MASS SPECTROMETRIC IDENTIFICATION OF AMINO ACIDS

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Received November 18, 1959

The advent of paper chromatography and the perfection of ion exchange methods have greatly advanced the identification, separation, determination and structure elucidation of amino acids using such very small amounts as normally encountered in the work with natural material. However, with the ever increasing number of amino acids found in nature, their identification via R_f values becomes more and more difficult and the same is true for their separation.

With these problems in mind, we have investigated the applicability of mass spectrometry in this field. Since mass spectra are quite complex and reproducible, they are one of the best criteria available for the identification of organic compounds and the determination of purity. Furthermore, a close relationship between structure and mass spectra was to be expected, which could be used to determine the structure of new amino acids, in much the same way as it was done for polyamino alcohols derived from small peptides (Biemann, Gapp and Seibl, 1959).

As volatile derivatives of amino acids, we have chosen the ethyl esters. They can be prepared easily on a micro scale and are also less susceptible to diketopiperazine formation than the methyl esters. All the spectra discussed in this paper were determined on distilled samples of the esters (except ornithine, see below), using one to two micromoles.* However, even the amount

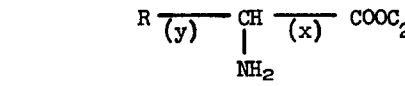
* The spectra were determined with a CEC 21-103C mass spectrometer, equipped with a heated inlet system operated at 140°.

of ester obtained starting with only 5-10 micrograms of methionine gave usable spectra.

In the following is outlined the general fragmentation pattern found in a considerable number of amino esters:

I. Monoamino-Monocarboxylic Esters

a) Aliphatic. The most important fragmentation takes place at (x),



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giving rise to a very strong peak at m/e R+29 (in the following referred to as "amine peak").** The structure of R can be deduced from other modes of decomposition of the molecular ion at certain bonds of R, as outlined for the isomeric leucines (Fig. 1). As in the fragmentation of hydrocarbons on electron impact, these molecules also break preferentially at highly substituted carbon atoms, e.g., leading to an M-43*** ion in leucine and M-29 in isoleucine. Norleucine, however, yields both these ions to a comparable extent, but not M-15, just as found in n-hydrocarbons. In addition, the low-mass part of the spectra of these isomers are quite different due to dissimilarities in the further decomposition of the $(R\text{-}CH\text{-}NH_2)^+$ fragment. Cleavage at (y) yields information about the environment of the α-carbon atom: An ion of mass 102 is formed from all esters of type I. It is, of course, absent in proline ethyl ester (Fig. 1). The spectra of the four isomeric amino butyric esters illustrate the usefulness of this fragment in combination with the "amine peak" for the differentiation of such isomers:

ethyl amino butyrate	α	β	γ	α-iso
"amine peak"	58	44	30	58
"ester peak"	102	116	130	116

** While this work was in progress, an abstract of a paper presented by C. O. Andersson before The Swedish Biochemical Society came to our attention, in which this type of fragment in the mass spectra of some amino acid methyl esters was mentioned. The author implied, however, that it would not be possible to distinguish between the isomeric leucines and valines (Andersson, 1958).

*** Denotes a mass of molecular weight minus 43 mass units.

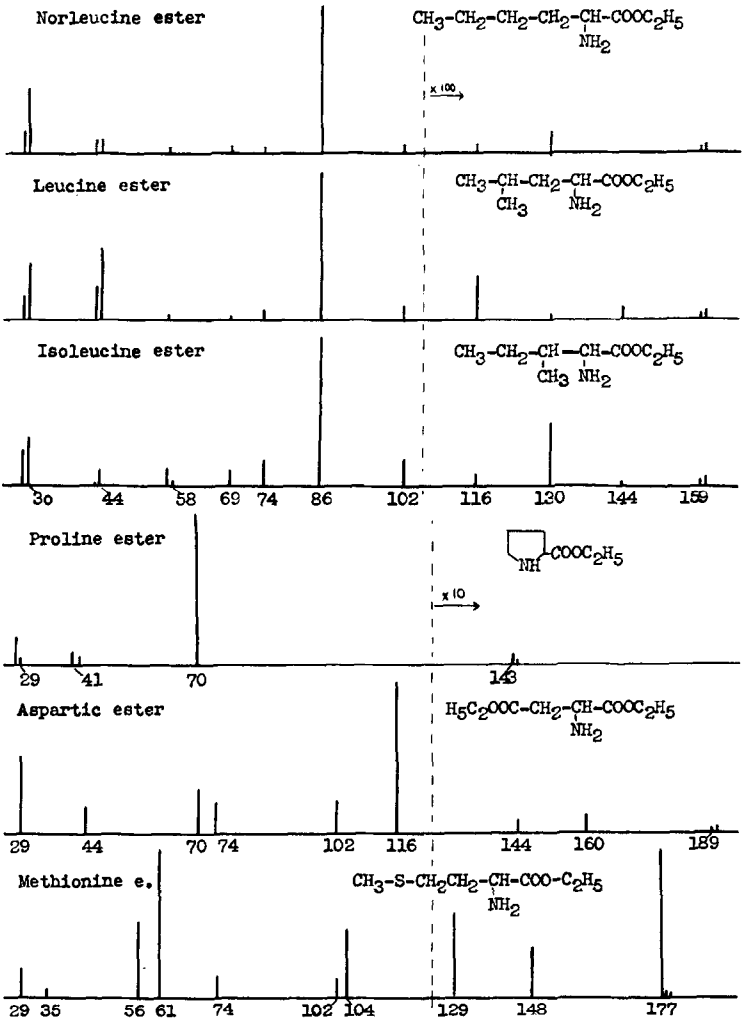
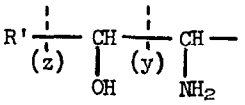


Fig. 1. Partial mass spectra of some amino acid ethyl esters.

The molecular weight is obtained from the corresponding peak, which is rather small (except in aromatic or sulfur-containing amino esters) but always accompanied by an M+1 peak, which can easily be recognized (Biemann, Gapp and Seibl, 1959).

b) Aromatic amino esters. These esters behave in much the same way on electron impact. Besides the more intense molecular ion already mentioned, they exhibit a prominent $(ArCH_2)^+$ peak.

c) β-Hydroxy-α-amino esters. The presence of the



grouping, in addition, gives rise to fragmentation at (z) leading to M-R' ions (e.g., 132 in threonine ethyl ester) and to some $(R'CHOH)^+$ via cleavage at (y) and retention of the positive charge at the oxygen-containing fragment.

d) Sulfur-containing amino esters. Relatively intense molecular ion peaks, an M+2 peak corresponding to the S^{37} isotope and fragments in the region of m/e 33-35 and 45-48 indicate the presence of sulfur. The elimination of CH_3SH from the methionine ester ion or its fragments yields mass 129 (M-48) and mass 56 (R-CH-NH_2 minus 48). The carbon-carbon bond next to sulfur is cleaved easily with retention of the charge at the S-containing fragment, as known for simple thioethers, which appears at m/e 61 in methionine ester (Fig. 1). Cysteine ester behaves similarly to serine ester with the corresponding shift in mass numbers.

II. Monoamino-Dicarboxylic Esters

These esters exhibit, in addition to cleavage at (x) and (y) in I, elimination of the elements of ethanol from the "amine fragment" leading to m/e 70 and 84 in aspartic ester (Fig.1) and glutamic ester, respectively.

III. Diamino-Monocarboxylic Esters

The "amine fragment" loses NH_3 predominantly, giving rise to an ion of mass 84 as the most intense peak in lysine ethyl ester. Since the cleavage at the carbon-carbon bond next to the second amine group also yields stable, N-containing fragments, information about the environment of this group is obtained (e.g., m/e 30 in lysine ester: $-CH_2NH_2^+$). If, however, the relative positions of the amino and carbethoxy groups are such that a six-membered ring can be formed, as in ornithine ester, the spectrum of the lactam is obtained.

By this method, it is also possible to identify the components of a complex amino acid mixture without prior separation. Here, the use of ionizing electrons of low energy (10-12 eV) is a distinct advantage: the "amine peaks" remain very intense (70-95% of all ions formed) whereas the other peaks almost disappear. Thus, a mixture of amino esters gives under those conditions a rather simple spectrum in which most of the intense peaks correspond to the

different $(R\text{-}CH\text{-}NH_2)^+$ fragments indicating the individual amino esters. The presence of an unexpected or unknown amino ester in a mixture could easily be recognized in such a spectrum. If it is then necessary to distinguish between isomers (like leucine and isoleucine), the mixture is also run at 70 eV and checked on some of the minor peaks characteristic of each compound.

A thorough interpretation of the mass spectrum of the ester of an hitherto unknown amino acid will allow the elucidation of its structure even if only a very small amount of material is available. For this purpose the use of more than one derivative (e.g., ethyl and butyl esters, N-alkylation etc.) is suggested to lend additional support to the correct interpretation of the fragment peaks.

Arginine and other guanidino compounds will not form volatile esters and could, therefore, not be determined in this way. We believe, however, that this is no serious limitation since these compounds can be readily detected by specific color reactions and separated by simple ion exchange or electrophoretic processes.

We have obtained excellent spectra of the ethyl esters of more than thirty amino acids, the more common ones of which are gly, ala, α-, β-, γ- and α-iso-amino butyric acid, val, n-val, leu, i-leu, n-leu, pro, phe, tyr, try, his, ser, thr, allo-thr, hy-pro, cys, met, asp, glu, lys and orn-lactam.

This investigation was supported by a research grant (RG-5472) from the National Institutes of Health, Public Health Service. A more detailed discussion of the spectra and experimental techniques will be published elsewhere.

References

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