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Optical Rotatory Dispersion Studies of Poly-L-tyrosine and Copolymers of L-Glutamic Acid and L-Tyrosine. Significance of the Tyrosyl Cotton Effects with Respect to Protein Conformation*

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The synthesis of high-molecular-weight (60,000–125,000) poly-L-tyrosine and copolymers of L-tyrosine and L-glutamic acid (random and block) is reported. Poly-L-tyrosine can exist in either a helical or a random-chain conformation in the un-ionized form. The optical rotatory dispersion (ORD) of helical poly-L-tyrosine (in 0.2 M NaCl, *p*H 11.2) over the wavelength range 500–227 mμ is recorded. Multiple Cotton effects are observed with peaks at 286 mμ, $[m']_{286} = 2650°$, and 254 mμ, $[m']_{254} = 4240°$, and a trough at 238 mμ, $[m']_{238} = -6410°$. Upon ionization of the phenolic hydroxyls of poly-L-tyrosine the positive peak of the Cotton effect at 286 mμ vanishes, the peak at 254 mμ diminishes, and simultaneously the trough at 238 mμ decreases. This probably represents a helix → random-coil transition. The b_o changes from +570 to +413 during this structural change. Ultraviolet-absorption spectra of the helical polypeptide indicate that the pK_a of the tyrosyl hydroxyl is much higher than that of the monomer, and a red shift of 2 mμ ($\lambda_{\max} = 277$ mμ) is observed. The ORD and ultraviolet spectra suggest that tyrosyl-tyrosyl interactions occur in the helical conformation. ORD and ultraviolet spectra are also reported for films of poly-L-tyrosine. The negative Cotton effect (trough, 238 mμ) further suggests that poly-L-tyrosine is a right-handed helix. The tyrosyl Cotton effect (peak, 286 mμ) is not observed in copolymers of L-tyrosine and L-glutamic acid until 20 mole % tyrosine is present. Although this Cotton effect is therefore unlikely to be observed in proteins, the b_0 and $[m']_{233}$ values can be significantly altered and the interpretation of such values, for proteins with high tyrosine contents, may be complex.

Optical rotatory dispersion (ORD)[1] measurements have become extensively used to determine the conformation of proteins (see Urnes and Doty, 1961; Blout, 1960; Fasman, 1963). The anomalous rotatory dispersion curves for many synthetic poly-α-amino acids and proteins in the helical conformation have been adequately interpreted by the equation proposed by Moffitt (Moffitt, 1956; Moffitt and Yang, 1956), where mrw = mean residue weight.

$$[m'] = \frac{\text{mrw}}{100} \cdot \frac{3}{n^2 + 2} \cdot [\alpha] = a_o \frac{\lambda_o^2}{\lambda^2 - \lambda_o^2} + b_o \left[\frac{\lambda_o^2}{\lambda^2 - \lambda_o^2}\right]^2$$

The constant, b_o, in this equation has been used as a measure of the helical content in polypeptides and proteins (Cohen and Szent-Gyorgyi, 1957; Blout and Karlson, 1958; Doty, 1960). The sign of b_o has been taken to signify the sense of helix. The value for b_o of approximately −630 has been found for the right-handed helical conformation of many synthetic poly-α-amino acids (Moffitt, 1956; Moffitt and Yang, 1956; also see Urnes and Doty, 1961) and several proteins, assumed to be completely helical (e.g., tropomyosin) (Cohen and Szent-Gyorgyi, 1957; Kay and Bailey, 1959).

Positive b_o values have been reported for several poly-α-amino acids; poly-L-tyrosine (Elliott *et al.*, 1957; Downie *et al.*, 1959; Coombes *et al.*, 1960; Katchalski, 1959; Fasman, 1962), poly-β-benzyl-L-aspartate (Blout and Karlson, 1958; Karlson *et al.*, 1960; Bradbury *et al.*, 1960), poly-*N*-benzyl-L-histidine (Norland *et al.*, 1963) and poly-L-tryptophan (Sela *et al.*, 1961). Left-handed helices of either L- or D-amino acids would be expected to have positive b_o values. Poly-β-benzyl-L-aspartate (b_o = +630) was shown to exist as a left-handed helix (Karlson *et al.*,

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[1] Abbreviation used in this work: ORD, optical rotatory dispersion.

TABLE I
POLYMERS AND COPOLYMERS OF L-GLUTAMIC ACID AND L-TYROSINE[a]

Sample No.	Mole Ratio[b] of *N*-Carboxyanhydrides, Glu:Tyr	$\eta(sp/c)$[c]	$[\eta]^{0.2\ M\ NaCl}_{pH\ 12.0}$[d]	mw_w[e]	dp_w[e]
6-204-11	9:1[f]	1.14	0.79	70,000	530
6-209-25	8:2[f]	1.39	0.73	67,000	490
10-161-25	95:5[g,h]	0.72	0.69 1.07[i]	60,000	460
11-248-19	47.5:5:47.5[g]	0.9	0.78 1.27[i]	69,000	530
11-429	0:100[j]	2.06	1.14	126,000	780
9-177	0:100		0.57[k]	62,000	380
32	100:0		1.31[i]	115,000	890

[a] Polymerization in benzene, conc = 1%, initiated with $NaOCH_3$, 0.366 N. [b] γ-Benzyl-L-glutamate-*N*-carboxyanhydride–*O*-acetyl-L-tyrosine-*N*-carboxyanhydride or *O*-carbobenzyloxy-L-tyrosine-*N*-carboxyanhydride, as indicated. [c] Specific viscosity before removal of blocking groups in 0.2% dichloroacetic acid. [d] Intrinsic viscosity of unblocked polymers. [e] Estimated from the molecular weight calibration of Idelson and Blout (1958b). [f] *O*-Acetyl-L-tyrosine-*N*-carboxyanhydride, *A/I* 200. [g] *O*-Carbobenzyloxy-L-tyrosine-*N*-carboxyanhydride, *A/I* 50. [h] This composition was found by use of the Spinco amino acid analyzer. [i] At *p*H 7.0. [j] 4% dioxane, *A/I* = 200. [k] At *p*H 10.5.

1960; Bradbury *et al.*, 1960). Despite the positive b_o value found for poly-L-tyrosine, it has been demonstrated, although not unambiguously, that this polypeptide is a right-handed helix (Fasman, 1962). This anomalous behavior has been attributed to the contribution of the chromophoric side chains on the β-carbon (Elliott *et al.*, 1957; Coombes *et al.*, 1960), wherein the primary helical arrangement of the peptide bonds necessarily imposes a secondary helical arrangement on the chromophoric aromatic residues, allowing for new tyrosyl-tyrosyl interactions between residues one helix turn apart, which presumably have such a large contribution to the ORD that the peptide contribution is obscured (see also Goodman *et al.*, 1963, for side-chain-chromophore effects).

It can readily be appreciated that the presence of such chromophores might greatly alter the interpretation of b_o values observed in proteins. However if such ORD studies could be extended into the near and far ultraviolet (all above-mentioned studies were performed at wavelengths greater than 365 mμ) it might be possible to observe several phenomena which would help clarify the influence of such chromophores on the ORD of polypeptides. These are: (1) As one of the main absorption bands of tyrosine lies at 275 mμ, it might be expected that a Cotton effect (see Kuhn, 1958; Urnes and Doty, 1961; Djerassi, 1960) would be found in the region of this absorption band in tyrosine-containing polypeptides if this chromophore were situated in a disymmetric environment, i.e., a helical conformation, which would vanish in the random-chain structure. (2) The observation of the Cotton effects, due to the peptide chromophore transitions, at 233 mμ ($n \rightarrow \pi^*$) and 190 mμ ($\pi \rightarrow \pi^*$) (Simmons *et al.*, 1961; Blout *et al.*, 1962, 1963) would further clarify the helical sense if such Cotton effects were free of any tyrosine chromophoric contributions.

This paper reports such studies, namely the Cotton effects of poly-L-tyrosine and copolymers of L-tyrosine and L-glutamic acid. The latter studies were performed to investigate the problem of how large a mole % of tyrosine in a polypeptide is necessary to be detectable by ORD and whether this might possibly be an observable phenomenon in proteins. This was investigated, since Simmons and Blout (1960) have suggested this as a possibility, while Urnes and Doty (1961) have doubted that such phenomenon would be observable in proteins. A preliminary report of this work has been presented (Fasman *et al.*, 1962).

Synthesis.—High-molecular-weight poly-L-tyrosine used in this study was prepared via two methods: (1) The polymerization of *O*-carbobenzyloxy-*N*-carboxy-L-tyrosine anhydride (Katchalski and Sela, 1953, 1958) in benzene with sodium methoxide as initiator (Blout and Karlson, 1956) with subsequent removal of the *O*-carbobenzyloxy group by the HCl-HBr method (Fasman *et al.*, 1961); (2) the polymerization of *O*-acetyl-*N*-carboxy-L-tyrosine anhydride (Bailey, 1950; see Katchalski and Sela, 1958, for review of *N*-carboxy anhydride preparations) in dioxane with sodium methoxide as initiator and subsequent removal of the *O*-acetyl group with equimolar amounts of sodium methoxide. Poly-L-tyrosine derived from the *O*-carbobenzyloxy derivative was used in the majority of the experiments in this report. Polymers with weight-average molecular weights estimated to be approximately 60,000–125,000 were obtained (see Table I). The conformation of the polymer obtained from the synthesis is probably dependent on the solvent and the method of unblocking. In benzene the interaction of the tyrosyl residues is minimal, and upon addition of a strong acid the hydrogen bonding of the backbone is disrupted and the polymer assumes a random structure. The aromatic residues consequently are entangled in various stereo positions and the polypeptide remains as a random coil upon isolation. It can be converted to the helical structure as follows:

The polymer was dialyzed in the sodium-salt-soluble form, versus 0.01 N HCl at 40° to obtain a material which gave high positive b_o values (~ +600). Unless this was done low b_o values were often obtained.

The *raison d'etre* for this treatment is as follows: If a strong acid is added to the sodium salt of the polymer (ionized phenol—a random coil) the polymer becomes un-ionized, but this occurs so rapidly that, as the aromatic residues do not have the rotational energy at room temperature or sufficient time to align in a stacked array necessary for the helical conformation, the polymer retains the random structure. The aromatic residues consequently became entangled in various stereo positions. However, when the polymer in the random ionized form is converted to the acid form slowly by dialysis against an acidic solution at an elevated temperature, the polymer becomes helical. This occurs because the phenolic residues slowly become un-ionized at an elevated temperature where they have sufficient rotational energy and time to slowly align themselves into the helical conformation. When solutions are then made of the polymer it is important to raise the *p*H slowly to the desired value, as once the solution is made too basic and the polymer is random, one cannot simply back-titrate with acid and obtain the helical form. This behavior of poly-L-tyrosine probably accounts for the great variety of b_o values reported in the literature, varying from +300 to +600. It has also recently been found that samples yielding low b_o values (100) in dimethylformamide can be converted to the helical conformation ($b_o = 450$) by heating the solution and then cooling (Lehrer and Fasman, 1964). The helical conformation in these solutions was also detected by the presence of an excimer band in the fluorescence-emission spectra.

The random copolymers, copoly-L-glutamic acid:L-tyrosine were prepared by mixing the appropriate *O*-carbobenzyloxy-*N*-carboxy-L-tyrosine anhydride or *O*-acetyl-*N*-carboxy-L-tyrosine anhydride and γ-benzyl-*N*-carboxy-L-glutamate anhydride (Blout and Karlson, 1956) in the desired ratio in benzene and initiating the polymerization with sodium methoxide (see Table I). The γ-benzyl and *O*-carbobenzyloxy groups were removed by the HCl-HBr treatment and the *O*-acetyl group in aqueous solution at *p*H 11.5 (see Materials and Methods). The block copolymer,[2] copoly-(L-glutamic acid)-(L-tyrosine)-(L-glutamic acid), 47.5:5:47.5, was prepared by initiating the γ-benzyl-*N*-carboxy-L-glutamate anhydride with sodium methoxide, allowing the polymerization to proceed for 24 hours until complete, then adding the *O*-carbobenzyloxy-*N*-carboxy-L-tyrosine anhydride and allowing this to add on to the chain for 24 hours, and finally adding γ-benzyl-*N*-carboxy-L-glutamate anhydride and allowing this to add on terminally (Gratzer and Doty, 1963). The polymer was unblocked by the HCl-HBr treatment. This produced a polymer which sandwiched a tyrosine block between two glutamic acid blocks. All polymers used were in the mw range of 60,000–125,000.

MATERIALS AND METHODS

All melting points are corrected. L-Tyrosine was obtained from Nutritional Biochemicals Corp. Distilled water was used throughout. Dioxane was purified by the Fieser (1941) procedure; dimethyl sulfoxide was obtained from Crown Zellerbach and purified by distillation from KOH under vacuum (2–3 mm Hg), bp 38°.

Optical Rotatory Dispersion (ORD).—Measurements of the optical rotation were made with a Bendix-Ericsson Polarimetric 62[3] automatic recording spectropolarimeter. This instrument is estimated to read ±0.0002° in the wavelength range 600–185 mμ. The recorder attachment was a Sargent recorder Model SR. The dispersions were performed with a slit of 1 mm at both entrance and exit, and fused-quartz cells of 0.1 and 0.01 dm length, selected for minimal birefringence, were used.[4] The ORD data are expressed in terms of [*m*′] (see Urnes and Doty, 1961; Fasman, 1963), the reduced mean residue rotation defined as $[m']_\lambda = [\alpha]_\lambda \times (\text{mrw}/100) \times [3/(n^2 + 2)]$, where $[\alpha]_\lambda$ is the specific rotation at wavelength λ, mrw is the mean residue weight, and *n* is the refractive index of the solvent at wavelength λ. The refractive indices as a function of wavelength of the solvents were obtained from those tabulated by Fasman (1963). To obtain b_o values the dispersion data were plotted according to the Moffitt equation (Moffitt, 1956), $[m'] = [a_o\lambda_o^2/(\lambda^2 - \lambda_o^2)] + [b_o\lambda_o^4/(\lambda^2 - \lambda_o^2)^2]$; plotting $[m'](\lambda^2 - \lambda_o^2)$ versus $1/(\lambda^2 - \lambda_o^2)$. The b_o was derived from the slope usually using $\lambda_o = 2120$ A (Moffitt and Yang, 1956). All rotations were measured at 24°.

Preparations of Solutions for ORD Measurements.—The polymer was weighed out in a 10-cc volumetric flask (e.g., 10 mg), approximately 6 cc of H_2O or 0.2 M NaCl were added. Sodium hydroxide (0.1 N) was added dropwise with stirring until all the polymer was dissolved (*p*H 11.2). The *p*H was then adjusted by slowly adding 0.1 N or 0.01 N NaOH to the desired *p*H and bulking up to volume.

The *p*H of the solution was measured with a Beckman *p*H meter, Model G, by means of a drop electrode, Beckman No. 40316 glass, No. 39270 reference.

The concentrations used were: 0.2 or 0.1% in a 1-cm cell for the wavelength range 556–333 mμ; 0.1% in a 1-mm cell for the wavelength range 400–262 mμ; 0.02% in a 1-mm cell for the wavelength range 286–227 mμ.

Films.—The films were prepared by placing a drop of a dimethyl sulfoxide solution of the polymer on the outside optical surface of a 1-mm quartz cell and rotating slowly in a vacuum desiccator until dry.

Absorbance Measurements.—Optical-density measurements were made on a Cary Model 14 recording spectrophotometer, with the same cells as used for the ORD measurements.

Viscometry.—Intrinsic viscosities, [η], were determined in Ubbelohde viscometers that had bulbs large enough to make a 10-fold dilution. Reduced specific viscosities were determined in Cannon-Ostwald-Fenske viscometers. Viscometers were chosen so that the flow time of the solvent was not less than 90 seconds. All measurements were made at 25 ● 0.1°.

*O-Carbobenzyloxy-N-carboxy-*L*-tyrosine Anhydride.*—This was prepared according to the method of Katchalski and Sela (1953) with improvements described by Katchalski and Sela (1958).

*O-Acetyl-N-carboxy-*L*-tyrosine Anhydride.*—*O*-Acetyl-L-tyrosine HCl (Bretschneider and Biemann, 1950) (8.8 g.) was suspended in 90 ml of dry dioxane. Phosgene was bubbled in with stirring, while the temperature was maintained at 45°. After 4 hours all the material had dissolved and nitrogen was bubbled in to remove excess phosgene. The solvent was removed on a rotary evaporator keeping the temperature below 40°. The remaining oil was dissolved in 25 ml of anhydrous ethyl acetate and the solvent again was removed on the rotary evaporator. The oil was dissolved in 75 ml anhydrous ethyl acetate, 25 ml *n*-hexane was added to opalescence, the solution was filtered through Celite, an additional 50 ml *n*-hexane was added, and the solution was allowed to crystallize at −30°. Additional amounts of hexane were added totaling 400 ml. The crystals were filtered, wt = 7.47 g. The crystals were twice recrystallized from ethyl acetate–hexane, and yielded colorless crystals. The crystals were dried at −30° *in vacuo* (1 mm Hg); (5.87 g, 57% yield); mp 120° (reported 119–120°, Overell and Petrow, 1955; Bailey, 1950; Schlogl *et al.*, 1963).

*Poly-O-acetyl-*L*-tyrosine.*—*O*-Acetyl-*N*-carboxy-L-tyrosine anhydride (5 g) was dissolved in 25 cc dry

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[2] Although it has been reported (Idelson and Blout, 1958a) that methoxide-initiated polymers will not serve as initiators for further *N*-carboxyanhydride polymerization, the following evidence has been obtained indicating the "block" nature of the polymer reported herein: (1) if the *N*-carboxy-L-tyrosine anhydride had polymerized separately, e.g., not onto the end of a poly-γ-benzyl-L-glutamate; chain, the poly-L-tyrosine would be insoluble at *p*H 4.42; as it is soluble, there must be a solubilizing fraction present, i.e., the poly-L-glutamic acid fraction. (2) Fluorescence studies of the block copolymer indicate that many tyrosyl residues are contiguous, by the observation of an excimer-emission band (Lehrer and Fasman, 1964), while the random copolymer did not exhibit such fluorescence.

[3] Manufactured by the Bendix-Ericsson U. K. Ltd. and distributed in the United States by the Bendix Corp., Cincinnati 8, Ohio.

[4] Optical Cell Co., Brentwood, Md.

CH_2Cl_2 and 250 cc of dry benzene was added. The polymerization was initiated with sodium methoxide (0.250 cc, 0.392 N $NaOCH_3$; *A/I* 200) and allowed to proceed for 7 days. The resulting white gel was broken up and stirred in ether and centrifuged, and the ether procedure was repeated three times. The solid was suspended in dioxane and lyophilized, and the resulting white precipitate was dried at 100° *in vacuo*. Yield, 3.6 g, 97%. Specific viscosity in 0.2% dichloroacetic acid is 3.33.

*Poly-*L*-tyrosine* (via *O*-acetyl-*N*-carboxy-L-tyrosine anhydride).—Poly-*O*-acetyl-L-tyrosine (3.4 g) was suspended in 200 cc dimethylformamide. Sodium methoxide (53 cc of 0.392 N $NaOCH_3$ [1.1 molar equivalent]) was added and the solution was stirred overnight. The solution was poured into ether (500 cc) and centrifuged, and the precipitate was dissolved in 200 cc H_2O. The solution was dialyzed versus H_2O (*p*H 12) for several days and then lyophilized; yield, 2.1 g of poly-L-tyrosine Na salt. $[\eta]^{0.2\ M\ NaCl}_{pH\ 11.65} = 0.93$.

*Poly-O-carbobenzyloxy-*L*-tyrosine.*—*O*-Carbobenzyloxy-*N*-carboxy-L-tyrosine anhydride[5] (0.2 g) was dissolved in purified dioxane (5 cc) and initiated with sodium methoxide (7.8×10^{-3} ml of 0.373 N $NaOCH_3$, $A/I = 200$). After 3 days the viscous solution was poured into anhydrous ether (20 cc), yielding a white fibrous polymer. The polymer was washed three times with anhydrous ether and dried at 90° for 2 hours. Yield, 0.16 g, 92%. The specific viscosity in 0.2% dichloroacetic acid is 2.06.

*Poly-*L*-tyrosine.*—*O*-carbobenzyloxy-*N*-carboxy-L-tyrosine anhydride[5] (0.35 g) was polymerized as before in 8.7 ml dioxane. After 3 days the viscous opalescent solution was diluted to 1% by the addition of 25.3 ml of dioxane. Dry HCl was bubbled into the solution for 5 minutes; precautions were taken to eliminate H_2O. After 1 minute the opalescent solution had cleared. HBr was then passed through the solution for 10 minutes; precipitation began after 5 minutes, and the solution became yellow. The reaction mixture was allowed to stand overnight. The HBr was removed by suction on the water aspirator while the solution was stirred, and after 1 hour the precipitate dissolved. Anhydrous ethyl ether (5 × dioxane volume) was added, causing a yellow oil to separate. The supernatant was decanted and the oil was dissolved in H_2O (10 cc), while the *p*H was maintained at 12.5 with 2 N NaOH. The solution was dialyzed versus 0.01 N HCl and a white precipitate slowly formed. The dialysis was continued for 8 hours with three changes of the acidic solution. The suspension was lyophilized and dried *in vacuo* (1 mm Hg) at 50° for 2 hours. Yield, 115 mg of a light-yellow polymer, 69% yield, $[\eta]^{0.2\ M\ NaCl}_{pH\ 12.2} = 1.14$. Some studies were performed with a sample of poly-L-tyrosine purchased from Pilot Chemicals, Inc.

*Conversion of Poly-*L*-tyrosine to the Acid-Helical Form for Optical-Rotatory-Dispersion Studies.*—Poly-L-tyrosine (500 mg) was suspended in 50 cc H_2O and 1 N NaOH was added until the material dissolved. The solution was placed in a dialysis bag and dialyzed versus 0.01 N HCl at 40°. Slowly a white precipitate formed. The solution was lyophilized and the white powder was dried at 50° *in vacuo* (1 mm Hg) for 2 hours.

Copolymers of L*-Glutamic Acid and* L*-Tyrosine.*—A typical polymerization for a 9:1, L-Glu–L-Tyr copolymer was carried out as follows: γ-Benzyl-*N*-carboxy-L-glutamate anhydride (Blout and Karlson, 1956) (3.62 g, 13.7×10^{-3} moles) was dissolved in dry benzene (362 cc) by warming, *O*-acetyl-*N*-carboxy-L-tyrosine anhydride (0.38 g, 1.52×10^{-3} moles) dissolved in dioxane (2 cc) by warming was added, and the polymerization was initiated with sodium methoxide (0.207 ml, 0.366 N $NaOCH_3$, $A/I = 200$). After 5 days a small sample was removed to obtain the viscosity of the blocked polymer. HBr, purified by passing over Cu wire and through Tetralin, was bubbled through the viscous solution for 20 minutes, and precautions were taken to exclude moisture. A white spongy precipitate appeared on standing overnight. The supernatant was siphoned off and the precipitate was washed twice with benzene. The excess HBr was removed by suction on a water aspirator ($CaCl_2$ drying tube was used to prevent moisture entering the reaction flask). The polymer was then extracted for 5 hours with acetone in a Soxhlet apparatus, the acetone being changed every hour. The yield of copoly-L-glutamic acid-*O*-acetyl-L-tyrosine was 1.75 g.

Deacetylation.—Copoly-L-glutamic acid–*O*-acetyl-L-tyrosine (1.65 g) was suspended in H_2O and NaOH (1 N) was added to bring the *p*H to 11.5. The *p*H slowly drifted to lower values and NaOH (1 N) was added to maintain the *p*H of 11.5. When no further base was required to maintain the *p*H, the clear solution was stirred overnight. The solution was dialyzed versus 0.01 N HCl for 2 days, causing a white precipitate to form, and then the solution was lyophilized. The resulting white fluffy powder was dried at 40° *in vacuo* (1 mm Hg). Yield, 1.56 g, $[\eta]^{0.2\ M\ NaCl}_{pH\ 11.7} = 0.79$.

The copoly-L-glutamic acid–L-tyrosine, 8:2, was prepared in a similar fashion.

*Copoly-*L*-glutamic Acid–*L*-Tyrosine, 95:5.*—*O*-Carbobenzyloxy-*N*-carboxy-L-tyrosine anhydride (0.152 g, 4.45×10^{-4} moles) and γ-benzyl-*N*-carboxy-L-glutamate anhydride (2.22 g, 8.46×10^{-3} moles) were dissolved in dry benzene (250 cc) by warming. The solution was cooled to room temperature and the polymerization was initiated with sodium methoxide (0.25 ml 0.355 N $NaOCH_3$, $A/I = 100$). After 4 days a sample of the slightly viscous solution was removed for the determination of viscosity of the blocked polymer. Dry HCl was bubbled through the remaining solution for 15 minutes and then HBr for 30 minutes, while care was taken to exclude moisture. A precipitate slowly formed. The solution was allowed to stand overnight, the supernatant was decanted, and the excess solvent and HBr were removed by suction on a water aspirator for 1 hour. The polymer was extracted for 3.5 hours with anhydrous ether, with repeated changes of ether. The polymer was dried at 40° *in vacuo* (1 mm Hg); yield, 0.95 g, 86% $[\eta]^{0.2\ M\ NaCl}_{pH\ 7.3} = 1.07$.

*Block Copoly-(*L*-glutamic Acid)–(*L*-Tyrosine)–Glutamic Acid), 47.52:5:47.5.*—γ-Benzyl-*N*-carboxy-L-glutamate anhydride (1.0 g, 3.8×10^{-3} moles) was dissolved in dry benzene by warming (freshly distilled from CaH_2, 100 ml) and the polymerization was initiated by the addition of sodium methoxide (0.22 ml of 0.345 N $NaOCH_3$, $A/I = 50$). After 24 hours *O*-carbobenzyloxy-*N*-carboxy-L-tyrosine anhydride (0.137 g, 4.0×10^{-4} moles), dissolved in benzene (13.6 ml) by warming, was added to the polymerization mixture. Twenty-four hours later γ-benzyl-*N*-carboxy-L-glutamate anhydride (1.0 g), dissolved in benzene (100 ml) by warming, was added to the solution. One day later a small sample (10 ml) was removed and poured into hexane (50 ml), causing a white fibrous precipitate to form. This was washed with hexane and dried at 100° for 2 hours *in vacuo*, ($\eta sp/c$) = 0.9 (0.2% dichloroacetic acid). On the following day anhydrous HCl was bubbled into the solution for 10 minutes, and then HBr was bubbled in for 20 minutes, with careful

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[5] Purchased from Pilot Chemicals, Inc. and recrystallized.

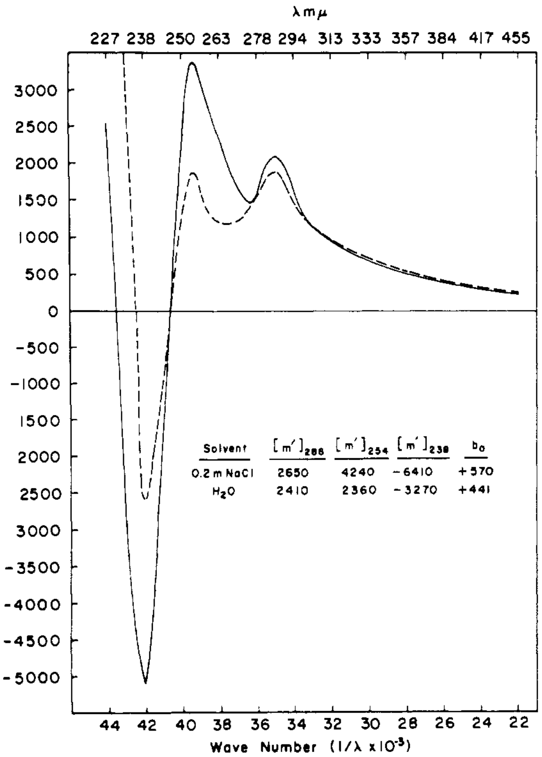

FIG. 1.—ORD curves of poly-L-tyrosine at pH 11.2. In 0.2 M NaCl, ———; in H_2O, ---------. The b_0 calculated over the wavelength range 500–322 mμ, with λ_c = 212 mμ. Concentrations used: 0.2% in a 1-cm cell for wavelength range 500–322 mμ, 0.1% in 1-mm cell from 322–227 mμ, and 0.02% in 1-mm cell from 286–227 mμ. $[m']$ = mean residue rotation. Each curve is an average of six separate experiments. Temperature, 24°.

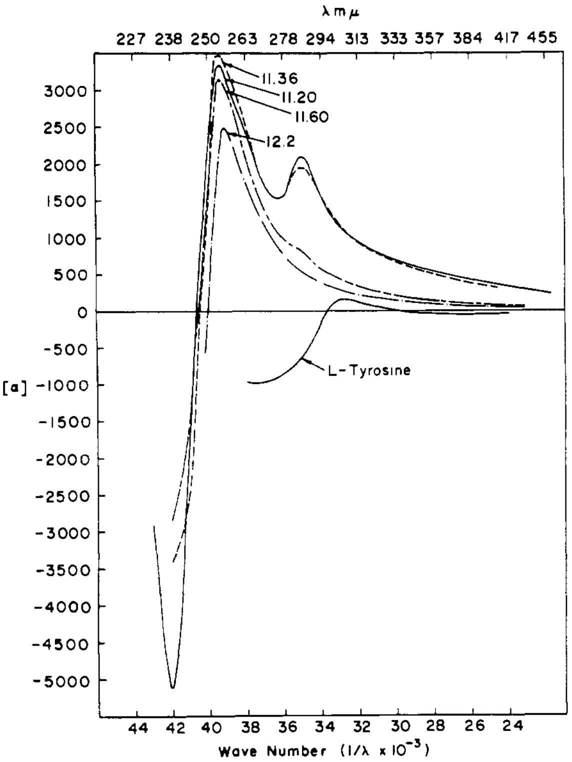

FIG. 2.—ORD curves of poly-L-tyrosine as a function of pH in 0.2 M NaCl. pH 11.20, ———; pH 11.36, ---------; pH 11.6, — – — –; pH 12.2, –·–·–·. L-Tyrosine pH 11.2, ———, as indicated. Concentrations used: 0.1% in a 1-cm cell for wavelength range 500–322 mμ; 0.1% in 1-mm cell for wavelength range 322–257 mμ; 0.02% in 1-mm cell for wavelength range 286–227 mμ. Temperature, 24°.

exclusion of moisture. The polymer precipitated out of solution after a short time and the next day the supernatant was decanted. Excess HBr and solvent were removed, with the exclusion of moisture, by suction on a water aspirator for 2.5 hours. The polymer was extracted with anhydrous ether for 3 hours, the ether being changed several times during this period. The polypeptide was dried *in* high *vacuo* for 2 hours at 40°. Yield, 0.72 g, $[\eta]^{0.2\text{ M NaCl}}_{p\text{H } 6.9}$ = 1.27.

RESULTS AND DISCUSSION

The ORD of poly-L-tyrosine in 0.2 M NaCl and in H_2O at pH 11.2 from 500 to 227 mμ is seen in Figure 1. These curves represent the average values of six experiments. Multiple Cotton effects are seen in this wavelength range. A positive Cotton effect with a maximum at 286 mμ is observed, with a mean residue rotation, $[m']_{286}$ = 2650° (0.2 M NaCl). The second positive peak is observed at 254 mμ with $[m']_{254}$ = 4240°; however, it is not obvious whether this is a true Cotton effect or is rather due to the rotation increasing at lower wavelengths and then being inverted owing to the large negative Cotton effect with a trough at 238 mμ, with $[m']_{238}$ = −6410°. The inflection point lies between 244 and 238 mμ. The circular-dichroism study in the following paper (Beychok and Fasman, 1964) demonstrates that this is a true Cotton effect. The b_o value calculated for the wavelength range 500–322 mμ is +570. The Cotton effects observed in the 0.2 M NaCl solutions are larger than in the pure-water solutions (see insert Fig. 1 for calculated values). This is the opposite to what might be anticipated, as it has been shown (Katchalski and Sela 1953) that the apparent pK_a of poly-L-tyrosine is lower in 0.2 M NaCl than in H_2O. As the helical content is presumably dependent on the degree of ionization, as in the case of poly-L-glutamic acid (Doty *et al.*, 1957; Idelson and Blout, 1958b; Fasman *et al.*, 1964; Applequist and Breslow, 1963), there would be less ionization of the phenolic hydroxyl in H_2O than in 0.2 M NaCl, and therefore one would anticipate greater helicity. However, the b_o in H_2O is +441, while in 0.2 M NaCl it is +570 (both at pH 11.2), suggesting a higher helical content in 0.2 M NaCl. This would also be suggested by the greater $[m']_{238}$ in 0.2 M NaCl, if this Cotton effect behaves similarly to the $[m']_{233}$ reported by Simmons *et al.*, (1961). These implications are incorrect because comparisons of helicity, based on b_o values, obtained at different ionic strengths are misleading. The magnitude of b_o at constant ionic strength does empirically correlate with helicity (see below). The $[m']_{238}$ value is contributed to by nonpeptide transitions as well as peptide-peptide transitions. This problem is further discussed in the following paper (Beychok and Fasman, 1964). Measurements could not be made at lower pH values as the polymer precipitated out of solution. Previously, a +540° b_o value for the helical conformation, which dropped to +450 in the random coil (Coombes *et al.*, 1960), has been reported. From the work of Katchalski and Sela (1953) it is seen that the polymer which they studied at pH 11.2 was approximately 80% ionized. However, the degree of polymerization was only 30, and it is possible that it was too short to take up a helical conformation or perhaps it was in the random conformation for reasons discussed earlier in this paper. Therefore, the pK_a of 9.8 reported is probably that of a random polymer. In the present work it is suggested that the pK_a of poly-L-tyrosine in the helical conformation is

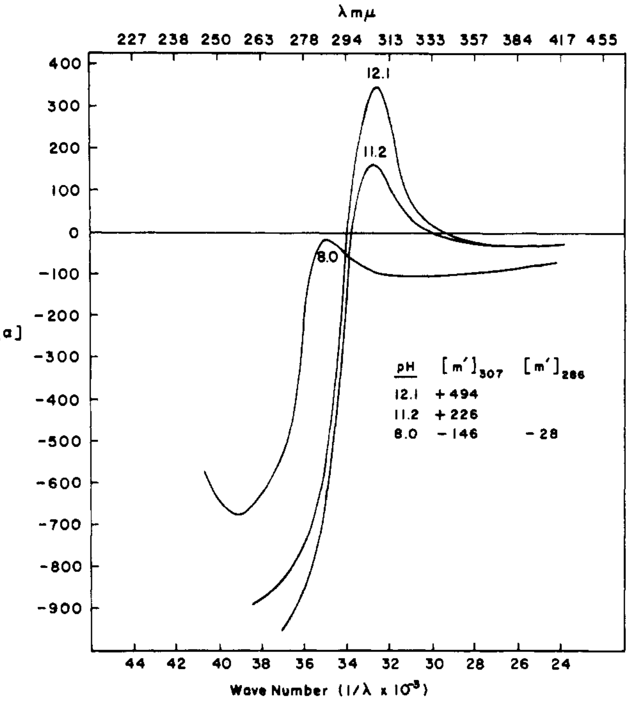

FIG. 3.—ORD curves of L-tyrosine as a function of *p*H in 0.2 M NaCl. *p*H 8.0, 11.2, and 12.1 as indicated. $[m']$, mean residue rotations (see Materials and Methods). Concentrations used: 0.1% in 1-cm cell for wavelength range 417–322 mμ; 0.1% in 1-mm cell for wavelength range 322–238 mμ. Temperature, 24°.

TABLE II
OPTICAL ROTATORY PARAMETERS OF POLY-L-TYROSINE AS A FUNCTION OF *p*H IN 0.2 M NaCl AT 24°

*p*H	$[m']_{286}$[a]	$[m']_{254}$[a]	$[m']_{238}$[a]	b_o[b]	a_o[b]
11.2	2650	4240	−6410	570	1044
11.36	2460	4410	−4280	550	1074
11.60	1038	3920	−3580	413	231
>13.0	688			420	133
L-Tyrosine at *p*H 11.2, $[m']_{286}$ = −888					

[a] $[m']$ is the mean residue rotation in deg cm²/decimole.
[b] b_o and a_o were calculated from the Moffitt equation with λ_o = 212 mμ over the wavelength range 417–322 mμ. Concentrations were between 0.1 and 0.2% in a 1-cm cell.

much higher than that previously reported. The polymer used in this study appears to be helical at *p*H 11.2 (as indicated from the Cotton effects and the b_o value), and this suggests that in high-molecular-weight helical samples the pK_a would be much higher than 9.8. This is also shown by the titration curve of Coombes *et al.* (1960), where the midpoint of the transition, as judged by $[\alpha]$, is approximately 11.3. However, if this transition occurs between 40 and 50% ionization as in poly-L-glutamic acid (Doty *et al.*, 1957; Applequist and Breslow, 1963), the pK_a lies at a still higher value. From Figure 4 it can be estimated that the pK_a is approximately 11.5. The higher pK_a is also evident from the spectrophotometric titration data in Figure 5, which show that at *p*H 11.2, in 0.2 M NaCl the tyrosyl hydroxyls thus appear to be essentially un-ionized in the polymer, while L-tyrosine is completely ionized. The measurement of the pK_a values by direct titration of helical and random poly-L-tyrosine is currently being made.

The effect of *p*H on the various Cotton effects of poly-L-tyrosine in 0.2 M NaCl is seen in Figure 2. Also listed in Table II are b_o, a_o, $[m']_{286}$, $[m']_{254}$ and $[m']_{238}$ values of poly-L-tyrosine calculated from these curves. The change in rotatory parameters in going from *p*H 11.2 to >13 probably represents a helix → random-coil transition (L-tyrosine at *p*H 11.2 is also shown for comparison). The positive peak of the Cotton effect at 286 mμ remains relatively unchanged on raising the *p*H from 11.2 to 11.36; however, at *p*H 11.6 it is much lower and at 12.2 it vanishes. The positive peak of the Cotton effect at 254 mμ changes slightly at *p*H 11.36, diminishes a little at *p*H 11.6, and diminished still further at *p*H 12.2 as compared to the *p*H 11.2 solution. The trough of the Cotton effects at 238 mμ slowly decreases with increasing *p*H. At *p*H 12.2 a trough exists at 238 mμ, but the accuracy of the measurement was poor owing to high absorption; therefore it was not placed on the graph. However, it does not decrease as much as might be expected for a helix → random-coil transition, as found in poly-L-glutamic acid, $[m']_{233}$ −18,000° → −2,000° (Yang and Samejima, 1963), −13,000° → −2,000° (Simmons *et al.*, 1961). However, L-tyrosine (Figs. 2 and 3) has an appreciable negative rotation at lower wavelengths. Here again the values at lower wavelengths could not be obtained owing to high absorption. For comparison the ORD of L-tyrosine as a function of *p*H is given in Figure 3, and the $[m']_{307}$ and $[m']_{286}$ values are listed. The $[\alpha]$ becomes increasingly negative at lower wavelengths for the un-ionized phenolic species (−700°). As the *p*H is raised to higher values, causing phenolic hydroxyl ionization, a positive Cotton effect appears with peak at 303 mμ and the negative rotation at lower wavelengths becomes larger. This is in contrast to the polymer which has a lower $[\alpha]_{303}$ on ionization. The rotations of the monomer are relatively much smaller than those found in the polymer.

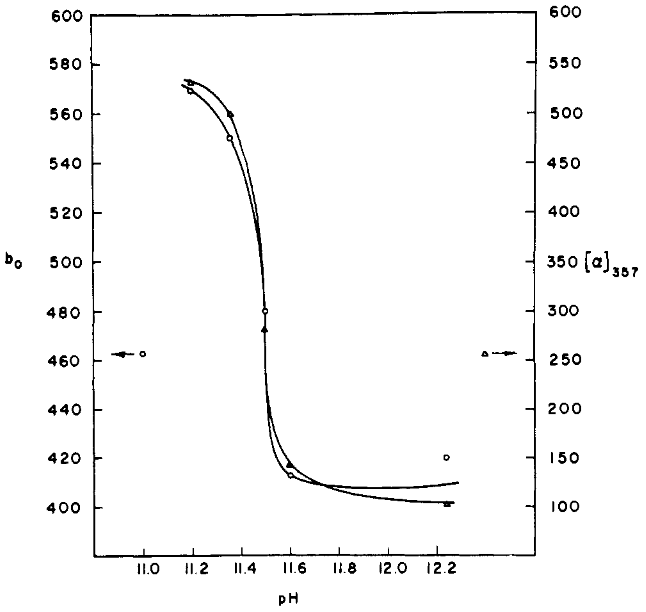

FIG. 4.—b_o and $[\alpha]_{357}$ as a function of *p*H for poly-L-tyrosine in 0.2 M NaCl. b_o was calculated from the Moffitt equation with λ_c = 212 mμ over the wavelength range 500–322 mμ.

A correlation of the b_o values for poly-L-tyrosine in 0.2 M NaCl with the above-mentioned Cotton effects can be made by the plot of b_o versus *p*H in Figure 4. The b_o of the helical form is found to be +570 and that of the random coil +413 (also see Table II). A plot of $[\alpha]_{357}$ versus *p*H is also seen in this figure. The transition as indicated by the two parameters, b_o and $[\alpha]_{357}$, is identical. It is obvious that other factors besides

TABLE III
SPECTRAL PROPERTIES OF L-TYROSINE, POLY-L-TYROSINE, AND RELATED COMPOUNDS

	Acid		Base		
	λ_{max} (mμ)	$\epsilon \times 10^{-3}$	λ_{max} (mμ)	$\epsilon \times 10^{-3}$	Ref.[a]
Tyrosine	275	1.34	293.5	2.33	(1)
	223	8.20	240	11.05	
	275	1.40	293	2.39	(2)
	275	1.29	295	2.30	(3)
N-Trimethyl-L-tyrosine	274	1.34	292	2.35	(2)
Poly-L-tyrosine	274.5	1.400	293.5	2.130	(4)
			242	9.800	
Glycyl-L-tyrosine	275	1.375	293	2.325	(5)
Poly-L-tyrosine	277	1.610	294	2.250	(6)
	282	1.080	243	10.100	

[a] References are as follows: (1) Beaven and Holiday (1952). (2) Martin *et al.* (1958). (3) Edsall and Wyman (1958). (4) Katchalski and Sela (1953). (5) Ref. 3, above. Acid, *p*H 4.6; base, *p*H 13. (6) This work. Acid, *p*H 11.2; base, *p*H >13.

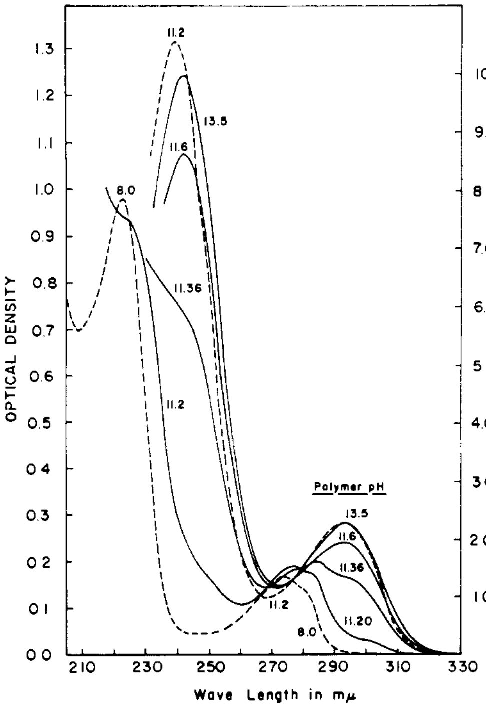

FIG. 5.—The far-ultraviolet-absorption spectra of poly-L-tyrosine and L-tyrosine as a function of *p*H in 0.2 M NaCl. Poly-L-tyrosine-absorption curves, ———, at *p*H 13.5, 11.6, 11.36, and 11.20 as indicated. L-Tyrosine, ---------, at *p*H 11.2 and 8.0 as indicated. Concentrations: 0.02% for poly-L-tyrosine and 0.0222% for L-tyrosine in a 1-mm cell. Temperature, 24°.

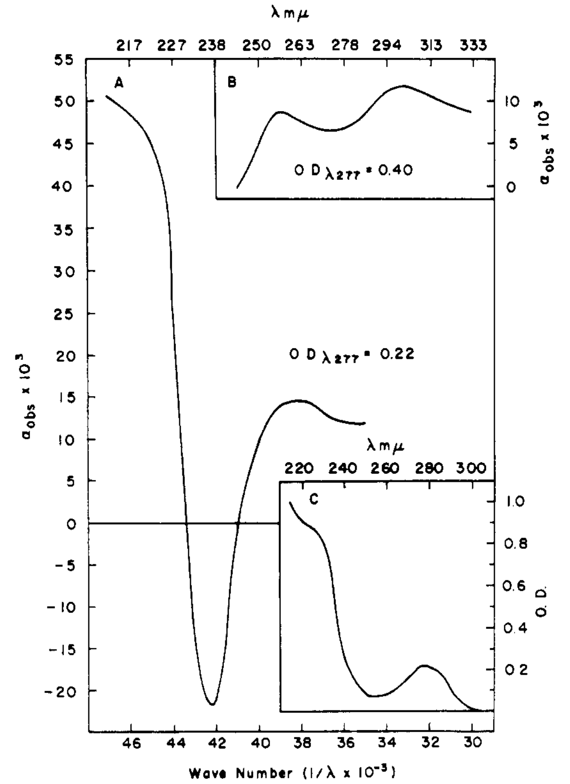

FIG. 6.—ORD and ultraviolet-absorption spectra of poly-L-tyrosine films. (A) ORD of a film with OD of 0.22 at 277 mμ. (B) ORD of a film with OD of 0.40 at 277 mμ. (C) Ultraviolet-absorption spectra of a poly-L-tyrosine film.

the transitions of the peptide chromophore arranged in a helical array are contributing to the b_0 value.

Ultraviolet-absorption spectra (Fig. 5) of poly-L-tyrosine were obtained with the solutions (0.2 M NaCl) used for the ORD versus *p*H curves (Fig. 2). The absorption curve obtained at *p*H 11.2 in the present study is similar in shape to that found for L-tyrosine at *p*H 8.0 for the un-ionized phenolic group, also shown in the figure. The reported spectral properties of L-tyrosine and derivatives, the poly-L-tyrosine studied by Katchalski and Sela (1953), and that used in this paper are seen in Table III. The absorption spectrum for poly-L-tyrosine in this study at *p*H 11.2 indicates that there is little ionization. The $\epsilon_{max} = 1610$ is slightly higher than that obtained for the monomer; however, a more significant observation is the red shift to $\lambda_{max} = 277$ from 275 mμ. The fact that this λ_{max} is representative of the un-ionized species is seen (insert C in Fig. 6) in the spectra taken from a film of the totally un-ionized poly-L-tyrosine, cast from dimethyl sulfoxide. On raising the *p*H of the poly-L-tyrosine solution above 13, the spectrum becomes identical to that found for the monomer. Two new shoulders are visible in the spectrum at *p*H 11.2, and at $\lambda = 252$ mμ and the other at 300 mμ. The shift of the λ_{max}, and the increase in ϵ_{max} are probably caused by tyrosyl-tyrosyl interactions in the helical conformation. In the helical conformation there is probably interaction between tyrosyl residues which are stacked in a helical array forming a secondary helical structure.

The high absorption of poly-L-tyrosine in aqueous solution prevented accurate rotatory measurements at low wavelength. It was also necessary to have a small degree of ionization because the completely un-ionized polypeptide was not soluble. Therefore an attempt was made to measure the ORD of films of the acid form

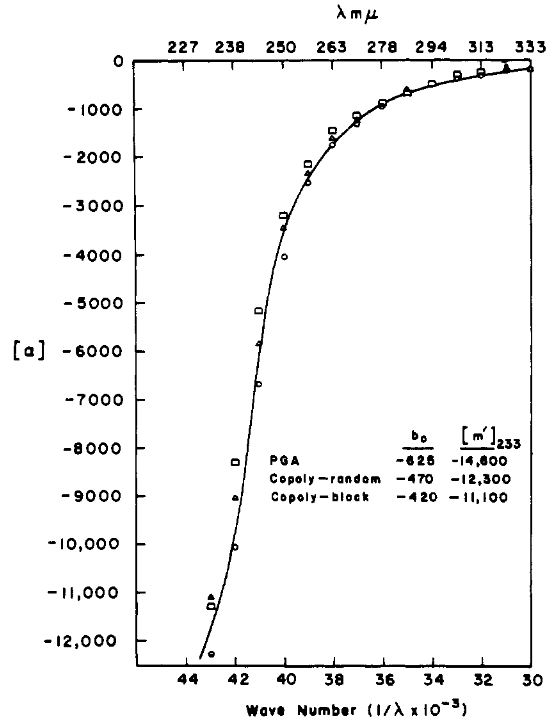

FIG. 7.—ORD curves of poly-L-glutamic acid and copolymers of L-glutamic acid and L-tyrosine in aqueous media. Poly-L-glutamic acid, *p*H 4.4, 0.04% in a 5-mm cell, □; copoly-L-glutamic acid–L-tyrosine, 95:5 (random copolymer), *p*H 4.35, 0.2% in 1-mm cell, ○; copoly-(L-glutamic acid)–(L-tyrosine)–(L-glutamic acid) 47.5:5:47.5 (block copolymer), *p*H 4.42, 0.2% in 1-mm cell, △. b_0 values obtained from the Moffitt equation, with λ_c = 216 mμ over the wavelength range 294–250 mμ. $[m']$ is the mean residue rotation (see Materials and Methods). Temperature, 24°. PGA = poly-L-glutamic acid.

TABLE IV
OPTICAL ROTATION (α_{obs}) OF FILMS OF POLY-L-TYROSINE CAST FROM DIMETHYL SULFOXIDE

Film	λ (mμ)[a]	α_{obs}[a] × 10³	$\alpha_{obs_{222}}$ × 10³	$\Delta\alpha_{obs}$[b] × 10³
1	233	−40.3	1.82	42.1
2	233	−41.0	6.82	47.8
3	236	−28.0	16.4	44.4
4	238	− 7.8	78.9	86.7
5	238	− 8.3	84.4	92.7
6	237	−23.3	45.9	69.2

[a] The wavelength at the bottom of the trough. [b] $\alpha_{obs_{222}} - \alpha_{obs_{233}}$.

(un-ionized) cast from dimethyl sulfoxide. It was hoped that lower wavelengths could be investigated as absorption losses owing to solvent would be eliminated. Earlier work on ORD of films has emphasized the inherent problems involved in this method (Downie *et al.*, 1959; Elliott *et al.*, 1962) and similar difficulties were encountered in this study.

In Figure 6 is seen a representative set of ORD curves from two films (Fig. 6A,B) and a typical ultraviolet spectrum for such a film (Fig. 6C). A new shoulder (positive) is observed at 222 mμ (Fig. 6A). ORD curves varying in the relative magnitude of the Cotton effects could be obtained with the same film by slight rotation of the film. In Table IV are listed the α_{obs} for the negative Cotton effect near λ_{238} mμ (also note a slight displacement of the minimum in the various films) and the α_{obs} for the shoulder of the positive Cotton effect at 222 mμ for the same film which had an OD of 0.22 at λ_{277}. Also listed is the $\Delta\alpha_{obs}$ between these two wavelengths. It can be seen that $\Delta\alpha_{obs}$ varies from 42.1 to 92.7 × 10^{-3} degrees. The value of α_{obs} = 0 at 250 mμ was arbitrarily set, this value having been taken from the aqueous solution ORD curves. It is highly probable that there is a slight orientation, despite great efforts to avoid it, and this accounts for the observed variations in the α_{obs}. The same overall character of the Cotton effects near 290, 250, and 238 mμ is seen in the films (Fig. 6A,B) as is found in solution with a slight shift to longer wavelengths of the first two in the films. A new peak (or shoulder) is observed between 222 and 217 mμ, a region unattainable in solution. As tyrosine has a strong absorption band at 223 mμ, it is difficult to determine whether this Cotton effect is due to this electronic transition, or whether it is the shoulder reported for helical polypeptides at 210 mμ (Blout *et al.*, 1962) and observed in this laboratory between 217 and 213 mμ for the helical form of poly-L-glutamic acid. The ultraviolet-absorption spectra of a poly-L-tyrosine film (see insert Fig. 6C) show the λ_{max} at 277 mμ for this totally un-ionized species, thus confirming that the polymer in the aqueous solution at *p*H 11.2, also having a λ_{max} = 277 mμ, was probably in a highly un-ionized state. It is now more understandable why the Cotton effect at 238 mμ does not reach the value associated with the helical form of poly-L-glutamic acid, $[m']_{233}$ = −13,000°. The electronic transition of the peptide bond occurs in the region of several transitions of the aromatic ring and each contributes to the rotatory properties, producing a complex dispersion curve. This is illustrated in the following paper where it is shown that several positive dichroism bands occur in this region (Beychok and Fasman, 1964).

Optical Rotatory Dispersion (ORD) of Copolymers.—As has been previously mentioned, the problem of whether Cotton effects due to aromatic residues in polypeptide helical conformations can be detected by ORD has not been unequivocally answered. To investigate this problem several copolymers of L-glutamic acid and L-tyrosine, containing various mole per cents of L-tyrosine, were investigated. In Figure 7 are seen the ORD curves for several of these polymers. The ORD curve of poly-L-glutamic acid at *p*H 4.4 (helical, b_o = −625, $[m']_{233}$ = −14,600°), and the random copoly-L-glutamic acid-L-tyrosine, 95:5, are seen to be very similar. The value of λ_c = 216 mμ was used in calculating b_o for the wavelength range 294–250 mμ. This was necessary to obtain straight-line Moffitt-Yang plots, in agreement with the work of Urnes and Doty (1961). There is no indication of a tyrosine Cotton effect at 286 mμ in the random copolymer (b_o = −470, $[m']_{233}$ = −12,300°). Thus it appears that a 5 mole % tyrosine content is not sufficient to be detectable by this Cotton effect, although both b_o and $[m']_{233}$ are decreased. However, the tyrosine residues are greatly diluted out in the random copolymer and probably cannot interact with each other along the secondary helical side-chain structure, which is perhaps a necessary requirement for this Cotton effect. Therefore a block copolymer[2] was made of copoly-(L-Glu)$_{47.5}$–(L-Tyr)$_5$–(L-Glu)$_{47.5}$ with a block of tyrosine between two glutamic acid blocks, allowing for tyrosyl-tyrosyl interactions, and it is seen that, likewise, no Cotton effect is observable (Fig. 7). The polypeptide is helical by the criteria of b_o = −420, and $[m']_{233}$ = −11,100°, although again these values are less than observed for poly-L-glutamic acid. Thus the 5% tyrosine block did not contribute sufficient rotation to be observable as a Cotton effect. The Cotton effect of poly-L-tyrosine at λ = 286 mμ has a residue rotation of +2650°, while poly-L-glutamic acid has a $[m']_{286}$ = +1200°. Therefore a 5% contribution due to tyrosine would yield a rotation of +133°. This is 11% of the observed poly-L-glutamic acid rotation at 286 mμ and one would expect this to be measurable; however, very little change in rotation is observed.

As these two polymers showed no Cotton effects, a series of copolymers containing higher tyrosine contents were synthesized. These polymers were insoluble in aqueous solutions at the acid *p*H necessary to have them helical so they were studied in dimethyl sulfoxide. In Figure 8 are reproduced the ORD curves for poly-L-glutamic acid, and two random copolymers of L-glutamic acid–L-tyrosine, 9:1 and 8:2. The curve for the 10% tyrosine copolymer is displaced to higher rotations, but no Cotton effect at 286 mμ is observed. However, in the 8:2, L-Glu–L-Tyr copolymer the Cotton effect begins to be discernible. The b_o (λ_c = 212 mμ), as indicated on the figure, showed the same change with tyrosine content as previously reported (Fasman, 1962) (poly-L-tyrosine in dimethyl sulfoxide in the helical conformation, b_o = +490, in the random coil +200), and can be taken to indicate a 100% helical conformation. The degree of randomness is not known for these copolymers. However, it is certainly more feasible to have a tyrosyl-tyrosyl interaction in higher tyrosine-containing polymers than in lower ones.

CONCLUSIONS

The ORD of helical poly-L-tyrosine (un-ionized form) displays multiple Cotton effects in the wavelength range 500–227 mμ. Two peaks (positive Cotton effects) with maxima at 286 and 254 mμ, respectively, are found with $[m']_{286}$ = 2650° and $[m']_{254}$ = 4240°. The first peak is probably due to the absorption band at 275 mμ found in L-tyrosine. A negative Cotton effect with trough at 238 mμ $[m']_{238}$ = −6410° is reported. This Cotton effect is mainly due to the peptide transitions, with trough reported at 233 mμ for many synthetic polypeptides. The reason for the shift of the trough from 233 to 238 mμ is discussed in the following paper (Beychok and Fasman, 1964). Under conditions of maximal helicity, at 0.2 M NaCl, the b_0 of +570 is found, which agrees with the value of +540 (Coombes *et al.*, 1960) that has been reported previously. The tyrosyl Cotton effect peak, $[m']_{286}$, disappears upon raising the *p*H from 11.2 to 12.2, the b_o drops to 413, and the $[m']_{238}$ also decreases during this transition. These rotatory changes are probably caused by a helix → random-coil transition of the peptide backbone. The $[m']_{238}$ does not decrease as much as might be expected for such a transition; but as indicated in this and the following paper, the trough at 238 mμ is contributed to by a tyrosyl Cotton effect, which persists in the random coil. A Cotton effect is not observed at 294 mμ, where the new absorption band lies for the ionized polymer, thus indicating that the helical backbone has been destroyed. The helical conformation of poly-L-tyrosine (un-ionized) in organic solvents has b_o values of +450 and +490 in dimethylformamide and dimethyl sulfoxide, respectively, and +100 and +200 in the same solvents in the random structure. Thus the b_o value in the random un-ionized polymer is lower than that found in aqueous solution (+413) for the ionized species. The peptide $n - \pi^*$ transition that is found at 225 mμ is situated among other electronic transitions of the aromatic ring, and consequently yields a complex dispersion curve. However, the monomer itself exhibits a Cotton effect in this region. The monomer has a positive Cotton effect, $[m']_{286}$ = −28°, in the un-ionized form (*p*H 8.0); however, it displays a larger positive Cotton effect, peak at 307 mμ, $[m']_{307}$ = +494°, upon ionization. In the polymer the Cotton effect, peak at 254 mμ, is retained upon ionization and no peak is seen at 307 mμ. The ORD of films of poly-L-tyrosine also indicate a shoulder at 222 mμ.

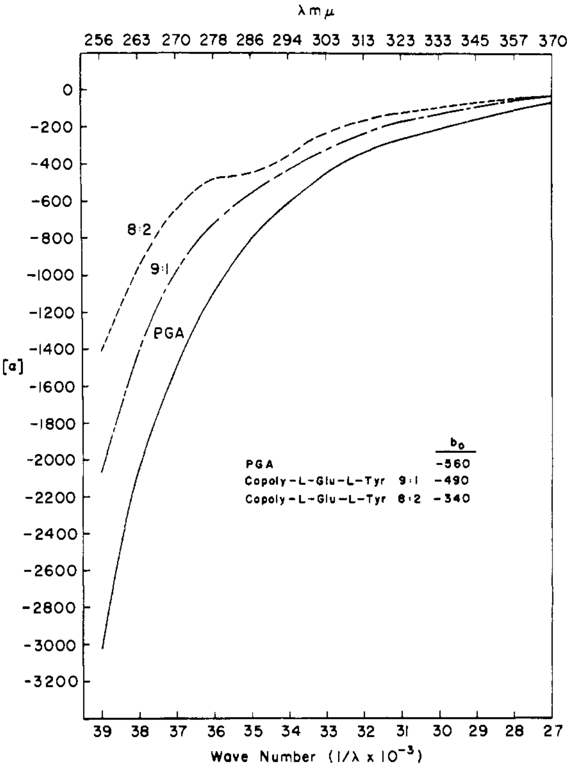

FIG. 8.—ORD curves of poly-L-glutamic acid and random copolymers of L-glutamic acid and L-tyrosine in dimethyl sulfoxide. Poly-L-glutamic acid, ———, concentration 0.2% in 1-cm cell. Copoly-L-glutamic acid–L-tyrosine, 9:1, — — — —, 0.2% in 1-mm cell. Copoly-L-glutamic acid–L-tyrosine, 8:2, ---------, 0.2% in 1-mm cell. b_0 calculated from the Moffitt equation, with λ_c = 212 mμ over the wavelength range 400–294 mμ. PGA = poly-L-glutamic acid.

Thus the approximate magnitude and the sign (negative) of the Cotton effect at 238 mμ observed in poly-L-tyrosine in the helical conformation support the previous contention (Fasman, 1962) that this polypeptide assumes the right-handed helical conformation. The circular-dichroism study in the following paper (Beychok and Fasman, 1964) also confirms that this sense of helix is right-handed. This study also indicates that there is overlap between the individual circular-dichorism bands and consequently each produces an effect on the other, thus complicating the interpretation.

Ultraviolet-absorption spectra indicate that the absorption maximum is shifted by 2 mμ to a longer wavelength (277 mμ) for the helical poly-L-tyrosine in solution and of films as compared to the monomer. Two new shoulders are also visible at 252 mμ and 300 mμ. This shift of λ_{max} is probably due to tyrosyl-tyrosyl interactions in the helical conformation.

ORD and ultraviolet-absorption spectral data are presented which suggest that the pK_a of high-molecular-weight helical poly-L-tyrosine lies at a higher value (pK_a = 11.5) than that found for lower molecular weight, presumably random-coil poly-L-tyrosine (Katchalski and Sela, 1953). Instances of an elevated pK_a for tyrosine ionization have been reported for many proteins (Steinhardt and Beychok, 1964) and have been interpreted in terms of inaccessibility or buried residues. However, in this model system, despite the apparently elevated pK_a, the phenolic OH is exposed to solvent. The studies on copolymers containing tyrosine indicate that at least 20 mole % of tyrosine is necessary in a copolymer to be detectable by the 286-mμ Cotton effect. Thus it appears likely that tyrosyl-tyrosyl interactions are necessary for this effect.

As such a high per cent of tyrosine is required to be detectable by ORD, it is considered unlikely that such Cotton effects would be seen frequently in proteins. Nevertheless, if the sequence of amino acids or the folding was such as to allow for the correct interaction between the tyrosines present, such Cotton effects may be observable. However, two conclusions can be drawn concerning the interpretation of ORD data of proteins which have a relatively large tyrosine content. The b_o values will be less negative but this is not an indication of a lower helical content. The $[m']_{233}$ value will also be less negative and again this is not indicative of a lower helical content. Whereas, in poly-L-tyrosine, a higher positive b_o correlates at constant ionic strength with increased helicity, it is worthwhile to point out that chromophore bands associated with tryptophan or histidine residues may contribute to b_o and $[m']_{233}$ in quite different ways than those associated with tyrosine residues. Thus extreme care should be exercised in the interpretation of ORD data for proteins containing a large number of amino acids with chromophoric side chains.

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