Conformational Aspects of Polypeptide Structure. XIX. Azoaromatic Side-Chain Effects[1,2]

Murray Goodman and Aaron Kossoy[3]

Contribution from the Department of Chemistry of the Polytechnic Institute of Brooklyn, Brooklyn, New York 11201. *Received June 14, 1966*

Abstract: A series of azopolypeptides derived from L-*p*-(phenylazo)phenylalanine and γ-benzyl-L-glutamate were prepared. We measured the absorption spectra and optical rotatory dispersions of these substances. In dioxane a positive Cotton effect is observed with a peak at 360 mμ. This phenomenon arises from interactions between the side-chain azoaromatic chromophores and the asymmetric centers in the ordered peptide main chain. In trifluoroacetic acid, another positive Cotton effect is observed with a peak at 425 mμ. We attribute this anomalous dispersion to the interactions among the highly ordered protonated azoaromatic side chains arrayed about the asymmetric peptide main chain.

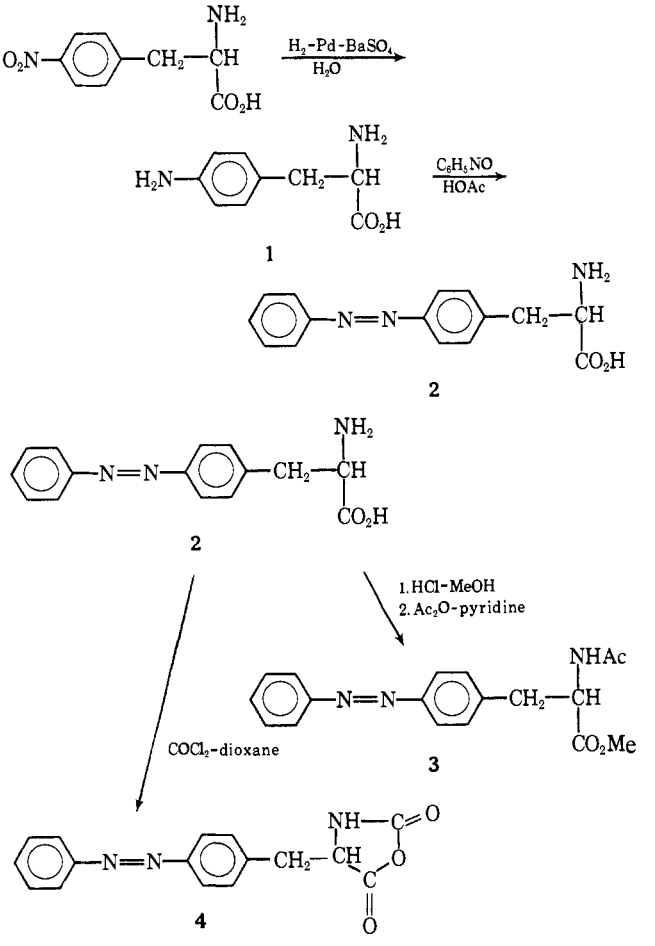
Amino acid side chains are a major factor affecting polypeptide conformations in solution.[4] Interactions between chromophores in the side chain and the optically active centers in the polypeptide main chain can be elucidated by optical rotatory dispersion[5] and circular dichroism[6] studies. Such effects have been observed with poly-L-tyrosine,[7] copolymers containing poly-β-(*p*-nitrobenzyl)-L-aspartate,[8] and the helical porphyrin *d*-urobilin.[9]

We prepared a series of polypeptides derived from L-*p*-(phenylazo)phenylalanine and γ-benzyl-L-glutamate in order to study the effect of an azoaromatic group in the side chain of a polypeptide.[10] In this paper we describe the synthesis and stereochemical properties of these materials.

Results and Discussion

The starting material for the synthesis, L-*p*-nitrophenylalanine, was catalytically reduced to L-*p*-aminophenylalanine (**1**) by the method of Bergmann.[11] The latter compound was then condensed in acetic acid with nitrosobenzene (prepared by the method of Vogel[12]) to yield L-*p*-(phenylazo)phenylalanine (**2**). This azoaromatic amino acid was treated successively with methanolic hydrochloric acid and acetic anhydride–pyridine to obtain the amide ester model compound **3**. In addition, compound **2** was allowed to react with phosgene in order to prepare the corresponding N-carboxy anhydride (**4**).[13] The synthesis is outlined in Scheme I. The N-carboxy anhydride of L-*p*-(phenylazo)phenylalanine was homopolymerized and was mixed with varying quantities of γ-benzyl-L-glutamate N-carboxy anhydride[14] to form copolymers. Sodium

Scheme I



(1) For the previous paper in this series, see M. Goodman, M. Langsam, and I. G. Rosen, *Biopolymers*, 4, 305 (1966).

(2) We gratefully acknowledge support for this research by grants from the National Science Foundation (Grant No. GB-2896) and the National Institutes of Health (Grant No. RG-08974).

(3) From a thesis submitted by A. Kossoy to the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the Ph.D. degree.

(4) For the relation of the side chain to polypeptide conformation in solution see E. R. Blout in "Polyamino Acids, Polypeptides and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p 275 ff.

(5) (a) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961); (b) J. T. Yang, *Tetrahedron*, 13, 143 (1961).

(6) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, 87, 218 (1965).

(7) (a) G. D. Fasman, E. Bodenheimer, and C. Lindblow, *Biochemistry*, 3, 1665 (1964); (b) S. Beychok and G. D. Fasman, 3, 1675 (1964); (c) Y. H. Pao, R. Longworth, and R. L. Kornegay, *Biopolymers*, 3, 516 (1965).

(8) M. Goodman, A. M. Felix, C. M. Deber, A. R. Brause, and G. Schwartz, *ibid.*, 1, 371 (1963).

(9) A. Moscowitz, *Proc. Natl. Acad. Sci. U. S.*, 52, 1190 (1964).

(10) The phenylazoalkyl chromophore in various glucosides was recently shown to be optically active; see E. O. Bishop, G. J. F. Chittenden, R. D. Guthrie, A. F. Johnson, and J. F. McCarthy, *Chem. Commun.* 93 (1965).

(11) E. D. Bergmann, *J. Am. Chem. Soc.*, 74, 4947 (1952).

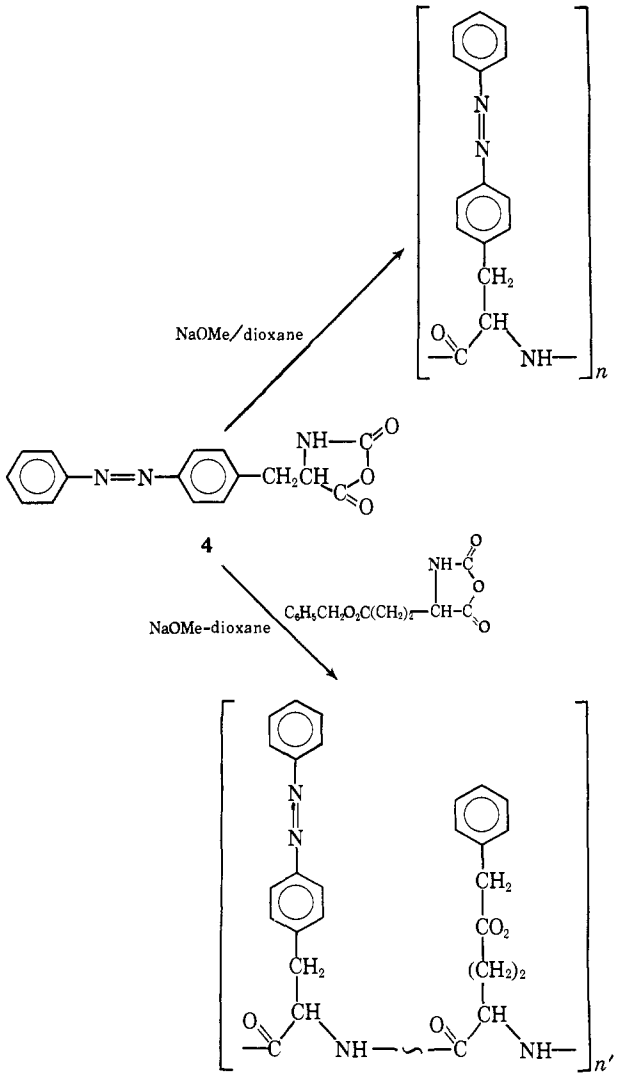
(12) A. I. Vogel, "Practical Organic Chemistry," 3rd ed, Longmans, Green, London, 1957, pp 629–631.

(13) A. C. Farthing, *J. Chem. Soc.*, 3213 (1950).

(14) Kindly provided by Dr. J. Hutchison of the Polytechnic Institute of Brooklyn.

methoxide was employed as the initiator.[15] These procedures are illustrated in Scheme II.

Scheme II



Molecular weights of selected polypeptides were determined in dichloroacetic acid by ultracentrifugation using the method of Yphantis.[16] The data obtained are shown in Table I. Dilution of the sample causes an apparent increase in the observed molecular weight. This behavior is typical of polyelectrolytes[17] and provides evidence that azoaromatic residues are protonated in strong acid (*cf.* discussion of ultraviolet spectra below).

The ultraviolet and visible spectra of *trans*-azobenzene, the amide ester **3**, and the polymeric materials are tabulated in Table II. The major electronic transitions of these substances are essentially those of *trans*-azobenzene itself. In nonacidic solvents the band for *trans*-azobenzene in the 325-mμ region is attributed to π–π* transitions[18] and the band in the 425-mμ region is due to n–π* transitions.[18] When the solvent is strongly acidic and the azo linkage is protonated the π–π* transition is shifted to approximately 420 mμ.[19]

Table I. Molecular Weights of Some Azopolypeptides in Dichloroacetic Acid

Mole % L-*p*-(phenylazo)-phenylalanine in polymer	*C*	Mol wt × 10^{-4}
0.0	0.366[a]	3.06
	0.195	2.81
	0.117	3.15
	0.062	2.76
14.0	0.431[a]	2.22
	0.224	2.49
	0.112	2.91
	0.055	3.38
25.6	0.609[a]	1.44
	0.311	2.06
	0.107	2.92
	0.038	4.74
49.7	0.627[a]	1.90
	0.402	2.21
	0.147	4.27
	0.094	5.45
79.9	0.706[a]	*b*
	0.350	2.42
	0.217	2.32
	0.103	3.55

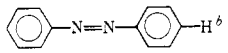
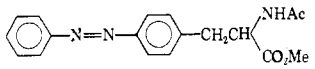
[a] These numbers are equal to c_0: $c_0 = [(A \tan\theta)/(aLm_1m_2\Delta nE^2)] \cdot (X/X_0)^2$ (R. Trautman and V. N. Schumaker, *J. Chem. Phys.*, **22**, 551 (1954)), where A is the measured area under the boundary, a is the cell thickness (in cm) along the optical path, L is the optical lever arm in cm, θ is the angle of the schlieren analyzer, m_1 and m_2 are the magnification factors of the camera and cylindrical lenses, E is the magnification of the photographic enlarger, and Δn = difference in refractive index between solvent and 1% solution of protein. X_0 is the position of the boundary at zero time and X is the boundary position at the time of recording. The three numbers below c_0 for each copolymer are dilutions by weight. [b] Very high absorption at this concentration precluded measurement.

We used the visible spectra of the polymers in trifluoroacetic acid to determine copolymer composition. Assuming the Beer–Lambert law, one should obtain a linear relationship between the mole % of azo residues and the specific absorptivities (optical density per gram) of the copolymers. The specific absorptivities were measured at 430 mμ and plotted against the mole % of L-*p*-(phenylazo)phenylalanine N-carboxy anhydride polymerized (Figure 1). This analytical technique is quite important because some of the azopolypeptides give noncombustible residues under standard microchemical analysis conditions (see Experimental Section and Table IV).

Optical rotatory dispersion studies in dioxane were carried out using poly-γ-benzyl-L-glutamate[14] and the soluble copolymers (Figures 2 and 3). Our results indicate that the copolymer curves are composites of a positive azoaromatic side-chain Cotton effect with a peak at 360 mμ superimposed on the rotatory dispersion effects derived from the main chain peptide chromophores. The n–π* transitions of peptide groups[6] give rise to a large negative Cotton effect at 233 mμ.[20] The extrapolated curve of the dioxane-insoluble homopolymer shows a rotation of approximately −10,700° at 233 mμ. We believe that this value indicates that the main chain is most probably helical throughout the

(15) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **78**, 941 (1956).
(16) D. A. Yphantis, *Ann. N. Y. Acad. Sci.*, **88**, 586 (1960).
(17) H. Morawetz, "Macromolecules in Solution," Interscience Publishers, Inc., New York, N. Y., 1965, pp 335–336.
(18) A. E. Gillam and E. S. Stern, "An Introduction to Electronic Absorption Spectroscopy," Arnold, London, 1957, p 271.
(19) H. H. Jaffé, S. J. Yeh, and R. W. Gardner, *J. Mol. Spectry.*, **2**, 1120 (1958).
(20) N. S. Simmons and E. R. Blout, *Biophys. J.*, **1**, 55 (1960).

Table II. Ultraviolet and Visible Absorption Spectra

Structures	$\lambda_{max}^{dioxane}$, mμ (log ε)	λ_{max}^{TFA},[a] mμ (log ε)
	A. Monomeric Models	
 [b]	228 (4.20), 317 (4.32), 438 (2.65)	292 (3.64), 419 (4.01)
	228 (4.20), 324 (4.25), 438 (3.23)	300 (3.83), 427 (4.21)
Mole % azo residue content[c]		
	B. Polymers and Copolymers	
0.0	258s[d] (2.63)	429 (2.24)
14.0	229s[d] (3.44), 325 (3.47), 438 (2.08)	300s[d] (2.88), 430 (3.57)
25.6	228 (3.48), 325 (3.76), 438 (2.24)	300 (3.08), 428 (3.75)
33.1		312s[d] (3.33), 428 (3.84)
37.9	229 (3.94), 323 (4.07), 438 (2.58)	305s[d] (3.12), 430 (3.98)
49.7	228 (3.95), 324 (4.07), 438 (2.59)	300s[d] (3.27), 428 (4.07)
79.9	Insoluble	310s[d] (3.47), 425 (4.31)
100.0	Insoluble	306s[d] (3.63), 425 (4.42)

[a] The cutoff point in a 1-cm cell *vs.* itself is 255 mμ. [b] The azobenzene spectra are included here, despite the numerous results reported in the literature, for comparison with the amide ester **3** and polymers. [c] Determined by absorption spectroscopy (see Figure 1). [d] Partially resolved shoulder.

copolymer series. Support for this explanation can be obtained from the optical rotatory dispersion of the amide ester **3** and the soluble azopolypeptides (Figures 2 and 3). This model compound exhibits small positive peaks at 233 and 350 mμ, although the entire rotatory dispersion curve is shifted to negative values. For the azopolypeptides, the magnitude of the positive Cotton effect in the 360-mμ region is greatly enhanced relative to the amide ester (Figures 2 and 3) while the negative Cotton effect at 233 mμ remains essentially constant relative to poly-γ-benzyl-L-glutamate (Figures 3 and 4).

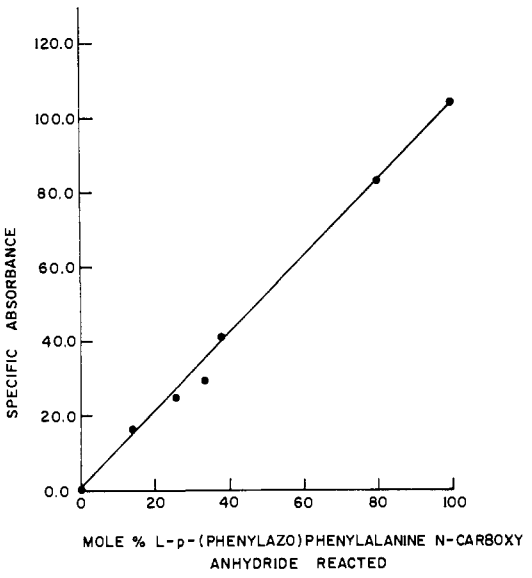


Figure 1. Specific absorptivities of azopolypeptides in trifluoroacetic acid at 430 mμ *vs.* mole % of L-*p*-(phenylazo)phenylalanine N-carboxy anhydride reacted.

A nonlinear dependence in dioxane of the corrected residue rotations (R′) on copolymer composition is observed at 233 and 360 mμ (Figure 4). This relationship shows that the origins of the Cotton effects derived from the azo chromophores arise both from interactions among the side-chain chromophores and between the azoaromatic group and the asymmetric center in a given residue (side-chain–main-chain effect).[21]

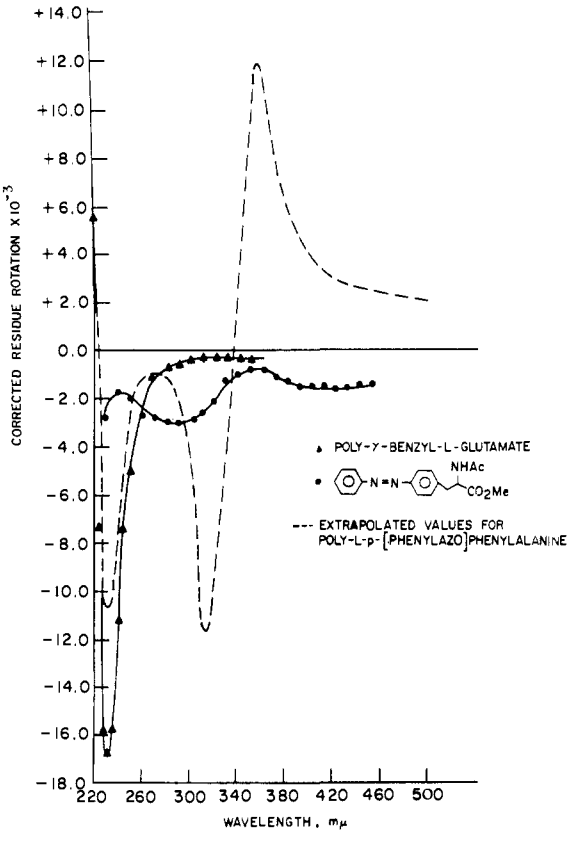


Figure 2. Optical rotatory dispersion of azo compounds in dioxane.

Our results in dioxane show that the azoaromatic chromophore is asymmetric by induction from the

(21) D. F. Bradley, M. Goodman, A. M. Felix, and R. Record, *Biopolymers*, 4, 607 (1966).

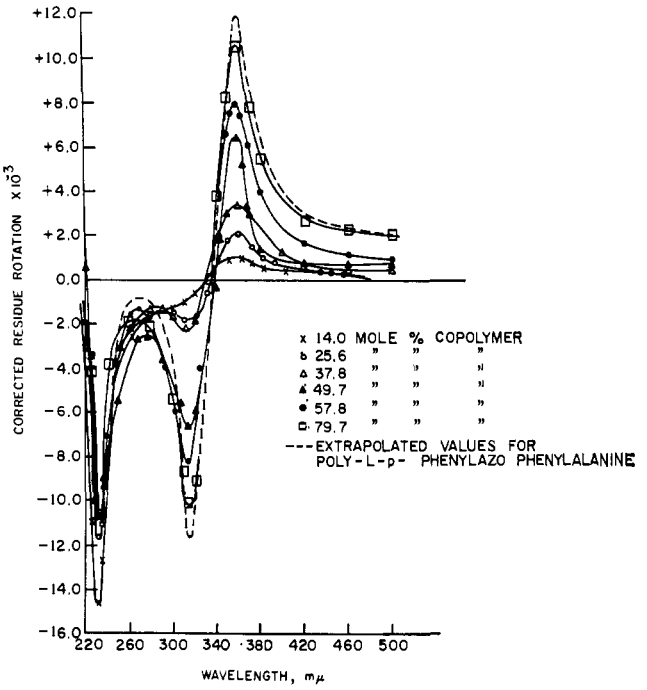


Figure 3. Optical rotatory dispersion of azopolypeptides in dioxane.

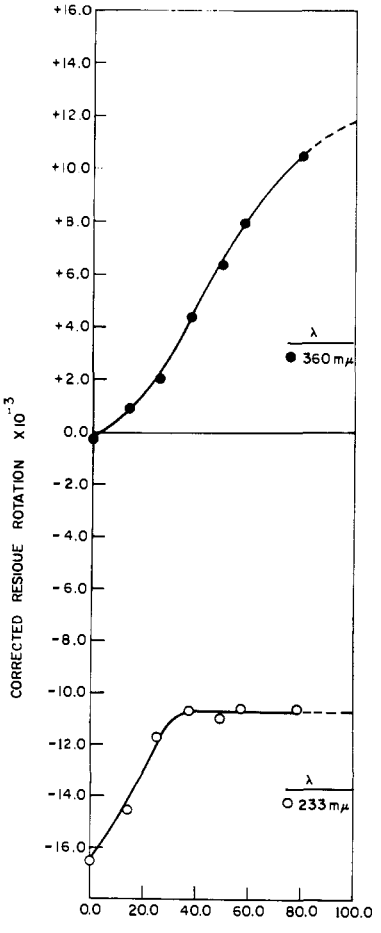


Figure 4. Dependence in dioxane of corrected residue rotations on copolymer composition.

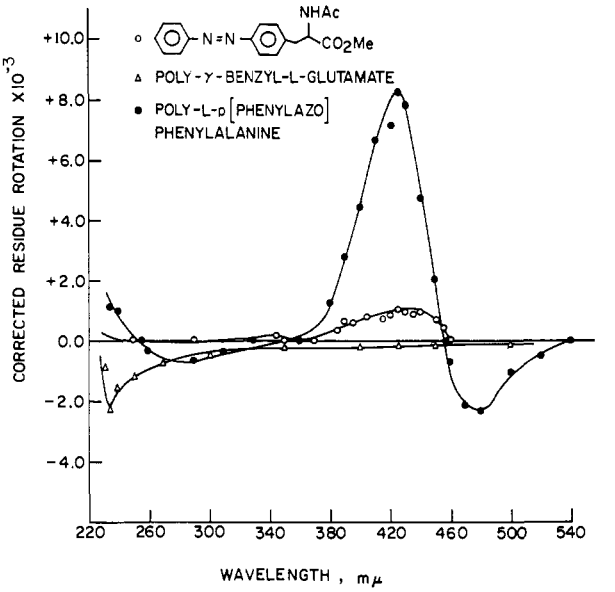


Figure 5. Optical rotatory dispersion of azo compounds in trifluoroacetic acid.

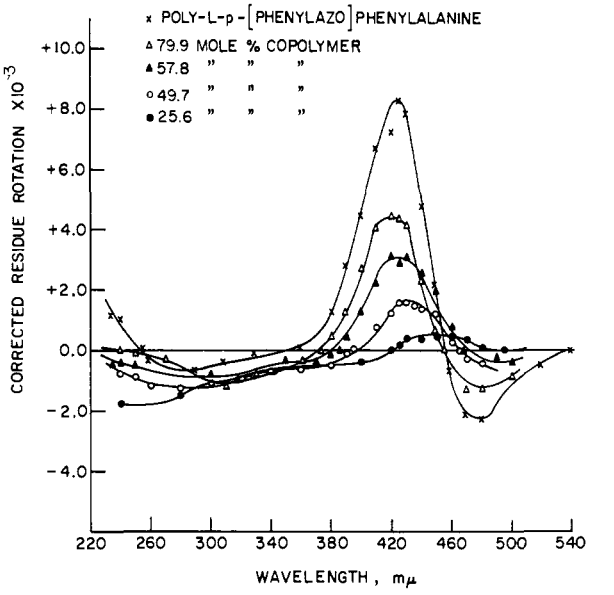


Figure 6. Optical rotatory dispersion of azopolypeptides in trifluoroacetic acid.

nearby asymmetric centers. The positive Cotton effect in trifluoroacetic acid arises from the conjugate acid of the azoaromatic chromophore and is therefore shifted to the 425-mμ region (Figures 5 and 6). The conjugate acid of the azoaromatic chromophore also exhibits induced asymmetry. Further, the appearance of the Cotton effect in trifluoroacetic acid is strong evidence for a high state of ordering in this strongly hydrogen-bonding solvent. The residue rotations of the azopolypeptides at 425 mμ show a dependence on the square of the mole per cent of azo residues (Figure 7). This fact, together with the shape of the Cotton effect, indicates the greater importance of side-chain–side-chain interactions in trifluoroacetic acid and suggests that these interactions bear a close resemblance to exciton band phenomena.[7c,22]

(22) I. Tinoco, *J. Am. Chem. Soc.*, **86**, 297 (1964).

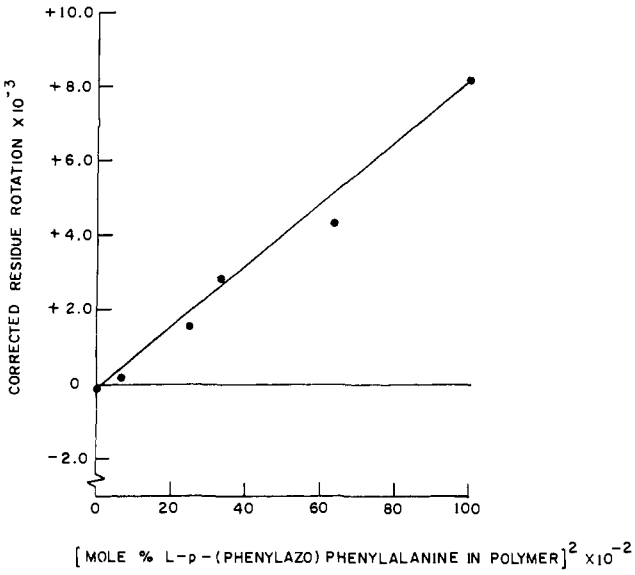


Figure 7. Dependence in trifluoroacetic acid of corrected residue rotations on copolymer composition.

Preliminary circular dichroism measurements[23] were carried out on several azopolypeptides. They show dichroic absorptions in dioxane and trifluoroacetic acid which correspond to the Cotton effects observed in these solvents. We are at present investigating the effect of photoisomerization on azopolypeptide conformations in solution. It should be noted that the photochemical properties of some azoaromatic vinyl polymers were used to obtain information on conformation.[24]

Experimental Section[25]

1. Commercial Materials and Solvents. Azobenzene was purchased from Distillation Products Industries and recrystallized several times from aqueous ethanol. L-*p*-Nitrophenylalanine was obtained from Cyclo Chemical Corp. and used without further purification. Anhydrous hydrochloric acid and phosgene were obtained from the Matheson Co. Dichloroacetic acid (Fisher, purified) and trifluoroacetic acid (Distillation Products Industries) were used without further purification. Dioxane used for polymerizations and hexane (Brothers Chemical Co.) were refluxed over and distilled from sodium. Dioxane used for optical activity measurements (Matheson Coleman and Bell, "Spectroquality" Grade) was passed through a column of alumina just prior to use[26] in order to remove water and peroxides. Ethyl acetate (Brothers Chemical Co.) was washed with 5% sodium carbonate and saturated calcium chloride solution, dried over anhydrous potassium carbonate, filtered, and distilled from phosphorous pentoxide.[27]

2. Preparation of Compounds. Nitrosobenzene. The method employed was that of Vogel.[12] Yields ranged from 50 to 60% (lit.[12] 65%). Recrystallization from ethanol gives a white solid (which turns green on heating) that has $\bar{\nu}_{max}^{Nujol}$ 118, 947, 777, 760, 688 cm^{-1}; λ_{max}^{EtOH} 217 mμ (log ε 3.79), 281 (3.99), 304 (3.86) [lit.[28] gives λ_{max}^{EtOH} 279 mμ (4.1), 300 mμ (3.9)].

L-*p*-Aminophenylalanine (1). The amino acid L-*p*-nitrophenylalanine (10.0 g, 0.048 mole) was reduced by catalytic hydrogenation in water following the procedure of Bergmann.[11] After recrystallization from aqueous acetone, the yields varied from 49 to 78% (lit.[11] 87%) of material with mp 235.0–237.0°. Infrared and ultraviolet spectra of the product show $\bar{\nu}_{max}^{Nujol}$ 3430, 3310, 1640 cm^{-1}; $\lambda_{max}^{H_2O}$ 283 mμ (3.11); $\lambda_{max}^{3N\ HCl}$ 256 mμ (2.68). The compound has $[\alpha]^{25}D$ −42.4° (*c*, 0.45; water).

L-*p*-(Phenylazo)phenylalanine (2). The procedure to prepare 4-methylazobenzene[29] was modified to prepare this compound. A typical experiment was as follows. To a stirred solution at 16–18° of L-*p*-aminophenylalanine (**1**) (4.4 g, 0.024 mole) in 50 ml of glacial acetic acid was added during a 40-min period a solution of freshly prepared nitrosobenzene (2.6 g, 0.024 mole). Immediate precipitation occurred. The mixture was diluted with 250 ml of distilled water and left standing at −5° overnight. The yellow precipitate was removed by filtration and dried over anhydrous calcium chloride *in vacuo* overnight. This procedure afforded 3.1 g (47% yield) of a yellow solid which has mp 219.7–220.5° dec (gas evolution). The spectra show $\bar{\nu}_{max}^{Nujol}$ 1630, 1600, 1520 cm^{-1}; $\lambda_{max}^{3N\ HCl}$ 229 mμ (3.98), 324 (4.21), 418 (3.20).

Anal. Calcd for $C_{15}H_{13}N_3O_2$: C, 66.90; H, 5.62; N, 15.55. Found: C, 66.76; H, 5.61; N, 15.60.

L-*p*-(Phenylazo)phenylalanine Methyl Ester Hydrochloride. To L-*p*-(phenylazo)phenylalanine (**2**) (5.4 g, 0.020 mole) suspended in 50 ml of anhydrous methanol was added anhydrous hydrochloric acid. The gas addition was carried out for 10 min. The solution was cooled to room temperature, concentrated under reduced pressure to *ca.* 15 ml, and diluted with 50 ml of anhydrous ether. The product was isolated by filtration, washed with ether, and dried overnight *in vacuo* over anhydrous calcium chloride. The yellow solid had $\bar{\nu}_{max}^{Nujol}$ 1720 cm^{-1} and was used without further purification in the next step.

N-Acetyl-L-*p*-(phenylazo)phenylalanine Methyl Ester (3). To the methyl ester hydrochloride (0.50 g, 0.0016 mole) was added 15 ml of pyridine and 3 ml of acetic anhydride (3.2 g, 0.032 mole). The mixture was stirred in an oil bath for 3 hr at 48–52° and then cooled and distilled at 21.8° (0.14 mm). The residue was washed three times with 10 ml of dry toluene. The resulting yellow solid was freed of the last traces of toluene *in vacuo* for 1 hr. The crude material melted at 143–149° and has $\bar{\nu}_{max}^{Nujol}$ 1720, 1640 cm^{-1}. Thin layer chromatography on silica gel using benzene–dioxane–acetic acid (50:50:2) as the developing solvent showed a single spot (R_f = 0.75). The compound was recrystallized from aqueous acetone and has mp 138.5–144.0°. The ultraviolet spectra show $\lambda_{max}^{dioxane}$ 228 mμ (4.20), 324 (4.25), 438 (3.23), and λ_{max}^{TFA} 300 mμ (3.83), 427 mμ (4.21).

Anal. Calcd for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.92. Found: C, 66.37; H, 5.71; N, 13.14.

L-*p*-(Phenylazo)phenylalanine N-Carboxy Anhydride (4). This substance was prepared from the amino acid **2** using the method of Farthing.[13] The crude material had $\bar{\nu}_{max}^{Nujol}$ 2230 (isocyanate), 1845, 1795, 1780 cm^{-1}; $\lambda_{max}^{dioxane}$ 228 mμ (4.20), 323 (4.38), 438 (2.82); λ_{max}^{TFA} 300 mμ (3.78), 427 mμ (4.53). Three recrystallizations in a drybox using anhydrous ethyl acetate–anhydrous hexane gives 3.4 g from 6.4 g of amino acid (48% yield). The material was stored at −20° until needed.

Poly-L-*p*-(phenylazo)phenylalanine. The N-carboxy anhydride **4** was polymerized by the procedure reported by Blout and Karlson.[15] After 1 week the polymer was precipitated by the addition of 100 ml of ether. The product was removed by filtration, redissolved, and reprecipitated with dioxane–ether three times. The orange solid was dried at 50° *in vacuo* over phosphorus pentoxide. Further purification was effected by Soxhlet extraction for 72 hr using refluxing dioxane and freeze drying the residue. (The yield is 1.3 g from 1.7 g of starting material; 90%.) The purified product has $\bar{\nu}_{max}^{Nujol}$ 1635 cm^{-1} and λ_{max}^{TFA} 306 mμ (shoulder) (3.63), 425 mμ (4.42).

Anal.[30] Calcd for $(C_{15}H_{13}N_3O_3)_n$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.07; H, 5.38; N, 15.40.

Copolymers of γ-Benzyl-L-glutamate and L-*p*-(Phenylazo)phenylalanine. These materials were prepared in an analogous manner to the homopolymer by using varying ratios of γ-benzyl-L-glutamate N-carboxy anhydride and L-*p*-(phenylazo)phenylalanine N-carboxy anhydride. The quantities used and yields obtained are tabulated in Table III and the analytical data in Table IV.

3. Apparatus and Measurements. Ultraviolet spectra were obtained using a Cary Model 14 ultraviolet–visible recording

(23) We thank Professor Sherman Beychok of the College of Physicians and Surgeons of Columbia University and Dr. Frank Bovey and his colleagues at the Bell Telephone Laboratories for these data.

(24) R. Lovrien and J. C. B. Waddington, *J. Am. Chem. Soc.*, **86**, 2315 (1964).

(25) Melting points are corrected. Microanalyses were carried out by Dr. Alfred Bernhardt of the Max Planck Institute, Mülheim, West Germany.

(26) L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed, revised, D. C. Heath, Boston, 1955, p 284.

(27) Reference 26, p 287.

(28) P. Ramart-Lucas and M. Martynoff, *Bull. Soc. Chim. France*, **16**, 905 (1949).

(29) F. K. Beilstein, "Handbuch der Organischen Chemie," Vol. 16, 1933, p 65.

(30) A residue of 1.55% of the original sample weight was obtained as a residue in the carbon–hydrogen determination.

Table III. Quantities Used and Yields Obtained for Polymers of L-*p*-(Phenylazo)phenylalanine and γ-Benzyl-L-glutamate

Mole % azo content[a]	γ-Benzyl-L-glutamate N-carboxy Anhydride mg	mmole	L-*p*-(Phenylazo)-phenylalanine N-carboxy Anhydride mg	mmole	*A*/*I*[b]	Yield mg	%
14.0	229	0.871	42.0	0.142	101	186	82
25.6	364	1.39	140	0.476	101	328	77
33.1	246	0.936	137	0.463	93.3	202	63
37.9	234	0.888	160	0.542	101	122	37
49.7	237	0.900	262	0.886	99.8	375	90
57.9	168	0.641	259	0.878	97.8	217	60
79.9	53.3	0.202	238	0.806	101	131	53

[a] Determined spectroscopically (see Results and Discussion). [b] Molar ratio of total anhydride to sodium methoxide initiator.

Table IV. Elemental Analysis of Polymers of L-*p*-(Phenylazo)phenylalanine and γ-Benzyl-L-glutamate

Mole % azo content	Calcd, % C	H	N	Found, % C	H	N	% residue of sample wt[a]
14.0	66.57	5.87	7.83	67.04	5.85	7.84	..
25.6	67.26	5.79	9.02	67.43	5.71	9.24	..
33.1	67.71	5.72	9.80	62.63	5.80	8.61	4.49
37.9	67.99	5.68	10.31	67.82	5.58	10.43	..
49.7	68.70	5.60	11.52	68.80	5.78	11.66	..
57.9	69.18	5.53	12.36	68.11	5.68	12.28	..
79.9	70.51	5.36	14.65	68.18	5.21	12.11	2.41

[a] Residues in the carbon and hydrogen analyses were reported for two of the copolymers.

spectrophotometer thermostated at 25.0 ± 0.5° and 1.00-cm fused silica cells. Infrared spectra were determined as Nujol mulls with Perkin-Elmer Models 21 or 137 spectrophotometers. Molecular weight determinations were carried out using a Beckman Model E analytical centrifuge. Optical rotatory dispersion data were obtained on a Cary Model 60 spectropolarimeter thermostated at 27.0 ± 0.5° with 0.100-mm (with TFA) and 1.00-cm (with dioxane) fused silica cells. Corrected residue rotations ([R′]) were calculated from the equation

$$[R'] = 3MRW[\alpha]^{T}_{\lambda}/10^{2}(n^{2} + 2)$$

where MRW = mean residue weight of copolymer and n = refractive index of solvent (uncorrected for wavelength).

Acknowledgment. We wish to thank Mr. Martin Falxa of our laboratories for his helpful participation in these researches and for carrying out some of the important rotatory dispersion measurements contained in this paper.

The Effect of Replacing One of the Hydrogens of the β-Carbon of the β-Mercaptopropionic Acid Residue in Deamino-oxytocin by a Methyl Group on Its Oxytocic and Avian Vasodepressor Activity[1]

Horst Schulz and Vincent du Vigneaud

Contribution from the Department of Biochemistry, Cornell University Medical College, New York, New York 10021. *Received July 25, 1966*

Abstract: The effect of replacement of one of the hydrogens on the β-carbon of the β-mercaptopropionic acid residue in the 1 position of the highly potent deamino-oxytocin by a methyl group has been investigated. A diastereoisomeric mixture of 1-L-β-mercaptobutyric acid-oxytocin and 1-D-β-mercaptobutyric acid-oxytocin was first prepared with the use of DL-β-benzylmercaptobutyric acid as the starting material. This mixture of diastereoisomeric analogs possessed approximately 40 units per mg of oxytocic and avian vasodepressor activities, representing about $^{1}/_{20}$th of the corresponding activities of deamino-oxytocin. Attempts to separate these diastereoisomers by countercurrent distribution and by partition chromatography on Sephadex G-25 were not successful, and in order to ascertain whether one or both of the diastereoisomeric analogs possessed biological activity it was decided to synthesize one of these diastereoisomers. DL-β-Benzylmercaptobutyric acid was subjected to resolution with the use of quinine, and the optically active β-benzylmercaptobutyric acid obtained thereby was shown to possess the D configuration. 1-D-β-Mercaptobutyric acid-oxytocin was then prepared and found to possess approximately 35 units per mg of oxytocic activity and 55 units per mg of avian vasodepressor activity. From the biological activities of the diastereoisomeric mixture of 1-D- and 1-L-β-mercaptobutyric acid-oxytocin and those of 1-D-β-mercaptobutyric acid-oxytocin, it is evident that replacement of one of the hydrogens on the β-carbon of the β-mercaptopropionic acid residue in the 1 position of the highly potent deamino-oxytocin results in a considerable decrease in oxytocic and avian vasodepressor activity.

It was recently found in this laboratory that replacement of the hydrogens on the β-carbon of the β-mercaptopropionic acid residue in the 1 position of the highly potent deamino-oxytocin[2] (Figure 1) by two methyl groups, as in deaminopenicillamine-oxytocin (1-β-mercaptoisovaleric acid-oxytocin), causes the total loss of oxytocic and avian vasodepressor activity.[3] In fact 1-deaminopenicillamine-oxytocin proved to be a highly

(1) This work was supported in part by Grant HE-01675 from the National Heart Institute, U. S. Public Health Service.

(2) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, 237, 1563 (1962); B. M. Ferrier, D. Jarvis, and V. du Vigneaud, *ibid.*, 240, 4264 (1965).

(3) H. Schulz and V. du Vigneaud, *J. Med. Chem.*, 9, 647 (1966).