala	44[b]	1.73	1.75	1.31	1.28	-	-	-	-
abu	58[b]	1.85	1.84	-	-	-	-	-	-
val	72[b]	1.87	1.83	-	-	-	-	1.60	1.65
leu	44[c]	-	-	-	-	1.41	1.38	-	-
ileu	69[c]	-	-	-	-	1.66	1.68	-	-
pro	70[b]	1.30	1.32	-	-	1.18	1.17	-	-
ser	60[b]	-	-	1.44	1.42	-	-	-	-
thr	74[b]	-	-	0.98	0.98	-	-	-	-
opro	86[b]	1.40	1.41	1.01	1.00	-	-	-	-
met	61[d]	-	-	-	-	1.43	1.44	-	-
phe	120[b]	1.00	1.00	-	-	-	-	1.00	1.03
tyr	107[e]	-	-	-	-	-	-	0.80	0.76
asp	116[b]	1.03	1.05	1.00	1.00	1.00	1.02	1.19	1.21
glu	84[c]	1.40	1.41	-	-	-	-	-	-
orn	69[f]	3.10	3.27	-	-	-	-	-	-

(a) m/e of peak used for calculation. (b) $(R\text{-}CH\text{-}NH_2)^+$ fragment of $R\text{-}CH(NH_2)CO_2C_2H_5$. (c) originates through further fragmentation of (b). (d) $(CH_3\text{-}S\text{-}CH_2)^+$. (e) $(HO\text{-}C_7H_6)^+$. (f) fragment of orn-lactam. (g) values represent molar ratios. (h) average of 2, (i) average of 4, (j) average of 3 determinations.

accuracy varies somewhat for the different amino acids and also depends on the particular composition of the mixture. At present, we are trying to improve the accuracy obtained with ornithine and tyrosine and to extend the method to some of the more common amino acids not listed in the table, like tryptophan, histidine, cysteine and, if possible, arginine.

We believe the main advantage of this new approach to be the speed of the analysis; each determination requires only 30-40 minutes instrument time, thus allowing the analysis of a larger number of samples than hitherto possible. This may be an important factor in many studies, e.g., following the total hydrolysis of a peptide, multiplicate analysis of mixtures to increase the confidence in the results obtained, determination of the amino acid composition of the large number of small peptides obtained in the partial hydrolysis of proteins, etc. Further-

more, new amino acids can be analyzed accordingly, once a small, pure sample for calibration is available.

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