SEPARATION OF PEPTIDE DERIVATIVES BY GAS CHROMATOGRAPHY COMBINED WITH THE MASS SPECTROMETRIC DETERMINATION OF THE AMINO ACID SEQUENCE

K. Biemann and W. Vetter

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

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Some time ago we showed that the amino acid sequence in small peptides can be deduced from the mass spectrum of the polyamino alcohol obtained from the peptide on reduction with lithium aluminum hydride (Biemann, Gapp and Seibl, 1959). The considerable volatility of these derivatives suggested the possibility of their separation by gas chromatography. The main advantage of this approach is speed and efficiency of separation and the fact that even rather small fractions can be easily collected in a form suitable for mass spectrometry without further treatment. Mixtures of peptides--for example, a partial hydrolyzate of a larger peptide--may thus be reduced as such and the polyaminoalcohols formed are subsequently separated in this manner.

Figure 1 illustrates this method on a mixture of five synthetic di-, tri and tetrapeptides (1-1.5 mg. of each in the form of their N-acetyl ethyl esters) which was reduced over night with an excess of $LiAlD_4$ in 1.5 ml. of tetrahydrofuran. After decomposition of the mixture with a small amount of water, the products were extracted three times with ether-methanol (10:1); the dried solution was evaporated almost completely; and the mixture was then injected into a gas chromatographic column at 260° (8% Apiezon L on Chromosorb W, pretreated with sodium hydroxide; 60 ml. He/min.). The fractions were collected in capillary tubes suitable for direct introduction into the mass spectrometer.

Fig. 1

Gas Chromatogram of Polyaminoalcohols

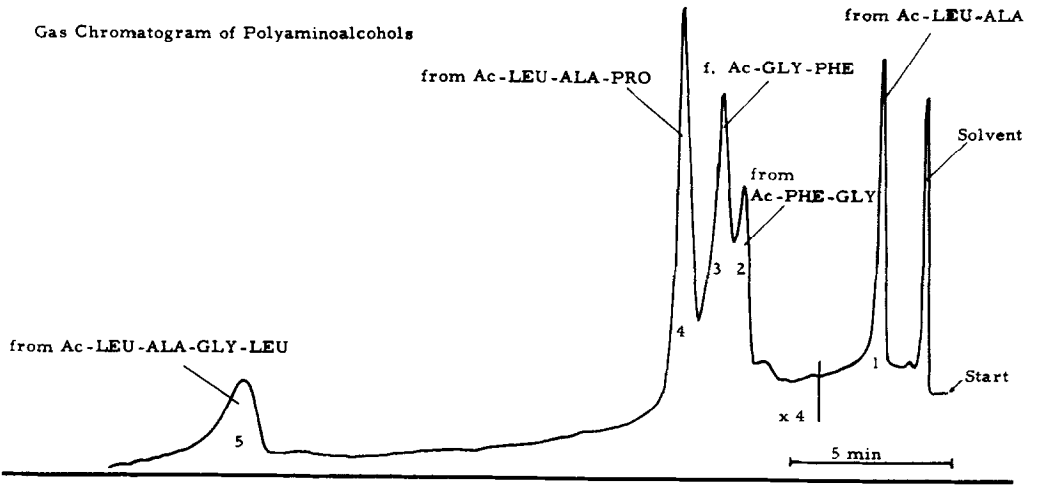


Fig. 2

Gas Chromatogram of Polyamines

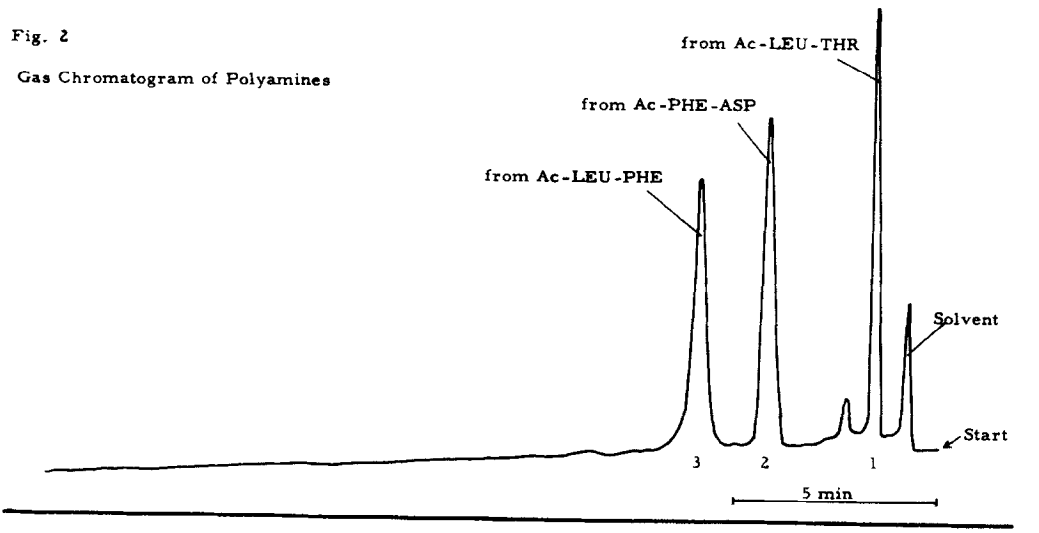
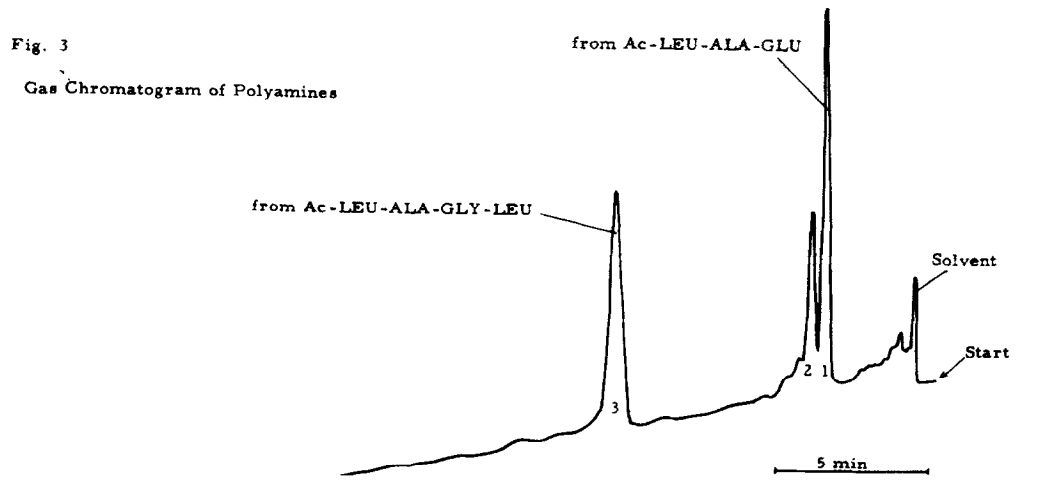


Fig. 3

Gas Chromatogram of Polyamines



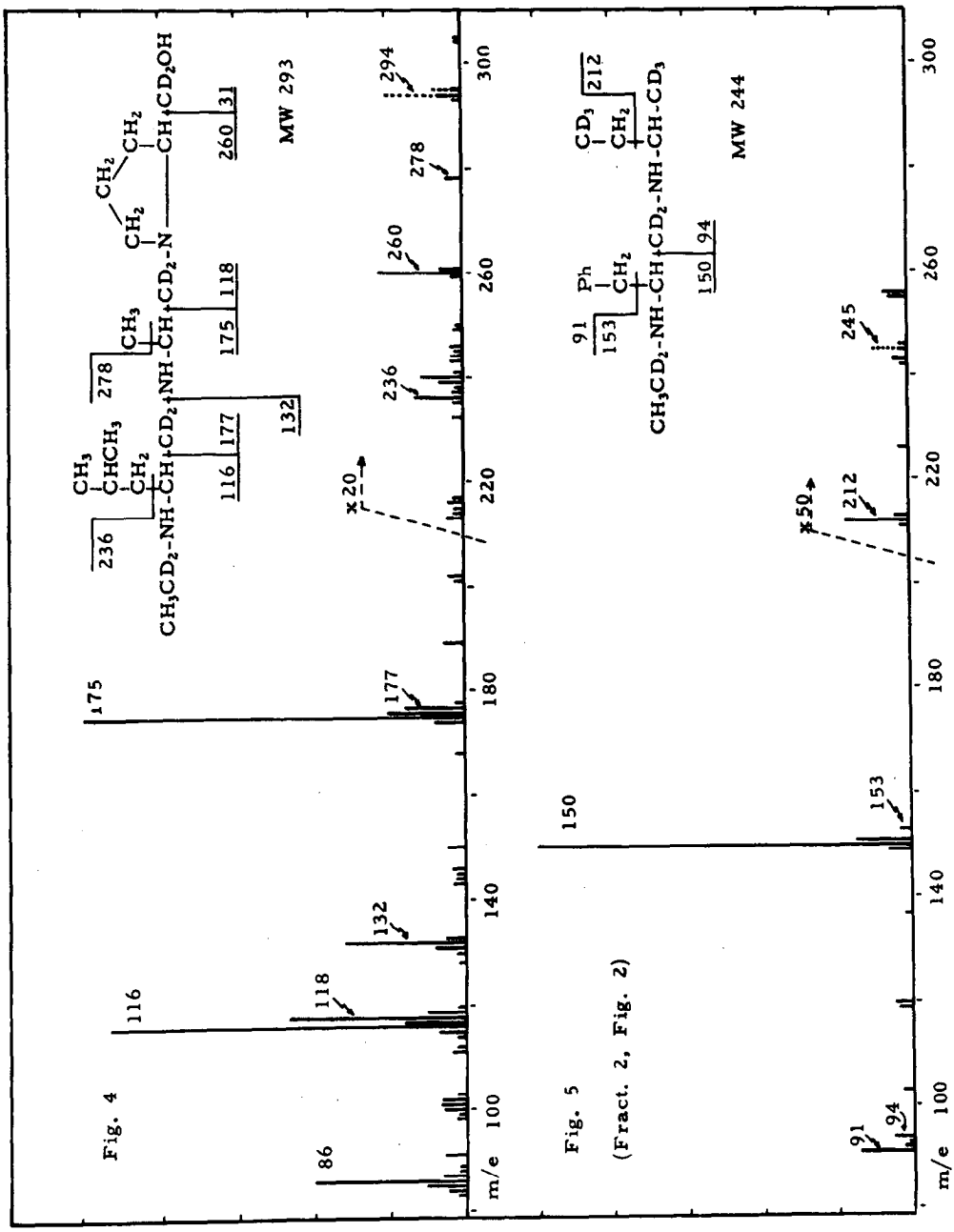


Fig. 4
MW 293
Fig. 5
(Fract. 2, Fig. 2)
MW 244
m/e

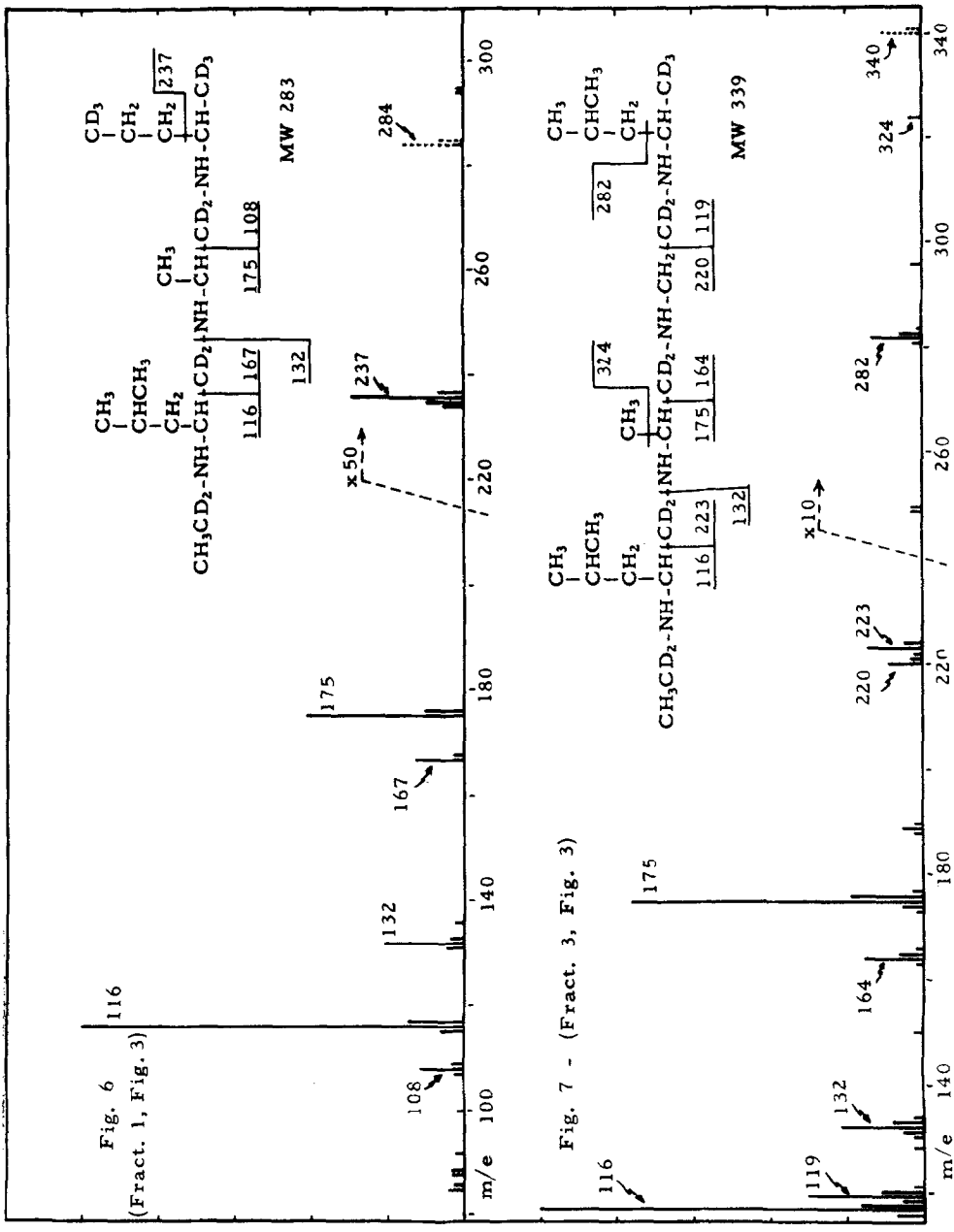


Fig. 6
(Fract. 1, Fig. 3)
MW 283
Fig. 7 - (Fract. 3, Fig. 3)
MW 339
m/e

Figure 4 shows the spectrum* of fraction 4 of Figure 1. It is unequivocally interpreted (Biemann, Gapp and Seibl, 1959) as due to the polyamino alcohol derived from Ac-leu-ala-pro. The spectra of the other fractions permitted their identification in the same manner. The spectrum of fraction 3 revealed some contamination from fraction 2 due to trailing of the gas chromatographic peaks; its contribution can, however, easily be subtracted from the mass spectrum and does not interfere with the interpretation.

The presence of hydroxy- or dicarboxylic amino acids in a peptide gives rise, however, to a polyamino alcohol with two or more hydroxyl groups. Such compounds emerge very late, if at all, from the gas chromatographic column. This was overcome by the elimination of all hydroxyl groups in a second reductive step. The polyamines thus obtained are much more volatile, have shorter retention times and less tendency to trail. They still retain the skeleton of the original peptide, and the mass spectrum of such a polyamine can be interpreted in terms of the amino acid sequence in a way analogous to the interpretation of the spectra of polyamino alcohols.

Figures 2 and 3 are gas chromatograms of such polyamines obtained from mixtures of the peptides (1-1.5 mg. of each) indicated in the figures. After reduction to the polyamino alcohols as described above, the product was dissolved in 0.5 ml. of chloroform and 0.5 ml. of thionylchloride added (the traces of water and methanol still present produce a small amount of HCl sufficient to convert the amines into the hydrochlorides), after 1.5 hrs. the mixture is evaporated, the residue reduced with $LiAlD_4$ in tetrahydrofuran and worked up as in the first reductive step.

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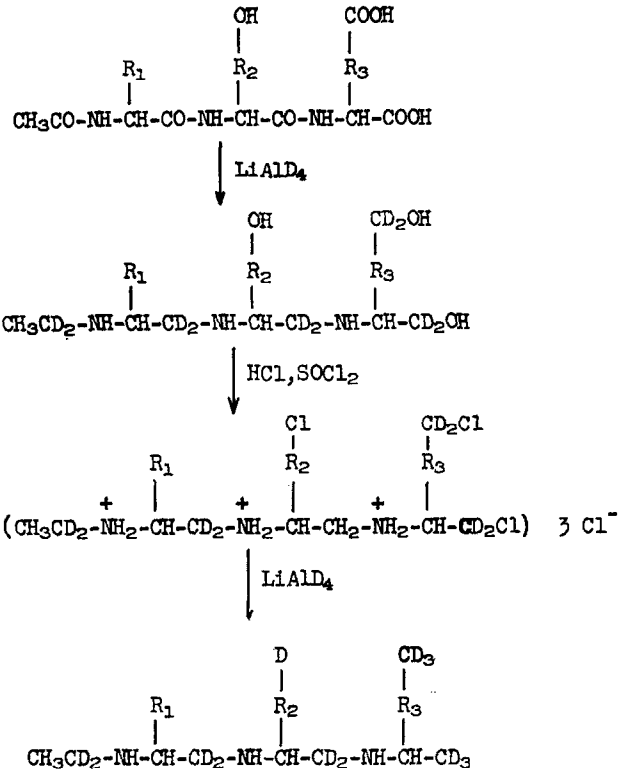
* All peaks are included which have at least 2% of the intensity of the highest peak. In the magnified regions (on the right of the slanted arrows) the minimum peak shown is decreased for the same factor. The dotted peaks indicate the increase in relative intensity of the M+1 peak and its isotope peak on switching the ion source from "focused" to "nonfocused." The spectra were determined with a CEC 21-103C mass spectrometer, equipped with a heated inlet system operated at 140°.

These reactions can be summarized as follows:

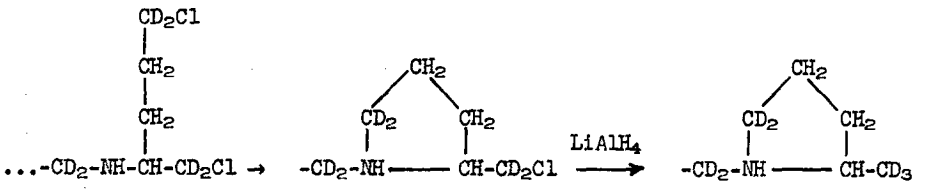


The mass spectra of some of the fractions are presented in Figures 5-7. From the most intense peaks the amino acid sequence in the parent peptide can be deduced in much the same way as it is done with the polyamino alcohols: The most important fragments are due to cleavage of carbon-carbon bonds next to nitrogen in the -N-C-C-N- groups and the loss of the side chains, in particular of the C-terminal one. The M+1 peak indicates the molecular weight which is one mass unit lower. It might be pointed out that we always find a small peak at M+11, or a doublet at M+11 and 12 in the deuterated derivatives.

The use of the deuteride instead of the hydride in these reductions is required for the differentiation of α-aminobutyric acid, threonine and aspartic acid which then yield the side-chains CH_3CH_2-, CH_3CHD- and CD_3CH_2-, respectively, or of valine and glutamic acid, alanine and serine, proline and hydroxyproline.

Fraction 2 of Figure 3 was found, on the basis of its spectrum, to be a

by-product of the reduction of the intermediary ω-chloro compound derived from Ac-leu-ala-glu which cyclized partly before reduction:



Also in this case the deuterium atoms present in the pyrrolidine ring make it possible to distinguish this product from the one derived from Ac-leu-ala-pro itself.

The results obtained thus far reveal considerable potentialities of this method for the speedy and facile determination of the amino acid sequence of di- to pentapeptides present in complex mixtures as they are encountered in certain phases of the determination of the primary structure of proteins.

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References

K. Biemann, F. Gapp and J. Seibl, J. Amer. Chem. Soc., 81, 2274 (1959).