has very nearly the same residue weight. The small increase in $\Delta[\alpha]$ observed on increasing the DCA concentration with both the helix and random coil is believed to be a solvent effect. Similar results have also been reported for the $\Delta[\alpha]$ change associated with the helix to random transition of other synthetic polyamino acids.[26]

The observation that the helix → random transition for poly-ε-carbobenzyloxy-L-lysine occurs at ~ 36% DCA in $CHCl_3$, while that for poly-γ-benzyl-L-glutamate occurs at ~ 68% DCA may be interpreted as indicating a helix of lower stability for the lysine polymer. These data support Applequist's proposal[15] of a "bent-helix" structure necessary to accommodate his light scattering, viscosity and sedimentation data. Thus poly-ε-carbobenzyloxy-L-lysine is pictured as a flexible rod rather than a rigid rod.

It may be noted that a helix of lower stability than either of the above mentioned has been reported.[27] The poly-β-benzyl-L-aspartate helix was destroyed in chloroform solution by the addition of 5–8% DCA. Deuterium exchange supports this observation, since the rate of D-exchange was found to be much faster for poly-β-benzyl-L-aspartate than for poly-γ-benzyl-L-glutamate.[28]

The observation that the stability of three uncharged α-helical synthetic polypeptides can vary so greatly in the same solvent system re-emphasizes that the stability of any particular polypeptide helix depends to a large extent on the nature of the side group attached to the α-carbon. Not only can the side chain determine the relative stability of the α-helix, but recent studies have shown that certain side chains prevent helix formation.[29]

(26) E. R. Blout in "Optical Rotatory Dispersion," by C. Djerassi, McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 244.

(27) R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, THIS JOURNAL, **82**, 2268 (1960).

(28) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature*, **183**, 1736 (1959).

(29) E. R. Blout, C. de Loze, S. M. Bloom and G. D. Fasman, THIS JOURNAL, **82**, 3787 (1960).

The effect of the side chain interactions in the stabilization of the helix in water-soluble synthetic polypeptides has been discussed previously.[30,31]

It is of interest to note that in the same solvent system the stability of the poly-ε-carbobenzyloxy-L-lysine α-helix is comparable to the stability of the β-structure of poly-O-acetyl-L-serine.[32] These hydrogen-bonded conformations of the two polypeptides were found to be destroyed by the addition of approximately 35–40% DCA to chloroform solutions. Thus it appears that the stabilization energy (which is a composite of several factors, such as the "residue" stabilization energy and the peptide hydrogen bonding stabilization energy) is of the same order of magnitude for both the α-helical and β-conformations in the above cases.

Summary

1. Poly-ε-carbobenzyloxy-L-lysine has been synthesized with a DP_w over 5,000. This corresponds to a weight average molecular weight greater than 1,300,000. 2. Poly-L-lysine·HCl has been prepared by removal of the blocking group from poly-ε-carbobenzyloxy-L-lysine with minimal peptide degradation. 3. The transition from the helical conformation to a random coil has been demonstrated in solution for poly-ε-carbobenzyloxy-L-lysine. 4. Evidence is presented that indicates that the stability of the helical structure of poly-ε-carbobenzyloxy-L-lysine in solution is weaker than that of poly-γ-benzyl-L-glutamate.

Acknowledgments.—We are pleased to acknowledge the valuable assistance of Miss Carole Lindblow in the preparative work and optical rotatory studies. We are indebted to Dr. C. de Loze for the infrared spectra and to Mr. K. Norland for fruitful discussions.

(30) J. A. Schellman, *Compt. rend. trav. Lab. Carlsberg, Ser. chim.*, **29**, No. 15 (1955).

(31) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

(32) G. D. Fasman and E. R. Blout, THIS JOURNAL, **82**, 2262 (1960).

[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION AND HARVARD MEDICAL SCHOOL, BOSTON 15, MASS.]

The Infrared Spectra of Polypeptides in Various Conformations: Amide I and II Bands[1]

BY T. MIYAZAWA[2] AND E. R. BLOUT

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The α-helix, the antiparallel-chain extended conformation and the parallel-chain extended conformation of polypeptides show both parallel and perpendicular dichroism in their amide I and II infrared absorption bands. The frequencies of the amide I and II bands of these conformations (as well as those of the random coil conformation) are characteristic and now have been shown to be explicable in terms of vibrational interactions between adjacent peptide groups in the chain and through hydrogen bonds. In particular, the amide I band (1695 cm.$^{-1}$) of the antiparallel-chain pleated sheet may be used in structure diagnoses of extended polypeptide chains. The amide I transition moment of the antiparallel-chain pleated sheet may be estimated from the intensity ratio of the parallel and perpendicular bands. The directions of the amide I and II transition moments of the α-helix of poly-γ-benzyl-L-glutamate are estimated to be inclined from the helix axis by 29–34° and 75–77°, respectively. The apparent dichroic ratios of the perpendicular amide I band (1630 cm.$^{-1}$) of the pleated sheet and the perpendicular amide II band (1545 cm.$^{-1}$) of the α-helix indicate the degree of orientation of the fiber axes. The interpretations of the infrared spectra of several proteins have been revised.

Several years ago some empirical correlations were established between the characteristic infrared bands of polypeptides at *ca.* 1650 cm.$^{-1}$ (amide I) and *ca.* 1550 cm.$^{-1}$ (amide II) and the conformation of these polypeptides.[3] Further it has been found[4]

(1) (a) This is Polypeptides. XXXI; for the preceding paper in this series see G. D. Fasman, M. Idelson and E. R. Blout, THIS JOURNAL, **83**, 709 (1961). (b) Alternate address of E. R. Blout: Research Division, Polaroid Corp., Cambridge 39, Mass.

(2) Institute for Protein Research, Osaka University, Kitaku, Osaka, Japan.

TABLE I

OBSERVED AND CALCULATED FREQUENCIES (CM.$^{-1}$) OF THE AMIDE I AND II BANDS OF POLYPEPTIDES IN VARIOUS CONFORMATIONS

Conformation	Designation	Theoretical frequency	Amide I frequencies ν_{obsd}[b]	Amide I frequencies ν_{cal}[c]		Amide II frequencies ν_{obsd}[b]	Amide II frequencies ν_{cal}[c]	
Random coil		ν_0	1656(s)	1658		1535(s)	1535	
α-Helix	$\nu \parallel$ (o)	$\nu_0 + D_1 + D_3$	1650(s)	(1650)	$D_1 = 10$	1516(w)	(1516)	$D_1 = -21$
	$\nu \perp (2\pi/n)$[a]	$\nu_0 - 0.17D_1 + 0.50D_3$	1652(m)	1647	$D_3 = -18$	1546(s)	1540	$D_3 = 2$
Parallel-chain pleated sheet	$\nu \parallel$ (o,o)	$\nu_0 + D_1 + D'_1$	1645(w)	1648		1530(s)	1530	$D_1 = -10$
	$\nu \perp (\pi$,o)	$\nu_0 - D_1 + D'_1$	1630(s)	1632		1550(w)	1550	$D'_1 = 5$
Antiparallel-chain pleated sheet	$\nu \parallel$ (o,π)	$\nu_0 + D_1 - D'_1$	1685(w)	(1685)	$D_1 = 8$ $D'_1 = -18$	1530(s)	(1530)	$D_1 = -10$
	$\nu \perp (\pi$,o)	$\nu_0 - D_1 + D'_1$	1632(s)	(1632)			1540	$D'_1 = -5$
	$\nu \perp (\pi,\pi)$	$\nu_0 - D_1 - D'_1$		1668			1550	
Nylon 66		$\nu_0 + D'_1$	1640(s)	(1640)		1540(s)	(1540)	$D'_1 = 5$

[a] n is the number of peptide groups per turn of the helix. [b] The letter symbols in the parentheses indicate observed intensities; s = strong, m = medium, w = weak. [c] The values in parentheses were used in the calculations of D_1, D'_1 and D_3.

that synthetic polypeptides in a folded (helical) conformation exhibit the amide I band at *ca.* 1655 cm.$^{-1}$ and the amide II band at 1540 cm.$^{-1}$, whereas those in an extended conformation show these bands at *ca.* 1630 and 1520 cm.$^{-1}$, respectively. However, these correlations do not apply to polyglycine II,[5] unoriented films of sodium poly-α,L-glutamate[6] and some proteins.[7]

In this paper we seek to establish a basis for correlating the observed amide I and II frequencies and dichroisms with the α-helical, parallel-chain pleated sheet, antiparallel-chain pleated sheet and random conformations of polypeptides and proteins. In a previous study it was found that polypeptides in the α-helical conformations show two amide II bands and those in the antiparallel-chain pleated sheet exhibit two amide I bands.[8] The treatment in the present paper assumes that the frequency splittings observed for the amide I and II bands are mostly due to the vibrational interactions among peptide groups in the chain and through hydrogen bonds.

Previously the localized vibrations, such as the amide I and II vibrations,[9] have been treated by first-order perturbation theory.[8] A general equation for the frequency of a localized vibration of an infinite helical chain has been derived

$$\nu(\delta,\delta') = \nu_0 + \underset{\text{intra}}{\sum_s} D_s \cos(s\delta) + \underset{\text{inter}}{\sum_{s'}} D_s' \cos(s'\delta') \quad (1)$$

where the first term ν_0 is the unperturbed frequency, and the second and third terms are due to the intrachain and interchain vibrational interactions, respectively, δ is the phase angle between adjacent group motions in the chain and the coefficient D_s is determined by the potential and kinetic energy interactions between the sth neighbors in the chain; δ' and D_s' pertain to the interactions through interchain hydrogen bonds and have meanings similar to those of δ and D_s, respectively. Thus taking into account only the adjacent group interactions, equations were derived[8] for the α-helix,[11] the parallel-chain pleated sheet[12] and the antiparallel-chain pleated sheet.[12]

In the present study the results of the calculations from eq. 1 will be used to interpret the observed spectra of the random, helical and pleated sheet conformations of synthetic polypeptides. In Table I (above) there is a summary of the observed and calculated frequencies of the amide I and amide II bands of the various polypeptide conformations. Finally, use will be made of these calculated frequencies to assign conformations to other synthetic polypeptides and to some proteins whose infrared spectra have been reported previously.

Extended Conformations

It has been recognized that polypeptide chains may exist in extended conformations as well as in folded conformations. In the fully extended conformation of polypeptides interchain hydrogen bonds may be formed satisfactorily only when the polypeptide chains are antiparallel. However, in this conformation steric hindrance between β-carbon atoms of adjacent chains is appreciable. Thus polypeptide chains tend to exist in more or less buckled conformations as either the parallel-chain pleated sheet or the antiparallel-chain pleated sheet.[12]

In order to understand the infrared spectra of polypeptides in extended conformations it is useful to study Nylon 66, a synthetic polymer containing secondary amide groups separated from each other by four or six methylene groups and which has been shown by X-ray diffraction to exist in a fully extended conformation. Nylon 66 is a useful model compound since it provides a simple example where

(3) A. Elliott and E. J. Ambrose, *Nature*, **165**, 921 (1950).

(4) A. Elliott, *Proc. Roy. Soc.* (*London*), **A221**, 104 (1953); E. J. Ambrose and A. Elliott, "Proc. Third International Congress of Biochemistry," Academic Press, Inc., New York, N. Y., 1956.

(5) A. Elliott and B. R. Malcolm, *Trans. Faraday Soc.*, **52**, 528 (1956).

(6) A. Elliott, W. E. Hanby and B. R. Malcolm, *Disc. Faraday Soc.*, **25**, 167 (1958).

(7) M. Beer, G. B. B. M. Sutherland, K. N. Tanner and D. L. Wood, *Proc. Roy. Soc.* (*London*), **A249**, 147 (1959).

(8) T. Miyazawa, *J. Chem. Phys.*, **32**, 1647 (1960).

(9) A normal coördinate treatment was made of the characteristic vibrations of simple monosubstituted amides.[10] It was learned from this calculation that the Cartesian displacements of the α-carbon atoms are negligible as compared with those of the C, O, N and H atoms of the peptide group; in other words, the vibrational motions are localized in the peptide group.

(10) T. Miyazawa, T. Shimanouchi and S. Mizushima, *J. Chem. Phys.*, **29**, 611 (1958).

(11) L. Pauling and R. B. Corey, THIS JOURNAL, **72**, 5349 (1950); *Proc. Natl. Acad. Sci.*, **37**, 235, 241 (1951).

(12) L. Pauling and R. B. Corey, *ibid.*, **37**, 729 (1951).

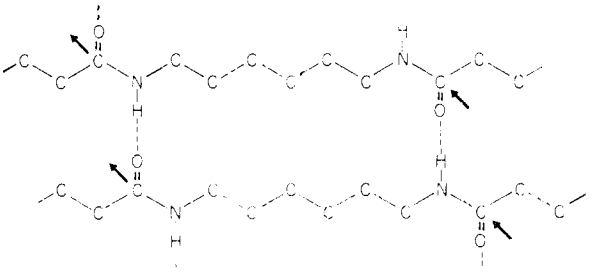

Fig. 1.—The structure of the crystalline region of oriented Nylon 66 and the infrared-active vibrational mode. The arrows represent the transition moments associated with the in-plane vibrations of the peptide groups.

only interchain peptide group interaction exists without any intrachain action.

Nylon 66.—The structure of the crystalline region of oriented Nylon 66 has been determined by Bunn and Garner[13] who found that this polyamide assumes the parallel-chain extended conformation and a unit cell contains two peptide groups from *one* chain (see Fig. 1). Thus for infrared active vibrations adjacent groups through hydrogen bonds move in phase ($\delta' = 0$). On the other hand, each peptide group is separated from others in the chain by four or six methylene groups and intrachain vibrational interactions may be neglected ($D_s = 0$). Taking into account only the adjacent group interactions through interchain hydrogen bonds, the frequency of a localized vibration is found to be

$$\nu = \nu_0 + D_1' \qquad (2)$$

There are two peptide groups in a unit cell of Nylon 66, but, because of the center of symmetry, only centrosymmetric vibrations are active in the infrared. The amide I and II bands of highly crystalline Nylon 66 are observed[14] at 1640 and 1540 cm.$^{-1}$, respectively.

Antiparallel-chain Pleated Sheet.—Localized vibrations of polypeptides in the antiparallel-chain pleated sheet have been treated[8] and it has been found that there are three kinds of infrared active vibrations. The theoretical frequencies are shown in Table I and schematic representation of the vibrational modes are shown in Fig. 2, where the arrows represent the transition moment associated with the in-plane vibrations of the peptide group; the phase angles in parentheses after the ν represent in-phase motions (o), and out-of-phase motions (π) relative to C–N direction. The ν(o, π) vibration gives rise to a band with parallel dichroism whereas the $\nu(\pi, o)$ and $\nu(\pi, \pi)$ vibrations give rise to bands with perpendicular dichroism.

Amide I Frequencies.—Two amide I bands, 1685 and 1632 cm.$^{-1}$, have been observed for polyglycine I.[5,15] The weak parallel band at 1685 cm.$^{-1}$ has been assigned to the ν(o, π) amide I vibration of the antiparallel-chain pleated sheet[8] and the strong perpendicular band at 1632 cm.$^{-1}$ to the $\nu(\pi, o)$ amide I vibration of this pleated sheet.

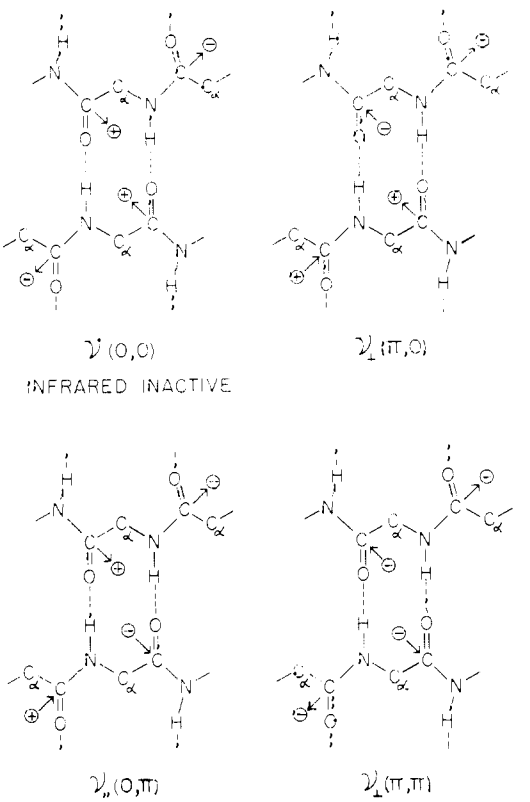

Fig. 2.—The vibrational modes of the antiparallel-chain pleated sheet. The arrows represent the components of the transition moments of the peptide groups in the plane of the paper. The plus or minus signs represent the components of the transition moments perpendicular to the plane of the paper, the former pointing upward and the latter pointing downward. The numbers in parentheses after the ν's indicate the phase angle (in radians) of the motions, o being in-phase and π out-of-phase. The first number represents the phase angle of the adjacent intrachain peptide group and the second that of the phase angle of the interchain peptide group.

The perpendicular $\nu(\pi, \pi)$ band has not been observed. In fact its intensity is expected to be even weaker[16] than the weak band ν(o, π) at 1685 cm.$^{-1}$.

The characteristic frequencies of polyglycine I may now be discussed in terms of vibrational interactions among adjacent groups (see Table I). In principle, at least, the values of ν_0, D_1 and D_1' may be calculated if the three frequencies ν(o, π), $\nu(\pi, \pi)$ and $\nu(\pi, o)$ were known. Unfortunately, as noted above, the $\nu(\pi, \pi)$ amide I band has not been observed. However, if it is assumed that the values of ν_0 and D_1' of polyglycine I are the same as those of Nylon 66,[17] the unknown constants may be calculated by equating the theoretical and observed frequencies of Nylon 66 and the ν(o, π) and $\nu(\pi, o)$ vibrations of polyglycine I (Table I). It

(13) C. W. Bunn and E. V. Garner, *Proc. Roy. Soc.* (*London*), **A189**, 39 (1947).

(14) C. H. Bamford, *et al.*, "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956, p. 147.

(15) E. R. Blout and S. G. Linsley, THIS JOURNAL, **74**, 1946 (1952); T. Miyazawa, T. Schimanouchi and S. Mizushima, *J. Chem. Phys.*, **24**, 408 (1956).

(16) The intensity ratio[8] of the $\nu(\pi,\pi)$ band and the $\nu(o,\pi)$ band is $\tan^2\phi$ where ϕ is the angle between the fiber axis and the plane of the peptide group. Since ϕ is about 30° the intensity of the $\nu(\pi,\pi)$ band will be about one-third of that of the $\nu(o,\pi)$ band.

(17) This assumption is reasonable since in both cases of polyglycine I and Nylon 66 the hydrogen bonds are nearly linear and the hydrogen bond strengths are nearly equal as judged from the NH stretching frequencies.

may be pointed out that the difference in the amide I frequencies of Nylon 66 (1640 cm.$^{-1}$) and polyglycine I (1632 cm.$^{-1}$) is due to the lack of the intrachain interactions (D_1 term) in Nylon 66.

The frequency splitting (53 cm.$^{-1}$) of the amide I bands of polyglycine I is largely due to the interchain vibrational interactions, since the value of D_1' is more than twice the value of D_1. The negative value of D_1' indicates that the potential energy increase due to the in-phase (o) motions of adjacent groups through hydrogen bonds is lower than that due to the out-of-phase (π) motions. In fact the C=O stretching displacement of a peptide group will favor the polar resonance form $^{+}NH{=}C{-}O^{-}$ of the adjacent group and will reduce the potential energy increase due to the C=O stretching displacement of the latter group.[18]

Amide II Frequencies.—The amide II band of polyglycine I at 1530 cm.$^{-1}$ shows parallel dichroism and is assigned to the ν(o, π) vibration. The two expected perpendicular bands, $\nu(\pi, o)$ and $\nu(\pi, \pi)$, have not been observed yet. Nevertheless the values of ν_0, D_1 and D_1' for the amide II vibration may be estimated from the observed frequencies of polyglycine I, Nylon 66 and incompletely deuterated poly-γ-benzyl-L-glutamate. In the last case, the observed amide II frequency (1535 cm.$^{-1}$) may be regarded as the unperturbed frequency ν_0.[8] Thus D_1' of Nylon 66 is calculated to be +5 cm.$^{-1}$. The interaction between the amide II vibrations through hydrogen bonds is caused by the displacement of the amide hydrogen atom in the plane of the peptide group but perpendicular to the NH···OC bond. The value of D_1' is expected to depend upon the angle between the planes of adjacent groups.[19] In the case of Nylon 66 adjacent peptide groups through hydrogen bonds are parallel to each other and as noted above D_1' is +5 cm.$^{-1}$. In the case of the antiparallel-chain pleated sheet, adjacent peptide groups are antiparallel and D_1' is assumed to be −5 cm.$^{-1}$. The value of D_1 is −10 cm.$^{-1}$ as calculated from the observed ν(o, π) frequency of polyglycine I.

Making use of D_1 and D_1' thus determined, the $\nu(\pi, o)$ and $\nu(\pi, \pi)$ amide II frequencies of polyglycine I are calculated to be 1540 and 1550 cm.$^{-1}$, respectively. The observed frequencies are shown in Table II.

Finally it may be remarked that the calculated $\nu(\pi, \pi)$ frequency of 1550 cm.$^{-1}$ is not much different from the strong perpendicular amide II frequency (*ca.* 1545 cm.$^{-1}$) of the α-helix. Accordingly a weak perpendicular amide II band around 1545 cm.$^{-1}$ in polypeptides and proteins does not necessarily indicate the presence of a small amount of the α-helix.

TABLE II

OBSERVED AMIDE I AND II FREQUENCIES (CM.$^{-1}$) OF EXTENDED CONFORMATIONS

Substance	Amide I $\nu_{\parallel}$	Amide I $\nu_{\perp}$	Amide II $\nu_{\parallel}$	Amide II $\nu_{\perp}$
Horsehair (steam-stretched)[a]	1645	1630	1530	1550
Silk (*Anaphe moloneyi*)[b]	1697	1630	1527	1552
Silk gut (*Bombyx mori*)[c]	1695	1634	1520	
Polyglycine I[b]	1685	1632	1530	1550[i]
Poly-γ-methyl-L-glutamate[d]	1692	1627	1521	
Poly-L-alanine[e]	1695	1635	1520	
Sodium poly-α,L-glutamate[f]				
(β′)	1690	1625	1525	
(β″)	1685	1615	1525	
N-Deuterated sodium poly-α,L-glutamate[f] (β′)	1680	1620	..	..
(β″)	1675	1610	..	..
Poly-L-lysine hydrochloride[g]	1690	1625		
Poly-O-acetyl-L-serine[h]	1705	1635	1520	

[a] Ref 21. [b] Ref. 5. [c] Ref. 29. [d] Ref. 4. [e] A. Elliott, *Proc. Roy. Soc., London*, **A226**, 408 (1954). [f] Ref. 22. [g] E. R. Blout and H. Lenormant, *Nature*, **179**, 960 (1957). [h] Ref. 24. [i] This band may well be due to polyglycine II.

Amide I Transition Moment.—The transition moments of the ν(o, π) and $\nu(\pi, o)$ vibrations of the antiparallel-chain pleated sheet (Fig. 2) are parallel to the two-dimensional layer of the hydrogen bonded network. Therefore the apparent dichroic ratio observed for such a band serves as a direct measure of the degree of orientation of the fiber axis. If the incident radiation is perpendicular to this two-dimensional layer, the intensity ratio of those two bands is[8]

$$I(o,\pi)/I(\pi,o) = (\tan^2\theta - \tan^2\phi)^{-1} \qquad (3)$$

where θ is the angle between the transition moment of each group and the fiber axis and ϕ is the angle between the plane of the peptide group and the fiber axis. The value of $\tan^2\theta$ for the amide I vibration of the extended conformation is much larger than unity and $\tan^2\phi$ is almost negligible since the angle ϕ is approximately 30° for the antiparallel-chain pleated sheet.

Some complications are involved in applying eq. 3. Firstly, even if the fiber axes lie nearly parallel to the oriented film surfaces the two-dimensional layer may not be quite parallel to the film surfaces. Secondly, since the interactions through hydrogen bonds have been found to be appreciable in the case of the amide I vibration, it may not be quite valid to assume that the total transition moment is the vector sum of the transition moments of all the groups involved. Nevertheless taking due consideration of these complications, eq. 3 may be useful in studying the structure of the antiparallel-chain pleated sheet, especially in the case of relatively low molecular weight polypeptides since unidirectional orientation is not necessary.

Amide II Transition Moment.—It would be desirable to attempt to resolve the $\nu(\pi,\pi)$ band from the strong ν(o, π) band by highly orienting the fibers. Then intensity comparison of these bands would allow estimation of the angle ϕ between the fiber axis and the plane of the peptide group, since the intensity ratio[8] is equal to $\tan^2\phi$.

(18) The value of D_1' of polyglycine I may be compared with that of crystalline formic acid molecules which form hydrogen-bonded chain polymers. The out-of-phase C=O stretching frequency of formic acid is 94 cm.$^{-1}$ higher than the in-phase C=O stretching frequency (*cf.* R. C. Millikan and K. S. Pitzer, THIS JOURNAL, **80**, 3515 (1958)). In this case the frequency splitting is due only to intermolecular hydrogen bonds and the value of D_1' is equal to one-half the frequency splitting, that is, −47 cm.$^{-1}$. Since the hydrogen bonds in crystalline formic acid are appreciably stronger than those of polyglycine, the magnitude of D_1' appears to vary with the strength of the hydrogen bonds.

(19) On the other hand, the interaction between the amide I vibrations through hydrogen bonds is caused by the displacement of the carbonyl oxygen atom parallel to NH···O=C bond, and the value of D_1' will not depend upon the angle between the planes of adjacent groups.

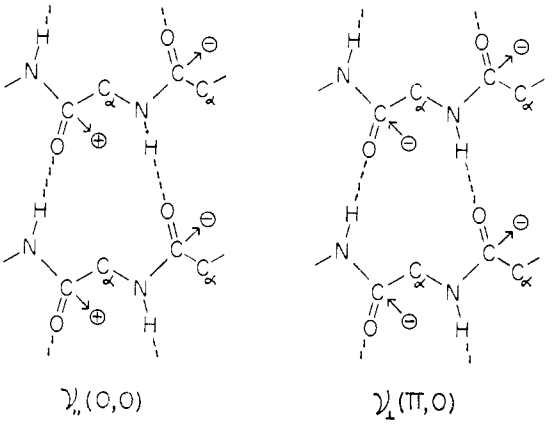

Fig. 3.—The vibrational modes of the parallel-chain pleated sheet. The arrows represent the components of the transition moments of the peptide groups in the plane of the paper. The plus or minus signs represent the components of the transition moments perpendicular to the plane of the paper, the former pointing upward and the latter pointing downward. The numbers in parentheses after the ν's indicate the phase angle (in radians) of the motions, o being in-phase and π out-of-phase. The first number represents the phase angle of the adjacent intrachain peptide group and the second that of the phase angle of the interchain peptide group.

Parallel-chain Pleated Sheet.—Localized vibrations of polypeptides in the parallel-chain pleated sheet have been treated[8] and the theoretical frequencies and schematic representation of the vibrational modes are shown in Table I and Fig. 3. The ν(o, o) vibration gives rise to a parallel band whereas the $\nu(\pi, o)$ vibration gives rise to a perpendicular band.

The fiber repeat period of β-keratin (6.6 Å.)[20] is much shorter than the value expected for the fully extended conformation, but agrees closely with the value (6.5 Å.) calculated for the parallel-chain pleated sheet.[12] Thus Pauling and Corey suggested that β-keratin is an example of the parallel-chain pleated sheet. Assuming that it is valid to use the ν_0 and D_1 of polyglycine I and D_1' of Nylon 66, the amide I and II frequencies of β-keratin have been calculated as shown in Table I.

The observed infrared spectrum of β-keratin (steam-stretched horsehair)[21] shows the parallel amide I band at 1645 cm.$^{-1}$ and the perpendicular amide I band at 1630 cm.$^{-1}$, in good agreement with the calculated values of 1648 and 1630 cm.$^{-1}$, respectively (Table I). The frequency splitting of the amide I bands of β-keratin is due only to the intrachain interactions and amounts to 15 cm.$^{-1}$. The amide II bands of β-keratin (parallel and perpendicular) were observed[21] at 1530 and 1550 cm.$^{-1}$, respectively, although the latter frequency is not quite definite because of possible contamination by a folded (helical) conformation. Nevertheless the frequency agreements are quite satisfactory.

Criteria for Distinguishing between Antiparallel- and Parallel-chain Extended Conformations.—On the basis of calculated frequencies[8] and experimentally observed frequencies reported in this paper for known conformations of polypeptides and proteins (Table II) it is now possible to distinguish between parallel- and antiparallel-chain extended conformations. Both conformations show a perpendicular amide I band $\nu(\pi, o)$ at 1630 ± 5 cm.$^{-1}$. However, the parallel amide I band $\nu(o, \pi)$ at 1695 ±10 cm.$^{-1}$ is observed only for antiparallel-chain pleated sheets and is characteristic of this conformation.

In spectra where the strong perpendicular amide I band is observed at 1630 ± 5 cm.$^{-1}$ the absence of the 1695 cm.$^{-1}$ band suggests that such spectra are due to parallel-chain pleated sheet conformations. The parallel amide I band at 1645 cm.$^{-1}$ will substantiate this conclusion. However, if there is a substantial amount of an α-helical conformation present, a strong parallel amide I band will appear at 1650 and the absolute assignment may be difficult.

In the case of steam-stretched horsehair (Table II) the amide II frequencies at 1530 and 1550 cm.$^{-1}$ allow the unequivocal assignment to the parallel-chain pleated sheet conformation. Therefore, by using both amide I and amide II frequencies, it is possible to differentiate between parallel-chain pleated sheets, antiparallel-chain pleated sheets and helical conformations.

Random Coil Conformation

The polypeptide chains of high molecular weight sodium poly-α,L-glutamate in oriented films exist in the extended conformation or in a folded conformation.[22] If, however, a film is cast from aqueous solution in a dry atmosphere without unidirectional orientation, quite a different film may be obtained with the amide I band around 1660 cm.$^{-1}$. X-Ray diffraction and optical rotation studies have been made by Elliott, *et al.*,[6] and it was suggested that this polypeptide existed in the random coil conformation in such films. Our infrared studies on sodium α,L-polyglutamate and poly-L-lysine hydrohalides have shown that the amide I band is observed at *ca.* 1655 cm.$^{-1}$ for the random coil conformation whose peptide groups are hydrogen bonded with others.[23] The amide II band of the random coil of sodium poly-α,L-glutamate is obscured by the strong band due to the ionized carboxyl group (1575 cm.$^{-1}$); however, a shoulder is observed around 1535 cm.$^{-1}$. The amide II band of the random coil of poly-L-serine[24] has been observed at 1535 cm.$^{-1}$.

The amide I and II frequencies observed for the random coil conformation are nearly the same as the calculated unperturbed frequencies 1658 and 1535 cm.$^{-1}$, respectively, as shown in Table I. In fact, if the conformation is quite random, the frequency shifts due to the interchain interactions as well as the intrachain interaction will average zero.

The α-Helix Conformation

The α-helix[11] has been established as one of the basic structures of polypeptide chains. As may be

(20) C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby and I. F. Trotter, *Nature*, **171**, 1149 (1953); **173**, 27 (1954).

(21) K. D. Parker, quoted in ref. 14, p. 405.

(22) H. Lenormant, A. Baudras and E. R. Blout, THIS JOURNAL, **80**, 6191 (1958).

(23) E. R. Blout and T. Miyazawa (to be published).

(24) G. D. Fasman and E. R. Blout, THIS JOURNAL, **82**, 2262 (1960).

Table I, two frequencies are expected, $\nu_{\parallel}$ (o) and $\nu\perp(2\pi/n)$, for the amide I and amide II bands of this conformation. The calculated frequencies for these bands are shown in the table. In the present study we have used high molecular weight poly-γ-benzyl-L-glutamate, whose spectra has been reported[25] and conformation observed by X-ray diffraction, as a model for a helical polypeptide. The infrared spectra of oriented films of this polypeptide prepared from chloroform solution were measured using a Perkin–Elmer model 21 spectrometer and a silver chloride polarizer. The data for the region 1500–1800 cm.$^{-1}$ are shown in Fig. 4.

The parallel amide I band and the perpendicular amide I band are observed at 1650 and 1652 cm.$^{-1}$, respectively. With the electric vector perpendicular to the orientation direction, the strong perpendicular amide II band is observed at 1546 cm.$^{-1}$ and another weak band is observed at 1498 cm.$^{-1}$. With the electric vector parallel three peaks of nearly the same intensity are observed at 1546, 1516 and 1498 cm.$^{-1}$. The 1516 cm.$^{-1}$ band has been assigned to the parallel amide II vibration.[8] While the two amide II bands disappear on deuteration, the 1498 cm.$^{-1}$ band persists. This band is assigned to a ring stretching vibration of the monosubstituted benzene ring. The bands around 1730 cm.$^{-1}$ are due to the ester C=O stretching modes.[26]

The amide I and II frequencies of the α-helix of poly-γ-benzyl-L-glutamate will now be discussed in terms of the vibrational interactions. The theoretical frequencies are given in Table I where D_1 and D_3 are the constants pertaining to the adjacent group interactions in the chain and through intrachain hydrogen bonds, respectively. For the amide I vibrations, the value of D_3 may be assumed to be the same as D_1' of Nylon 66; D_1 is determined from the parallel amide I frequency and the value of the perpendicular amide I frequency is calculated to be 1647 cm.$^{-1}$. This calculated value agrees quite well with the observed value of 1652 cm.$^{-1}$. For the amide II vibration D_3 has been assumed to be D_1' (of Nylon 66) times cos 60°, since in the α-helix the planes of adjacent peptide groups through intrachain hydrogen bonds make an angle of *ca.* 60°; D_1 is determined as −21 cm.$^{-1}$ from the parallel amide II frequency (of poly-γ-benzyl-L-glutamate) and then the parallel amide II frequency is calculated to be 1540 cm.$^{-1}$ in good agreement with the observed value of 1546 cm.$^{-1}$.

In addition to the spectral studies on oriented films of poly-γ-benzyl-L-glutamate, unoriented films of high molecular weight poly-α,L-glutamic acid in the α-helical conformation were cast from water–dioxane solution and the amide I and II frequencies were measured. Their frequencies are listed in Table III. In the same table the characteristic amide I and II frequencies of several proteins are also shown. In all these cases the characteristic amide I bands (1630 cm.$^{-1}$) of the extended conformations were not observed, but the characteristic amide I and II bands of the α-helical conformation were observed.

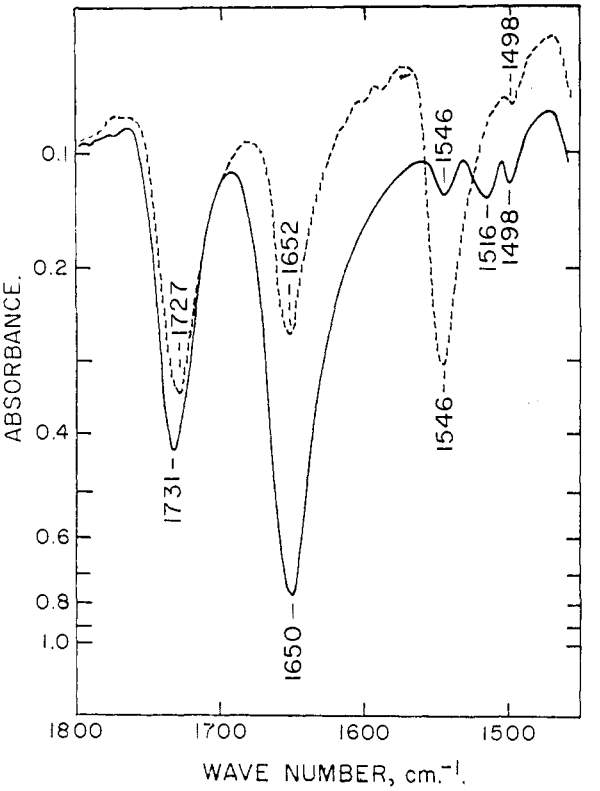

Fig. 4.—Polarized infrared spectra of poly-γ-benzyl-L-glutamate (film unidirectionally oriented from chloroform solution): —, electric vibration direction parallel to orientation direction; – – –, electric vibration direction perpendicular to orientation direction.

TABLE III

OBSERVED AMIDE I AND II FREQUENCIES (CM.$^{-1}$) OF THE α-HELIX

Substance	Amide I $\nu_{\parallel}$	Amide I $\nu_{\perp}$	Amide II $\nu_{\parallel}$	Amide II $\nu_{\perp}$
Poly-γ-benzyl-L-glutamate[a]	1650	1652	1516	1546
Poly-α,L-glutamic acid[a]	1650	1650	1515	1550
Elephant hair[b]	1660	1660	1515	1545
Horsehair[c]	1650	1645	1520	1550
Epidermis[d]	1655	1655	1520	1545

[a] The present study. [b] Ref. 29. [c] Ref. 21. [d] Ref. 30.

Finally we should make some remarks regarding the spectra of the polypeptide conformations that have been discussed above. For example, it has been observed that the dichroism of the 1630 cm.$^{-1}$ band of extended conformations is much higher than that of the 1650 cm.$^{-1}$ band of the α-helix. This is now explicable because the former band is due only to a perpendicular vibration whereas the latter is due to both a parallel vibration and a perpendicular vibration. Secondly, the frequency of the strong

(25) E. R. Blout and A. Asadourian, THIS JOURNAL, **78**, 955 (1956).

(26) It should be noted that the peak frequency is 1731 cm.$^{-1}$ if observed with the electric vector parallel to the helix axis whereas the peak frequency is 1727 cm.$^{-1}$ if observed with the electric vector perpendicular to the helix axis. Since all the ester groups in the α-helix are presumably equivalent, the frequency splitting is suggestive of a weak vibrational interaction among ester groups which are oriented in some regular manner with respect to the backbone polypeptide chain. It may be mentioned that the bands, 730 and 687 cm.$^{-1}$, due to the out-of-plane C–H bending modes of the benzene ring have appreciable perpendicular dichroism.[28] This indicates that the benzene rings in this polypeptide are oriented nearly parallel to the helix axis. Furthermore, many bands in the fingerprint region are highly dichroic. These observations suggest that the side groups of poly-γ-benzyl-L-glutamate are oriented in a regular manner around the helix axis.

parallel amide II band of extended conformations is not much different from that of the weak parallel amide II band of the α-helix. Therefore, the presence of a weak parallel band around 1520 cm.$^{-1}$ does not necessarily indicate the presence of a small amount of the extended conformation but rather may be due to an α-helical conformation. Lastly, it is now clear that the amide I and amide II frequencies of the conformations noted above may be explained primarily in terms of adjacent group interactions in the chain and through hydrogen bonds. The frequency difference of the amide I bands of the α-helix and extended conformations were discussed by Krimm,[27] but since at that time only one amide I band was recognized for each conformation, the significance of the vibrational interactions was not evident.

Direction of Amide II Transition Moment.—The intensity comparison of the two amide II bands of the α-helix allows estimation of the direction of the amide II transition moment with respect to the helix axis on the assumption that the total transition moment is the vector sum of the transition moments of all the groups involved. This assumption is considered to be appropriate for the amide II vibration of the α-helix since the interaction term through hydrogen bonds ($D_3 = +2$ cm.$^{-1}$) is almost negligible. Since the polypeptide chains of poly-γ-benzyl-L-glutamate are known to lie in the plane of oriented films[28] the intensity ratio of the perpendicular band and the parallel band is equal to $(1/2)\tan^2\theta$. Taking into account the band widths the intensity ratio is estimated to be 7–9 and θ is thus calculated to be 75–77°. This value does not agree too well with the value 89° calculated[7] using the atomic coördinates given by Pauling and Corey. In fact if the latter value should be correct, the parallel amide II band would be almost undetectable.

Direction of Amide I Transition Moment.—The parallel amide I band and the perpendicular amide I band of the α-helix overlap each other (*cf.* Fig. 4). However, the angle θ may be estimated from the apparent dichroic ratio if the error due to imperfect orientation of the helix axes is corrected. This correction may be carried out by referring to the apparent dichroism of the strong amide II band at 1546 cm.$^{-1}$. This band is due to the transition moment exactly perpendicular to the helix axis, and if the helix axes lie in the plane of the oriented film the apparent dichroic ratio will be equal to

$$R_\perp = \left(\int_0^{2\pi} g(\Psi)\cos^2\Psi d\Psi\right) \Big/ \left(\int_0^{2\pi} g(\Psi)\sin^2\Psi d\Psi\right) \quad (4)$$
$$= 1/a$$

where Ψ is the angle between the helix axis and the orientation direction and $g(\Psi)$ is the distribution function pertaining to the orientation of the helix axes. For a parallel band, however, the apparent dichroic ratio will be reversed and equal to $R_\parallel = a$. When the perpendicular band and parallel band overlap each other as in the case of the amide I bands of the α-helix the apparent dichroic ratio will be equal to

$$R = (aI_\parallel + I_\perp)/(I_\parallel + aI_\perp) \quad (5)$$

where $I_\parallel$ and $I_\perp$ are the intensities of the parallel band and the perpendicular band, respectively. The value of a in eq. 4 was found to be 0.1_0–0.1_5 from the apparent dichroic ratio of the perpendicular band at 1546 cm.$^{-1}$. The apparent dichroic ratio of the composite amide I band was observed to be 0.3_0–0.3_5, and by the use of eq. 5 the corrected intensity ratio $I_\perp/I_\parallel$ of the amide I bands was found to be 0.1_5–0.2_5. From this value the angle θ for the amide I vibration of the α-helix was estimated to be 29–34°. Although this value of θ is an approximate one because of the vibrational interaction through hydrogen bonds, it agrees quite well with the value[7] of 30° calculated from the atomic coordinates of Pauling and Corey.

Proteins

In discussing the infrared spectra of proteins it should be mentioned that the significance of vibrational interactions appears to have been unrecognized up to this time and thus a conformation has been correlated with only one amide I (or II) frequency. In the following section the infrared spectra of several proteins will be interpreted in the light of conclusions derived in the preceding sections.

Silk Gut (*Bombyx mori*).—Three amide I bands have been observed[29] at 1695, 1660 and 1634 cm.$^{-1}$; the perpendicular band at 1634 cm.$^{-1}$ has already been assigned to the extended conformation. The parallel band at 1695 cm.$^{-1}$ was once considered to be due to some combination band[5]; however, this is now assigned to the $\nu(O, \pi)$ vibration of the antiparallel-chain pleated sheet. The non-dichroic band at 1660 cm.$^{-1}$ was formerly considered to be due to the α-helix in the amorphous region; however, on the basis of the foregoing discussion of polypeptide spectra, this is more reasonably assigned to the random conformation.

Silk (*Anaphe moloneyi*).—Infrared spectra of *Anaphe moloneyi* silk (cast from trifluoroacetic acid and rolled) have been measured.[5] The two amide I bands, 1697 and 1630 cm.$^{-1}$, and the amide II band at 1527 cm.$^{-1}$ are now assigned to the antiparallel-chain pleated sheet. The band at 1650 and 1537 cm.$^{-1}$ may be assigned to the random conformation. The amide II band at 1552 cm.$^{-1}$ may be due to the $\nu(\pi, \pi)$ vibration of the antiparallel-chain pleated sheet.

Water-soluble Silk.—Water-soluble *Bombyx mori* silk (cast on mercury at 100°)[29] shows the amide I band at 1660 cm.$^{-1}$ and the amide II band at 1535 cm.$^{-1}$ at the same frequencies observed for synthetic polypeptides in the random coil conformation.

α-Keratin.—Infrared spectra of natural horsehair have been measured by Parker.[21] The frequencies and dichroism of the amide I and II bands (Table III) are similar to those of the α-helix of the poly-γ-benzyl-L-glutamate. Previously the parallel amide II band at 1520 cm.$^{-1}$ has been considered to be due to the extended conformation even though neither the perpendicular amide I band (1630 cm.$^{-1}$) nor the X-ray reflection due to the extended conformation were observed. The band at 1520 cm.$^{-1}$

(27) S. Krimm, *J. Chem. Phys.*, **23**, 1371 (1955); also see C. G. Cannon, *ibid.*, **24**, 491 (1956).

(28) Reference 14, p. 350.

(29) E. J. Ambrose and A. Elliott, *Proc. Roy. Soc.* (*London*), **A206**, 206 (1951).

is now more reasonably assigned to the parallel amide II vibration of the α-helix. A weak shoulder is observed around 1660 cm.$^{-1}$ which may be assigned to the random conformation in the amorphous region. Infrared spectra similar to those of horsehair have also been observed for elephant hair[29] and epidermis.[30]

Summary

(1) The amide I and II frequencies of the α-helix, the parallel-chain pleated sheet, the antiparallel-chain pleated sheet, and the random conformations of polypeptides have been explained in terms of vibrational interactions between adjacent peptide groups in the chain and through hydrogen bonds. (2) Parallel-chain pleated sheet conformations can be distinguished from antiparallel-chain pleated sheet conformations since the former exhibit the parallel amide I band at 1645 cm.$^{-1}$, while the latter show the band at 1695 cm.$^{-1}$. The perpendicular amide I band (*ca.* 1630 cm.$^{-1}$) and the parallel amide II band (*ca.* 1525 cm.$^{-1}$) are common to both these conformations. (3) Using the above criteria many extended polypeptide chains (except β-keratin) were found to be in the antiparallel-chain pleated sheet conformation. (4) The random coil conformation shows the amide I and II bands *ca.* 1660 and *ca.* 1535 cm.$^{-1}$, respectively. (5) Both the parallel and perpendicular amide I bands of the α-helix are observed at *ca.* 1650 cm.$^{-1}$ whereas the parallel and perpendicular amide II bands are observed at *ca.* 1520 and *ca.* 1550 cm.$^{-1}$, respectively. (6) The directions of the amide I and II transition moments of the α-helix of poly-γ-benzyl-L-glutamate were estimated to be inclined from the helix axis by 29–34° and 75–77°, respectively. (7) On the basis of the infrared spectra–conformation correlations described in this paper it has been possible to revise the interpretation of the amide I and II bands of several proteins.

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(30) K. M. Rudall in *Adv. Protein Chem.*, **7**, 281 (1952).

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

On the Peptides of L-Lysine[1,2]

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By carbobenzoxylation of N^{ϵ}-monobenzylidene-L-lysine (I) followed by acidification benzaldehyde is split off and pure α-monocarbobenzoxy-L-lysine (IIIa) is formed. By the action of dry hydrogen bromide dissolved in glacial acetic acid on N^{ϵ}-trityl-N^{α}-carbobenzoxy-L-lysine methyl ester only the carbobenzoxy group is eliminated and crystalline dihydrobromide of pure N^{ϵ}-trityl-L-lysine methyl ester (V) is formed; on treatment of N^{α},N^{ϵ}-ditrityl-L-lysine methyl ester with two equivalents of hydrogen chloride in methanol only the N^{α}-trityl group is split off and the crystalline dihydrochloride of the same N^{α}-trityl derivative V is formed. Compounds III and V are valuable intermediates for the preparation of different types of lysine peptides, III for the synthesis of ϵ-peptides and V for the synthesis of α-as well as mixed α,ϵ-peptides of lysine. Several such peptides were prepared in this way.

Introduction

It has been demonstrated that in some natural products, as in biocytin[3] and bacitracin,[4,5] the ϵ-amino group of L-lysine participates in the formation of an amide bond. However, until recently, no evidence was available for such a peptide linkage in proteins[6] involving the ϵ-amino group of L-lysine. The isolation of N^{ϵ}-(glycyl-α-glutamyl)-L-lysine after partial hydrolysis of collagen[7] suggests that, at least in some proteins, L-lysine may serve as a branching point of the polypeptide chain. For the elucidation of this unusual facet of protein structure and for the synthesis of bacitracin analogs, the availability of α- and ϵ-peptides, as well as of mixed α,ϵ-peptides of L-lysine would be very useful.

The preparation of α-peptides of L-lysine starting with N^{ϵ}-carbobenzoxy-L-lysine[8a] or N^{ϵ}-formyl-L-lysine[8b] presents no difficulty. ϵ-Carbobenzoxy-L-lysine was first prepared by selective decarbobenzoxylation[8a] of dicarbobenzoxy-L-lysine, and subsequently by direct carbobenzoxylation of the L-lysine copper complex.[9] The latter method of protection of the α-amino group served in a few cases for the introduction of amino acid residues directly at the ϵ-position.[10,11] A rather tedious way for the synthesis of ϵ-peptides is through the use of N^{α}-tosyl-L-lysine as starting material,[7,12] prepared in turn from N^{ϵ}-carbobenzoxy-L-lysine. After the

(1) A summary of this paper was presented at the 2nd European Peptide Symposium, Munich, Ger., September, 1959. Abstracted in part from the doctoral dissertation of Mrs. B. Bezas, Faculty of Natural Sciences (Chemistry Section), University of Athens, Greece, May, 1960.

(2) This investigation was supported by a grant from the Royal Helenic Research Foundation.

(3) L. D. Wright, L. G. Gresson, R. H. Skeggs, R. T. Wood, R. L. Peck, D. E. Wolf and K. Folkers, *J. Am. Chem. Soc.*, **72**, 1048 (1950), **74**, 1996 (1952).

(4) L. C. Craig, W. Hausmann and J. R. Weisiger, *ibid.*, **77**, 723 (1955); *J. Biol. Chem.*, **199**, 865 (1952).

(5) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **53**, 604 (1953); I. M. Lockhart and E. P. Abraham, *ibid.*, **62**, 645 (1956).

(6) P. Desnuelle in H. Neurath and K. Bailey, "The Proteins," Vol. I, Academic Press, Inc., New York, N. Y., 1953, p. 134.

(7) G. L. Mechanic and M. Levy, *J. Am. Chem. Soc.*, **81**, 1889 (1959).

(8) (a) M. Bergmann, L. Zervas and W. F. Ross, *J. Biol. Chem.*, **111**, 245 (1935); (b) K. Hoffmann, E. Stutz, G. Spuhler, H. Yajima and E. T. Schwarz, *J. Am. Chem. Soc.*, **82**, 3727 (1960).

(9) F. Sanger and A. Neuberger, *Biochem. J.*, **37**, 515 (1943).

(10) D. Theodoropoulos, *J. Org. Chem.*, **23**, 140 (1958).

(11) D. L. Swallow and E. P. Abraham, *Biochem. J.*, **70**, 359 (1958).

(12) D. Theodoropoulos and L. C. Craig, *J. Org. Chem.*, **21**, 1376 (1956).