of racemization relative to ring size are being determined.

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RECEIVED JUNE 25, 1962

MASS SPECTRA OF ORGANIC MOLECULES. II.[1] AMINO ACIDS[2]

Sir:

In our efforts to extend the applicability of mass spectrometry to organic molecules of extremely low volatility, we have been able to determine the mass spectra of amino acids without prior conversion to more volatile derivatives required for our earlier work.[1,3] Using the same technique which had made it possible to obtain mass spectra of nucleosides,[4] volatilization of the sample directly into the ion source and close to the ionizing electron beam gave excellent spectra of free amino acids and even their hydrochlorides.[5]

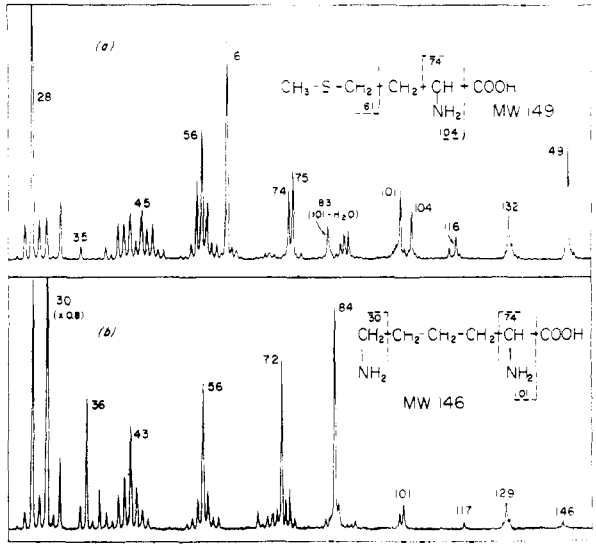
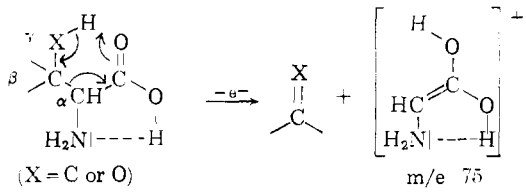


Fig. 1.—Reproductions of mass spectra of amino acids: (a) methionine; (b) lysine monohydrochloride (mass 28 and 32 due to air; mass 30 in (b) 1.25 × as abundant as shown).

These spectra[6] (determined with samples ranging from 0.25–10 μg.) were quite similar to those of the corresponding ethyl esters,[1] indicating that free amino acids exist in the gas phase as the undissociated amino carboxylic acids or possibly hydrogen-bonded forms thereof. It will, therefore, suffice to discuss only those peaks not present as such in the mass spectra of the amino acid ethyl esters.

The lack of the ester group leads to a shift of 28 mass units in peaks due to the elimination of a group other than the acid moiety (*e.g.*, *m/e* 102 in ethyl esters *vs.* *m/e* 74 in the acids). There seem to be only two new modes of fragmentation: First, there is always found a peak of significant intensity at *m/e* 45 corresponding to the carboxyl group while the corresponding one at *m/e* 73 in ethyl esters is absent. This we attribute to the stability of a positively charged carboxyl fragment ($O{=}C{-}O^{+}{-}H$) which is equivalent to a protonated carbon dioxide molecule while the corresponding carbethoxy ion of mass 73 ($O{=}C{-}O^{+}{-}C_2H_5$) is energetically less favored.

The second difference is found in the presence of a peak at *m/e* 75 in the spectra of a number of amino acids, namely, all those containing a hydrogen atom in a γ-position. This rearrangement is well known[7] for fatty acids and their esters, but is not observed in α-amino esters, because of the availability of the free electron pair on nitrogen.[1] In the free acids hydrogen bonding seems to decrease this effect, thus favoring this rearrangement which is very sensitive to the electron density at the atom attached to C_α.[8]



The mass spectrum of methionine (Fig. 1a) illustrates the similarity to that of the ester.[1] The peaks at mass 74, 101, 132, and 149 (mol. wt.) are those which occur 28 mass units higher in the ethyl ester. Fragments of mass 75, 57, and 45 correspond to the additional fragmentation modes discussed above; (*m/e* 75 is only partly due to CH_3-S-CH_2-CH_2^+, as evidenced by deuteration experiments).

It is thus possible to interpret the mass spectra of free amino acids based on the behavior of amino esters under electron impact as discussed previously.[1] Glutamic acid appears to dehydrate to pyroglutamic acid prior to sublimation, but hydroxyamino acids vaporize without decomposition.

The fragmentation processes discussed here and earlier[1] are corroborated by the mass spectra of N^{15}-labeled amino acids and of N,O-perdeuterio derivatives which can easily be obtained.[9]

Even salts are amenable to this technique if both the corresponding base and acid are sufficiently volatile and thermally stable at the

(1) Paper I: K. Biemann, J. Seibl and F. Gapp, *J. Am. Chem. Soc.*, **83**, 3795 (1961).

(2) This investigation was supported by a grant from the National Aeronautics and Space Administration (NsG 211-62). We wish to thank Mr. M. Munroe for invaluable help with the instrumentation.

(3) K. Biemann, J. Seibl and F. Gapp, *Biochem. Biophys. Res. Communs.*, **1**, 307 (1959).

(4) K. Biemann and J. A. McCloskey, *J. Am. Chem. Soc.*, **84**, 2005 (1962).

(5) W. L. Baun and D. W. Fischer [*Anal. Chem.*, **34**, 294 (1962)] reported the introduction of free amino acids into a spark source mass spectrometer. Our spectrum of lysine hydrochloride (Fig. 1b) indicates that the much more gentle conditions of sublimation of the sample at relatively low temperatures into an electron beam of 70 ev. as contrasted to sparking at high frequency with 100 Kv. lead to spectra which are much more characteristic of the original molecule.

(6) For experimental conditions see footnote 6 in reference 4. Samples were vaporized at temperatures between 80° and 200°.

(7) F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959).

(8) For a detailed discussion of the interpretation of mass spectra see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962. Chap. 8.

(9) For experimental details see footnote 9 in reference 4.

temperature of their dissociation. The resulting mass spectrum shows contributions of the free base and the free acid superimposed. Figure 1b is the mass spectrum obtained from lysine monohydrochloride. The peaks in the region of *m/e* 35 through 39 in Fig. 1b represent hydrochloric acid while the rest of the spectrum is due to free lysine which again illustrates the similarity to the spectrum of its ethyl ester.[1] Histidine dihydrochloride and cysteine hydrochloride also give rise to good spectra.

The simplicity, speed, and sensitivity of the method described make it a valuable tool for the identification and characterization of extremely small amounts of amino acids.

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RECEIVED MAY 25, 1962

NEW COTTON EFFECTS IN POLYPEPTIDES AND PROTEINS[1,2]

Sir:

Recently we have described the presence of a Cotton effect in the optical rotatory dispersion curves of α-helical polypeptides and in both fibrous and globular proteins.[3,4,5] This Cotton effect, having an inflection point at 225 mμ, and a minimum (or trough) at 233 mμ has been shown to be conformation-dependent. The original observations have been confirmed and extended by other workers using other proteins.[6,7]

In this communication we report the finding in the far ultraviolet of (a) a new, large, positive Cotton effect characteristic of helical polypeptides and proteins, and (b) a weak, negative Cotton effect in the random form of polypeptides. The new "helical" Cotton effect has a maximum (or peak) at approximately 198 mμ and an inflection point at about 190 mμ; some data are shown in the figures. Like the 225 mμ Cotton effect, the magnitude of the 190 mμ Cotton effect is conformation-dependent and is shown with the data from the helical and random forms of polyglutamic acid (Fig. 1). The data for the helical form (Fig. 1, curve A) also reveal an inflection point at about 215 mμ. This is the place where the negative 225 mμ Cotton effect would turn down if it were not for the presence of the ascending limb of the positive 190 mμ Cotton effect which evidently dominates the rotation around these wave lengths.

The *helical form* of poly-α,L-glutamic acid *shows a positive 190 mμ Cotton effect* with a peak

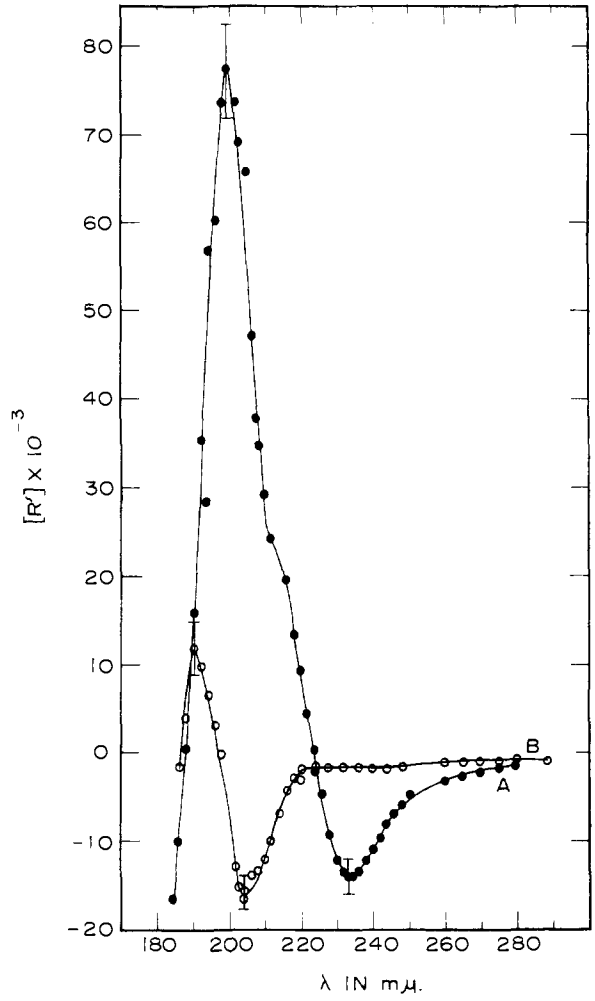

Fig. 1.—Curve A, ●-●-●, the ultraviolet optical rotatory dispersion of the helical form of poly-α,L-glutamic acid in water solution, pH 4.3. A 1-mm. cell was used. Concentrations ranged from 0.0137 to 0.0456%. Curve B, O-O-O, the ultraviolet optical rotatory dispersion of the random coil form of poly-α,L-glutamic acid (sodium salt), pH 7.1 in water solution. A 1-mm cell was used. Concentrations ranged from 0.0176 to 0.400%. The vertical lines at the peaks and troughs indicate the range of experimental uncertainty. All rotatory dispersion measurements were obtained with a newly designed spectropolarimeter (O. C. Rudolph and Sons, Model 220/200/1012/658-313/100) which employs a double prism monochromator. [R'] was calculated using the refractive index of water at 240 mμ.

residue rotation, [R'], of about 80,000; in striking contrast, the *random coil form* of this polypeptide not only *shows* a much lower rotation in this spectral region but also the presence of *a weaker negative Cotton effect* with a trough around 204 mμ, an inflection point at 197 mμ, and a peak around 190 mμ (Fig. 1, curve B). Possibly the 197 mμ Cotton effect is characteristic of a peptide bond from an asymmetric α-amino acid without superimposed conformational effects. Thus it seems likely that the large positive Cotton effect observed in the helical form of the polypeptide contains buried under it the smaller negative Cotton effect seen with the random material.

(1) This is Polypeptides XXXVIII. For the preceding paper in this series see S. M. Bloom, G. D. Fasman, C. de Lozé and E. R. Blout, *J. Am. Chem. Soc.*, **84**, 458 (1962). Alternate address for E. R. Blout, Chemical Research Laboratories, Polaroid Corporation, Cambridge 39, Massachusetts.

(2) This work was supported in part by U. S. Public Health Service Grant A2558, and in part by the Office of the Surgeon General, Department of the Army.

(3) N. S. Simmons and E. R. Blout, *Biophys. J.*, **1**, 55 (1960).

(4) N. S. Simmons, C. Cohen, A. G. Szent-Gyorgyi, D. B. Wetlaufer and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 4766 (1961).

(5) S. Beychok and E. R. Blout, *J. Mol. Biol.*, **3**, 769 (1961).

(6) B. Jirgensons, *Arch. Biochem. and Biophys.*, **96**, 314 (1962).

(7) S. B. Zimmerman and J. A. Schellman, *J. Am. Chem. Soc.*, **84**, 2259 (1962).