[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Infrared Spectra of Synthetic Polypeptides Prepared by the Lossen Rearrangement

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Infrared spectra have been obtained for polyglycine, poly-α-aminobutyric acid, polynorleucine, poly-5-aminovaleric acid, poly-2-amino-4-pentenoic acid, polyphenylalanine and polytryptophan, all prepared by the Lossen rearrangement. The solvents used for polymerization as well as for casting films have definite effects on the absorption, and hence presumably on the configuration of the polypeptide chain. Polymers with bulky side chains also show differences from those with small side chains.

In view of the wide interest in the infrared spectra of polypeptides[1] it has seemed worthwhile to examine the absorption bands of some of these synthetic polymers prepared recently by a novel procedure.[2] Of particular interest has been a comparison of spectra of a single poly-(amino acid) synthesized in different reaction media, especially in connection with small variations in the wave length of the carbonyl absorption band.

Experimental

The details of the syntheses of polyglycine, poly-α-aminobutyric acid, polynorleucine, poly-5-aminovaleric acid, poly-2-amino-4-pentenoic acid, polyphenylalanine and polytryptophan by the Lossen rearrangement have been described.[2-5] The starting compounds were all racemic.

Films of polypeptides were cast from formic acid (98–100%), from redistilled *m*-cresol and from ethanol. A solution containing 5–10 mg. of polypeptide in 0.5–1.0 ml. of solvent was spread on a silver chloride plate[6] and the solvent allowed to evaporate in a desiccator containing sodium hydroxide and phosphoric anhydride. Spectra were taken with a Beckman self-recording infrared spectrophotometer, model IR2T. Tracings of the same film taken at different times were reproducible to better than 0.02 μ. We are indebted to Mr. M. Knepp and Miss R. Guy for obtaining the spectra.

Results

The absorption spectra of all samples except polytryptophan have been summarized in abbreviated form, in Figs. 1–10, convenient for comparison of relative intensities and positions of the peaks. A full reproduction of the one automatic tracing is shown for polytryptophan in Fig. 11.

Discussion

In their general features the spectra of the polypeptides prepared by the Lossen rearrangement are comparable with those obtained by polymerization of azasuccinic anhydrides. Thus all of the polymers show the characteristic absorption of hydrogen-bonded N–H groups near 3.0 μ. Likewise all show a band near 3.25 μ, which has also been attributed to the N–H group.[1] It is pertinent to point out that a 3.25 μ peak appears even in the non-aromatic poly-(amino acids) and hence the assignment to N–H vibrations is indirectly confirmed. It is also of interest to note that polytryptophan, in contrast to all of the other polymers examined, shows an additional absorption at 2.89 μ. This band is certainly due to the non-hydrogen bonded N–H vibration of the nitrogen in the indole nucleus.

All of the polymers also show typical C–H peaks in the 3.4 μ region. On the whole the intensity of absorption in the polypeptides with large aliphatic side-chains is stronger at 3.4 than at 3.25 μ; in contrast with aromatic side chains, the absorption is stronger at 3.25 μ, as one would expect if these groups also contribute to the peak at this wave length.

Typical amide carbonyl absorption is shown in the 6 μ region. Most of the polypeptides whether polymerized in benzene or water, if soluble in formic acid, exhibit a C=O band at 6.1 μ. Ambrose and Elliott[7] have suggested that a peak at 6.1 μ is characteristic of the β or extended chain configuration of polypeptides and proteins; whereas carbonyl absorption at 6.0 μ is characteristic of the α or folded configuration. It would seem then that the polypeptide films cast from formic acid are all in the extended form.

It is also quite clear from the spectra examined that the configuration of a synthetic polypeptide depends on the solvent used for polymerization as well as for casting. Thus polyphenylalanine prepared in hot water and cast from formic acid showed a peak near 6.1 μ. A sample polymerized in boiling benzene was found to be insoluble in formic acid but soluble in *m*-cresol from which solvent the cast film showed a double peak at 6.02 and 6.11 μ, a result presumably indicative of a mixture of α and β configurations. A small fraction of the material polymerized in benzene was found to be soluble in benzene; the specimen cast from benzene showed a C=O band at 6.02 μ. These results thus agree with those of Ambrose and Elliott[7] in that the benzene-soluble material, which could hardly have an extended cross-linked configuration,[7] does show a 6.02 μ peak. In the same connection it is of interest to note that polytryptophan shows a peak at 6.0 μ, and simultaneously is soluble in ethanol.

In this example, too, the very bulky side chain would hinder cross linkages between backbone chains and favor a folded configuration. With polytryptophan steric interference by the indole side chains may block full hydrogen bonding within a folded arrangement; thus the amide NH absorption appears slightly below 3.0 μ, a position indicative of incomplete hydrogen bonding.

In contrast to the experience of Ambrose and Elliott[7] we have found that casting films from *m*-

(1) For a recent review see G. B. B. M. Sutherland, *Advances in Protein Chemistry*, **7**, 291 (1952).

(2) C. D. Hurd and C. M. Buess, THIS JOURNAL, **73**, 2409 (1951).

(3) C. D. Hurd and L. Bauer, *ibid.*, **73**, 4387 (1951).

(4) C. D. Hurd, C. M. Buess and L. Bauer, *J. Org. Chem.*, **17**, 865 (1952).

(5) L. Bauer, Ph.D. Dissertation, Northwestern University, 1952.

(6) I. M. Klotz, P. Griswold and D. M. Gruen, THIS JOURNAL, **71**, 1615 (1949).

(7) E. J. Ambrose and A. Elliott, *Proc. Roy. Soc. (London)*, **A205**, 47 (1951).

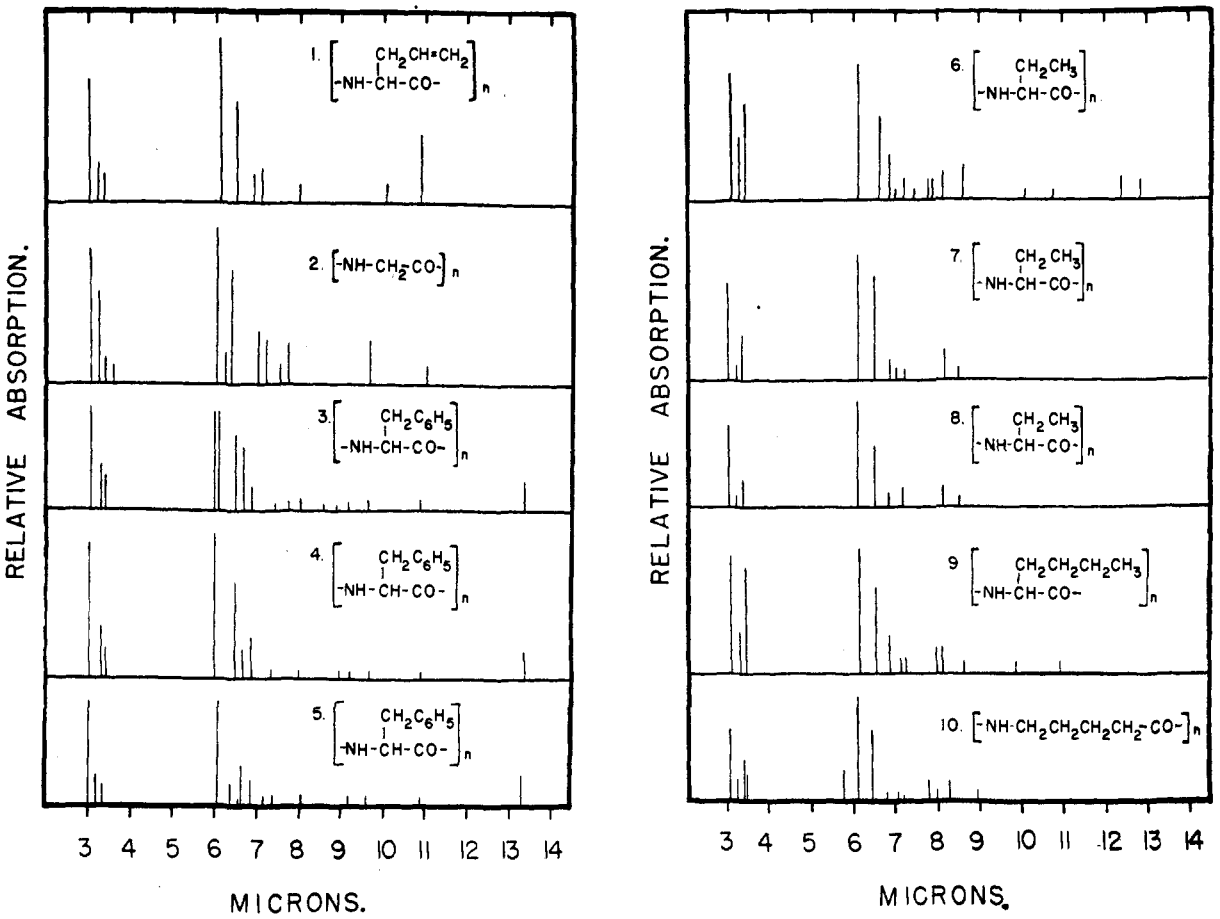

Fig. 1.—Infrared spectrum of poly-2-amino-4-pentenoic acid from the rearrangement[5] of sodium allylcarboxyaceto-(benzoylhydroxamate) in water, the film being cast from formic acid.

Fig. 2.—Infrared spectrum of polyglycine from the rearrangement[2] of sodium carboxyaceto-(benzoylhydroxamate) in benzene, the film being cast from formic acid.

Fig. 3.—Infrared spectrum of polyphenylalanine from the rearrangement[3] of sodium α-carboxy-β-phenylpropiono-(benzoylhydroxamate) in benzene, the film being cast from *m*-cresol.

Fig. 4.—Infrared spectrum of polyphenylalanine from the rearrangement[3] of sodium α-carboxy-β-phenylpropiono-(benzoylhydroxamate) in benzene, the film being cast from benzene.

Fig. 5.—Infrared spectrum of polyphenylalanine from the rearrangement[3] of sodium α-carboxy-β-phenylpropiono-(benzoylhydroxamate) in water, the film being cast from formic acid.

Fig. 6.—Infrared spectrum of poly-α-aminobutyric acid from the rearrangement[3] of sodium α-carboxybutyro-(benzoylhydroxamate) in benzene, the film being cast from *m*-cresol.

Fig. 7.—Infrared spectrum of poly-α-aminobutyric acid from the rearrangement[3] of sodium α-carboxybutyro-(benzoylhydroxamate) in benzene, the film being cast from formic acid.

Fig. 8.—Infrared spectrum of poly-α-aminobutyric acid from the rearrangement[3] of sodium α-carboxybutyro-(benzoylhydroxamate) in water, the film being cast from formic acid.

Fig. 9.—Infrared spectrum of polynorleucine from the rearrangement[2] of sodium α-carboxycapro-(benzoylhydroxamate) in water, the film being cast from formic acid.

Fig. 10.—Infrared spectrum of poly-5-aminovaleric acid from the rearrangement[4] of sodium 5-carboxyvalero-(benzoylhydroxamate) in toluene, the film being cast from formic acid.

cresol does not ensure a 6.0 peak. Thus poly-α-aminobutyric acid polymerized in benzene showed absorption at 6.12 μ whether cast from *m*-cresol or formic acid. The same peak was observed with a specimen polymerized in water and cast from formic acid.

An unexpected but definite shoulder has been observed at 5.8 μ with poly-5-aminovaleric acid, evidently due to a —COOH group. This may indicate that some of the polypeptide is not in the zwitterionic form, at least in the solid state. It is difficult to be certain, however, that there is no residual formic acid in the solid film, although none of the other films gave any indication of such a peak. A similar inflection has been reported for polyglycine by Blout and Linsley[8] in their infrared investigations of peptides.

Bands near 6.5 μ, due to N–H deformation frequencies, were observed in all specimens although not always in the exact positions near 6.45 and 6.58 μ which were suggested[7] for the α- and β-configurations, respectively. Polytryptophan, for example, with an absorption at 6.0 μ characteristic

(8) E. R. Blout and S. G. Linsley, THIS JOURNAL, **74**, 1946 (1952).

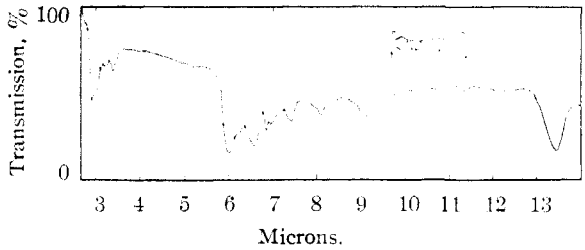

Fig. 11.—Infrared spectrum of polytryptophan from the rearrangement[5] of sodium α-carboxy-β-3-indolylpropiono-(benzoylhydroxamate) in water, the film being cast from ethanol.

of the α-form, has a peak at 6.58 μ ascribed[7] to the β-configuration. Likewise, poly-α-aminobutyric acid, polynorleucine and poly-5-aminovaleric acid, with bands at 6.12 ± 0.01 μ, show N–H peaks at the unexpected positions of 6.48, 6.50 and 6.45 μ, respectively. Absorption near 6.8 μ due to CH_2 groups has also been found consistently. Above 7 μ, characteristic double-bond absorption was observed at 10.88 μ with poly-2-amino-4-pentenoic acid, and a typical band for the benzene ring is present in the spectra of polyphenylalanine between 13.33–13.41 μ, and in that for polytryptophan at 13.46 μ. Consistent absorption appears in all of the polypeptides in the regions near 7 and near 8 μ, but no unequivocal assignments could be made for these peaks.

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A Study of Acid Catalysis in Ketone Acylations

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Six Lewis acids have been investigated as possible catalysts for the acylation of ketones with anhydrides to give β-diketones. Of these reagents, only two, anhydrous ferric chloride and anhydrous zinc chloride, are satisfactory. In the attempted acylation of acetophenone with acetic anhydride in the presence of aluminum chloride, only dypnone was obtained; while the use of either iodine or anhydrous stannic chloride as the catalyst gave benzoic acid instead of the expected benzoylacetone.

The Claisen acylation of ketones to give β-diketones has been effected by both acidic and basic condensing agents. Although the use of a number of bases such as sodium metal,[2] alkali alkoxides,[3] alkali amides,[4] substituted alkali amides[5] and sodium triphenylmethide[4] has been investigated, apparently only one acid, gaseous boron fluoride[6–8] has been studied.

Since Friedel–Crafts acylations have been effected not only by aluminum chloride,[9] but by a number of other Lewis acids such as iodine,[10] phosphorus pentoxide[11] and anhydrous ferric[12] and zinc chlorides,[13] it was of interest to determine whether general acid catalysis was applicable in the Claisen acylation of ketones with anhydrides.

To study the relative effectiveness of the various catalysts, the acylation of acetophenone with acetic anhydride, as shown in the following equation, was investigated in the presence of six Lewis acids.

$$C_6H_5COCH_3 + (CH_3CO)_2O \xrightarrow{\text{Lewis acid}} CH_3CO_2H + C_6H_5COCH_2COCH_3$$

No benzoylacetone was obtained using aluminum chloride. The isolation of the dehydrated ketol, dypnone (11.5%) in this experiment, confirms the earlier work of Calloway and Green,[14] who found that acetophenone is self-condensed by aluminum chloride. Phosphorus pentoxide was a poor catalyst for the reaction since it gave only a 7.5% yield of the product.

The use of either iodine or stannic chloride to effect the reaction gave none of the desired β-diketone. Instead, benzoic acid, in yields of 18 and 38%, respectively, and acetophenone were isolated from these attempted acylations. It is possible, as shown in the following scheme, that the benzoic acid arose from the iodine or stannic chloride-induced cleavage of benzoylacetone which was probably formed but not isolated.

$$C_6H_5COCH_2COCH_3 + SnCl_4 \longrightarrow C_6H_5\underset{\displaystyle OSnCl_4}{\overset{|}{C}}CH_2COCH_3 + C_6H_5COCH_2\underset{\displaystyle OSnCl_4}{\overset{|}{C}}CH_3$$

I II

$$I \xrightarrow{H_2O} C_6H_5CO_2H + CH_3COCH_3 + 4HCl + SnO_2 + 2H_2O$$

$$II \xrightarrow{H_2O} CH_3CO_2H + C_6H_5COCH_3$$

(1) This paper is based on a thesis presented by Bruno M. Perfetti to the graduate faculty of the University of Pittsburgh in partial fulfillment of the requirements for the Master of Science degree.

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(8) J. T. Adams and C. R. Hauser, *ibid.*, **67**, 284 (1945).

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(13) H. D. Hartough and A. I. Kosak, *ibid.*, **69**, 1012 (1947).

(14) N. O. Calloway and L. D. Green, *ibid.*, **59**, 809 (1937).