TABLE I

τ VALUES OF OLEFINIC PROTON IN *trans* AND *cis* (WITH RESPECT TO $-CH_3$ AND $-CO_2R$) TRISUBSTITUTED OLEFINIC ACIDS AND ESTERS[a]

CH_3-C-H ‖ $X-C-CO_2R$	*trans*	CH_3-C-H ‖ RO_2C-C-X	*cis*
CH_3-C-H ‖ CH_3-C-CO_2H	3.03	CH_3-C-H ‖ $HO_2C-C-CH_3$	3.82
CH_3-C-H ‖ CH_3-C-CO_2Me	3.28	CH_3-C-H ‖ $MeO_2C\ C-CH_3$	4.03
CH_3-C-H[b] ‖ $C_2H_5-C-CO_2H$	3.00	CH_3-C-H[b] ‖ $HO_2C-C-C_2H_5$	3.82
CH_3-C-H[c] ‖ $(CH_3)_2CH-C-CO_2Me$	3.3	CH_3-C-H[d] ‖ $HO_2C-C-CH_2-C(=CH_2)-CO_2H$	3.70
CH_3-C-H[e] ‖ $MeO_2C-CH_2-C-CO_2Me$	3.2	CH_3-C-H ‖ $MeO_2C-C-CH_2-C(=CH_2)-CO_2Me$	3.95
Dimethyl integerrinecate	3.24	Dimethyl senecate	3.99
		Dimethyl riddellate	3.96
		Dimethyl seneciphyllate	3.90

[a] All spectra were determined on a Varian high resolution spectrometer at 60 Mc. per second. We are thankful to Mr. T. Van Auken for providing the spectra of tiglic and angelic acids and their esters (run in CCl_4). All other spectra were obtained in $CDCl_3$ using tetramethylsilane as an internal standard. Values are expressed in parts per million on the "τ scale" as defined by Tiers (*J. Phys. Chem.*, **62**, 1151 (1958)). The peaks due to the single proton appeared in all case as quartets and the values are reported for the center of each quartet. All the compounds studied except α-ethylisocrotonic acid were isomerically pure. The sample of α-ethylisocrotonic acid available was a mixture of *cis* and *trans* compounds and consequently had quartets at 3.00 and 3.82 corresponding to *cis* and *trans* protons. We are grateful to Professor T. A. Geissman for a gift of senecic acid and to the A. P. Sloan Foundation for a grant which made this investigation possible. [b] E. Blaise and P. Bagard, *Ann. Chim.*, 11, 111 (1907). [c] R. Adams and B. L. Van Duuren, THIS JOURNAL, **74**, 5349 (1952). [d] Ref. 1. [e] M. C. Kloetzel, *ibid.*, **70,** 3571 (1948).

shift occurs in the τ value of the olefinic proton on changing the substituent X from $-CH_3$ to $-C_2H_5$ to $-CH(CH_3)_2$, etc. (4) The close agreement bebetween the values of the olefinic proton in methyl tiglate, methyl isopropylcrotonate, dimethyl ethylidenesuccinate and dimethyl integerrinecate indicates that in all these cases the proton is oriented *cis* to the carbomethoxyl groups. Similarly, the values for methyl angelate, dimethyl senecate, dimethyl α-methylene-α'-ethylideneglutarate (obtained from riddellic acid), dimethyl riddellate, and dimethyl seneciphyllate all have the same arrangement of groups about the double bond. (5) A small shift of the τ value of the olefin proton toward higher field side is observed in the ester of an acid.[8]

The sensitivity of this correlation of the *cis* and *trans* isomers is apparent by comparing the difference of the values of the ethylidenic protons in the *cis–trans* pair dimethyl senecate and dimethyl integerrinecate with the differences of the values of the pairs methyl angelate and methyl tiglate, and α-ethylcrotonic acid and α-ethylisocrotonic acid. Thus orientation of groups about the olefin linkage in the necic acids, assigned on the basis of ultraviolet spectra and melting points, is confirmed.

The shielding values of the methyl protons also could be used[4] for determining the stereochemistry of the double bond, but the single proton values are to be preferred due to their larger sensitivity to environmental changes. Between the *cis* and *trans* compounds the shift in the methyl proton values is about 0.2 p.p.m. whereas the shift in the olefinic proton values is about 0.8 p.p.m. There is some evidence also that n.m.r. spectroscopy provides a valuable tool for determining the compositions of mixtures of *cis* and *trans* trisubstituted olefinic acids.

(8) N. J. Leonard in "The Alkaloids," edited by R. H. F. Manske, Vol. VI, Chapter 3, Academic Press, New York, N. Y., 1960.

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THE DEPENDENCE OF THE CONFORMATIONS OF SYNTHETIC POLYPEPTIDES ON AMINO ACID COMPOSITION[1,2]

Sir:

Several years ago the α helix[3] was demonstrated by X-ray diffraction in a few synthetic polypeptides[4,5,6] and recently has been shown to be a basic

(1) This is Polypeptides. XXX. For the preceding paper in this series see E. Katchalski, G. D. Fasman, E. Simons, E. R. Blout, F. R. N. Gurd and W. L. Koltun, *Arch. Biochem. Biophys.*, **88**, 361 (1960). Alternate address for E. R. Blout, Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Massachusetts.

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

(3) L. Pauling, R. B. Corey and H. R. Branson, *Proc. Natl. Acad. Sci., U. S.*, **37**, 205 (1951), et seq.

(4) C. H. Bamford, W. E. Hanby and F. Happey, *Proc. Roy. Soc. (London)*, **206A**, 30 (1951); C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby and I. F. Trotter, *ibid.*, **141B**, 49 (1953); C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby and I. F. Trotter, *Nature*, **173**, 27 (1954).

(5) M. F. Perutz, *ibid.*, **167**, 1053 (1951).

(6) H. L. Yakel, *Acta Cryst.*, **6**, 724 (1953).

TABLE I

MOLECULAR AND INFRARED SPECTRAL PROPERTIES OF VARIOUS SYNTHETIC POLYPEPTIDES

Polypeptide	η_{sp}/c [a]	Estimated [b] DP$_w$	Estimated [b] MW$_w$	Amide I [c] Frequency (cm.$^{-1}$)	Amide I [c] Dichroism	Amide II [c] Frequency (cm.$^{-1}$)	Amide II [c] Dichroism	Structure [c]
Poly-L-valine	0.29	190	19,000	1638	⊥	1545	‖	β
Poly-L-leucine	.11	35	3,900	1650	‖	1543	⊥	α
Poly-S-methyl-L-cysteine	.24	140	16,000	1632	⊥	1540, 1525	‖	β
Poly-S-benzyl-L-cysteine	.49	365	77,000	1632	⊥	1524	‖	β
Poly-L-methionine	.22	135	18,000	1648	‖	1540	⊥	α
Poly-O-acetyl-L-serine	.14	130	16,800	1637	⊥	1520	‖	β
Poly-L-serine	.10 [d]	130	11,300	1653	..	1525–1535	..	Random
Poly-β-benzyl-L-aspartate	.30	190	39,000	1668	‖	1563	⊥	α
Poly-γ-benzyl-L-glutamate	.15	100	22,000	1645	‖	1542	⊥	α

[a] The reduced specific viscosities were determined at a concentration of 0.2% in either dichloroacetic acid or trifluoroacetic acid to assure that the polypeptides were in the random form. [b] The weight average degrees of polymerization (DP$_w$) and the weight average molecular weights (MW$_w$) were estimated from the correlation of the viscosities of the random form of poly-γ-benzyl-L-glutamate with light scattering and sedimentation measurements (see P. Doty, J. H. Bradbury and A. M. Holtzer, THIS JOURNAL, **78**, 947 (1956); J. C. Mitchell, A. E. Woodward and P. Doty, *ibid.*, **79**, 3955 (1957)). [c] Of oriented films. [d] Intrinsic viscosity in water.

structural unit of certain proteins.[7] Infrared dichroism also has been used to identify the α-helical conformation and to differentiate between it and extended intermolecularly H-bonded (β) structures.[8,9] We now wish to report the infrared results of recent work with synthetic polypeptides derived from several naturally occurring α-amino acids.[10] The data indicate that the conformation of each of these homopolypeptides is intrinsically dependent on its amino acid composition and, in particular, several non-α-helix-forming polypeptides are described.

Infrared spectra of oriented films were examined, and by determining the location and dichroism of the amide I (C=O stretching) frequencies and amide II (NH deformation coupled with CN stretching) frequencies assignments of the conformations of these polypeptides were made. The data are shown in the table. It should be noted that the conformation does not appear to depend on the molecular weight in the samples examined, since some high molecular weight polypeptides are α-helical while others of the same or higher degree of polymerization show only β or random conformations in the solid state.

On the basis of the results presented here we believe that there are two classes of α-amino acids, those which form helical (α) structures and those which form either random or β structures. The non-helix-forming polypeptides are of two types—first, those which are di-substituted by other than hydrogen on the β-carbon atom; second, those which have a heteroatom (oxygen or sulfur) attached directly to the β-carbon atom. The first type is exemplified by poly-L-valine. The fact that this polypeptide does not form an α-helical structure can be explained on steric grounds since molecular models of a right-handed α-helical conformation of this polyamino acid show marked steric interference of the side chains. It is important to note that poly-L-leucine (a homolog of poly-L-valine with one additional methylene group) has an α-helical conformation. Molecular models of poly-L-leucine indicate much less side chain to side chain steric interference in the α-helical structure.

The second class of non-helix-forming polypeptides is exemplified by those derived from L-cysteine and L-serine. From the data in the table it is apparent that these polypeptides in the oriented solid state exist in the β conformation. In these cases it is not possible to attribute the lack of formation of a helical structure to steric factors. It appears that with these materials the β form is the thermodynamically stable state. It is interesting that the next higher homolog of poly-S-methyl-L-cysteine, namely, poly-L-methionine, does form an α structure. As has been noted previously,[11] both the esters of poly-α,L-glutamic acid and the esters of its des-methylene homolog, poly-α,L-aspartic acid, form α structures.

Poly-O-acetyl-L-serine exists in a β conformation, but the water soluble poly-L-serine, derived from it, does not. We have been unable as yet to obtain an oriented specimen of poly-L-serine[12] and suggest that intramolecular hydrogen bonding between the amide group and the hydroxyl in the side chain may be sufficient to decrease the tendency toward the formation of either α helices or β structures so that this material exists in a random conformation.

Finally, it seems probable that the effects described here on the relationship between amino acid composition and polypeptide structure also are operative in protein structures. When a sequence of β or random-forming amino acids is contiguous to an α-helical section of a protein chain this may be the site where a loop or reversal of direction can occur. In addition, the presence of large amounts of β- or random-forming amino acids may explain the relatively low helix content postulated for certain globular proteins.[13] This

(7) J. C. Kendrew, R. E. Dickerson, B. E. Strandberg, R. C. Hart, D. R. Davies, D. C. Phillips and V. C. Shore, *Nature*, **185**, 422 (1960).

(8) E. J. Ambrose and A. Elliott, *Proc. Roy. Soc. (London)*, **A205**, 47 (1951); C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press Inc., New York, N. Y., 1956.

(9) In this paper we do not distinguish between the various extended conformations.

(10) We exclude from this discussion polypeptides derived from imino acids since α-helical structures are not possible with such substances.

(11) E. R. Blout and R. H. Karlson, THIS JOURNAL, **80**, 1259 (1958); E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature*, **183**, 1736 (1959); R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, THIS JOURNAL, **82**, 2268 (1960).

(12) G. D. Fasman and E. R. Blout, *ibid.*, **82**, 2262 (1960).

(13) P. Doty, "Proceedings of the Fourth International Congress of Biochemistry, Vienna," Pergamon Press, London, 1959, Vol. IX, p. 8.

suggestion is supported by preliminary data obtained with copolypeptides of helix-forming and β-forming amino acids.[14]

(14) R. K. Kulkarni, G. D. Fasman and E. R. Blout, to be published.

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THE NATURE OF THE SPECTRAL CHANGES ACCOMPANYING THE DEACYLATION OF MONOACETYL-α-CHYMOTRYPSIN[1]

Sir:

Evidence was presented[2] which indicated that two stable monoacetyl-α-chymotrypsin intermediates,[3] AC-I and AC-A, existed in the α-chymotrypsin catalyzed hydrolysis of *p*-nitrophenyl acetate and that AC-I was the direct intermediate in the catalytic reaction. Spectroscopic studies did not reveal acetyl-imidazolyl as intermediate in the deacylation of AC-I.[2] However, acetyl-imidazolyl has been implicated in the deacylation of monoacetyl-δ-chymotrypsin.[4] The difference spectrum of this acetyl enzyme at *p*H 8.9, where it deacylates, *versus* *p*H 3.5, showed a rapid increase in absorption at 245 mμ and a spectral peak with a maximum near 245 mμ.[4] It was noted[4] that the position of the peak and the rate of decrease in absorbancy are characteristic of acetyl-imidazole and its rate of hydrolysis. The nature of these spectral changes is an important consideration in attempts to understand chymotrypsin catalyzed reactions[5] and is the subject of this communication.

The difference spectra of AC-A, AC-I, monoacetyl-δ-chymotrypsin and, *significantly*, *α-chymotrypsin* at *p*H 9.0 *versus* *p*H 3.5 all show a maximum at approximately 245 mμ (Fig. 1). The *p*H dependence of ΔD at 245 mμ indicates that the principal component of these difference spectra is due to tyrosyl ionization. The difference spectrum of N-acetyl-L-tyrosine ethyl ester at *p*H 9.0 *versus* *p*H 3.5 is shown in Fig. 1. This spectrum, modified through incorporation of tyrosine into a protein and by *p*H dependent electrostatic effects on other chromophores, could easily explain the *p*H difference spectra observed with monoacetyl-chymotrypsin and chymotrypsin.

An absorption peak near 245 mμ is not observed in a difference spectrum of AC-A *versus* deacylated AC-A at a *p*H where AC-A is known to deacylate, providing that the *p*H and ionic strength of the solutions are the same (Fig. 1 inset). In these experiments an increase followed by a slow decrease in absorbancy near 245 mμ is observed with AC-A

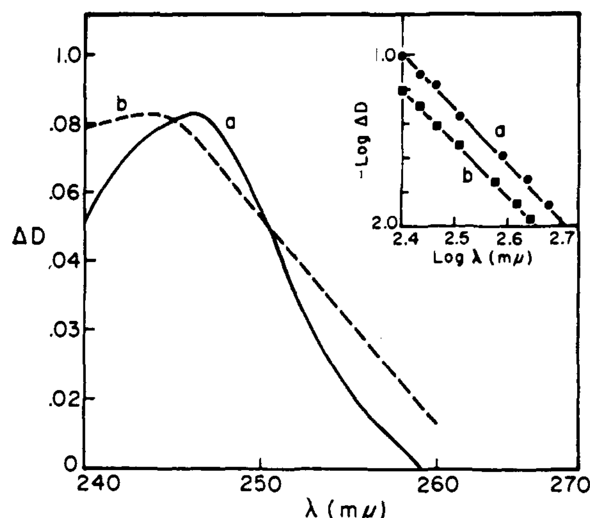

Fig. 1.—Ultraviolet *p*H difference spectra, *p*H 9.0 (0.033 *M* tris-(hydroxymethyl)-aminomethane-HCl buffer) *versus* *p*H 3.5 (HCl): curve a, α-chymotrypsin (6×10^{-5} *M*); curve b, N-acetyl-L-tyrosine ethyl ester (2×10^{-5} *M*). INSET: Ultraviolet and visible difference spectra of AC-A (monoacetyl-α-chymotrypsin) *versus* deacylated enzyme, *p*H 6.3 (0.05 *M* tris-(hydroxymethyl)-aminomethane-HCl, 0.05 *M* $CaCl_2$), 25 ± 0.5°; curve a, 1 minute after adjustment of *p*H of AC-A solution from *p*H 3.5 (HCl) to *p*H 6.3; curve b, 15 minutes after adjustment of AC-A solution to *p*H 6.3. All experiments were performed in presence of 0.05 *M* $CaCl_2$ to prevent the spectral changes at 245 mμ observed by Spencer and Sturtevant[5] with α-chymotrypsin. A Cary Model 14 self-recording spectrophotometer was used for these studies. Three times crystallized α-chymotrypsin, purchased from Worthington Biochemicals, was used. Enzyme concentrations were determined spectrophotometrically at 280 mμ by using $E_{1\%}^{280} = 20.0$ to relate extinction to enzyme concentration.[10] A molecular weight of 25,000 was assumed for α-chymotrypsin.

and monoacetyl-δ-chymotrypsin[4] (but not with AC-I).[2] However, the absorbancy changes are not only observed at 245 mμ but also in the visible region where chymotrypsin is known not to absorb[6] (Fig. 1 inset). It was observed[7,8] that absorbancy in difference spectra at wave length regions where the protein does not absorb can result from Rayleigh light scattering due to differences in molecular aggregation. In the visible region a log–log plot ΔD *versus* λ is linear, and extrapolation of this linear portion into the region of absorption allows correction of the scattering contribution.[8] The linearity of the plot in Fig. 1 (inset) indicates that the absorbancy at 245 mμ accompanying the deacylation of AC-A is due to light scattering. Accordingly, the rate of change in absorbancy is the same at all wave lengths between 240 mμ and 500 mμ in the *p*H range studied (*p*H 5.5–9.0) (Fig. 1 inset). It should be noted that both the association-dissociation of chymotrypsin[9]

(1) This work was aided by grants from the National Science Foundation, and the Office of Naval Research.

(2) G. P. Hess and M. A. Marini, *4th Intern. Congr. Biochem.*, p. 42, Vienna, (1958); M. A. Marini and G. P. Hess, THIS JOURNAL, **81**, 2594 (1959); *ibid.*, in press (1960); *Nature*, **184**, 113 (1959).

(3) A. K. Balls and H. N. Wood, *J. Biol. Chem.*, **219**, 245 (1956).

(4) G. H. Dixon and H. Neurath, THIS JOURNAL, **79**, 4558 (1957).

(5) G. H. Dixon, H. Neurath and J. F. Pechère, *Ann. Rev. Biochem.*, **27**, 489 (1958); A. Tinoco, Jr., *Archiv. Biochem. Biophys.*, **76**, 148 (1958); T. Spencer and J. M. Sturtevant, THIS JOURNAL, **81**, 1874 (1959); H. Gutfreund and B. R. Hammond, *Biochem. J.*, **73**, 526 (1959).

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