equivocal and is confirmed in the recent report by Krauch, Farid, and Schenck.[4]

Irradiation of Coumarin in Dioxane Containing Benzophenone. The procedure to be described here may be taken as typical of our rotating turntable experiments. Six Pyrex test tubes (21 mm) were charged with 2.20 g (15 mmoles) of coumarin and 45 ml of *p*-dioxane. The tubes were divided into sets of two, one set containing 0 mg, one set with 52 mg (0.28 mmole), and the third set with 260 mg (1.5 mmoles) of benzophenone. After degassing with N_2, the tubes were sealed and placed in a turntable which surrounded a Hanovia 450-w immersion well. The whole apparatus was immersed in a large water bath and the table made to rotate by a small motor. During the photolysis the temperature of the system reached *ca.* 32°. An aqueous solution of nickel sulfate (250 g of the hexahydrate in 500 ml of water) was pumped through the immersion well as a coolant and a cylindrical Corex filter placed inside the well.

The mercury lines emitted by the lamp (A) and the combined absorbances, in units of absorbance (log I_0/I), of the filter solution and the Pyrex glass are as follows: 2967 (0.37), 3025 (0.25), 3130 (0.16), 3340 (0.13), 3660 (1.30). (These numbers are for an annular space of 3.7 mm in the immersion well; this number may be as large as 5.6 mm according to the manufacturer's specifications.) Using the wattage of light emitted by the lamp at each wavelength (according to specifications), the absorbances given above, and the absorbances of coumarin and the various concentrations of benzophenone at 3660 A, one obtains a net absorption for coumarin of 95–99% of the light, depending on the dimensions of the annular space.

The quantum yields presented in the text were derived from the duration of the photolysis (96 hr) and the portion of light captured by each tube. The latter is a maximum of $^1/_9$ (the fraction of the total circumference of the table occupied per tube); we have arbitrarily reduced this by a factor of 2 to take into consideration reflection, lamp intensity fluctuation, etc. Thus, the ϕ's given in the text are based on $^1/_{18}$ of the photons theoretically being transmitted by the filter solution and Pyrex.

After completion of the irradiation, the tubes were opened and aliquots examined in the fashion described above.

Irradiation of Coumarin in the Presence of Piperylene. The irradiation in acetonitrile was conducted as described under "Analytical Procedure" (see above) except that the photolysis mixture contained 200 μl (2.0 mmoles) of distilled piperylene (Aldrich Chemical Co.). The photolysis in propyl bromide, conducted on the turntable, involved the use of 45 ml of solvent, 2.2 g (15 mmoles) of coumarin, and 75 μl (0.75 mmole) of piperylene. A control containing no piperylene was run simultaneously.

Acknowledgment. We are grateful to the National Science Foundation (Grant GP-04511) for partial support of this work. We also thank Mr. William Ferree for assistance in parts of this project.

The Conformation of Poly-L-tyrosine in Quinoline from Dielectric Dispersion Studies

Jon Applequist[1] and Tibor G. Mahr

Contribution from the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa, and the Department of Chemistry, Columbia University, New York, New York. *Received June 23, 1966*

Abstract: The dipole moments, rotational relaxation times, and parameters expressing the polydispersity have been determined for poly-L-tyrosines of molecular weights 26,000–68,000 in dilute quinoline solution from measurements of dielectric dispersion and loss in the frequency range 1–100 kc. The molecular weight dependence of the dipole moment and relaxation time demonstrate that the molecular conformation is that of a rigid α helix over the temperature range 15–50°. The sense of the helix was determined by observing the effect of bromine substitution at the 3 position upon the dipole moment. An analysis of the accessible side-chain conformations showed that bromination should result in an increase in dipole moment for a right-handed helix and a decrease for a left-handed helix. An observed decrease of 1.0 ± 0.1 D. per residue was within the range predicted for the left-handed helix. This assignment of helix sense is opposite to that made by Fasman and co-workers on the basis of optical rotatory evidence and therefore appears to call for a reexamination of the theoretical basis for interpretation of the latter.

The purpose of the study reported here was to examine the dielectric dispersion behavior of poly-L-tyrosine in dilute solution, and from this to gain information on the dipole moment, molecular shape, and polydispersity of this material. It was further expected that conditions could thereby be discovered under which the polymer is in the form of a rigid helix, and that by studying the effect of brominating the tyrosine side chains at the 3 position (*ortho* to the hydroxyl group) the sense of the helix could be determined. It will be shown that the necessary conditions were met in quinoline solutions, and that the observed effect of bromination is consistent only with a *left-handed* sense of helix. To our knowledge this assignment of helix sense is the first to be made for a polypeptide in solution on an absolute basis, *i.e.*, as distinguished from those methods which are capable of determining only whether two polypeptides have the same or opposite helix sense.

The sense of the α helix has been one of the most elusive aspects of this structure. Molecular models reveal no obvious reason why one sense should be preferred over the other for any of the amino acids capable of taking on the structure, yet it is known from the effect of the structure on optical activity[2] that a preference exists. The theoretical interpretation of the optical rotation has apparently remained inconclusive as to the absolute configuration,[3] and in fact it appears that a determination of helix sense by some absolute

---

[1] To whom inquiries should be addressed at Iowa State University.

[2] P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).

[3] See G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965), for a summary of the current status of the application of the theory.

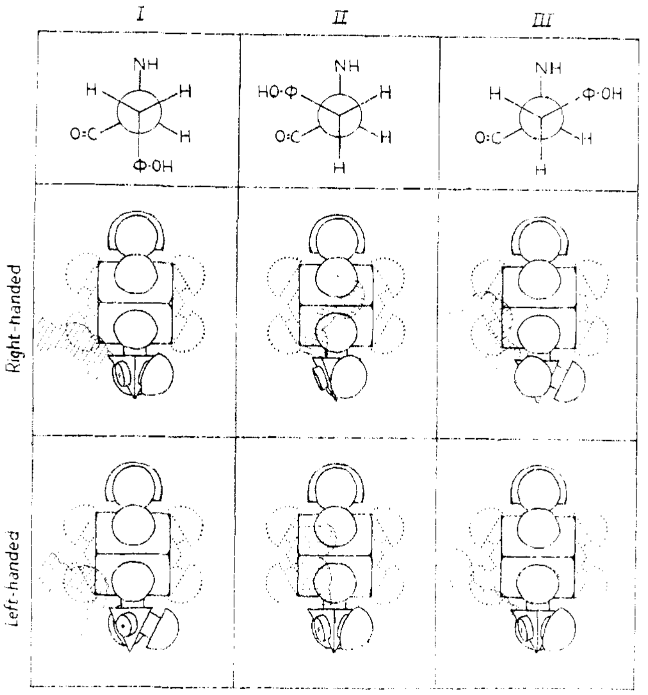


Figure 1. Courtauld models showing steric interactions between the L-tyrosyl side chain and the helix backbone (shaded area). The β carbon is at the bottom of each model and the OH group is at the top. The dotted outline shows the phenyl group in the plane of the paper.

method is required as a test of the validity of the approximations used in the theory of optical rotation.

A major step in the solution of this problem was the determination of the structure of crystalline myoglobin by Kendrew, *et al.*[4] It was found that a major part of the molecule consists of sections of a right-handed α helix. On the reasonable assumption that the same structure exists in myoglobin solutions, it then became possible to correlate optical rotatory behavior with helix sense.[5,6] This correlation has been applied to poly-L-tyrosine in aqueous solution by Fasman, Bodenheimer, and Lindblow,[7] and by Beychok and Fasman.[8] Their conclusion was that this material is in the form of a *right-handed* helix. Since we are led to the opposite conclusion from the present study, we find it necessary to question the basis on which the helix sense can be assigned from optical rotatory dispersion evidence. This point will be discussed on the last section of the paper.

A number of previous studies of dielectric dispersion of helical polypeptides in solution have appeared.[9–12] These have established the utility of the method in gaining information on the dipole moment, molecular shape, and polydispersity. None of the previous polypeptides studied have, however, contained side chains whose structure was such as to allow a determination of the helix sense from the measured dipole moments. The structural features that make this possible for poly-L-tyrosine will be discussed in the next section.

We call attention here to the related X-ray diffraction study of poly-3,5-dibromo-L-tyrosine in dimethylformamide solution by Brady, *et al.*[13] They conclude that the polymer forms an essentially rigid helix under the conditions of their study. Since poly-L-tyrosine is helical under the same conditions,[14] it appears that bromine substitution has little effect on the secondary structure. The same conclusion is reached in the present study on the basis of a somewhat more rigorous test.

Structural Considerations

In this section we analyze the structure of poly-L-tyrosine in the α-helical form in order to calculate the total dipole moment as a vector sum of the various group moments.

Figure 1 shows Newman projections of the C^α–C^β bond in the three conformations corresponding to the potential energy minima found in small molecules.[15] The drawings of Courtauld models (from Ealing Corp., Cambridge, Mass.) show the relative positions of the $CH_2C_6H_4OH$ group and the backbone of the helix (shaded area). The dotted outline shows the phenyl group in its position of maximum overlap with the backbone, and the solid outline shows the position of minimum overlap. For a right-handed helix it is seen that only conformation I can be achieved with little or no overlap, and likewise for conformation III in the left-handed helix.

Pao, *et al.*,[16] have considered the conformation about the C^α–C^β bond on the basis of models similar to ours, but have adopted the criterion that the phenyl ring should have maximum freedom of rotation about the C^β–C^γ bond, and have not considered the potential energy of rotation about the C^α–C^β bond. They conclude that the most favorable conformation for the left-handed helix is III, as we find also by our criteria. However, for the right-handed helix they find that C^β is rotated 45° clockwise from conformation III to give maximum freedom. This assignment appears dubious on consideration of the C^α–C^β rotational energy barrier. Assuming that the barrier height is 3 kcal,[15] their conformation would be 2.6 kcal above the minimum. If the range of rotation about the C^β–C^γ bond were increased as much as twofold on going from a staggered conformation to the Pao conformation, the entropy gain would be $R \ln 2$, giving a net free energy *increase* of $2.6 - RT \ln 2 = 2.2$ kcal. Thus the potential energy dominates the free energy, and the most probable conformation should be close to a potential energy minimum.

It will therefore be assumed in the following that there is negligible freedom of rotation about the C^α–C^β bond, and that its conformation is that of the least

---

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

(5) P. J. Urnes, K. Imahori, and P. Doty, *Proc. Natl. Acad. Sci. U. S.*, 47, 1635 (1961).

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

(7) G. D. Fasman, E. Bodenheimer, and C. Lindblow, *Biochemistry*, 3, 1665 (1964).

(8) S. Beychok and G. D. Fasman, *ibid.*, 3, 1675 (1964).

(9) A. Wada, "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p 131.

(10) E. Marchal and J. Marchal, *Bull. Ampère*, 9, 82 (1960).

(11) E. Marchal and J. Marchal, *ibid.*, 10, 63 (1961).

(12) S. Takashima, *Biopolymers*, 1, 171 (1963).

(13) G. W. Brady, R. Salovey, and J. M. Reddy, *ibid.*, 3, 573 (1965).

(14) J. D. Coombes, E. Katchalski, and P. Doty, *Nature*, **185**, 534 (1960).

(15) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y. 1960, p 130.

(16) Y. H. Pao, R. Longworth, and R. L. Kornegay, *Biopolymers*, 3, 519 (1965).

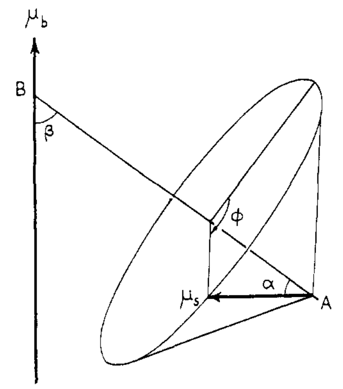


Figure 2. Geometry of the group moments in helical poly-L-tyrosine.

hindered potential energy minimum indicated in Figure 1.

The geometry of the significant group dipole moments is shown in Figure 2. μ_b is the component of the backbone moment per residue parallel to the helix axis, which is designated the z axis. (A small off-axis component of the backbone moment[9] is ignored, since its contribution is negligible in a long helix.) The direction of the side-chain group moment μ_s is specified by the angles α, β, and ϕ. The only degree of freedom is ϕ, which is the phase of rotation of μ_s about AB, the axis of the C^{β}–C^{γ} bond.

The probability of a given ϕ is proportional to exp $[-U(\phi)/kT]$, where $U(\phi)$ is the potential energy of internal rotation, k is Boltzmann's constant, and T is the absolute temperature. If we assume that AB is a rotational symmetry axis of order two or greater for the function $U(\phi)$, then the mean square dipole moment μ^2 of a helix with N residues is found by the appropriate averaging process[17,18] to be

$$\mu^2 = N^2(\mu_b + \mu_s \cos\alpha\cos\beta)^2 + N{\mu_s}^2\sin^2\alpha \qquad (1)$$

We will be concerned with cases where $N > 100$, for which the second term in eq 1 is negligible. The significance of the first term may then be understood as follows. The mean z component μ_r of the residue dipole moment becomes

$$\mu_r = \mu/N = \mu_b + \langle\mu_z\rangle \qquad (2)$$

where $\langle\mu_z\rangle$ is the average value of μ_z, the z component of the side-chain moment, whose instantaneous value is

$$\mu_z = \mu_s\cos\alpha\cos\beta + \mu_s\sin\alpha\sin\beta\cos\phi \qquad (3)$$

Upon averaging over ϕ under the condition stated above, the second term in eq 3 vanishes, and

$$\langle\mu_z\rangle = \mu_s\cos\alpha\cos\beta \qquad (4)$$

as is seen in eq 1.

In the present work we are interested in side chains whose polarity is equivalent to that of phenol and o-bromophenol. Both molecules are planar.[19,20] The relevant data for these compounds, obtained in benzene solutions, are given in Table I. For phenol $\alpha \cong 90°$, whence $\langle\mu_z\rangle \cong 0$. For poly-L-tyrosine it is therefore expected that $\mu_r \cong \mu_b$.

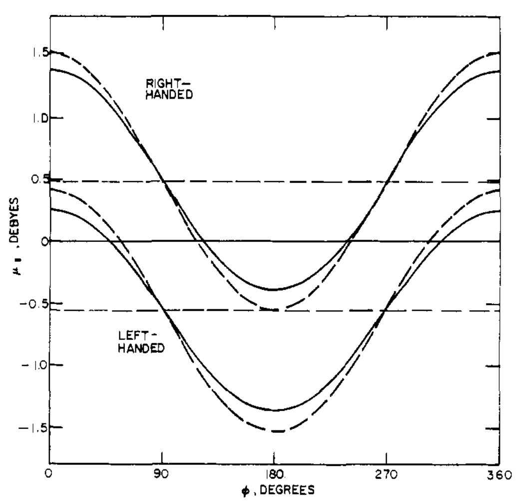
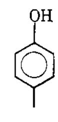
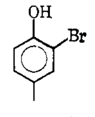
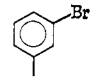


Figure 3. z-Component of the side-chain moment as given by eq 3, using the data in Table I for o-bromophenol (———) and for bromobenzene (- - - -); see text.

Table I. Side-Chain Group Dipole Moments

Group	μ_s, D.[a]	α, deg
	1.55	90[b]
	1.38	54.7[c]
	1.54	60[d]

[a] From ref 48. [b] Determined by decomposition of the group moments in p-chlorophenol and p-bromophenol. The Stark splitting in gaseous phenol is consistent with this assignment (H. Forest, personal communication). [c] Determined by decomposition of the group moments in o,p-dibromophenol (μ = 1.35 D. A. A. Antony, F. K. Fong, and C. P. Smyth, *J. Phys. Chem.*, **68**, 2035 (1964)). [d] From the geometry of the group.

Figure 3 shows the behavior of μ_z for 3-bromotyrosine as given by eq 3. The value of β was taken as 52.5° for the right-handed helix and 135.2° for the left-handed helix, as calculated from the atomic coordinates given by Pauling and Corey[21] and the direction of μ_b specified by Wada.[9,17] The solid curves in Figure 3 were calculated from the parameters for o-bromophenol in Table I. Since our experiments were carried out in quinoline rather than benzene, it might be argued that the intramolecular hydrogen bonding in o-bromophenol in benzene[20] would be disrupted in quinoline, thereby possibly changing the group moment. We estimate this effect by assuming that, under the conditions of interest, the OH and Br groups do not interact, and, therefore, that the net contribution of the side-chain moment is the sum of the

---

(17) A. Wada, *J. Chem. Phys.*, 31, 495 (1959).

(18) Equation 1 differs from Wada's eq 6, both because we are treating a somewhat different case and because, we suspect, some terms have been omitted from the latter.

(19) T. Kojima, *J. Phys. Soc. Japan*, **15**, 284 (1960).

(20) J. H. Richards and S. Walker, *Trans. Faraday Soc.*, **57**, 412 (1961).

(21) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci. U. S.*, **37**, 235 (1951).

contributions predicted for phenol and bromobenzene side-chain groups. Since the net contribution of a phenol group to $\langle\mu_z\rangle$ is very small, the significant quantity in this approximation is the bromobenzene moment, oriented as shown in Table I. The dashed curves in Figure 3 were calculated from these data. It is seen that μ_z is very insensitive to assumptions made about intramolecular hydrogen bonding. In particular, $\langle\mu_z\rangle$, shown by the horizontal dashed lines, is identical for both methods of calculating μ_z.

It is particularly important to note that the sign of $\langle\mu_z\rangle$ will be opposite for the two senses of the helix. This will apparently be true for any imaginable form of the function $U(\phi)$. A seeming exception would be a function with a strong potential energy minimum at $\phi = 180°$, which could cause $\langle\mu_z\rangle$ to be negative for a right-handed helix; or, likewise, such a minimum at 0° could cause $\langle\mu_z\rangle$ to be positive for a left-handed helix. The positions $\phi = 0$ and 180° are, however, almost exactly those at which the overlap between the side-chain and the helix backbone are maximal (see Figure 1). Therefore, it appears most likely that strong potential energy *maxima* will be associated with these conformations, rendering them relatively improbable.

Theoretical Background

Determination of Dipole Moment. Buckingham[22] has developed a theory of the dielectric constant of polar liquids, wherein each polar molecule is regarded as a homogeneously polarized ellipsoid embedded in a continuous medium having the bulk dielectric properties of the mixture. This model appears to be a good approximation to the system studied here. A modified form of Buckingham's equation is

$$\frac{4\pi\mathfrak{N}c\mu^2 fg}{9kTM} = \frac{(2\epsilon_s + 1)(\epsilon_s - n^2)}{3(2\epsilon_s + n^2)} - \frac{(2\epsilon_\infty + 1)(\epsilon_\infty - n^2)}{3(2\epsilon_\infty + n^2)} \tag{5}$$

where ϵ_s is the static dielectric constant of the solution, ϵ_∞ is the dielectric constant at some frequency where only the solvent contributes to the orientation polarization, n is the refractive index of the solution, c is the concentration of solute in g/cc, M is the molecular weight of the solute, $\mathfrak{N}$ is Avogadro's number, and f and g are given by

$$f = \frac{(2\epsilon_s + 1)[1 + A(n_2{}^2 - 1)]}{3[\epsilon_s + A(n_2{}^2 - \epsilon_s)]} \tag{6}$$

$$g = \frac{\epsilon_s[1 + A(n_2{}^2 - 1)]}{\epsilon_s + A(n_2{}^2 - \epsilon_s)} \tag{7}$$

where n_2 is the refractive index of the solute and A is the shape factor for the solute. For a prolate ellipsoid of revolution with axial ratio p greater than about 10, A is given sufficiently accurately by[23]

$$A = (\ln 2p - 1)/p^2 \tag{8}$$

Equation 5 follows from the assumption that Buckingham's theory for the static dielectric constant holds at the frequency where the dielectic constant is ϵ_∞ if one sets the effective solute dipole moment at that frequency equal to zero. This assumption is verified for our system by the observation that ϵ_∞ is very close to the static dielectric constant of pure quinoline and that the latter, in conjunction with Buckingham's equation, gives a dipole moment for quinoline (2.27 D.) in good agreement with the experimental value in the vapor state[24] (2.29 D.).

The quantity f enters the theory as the factor multiplying the true "gaseous" dipole moment μ to give the net moment of the solute molecule (polarized by the reaction field) plus the neighboring medium polarized by the solute molecule. The factor g takes into account the effects of the reaction field polarization of the solute and the cavity field correction of the orienting field to give the correct orientational energy of the solute molecules. For large axial ratios, where A approaches zero, it is seen from eq 7 that $g \cong 1$, corresponding to the fact that the reaction field vanishes and that the orienting component of the cavity field is identical with that of the applied field for a very thin rodlike particle whose dipole moment is parallel to the major axis. In this limit, f approaches $^2/_3$ for a fairly polar liquid ($\epsilon_s \gg 1$), and it is seen that an important effect of solvent polarization by the solute remains in this type of system.

Since A is small, an approximate value for n_2 may be used in eq 6 and 7 without sacrifice of accuracy, and we have taken the value $n_2 = 1.6$ found for crystalline tyrosine.[25]

A useful approximate form of eq 5 may be derived. Considering the limit of large p noted above and the limit of infinite dilution, where ϵ_s and ϵ_∞ both approach the dielectric constant of the pure solvent ϵ_0, eq 5 reduces to

$$\mu^2 = \frac{9kTM}{4\pi\mathfrak{N}b}\frac{\Delta\epsilon}{c} \tag{9}$$

where

$$b = 2 + n^2/\epsilon_0 \tag{10}$$

and $\Delta\epsilon = \epsilon_s - \epsilon_\infty$. Equation 9 was given by Oncley,[26] using other values for b. Wada's treatment[17] for solutions of rodlike particles gives eq 9 with $b = 3$. It is seen that this corresponds to the infinite dilution limit of the Buckingham treatment if $n^2 = \epsilon_0$, which is true only in a nonpolar solvent.

In the present study, dipole moments calculated from eq 9 agree to within 3% or better with those calculated from eq 5. All dipole moments reported here were, however, obtained from eq 5, with the aid of a computer (IBM 360/50 at the Iowa State University Computation Center). In these calculations n was taken as the value for pure quinoline at 587 mμ, given by the following relationship found to fit the data[27] over the temperature range $7° < t < 100°$.

$$n = 1.636 - 4.70 \times 10^{-4}t \tag{11}$$

Relaxation Times. According to the Debye theory of dielectric relaxation, the real and imaginary parts,

---

(22) A. D. Buckingham, *Australian J. Chem.*, **6**, 93, 323 (1953).

(23) Compare with E. G. Stoner, *Phil. Mag.*, **36** (7), 803 (1945).

(24) A. D. Buckingham, J. Y. H. Chau, H. C. Freeman, R. J. W. LeFevre, D. A. A. S. Narayana Rao, and J. Tardif, *J. Chem. Soc.*, 1405 (1956).

(25) "Handbook of Chemistry and Physics," 42nd ed, Chemical Rubber Publishing Co., Cleveland, Ohio, 1961.

(26) J. L. Oncley, *Chem. Rev.*, 30, 433 (1942).

(27) Landolt-Börnstein, "Zahlenwerte und Funktionen," Vol. 2, Part 8, 6th ed, Springer Verlag, Berlin, pp 5–656.

ϵ' and ϵ'', respectively, of the dielectric constant of the solution are related to the angular frequency ω of the applied field by[28]

$$\frac{\epsilon' - \epsilon_\infty}{\Delta\epsilon} = \frac{1}{1 + \omega^2\tau^2} \tag{12}$$

$$\frac{\epsilon''}{\Delta\epsilon} = \frac{\omega\tau}{1 + \omega^2\tau^2} \tag{13}$$

where τ is the so-called macroscopic relaxation time. There has been a considerable body of literature on the question of the relation between τ and the microscopic, or molecular, relaxation time. We make the assumption, supported by strong theoretical evidence,[29–31] that the two relaxation times are identical.

We further assume that the relaxation time of a rigid helix of radius b and length L is given sufficiently accurately by Perrin's equation[32] for the relaxation time of a prolate ellipsoid with the same minor and major axes, respectively

$$\frac{\tau T}{\eta_0} = \frac{\pi L^3}{6k[\ln (L/b) - {}^1\!/_2]} \tag{14}$$

where η_0 is the solvent viscosity. For poly-L-tyrosine and its brominated derivatives, we take $b = 9.5$ A, the approximate radius found from molecular models of the α helix.

There is a question as to whether the better macroscopic hydrodynamic approximation to a helix is a prolate ellipsoid or a cylinder. The model studies by Haltner and Zimm[33] and by Broersma[34] showed that the two cases differ significantly, and that the cylindrical model accounts more satisfactorily for the hydrodynamic properties of tobacco mosaic virus in water. However, this does not resolve the question for the present system, since the shortest dimension of the α helix is comparable to the size of the solvent molecules (about 9.5 A for the major axis of quinoline). Studies on the dielectric relaxation of small molecules suggest that, insofar as the relaxation time is sensitive to the shorter dimension, macroscopic hydrodynamic models are unreliable in such a situation.[35,36] In spite of this difficulty, a number of cases can be cited[9,37–40] where Perrin's equation or related treatments of the hydrodynamics of a prolate ellipsoid have shown rather good agreement with experimental data on rodlike macromolecules of relatively small dimensions.

Our objective is to determine L from the measured τ. An estimate of the maximum error in our results from application of eq 14 may be obtained from the fact that the length of a prolate ellipsoid is 10–12% greater than the length of a cylinder having the same minor axis and relaxation time, for the range of sizes of interest here, as determined by the equations of Perrin and Broersma. The actual error may be less, in view of the previous successes of the ellipsoidal model, and the fact that lengths calculated in this way for poly-L-tyrosine are more consistent with the α-helical structure than are lengths obtained from Broersma's equation.

Molecular Weight Distribution. Let $G(N)$ be the number-fraction of N-mers in the polymer system. For the case of a rigid helix, eq 12 and 13 are then replaced by[10,17]

$$\frac{\epsilon' - \epsilon_\infty}{\Delta\epsilon} = \sum_{N=1}^{\infty} \frac{N^2G(N)}{1 + \omega^2\tau_N{}^2} \Bigg/ \sum_{N=1}^{\infty} N^2G(N) \tag{15}$$

$$\frac{\epsilon''}{\Delta\epsilon} = \sum_{N=1}^{\infty} \frac{N^2G(N)\omega\tau_N}{1 + \omega^2\tau_N{}^2} \Bigg/ \sum_{N=1}^{\infty} N^2G(N) \tag{16}$$

where τ_N is the relaxation time of the N-mer. Marchal and Marchal[10,11] have shown that $G(N)$ may be determined uniquely from the observed dispersion data. The method is, however, subject to large errors, and it can be argued that any function $G(N)$ which reproduces the dispersion and loss curves, *via* eq 15 and 16, within experimental error is as good a representation of the molecular weight distribution as can be obtained. We therefore choose the function[41]

$$G(N) = \frac{r}{N\pi^{1/2}}e^{-r^2z^2} \tag{17}$$

where

$$z = \ln (N/N_0)$$

and where r and N_0 are constants, which may be adjusted to fit eq 15 and 16 to the experimental data, as will be demonstrated below.

The kth moment m_k of $G(N)$ may be calculated by representing N as a continuous variable

$$m_k = \int_0^\infty N^kG(N)\mathrm{d}N = N_0{}^ke^{k^2/4r^2} \tag{18}$$

Various averages of the degree of polymerization may thus be calculated: for example, the number-average $N_n = m_1$, the weight-average $N_w = m_2/m_1$, and the viscosity-average $N_v = (m_{a+1}/m_1)^{1/a}$, where a is the power of N which is proportional to the intrinsic viscosity.

Dispersion and loss curves calculated from eq 15 and 16 are shown in Figures 4 and 5, taking $N_0 = 200$ and various values of r. τ_N was calculated from eq 14, letting $L = 1.50N$ A, as required for the α helix. The calculations were done by computer, terminating the sums when 99.99% of the molecules had been included. The abscissas in Figures 4 and 5 are logarithmic in $\omega\tau_w$, where τ_w is the relaxation time of the species of weight-average N. Curves plotted in this way were found to be essentially independent of N_0. (This results from the fact that the summands in eq 15 and 16 can be reduced approximately to functions of the ratio N/N_0.) Therefore, the parameters r and τ_w may be determined by comparison of the experimental data with "standard" curves such as shown in Figures 4 and 5, for which N_0 was chosen arbitrarily.

---

[28] P. Debye, "Polar Molecules," Dover Publications, New York, N. Y., 1929, p 94.

[29] R. H. Cole, *Progr. Dielectrics*, **3**, 47 (1961).

[30] K. H. Illinger, *ibid.*, 4, 39 (1962).

[31] N. E. Hill, *Proc. Phys. Soc.* (London), **72**, 532 (1958).

[32] F. Perrin, *J. Phys. Radium*, (7) **5**, 497 (1934).

[33] A. J. Haltner and B. H. Zimm, *Nature*, **184**, 265 (1959).

[34] S. Broersma, *J. Chem. Phys.*, **32**, 1626 (1960).

[35] R. J. Meakins, *Trans Faraday Soc.*, **54**, 1160 (1958).

[36] R. D. Nelson, Jr., and C. P. Smyth, *J. Phys. Chem.*, **68**, 2704 (1964).

[37] P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).

[38] J. T. Yang, *ibid.*, **81**, 3902 (1959).

[39] J. T. Yang, *J. Polymer Sci.*, **54**, S14 (1961).

[40] S. Claesson, W. Kern, P. H. Norberg, and W. Heitz, *Makromol. Chem.*, **87**, 1 (1965).

[41] W. D. Lansing and E. O. Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935).

Table II. Analytical Data on Poly-L-tyrosine and Its Brominated Derivatives

Sample	X_{Br}[a]	C Calcd	C Found	H Calcd	H Found	N Calcd	N Found	Br Calcd	Br Found	$[\alpha]_{577}{}^{30}$
T-19	0	66.2	63.9	5.5	5.7	8.6	8.1			171.5
BT-1	0.2556	58.96	57.93	4.80	5.18	7.64	7.22	11.14	10.45	173.0
BT-2	0.4450	54.51	53.50	4.35	4.16	7.07	6.67	17.94	17.25	174.0
BT-3	0.5415	52.50	52.51	4.14	4.22	6.80	6.82	21.02	21.06	174.0
BT-4	0.6831					6.45	6.11	25.15	25.12	175.0

Elemental composition, %

[a] X_{Br} = number of bromine atoms per residue calculated from the elemental analysis to give least-squares deviation between calculated and found percentages.

The fortunate circumstance that τ_w is the reciprocal of an angular frequency near the steepest portion of the dispersion curve, and near the maximum in the loss curve, provides a sensitive method for determining the weight-average molecular weight M_w, using eq 14 and the assumption that the linear density is that of the α helix. It is also evident from Figures 4 and 5 that this determination is insensitive to errors in r. Wada[17] has shown that, if one sets $M = M_w$ in eq 9, then the dipole moment obtained is that of the species whose molecular weight is M_w. (This correspondence does not hold if one takes any other average for M.) After justifying the assignment of the α-helix structure, we will use molecular weights obtained from τ_w in eq 5. It is worth noting that: (1) the molecular weights, and therefore the dipole moments, determined in this way are probably the most *precise* obtainable; and (2) the experimental error in $\Delta\epsilon$ is largely self-canceling in the calculation of μ_r, by virtue of the propagation of this error in the values of μ and N_w obtained as described. Both the precision and accuracy of μ_r are thus significantly enhanced.

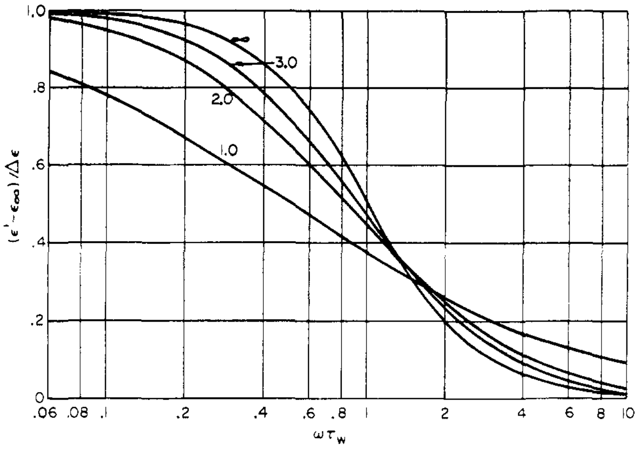


Figure 4. Theoretical dielectric dispersion curves for polydisperse systems of rodlike molecules whose dipole moments are proportional to their lengths and whose distribution is given by eq 17. Values of r are indicated on the curves. The case $r = \infty$ corresponds to a monodisperse system.

Experimental Section

Materials and Methods. Poly-L-tyrosine. Samples designated by the prefix T were obtained from Pilot Chemicals, Watertown, Mass. Sample J-25 was generously provided by Dr. J. D. Coombes before the material became commercially available. The purification is described below.

Quinoline. Matheson Refined or Fisher Reagent Grade quinoline was purified further by shaking 1500 g of quinoline with 50 g of barium oxide (Fisher Purified), followed by overnight standing and vacuum distillation of the decanted liquid at *ca.* 86° (0.3 mm), through an adiabatic 2.5-ft column packed with nichrome rings. The first and last 5% fractions were discarded. The procedure was repeated on the middle fraction, but substituting 30 g of alumina (Woelm neutral, grade I) for barium oxide, and this same procedure was again repeated on the middle fraction of the second distillate. The middle fraction of the third distillate was stored under a helium atmosphere in wash bottles wrapped in aluminum foil and fitted with Teflon stopcocks for passage of gas and liquid. Subsequent transfers of the liquid were carried out in a drybox. In this way the solvent was kept for 4–6 months without suffering discoloration or change in conductance. The lowest specific conductance attained was 3.0×10^{-10} mho/cm, which shows significant improvement over the value 10^{-8} mho/cm listed by Landolt-Börnstein.

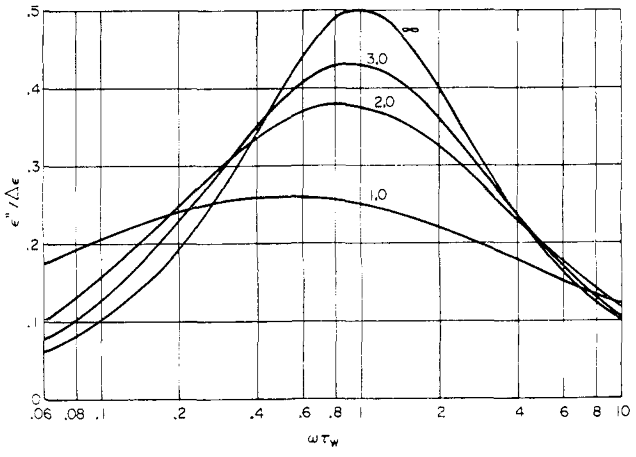


Figure 5. Theoretical loss curves for the same systems as in Figure 4.

Preparation and Purification of Solutions. Poly-L-tyrosine (0.2–0.5 g) or its brominated derivative was dissolved in 40–50 cc of purified quinoline by stirring overnight followed by heating for 2 hr at 60–65°. Ordinarily a fraction of up to 10% (or more for highly brominated samples) of the material remained undissolved and was removed by centrifugation at room temperature. Containers for solutions were foil-wrapped and glass-stoppered, and all transfer operations were carried out in a drybox. Solutions so prepared had specific conductances in the range 10^{-4}–10^{-5} mho/cm. The object of the further purification was to reduce the conductance to a level suitable for dielectric measurements. To the solution was added 3 g of alumina (Woelm neutral) which had been extracted with distilled water for 72 hr in a Soxhlet apparatus, oven-dried overnight at 140°, and finally ignited in a furnace at 800° for 4 hr. The suspension was three times alternately shaken (15–30 min) and centrifuged, after which the supernatant was removed and treated in the same manner with fresh alumina. After five or six such treatments with fresh alumina (using 1 g of alumina which had been mortar ground before the Soxhlet treatment in the fifth and sixth steps), the specific conductance of the supernatant solution was found by means of dip-in electrodes to have fallen below 10^{-8} mho/cm, and the solution was transferred to the capacitance cell for dielectric measurements. Subsequent optical rotation measurements showed that the fraction of the original polymer remaining in solution after this treatment was in the range 7–32%, tending to be higher for the lower molecular weight samples. While some impurities in the starting material are removed in the process, it is apparent that the alumina adsorbs a significant amount of the polymer, most likely resulting in a degree of molecular weight fractionation.

Poly-L-tyrosine was isolated for chemical analysis after the first treatment with alumina described above by precipitating with dry hexane, then five times alternately redissolving the centrifuged solid in 0.1 *M* NaOH (discarding a gummy residue) and reprecipitating by rapid addition of 0.1 *M* HCl. The final precipitate was shaken with ether to break the voluminous hydrogel, washed with ether, and dried overnight *in vacuo* at 60° over P_2O_5. The pulverized sample gave the analysis shown in Table II.

Bromination of Poly-L-tyrosine. A 1-g portion of poly-L-tyrosine, sample T-19, was dissolved in 10 cc of N,N-dimethylformamide (Eastman Spectrograde). A 0.12-g/cc solution of bromine (Baker reagent) in CCl_4 was added dropwise from a buret to the poly-L-tyrosine solution, with stirring in a water bath to maintain a temperature in the range 25–35°, until an amount had been added which would theoretically give the desired degree of bromination of the polymer. After continued overnight stirring, the polymer was precipitated with 0.1 *M* HCl, then purified as described above for hexane-precipitated poly-L-tyrosine. Analytical data are given in Table II.

Samples T-19, BT-2, and BT-4 were hydrolyzed to monomers with HCl and analyzed by the paper chromatographic method of Archer and Crocker.[42] (We are grateful to Bernard Mach for carrying out this analysis.) The major constituents were tyrosine (R_f 0.30) and, in the brominated samples, 3-bromotyrosine (R_f 0.40). 3,5-Dibromotyrosine (R_f 0.50) appeared as a trace constituent, along with an unidentified trace material (R_f 0.66). It is thereby established that bromination of a given residue more than once is largely excluded under the conditions used. This fact is of interest, although it offers no appreciable simplification in the interpretation of the dipole moment data.

The Dielectric Cell. The liquid under study was contained in a cell constructed of concentric platinum cylinders joined by metal-to-glass seals into a U-tube arrangement similar to that of de Vos.[43] The outer cylinder was connected to ground and was in contact with the thermostat liquid (Univolt 35 low permittivity transformer oil). The inner cylinder was connected to the "high" terminal of the measuring bridge by way of the inner terminal of a coaxial connector at the top of the cell. The outer shell of the coaxial connector was attached to a brass plate in which the cell was supported, and served as the "guard" point. The cell was calibrated with benzene (Fisher reagent) purified by the method of Leonard and Sutton,[44] and was found from measurements at 100 kc to have an air capacitance of 22.81 $\mu\mu$f, which was constant over the temperature range 20–60°.

Dielectric Measurements. Capacitance and dissipation factor measurements were carried out on a General Radio Type 1610-A capacitance measuring assembly, consisting of a Schering bridge with guard circuit, oscillator, frequency filter, amplifier, and null detector. The accessible frequency range for the systems studied here was 1–100 kc/sec. Measurements were made by the three-terminal substitution method. The filled dielectric cell was assumed to be equivalent to a resistance and capacitance in parallel in the calculation of the capacitance and dissipation factor. The limits of error were 0.1% in capacitance and about 2% in dissipation factor.

The apparent real dielectric constant ϵ'_{app} was calculated as the ratio of capacitance of the filled cell, corrected for lead capacitance, to the air capacitance given above. ϵ' was then obtained as $\epsilon' = \epsilon'_{\text{app}} - \epsilon'_{\text{p}}$, where ϵ'_{p} is the contribution from polarization capacitance, determined by interpolation of measurements on solutions of quinoline hydrochloride in quinoline having conductances in the same range as the polymer solutions. ϵ'_{p} was at most 8% of ϵ', and diminished with increasing frequency.

As used in eq 13 and 16, ϵ'' is the dipole relaxation contribution to the imaginary part of the dielectric constant, exlcuding the contribution from the conductance of the solution. Thus ϵ'' is related to the dissipation factor *D* by

$$D = \frac{\epsilon''}{\epsilon'} + \frac{1.80 \times 10^{12}\kappa}{\nu\epsilon'} \qquad (19)$$

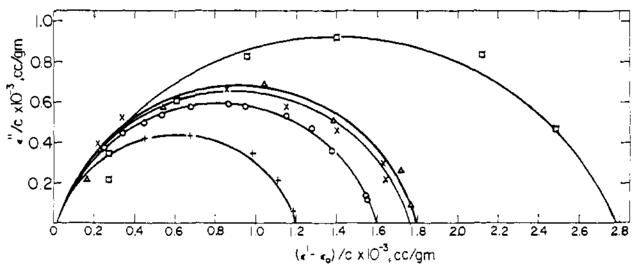


Figure 6. Cole–Cole arc plots for poly-L-tyrosines in quinoline at 30°. Samples: (+) T-15(c), (○) T-19, (×) T-14, (△) J-25, (□) T-6.

where κ is the specific conductance in mho/cm and ν is the frequency in cps. The second term on the right side of eq 19 was evaluated experimentally by assuming $\epsilon'' = 0$ at the lower limit of the frequency range. This assumption introduces some systematic error, but its effect on the final results is small. The limit of random error in ϵ'' near its maximum is about 5%, and becomes larger in the tails.

Regarding the magnitude of κ, it is of interest to convert the value found from eq 19 to an "equivalent" conductance Λ of the polymer, given by $\Lambda = M_n\kappa/c$. At 30° Λ was in the range 0.14–3.1 mhos cm²/mole, depending on the degree of success of the alumina treatment. If we assume that Cl^- is representative, in terms of mobility, of the major charge carriers present, and note that this ion would have an equivalent conductance of about 24 mhos cm²/mole when the aqueous solution value is corrected for the solvent viscosity,[45] then the lowest values of Λ would correspond to less than 0.01 charge carrier per polymer molecule. It is therefore reasonable to suppose that the polymer is a nonelectrolyte, as is necessary for the interpretation of the dipole moments. The conductance of the solutions most likely arises from remaining impurities in the sample, as well as from the conductance of the solvent.

Viscosity Measurements. Intrinsic viscosities $[\eta]$ were determined in quinoline at 25° by means of a Cannon–Ubbelohde semimicro viscometer having a solvent flow time of 380 sec, found by extrapolation of solution flow times to zero concentration. The concentration range covered was 0.17–1.0 g/dl.

Optical Rotation Measurements. Optical rotations were measured on a Rudolph manual spectropolarimeter, Model 200/S340/80AQ6, equipped with a John Fluke Model 412A photomultiplier voltage supply and a Hewlett-Packard Model 425AR DC-microvoltmeter and Hewlett-Packard Model 130C oscilloscope combined as detector. Solutions were contained in a 1-dm jacketed polarimeter tube. The rotatory dispersion was examined over the wavelength region 400–600 mμ, using zirconium and mercury arc lamps. Specific rotations of analyzed samples, listed in Table II, were determined at the 577-mμ mercury line at concentrations in the range 1.0–1.6 g/l. in quinoline. These values were then used to determine the concentrations of solutions purified as described above from their measured rotations. The precision in the latter was within 0.001°, corresponding to a precision of 0.5–2% in concentrations.

Results

Extrapolation of Dielectric Constants. ϵ_s and ϵ_∞ were obtained from a plot of ϵ'' *vs.* ϵ' by using a circular arc to extrapolate to zero ϵ''. The method is illustrated in Figure 6 for various poly-L-tyrosine samples. The ratios of dielectric constants to concentrations are shown in order to reduce all the data to a common basis. We have taken $(\epsilon_\infty - \epsilon_0)/c = 20$ cc/g for all samples at all temperatures, since this value was found from the data fitting the circular arc most precisely, those for sample T-19 at 30°. This was considered the most accurate means of establishing the smaller horizontal intercept of the arc, which thus acted as a fixed point in locating the entire arc. The values of ϵ_s and ϵ_∞ obtained in this manner are listed in Table III. The

---

[42] R. Archer and C. Crocker, *Biochim. Biophys. Acta*, **9**, 704 (1952).

[43] F. C. de Vos, *Rec. Trav. Chim.*, **69**, 1157 (1950).

[44] N. G. Leonard and L. E. Sutton, *J. Am. Chem. Soc.*, 70, 1564 (1948).

[45] H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed, Reinhold Publishing Corp., New York, N. Y., 1958, p 284.

Table III. Dielectric Properties and Dipole Moments of Poly-L-tyrosines and Brominated Derivatives in Quinoline

Sample[a]	Temp, °C	c, g/l.	ϵ_s	ϵ_∞	ν_w,[b] kc/sec	r	M_w[c]	μ, D.	μ_r, D.
T-15(a)	15	0.377	9.797	9.267	41.5	2.2	30,300 ± 660	942 ± 27	5.06 ± 0.08
	30	0.373	9.316	8.822	67.0	1.9	29,300 ± 470	919 ± 19	5.11 ± 0.08
	40	0.370	8.988	8.523	81.8	2.2	29,800 ± 630	916 ± 27	5.01 ± 0.08
	50	0.368	8.692	8.235	100	2.2	30,000 ± 670	927 ± 25	5.04 ± 0.09
T-15(b)	15	1.238	10.880	9.286	62.0	1.6	26,000 ± 1160	831 ± 21	5.22 ± 0.10
	30	1.224	10.275	8.839	88.5	1.9	26,400 ± 1300	819 ± 23	5.06 ± 0.11
	40	1.216	9.867	8.540	118	1.9	26,000 ± 570	794 ± 17	4.99 ± 0.06
	50	1.207	9.500	8.252	139	2.2	26,500 ± 460	793 ± 12	4.88 ± 0.05
T-15(c)	15	1.927	11.735	9.300	49.0	2.2	28,400 ± 460	866 ± 14	4.98 ± 0.04
	30	1.906	11.106	8.853	78.5	1.9	27,700 ± 530	846 ± 13	4.98 ± 0.05
	40	1.893	10.680	8.554	96.2	1.9	28,000 ± 560	843 ± 16	4.90 ± 0.05
	50	1.879	10.249	8.266	117	2.2	28,400 ± 780	834 ± 20	4.79 ± 0.07
T-19	30	1.240	10.797	8.840	28.0	1.9	40,800 ± 750	1202 ± 17	4.80 ± 0.05
T-14	15	1.156	11.550	9.284	14.1	1.6	45,400 ± 1300	1384 ± 39	4.97 ± 0.07
	30	1.143	10.843	8.838	22.6	1.6	44,200 ± 690	1321 ± 20	4.88 ± 0.05
	40	1.135	10.446	8.539	30.2	1.6	43,400 ± 1320	1300 ± 41	4.89 ± 0.07
	50	1.127	9.953	8.251	41.3	1.9	42,000 ± 1040	1228 ± 33	4.77 ± 0.06
J-25	30	0.157	9.098	8.818	12.7	2.7	54,700 ± 1510	1479 ± 109	4.41 ± 0.28
T-6	30	0.495	10.194	8.825	7.1	1.6	67,600 ± 1720	2063 ± 68	4.97 ± 0.10
	45	0.489	9.814	8.380	9.8	1.4	68,700 ± 1800	2190 ± 72	5.20 ± 0.10
BT-1	30	1.387	10.434	8.843	30.9	1.9	44,100 ± 1100	1064 ± 26	4.42 ± 0.06
BT-2	30	1.569	10.459	8.846	28.5	1.9	49,200 ± 1500	1064 ± 25	4.29 ± 0.07
BT-3	30	2.08	10.544	8.857	36.1	1.9	46,800 ± 2500	936 ± 40	4.11 ± 0.10
BT-3	30	1.15	9.885	8.838	36.1	1.9	46,800 ± 2200	972 ± 35	4.28 ± 0.10
BT-4	30	0.563	9.236	8.826	47.9	2.5	44,400 ± 1600	842 ± 35	4.12 ± 0.08
BT-4	30	0.383	9.099	8.823	47.9	2.5	44,400 ± 1400	837 ± 32	4.09 ± 0.08

[a] Letter in parentheses following sample number distinguishes separately purified batches. Molecular weight differences among batches are most likely due to partial fractionation during purification. [b] ν_w is the "critical" frequency of the weight-average species and is equal to $1/2\pi\tau_w$. [c] Determined from relaxation time.

limit of random error in ϵ_s due to the extrapolation procedure was estimated to be in the range 0.005–0.08 for the various samples.

The arcs shown in Figure 6 correspond to the empirical relationship of Cole and Cole[46]

$$\epsilon' - i\epsilon'' = \epsilon_\infty + \frac{\epsilon_s - \epsilon_\infty}{1 + (i\omega\tau)^{1-\alpha}} \tag{20}$$

where $i = (-1)^{1/2}$, τ is a characteristic relaxation time, and α is a parameter which increases from zero as the center of the arc is displaced downward from the horizontal axis. We find α to be in the range 0.10–0.25 for the polymers studied. For a macromolecular system this is indicative of a relatively narrow range of relaxation times, though by no means a monodisperse system.[47]

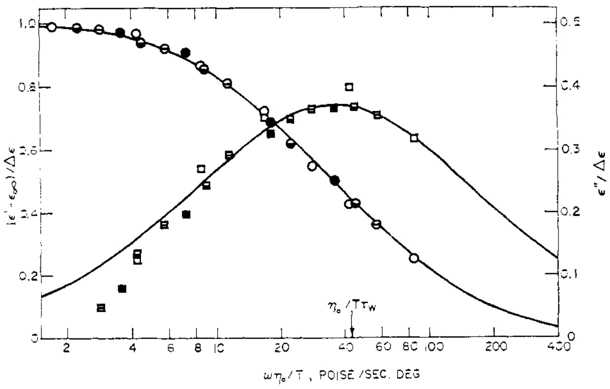


Figure 7. Dielectric dispersion (circles) and loss (squares) for poly-L-tyrosine T-15(c) in quinoline at various temperatures: (○, □) 15°, (⊖, ⊟) 30°, (◒, ⬓) 40°, (●, ■) 50°.

Determination of r and τ_w. Figure 7 shows the dispersion and loss curves for sample T-15(c) at four different temperatures. The frequency scale has been multiplied by the factor η_0/T in order to show, by the coincidence of the data, that the molecular shape is independent of temperature.

Figure 7 illustrates the general procedure used to determine r and τ_w. The dispersion of ϵ' was plotted on the scale shown and superimposed on the theoretical curve giving the best fit, in this case corresponding to $r = 1.9$.

The frequency at which $\omega\tau_w = 1$ was thus established, as shown by the arrow in Figure 7. As a check, the theoretical loss curve corresponding to the same parameters was then compared with the experimental ϵ'', and usually showed the quality of agreement seen in Figure 7. The loss curves could, of course, be used to obtain τ_w and r independently, but such values would be less reliable due to the greater uncertainty in ϵ''. The limits of random error in our procedure were estimated at about 10% in r and 3% in τ_w.

Tests for Helical Structure. Inasmuch as our interpretations of relaxation times and dipole moments involve the assumption of an α-helical structure, it is desirable to find independent support for this assumption. For the molecular weight range of interest in this study, relaxation times calculated from eq 14 are closely approximated by the relation

$$\tau T/\eta_0 \propto N^{2.65} \tag{21}$$

Furthermore, from eq 2 and 9

$$T\Delta\epsilon/c \propto N \tag{22}$$

---

(46) R. H. Cole and K. S. Cole, *J. Chem. Phys.*, **9**, 341 (1941).

(47) L. de Brouckère and M. Mandel, *Advan. Chem. Phys.*, **1**, 77 (1958).

Table IV. Molecular Weights and Intrinsic Viscosities of Poly-L-tyrosines

	$[\eta]$, dl/g			M_w	
Sample	0.2 M NaCl,[a] pH 11	Quinoline	Nominal mol wt[a]	From τ_w	Other measurement
T-15	0.15	0.175[d]	28,000	26,400 ± 1300	32,700 ± 4900[b]
T-19	0.3		49,000	40,800 ± 750	46,000 ± 2300[c,d]
T-14	0.28	0.608[e]	47,000	44,200 ± 690	36,200 ± 1700[b]
T-6	0.56	0.916[d]	68,000	67,600 ± 1700	74,200 ± 5700[b]

[a] Data supplied by Pilot Chemicals, Inc. No high accuracy is claimed for the molecular weights, since they are based on a rough calibration of the intrinsic viscosity. [b] From Yang's equation, using $[\eta]$ in quinoline. [c] Determined by light scattering in dimethylacetamide solution by M. L. Wallach and R. C. Miller, E. I. du Pont de Nemours and Co. [d] Determined on solutions untreated with Al_2O_3. [e] Determined on same solution used for dielectric dispersion measurements.

Thus

$$\tau T/\eta_0 \propto (T\Delta\epsilon/c)^{2.65} \qquad (23)$$

Equation 23 provides a test for a rigid helical structure on the basis of dielectric dispersion parameters. Figure 8 shows the appropriate quantities plotted on logarithmic scales, taken from the data in Table III for all poly-L-tyrosines at all temperatures, with the exception of sample J-25. A straight line with slope = 2.65 is shown for comparison. It is seen that the data are consistent with a helical structure that remains rigid over the ranges of temperature and molecular weight studied.

Independent molecular weight measurements provide further evidence for this conclusion. The available data are given in Table IV. The direct measure of M_w by light scattering for sample T-19 is reasonably consistent with that obtained from τ_w, allowing for a small extent of fractionation during purification for the latter measurement. Other values of M_w in Table IV were obtained from intrinsic viscosities in quinoline using a relationship due to Yang[39]

$$M_v = L_v{}^3/317.2[\eta][p^2/\nu(p)] \qquad (24)$$

where L_v is the length in Ångstroms of a molecule of viscosity-average molecular weight M_v, and $p^2/\nu(p)$ is a function tabulated by Yang. From our knowledge of the molecular weight distribution it was possible to obtain L_v from the appropriate relaxation time and to determine M_w as well as M_v. Rough agreement with molecular weights obtained for τ_w alone is seen in Table IV. Since the discrepancies appear to be largely attributable to experimental error, these results support the assumption that the molecular conformation is specifically that of the α helix.

Dipole Moment of Poly-L-tyrosine. Dipole moments are listed in Table III along with the total limits of random error calculated from the estimated errors in dielectric constants, concentrations, and relaxation times. Each μ and μ_r was calculated on the basis of M_w determined at the particular concentration and temperature of the experiment in order to take advantage of the self-cancellation of the errors in $\Delta\epsilon$ mentioned above. It is seen that variations in μ_r are largely accounted for by the estimated errors. The absence of a consistent trend with temperature or molecular weight suggests that any remaining discrepancies arise from systematic errors, which are in any case small. The constancy of μ_r is consistent with the conclusion that the molecule is a rigid helix over the range of temperature and molecular weight studied. The weighted mean of μ_r is 4.94 D., with a standard deviation of the mean of 0.014 D., both values having been calculated assuming that the standard deviations of individual measurements are approximated by the stated limits of error. The value 4.94 D. is thus a measure of the backbone moment μ_b, according to the discussion in the Structural Considerations section. This is approximately as expected, considering the dipole moment of N-methylacetamide in dioxane (4.40 D.)[48] and Wada's[9] prediction that the backbone moment should be in the range 4–5 D.

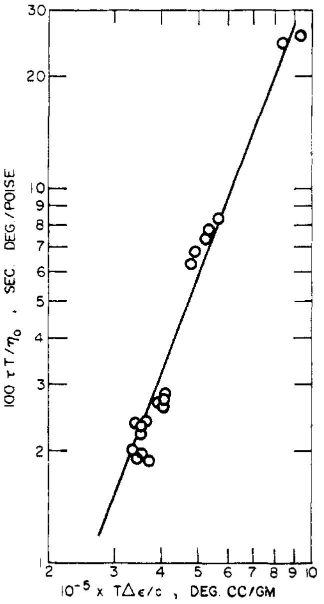


Figure 8. Relaxation time *vs.* dielectric increment, both corrected as shown for temperature dependence, for poly-L-tyrosines in quinoline. The solid line has the slope predicted by Perrin's equation.

Effect of Bromination. Dielectric dispersion curves for sample T-19 and its brominated derivatives are shown in Figure 9. It is seen that increasing bromination results in a marked decrease in ϵ_s, with little change in molecular weight. μ_r is, therefore, *decreased* by the addition of bromine, as shown in Table III and in Figure 10. The dashed lines in Figure 10 show the theoretical behavior of μ_r for the two senses of the helix as calculated from eq 2 and 4, letting μ_b take the value at the intercept of the experimental least-squares line, and using for the other quantities the same values as in Figure 3. From the slope of the experimental line, the

---

(48) A. L. McClellan, "Tables of Experimental Dipole Moments," W. H. Freeman Co., San Francisco, Calif., 1963.

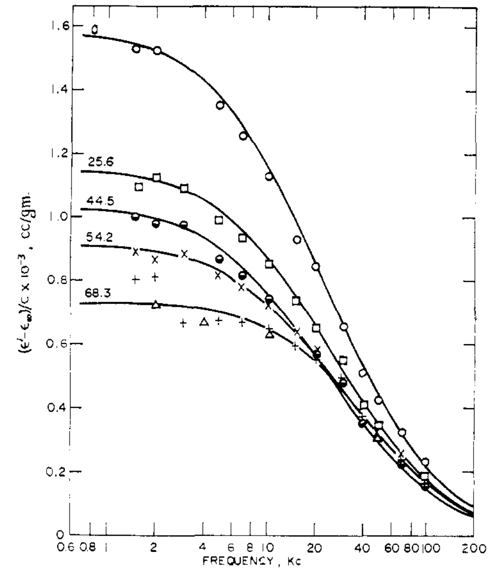


Figure 9. Dielectric dispersion curves for poly-L-tyrosine and its brominated derivatives in quinoline at 30°. The per cent of residues brominated is shown on each curve. The curves are theoretical, using parameters given in Table III. Samples: (○) T-19; (□) BT-1; (◒) BT-2; (×) BT-3, 2.08 g/l.; (+) BT-4, 0.563 g/l.; (△) BT-4, 0.383 g/l.

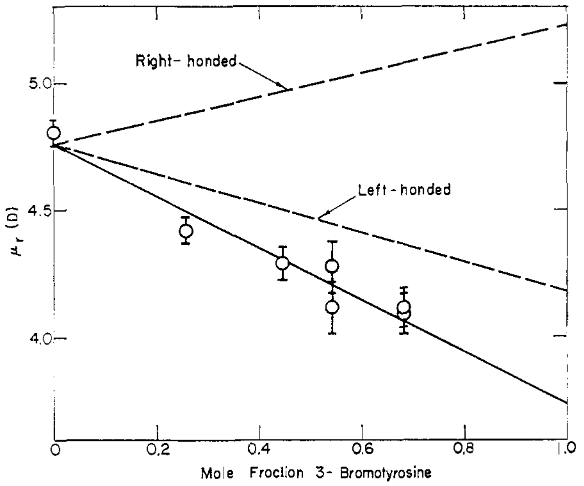


Figure 10. Residue dipole moment as a function of the degree of bromination of sample T-19. The dashed lines were calculated from eq 2 and 4. The solid line was determined from the experimental points by the method of least squares.

value of $\langle \mu_z \rangle$ for 3-bromotyrosine is found to be -1.0 ± 0.1 D. Reference to Figure 3 shows that this value is in the expected range for a left-handed helix. This agreement is interpreted as compelling evidence for assignment of the left-handed sense.

From the data in Table III it is observed that the molecular weight decreases slightly at high degrees of bromination, while the sharpness of the distribution remains essentially constant, increasing somewhat for sample BT-4. We would attribute this to removal of a high molecular weight fraction during isolation, rather than to degradation, since the latter should result in a broadening of the distribution, and since the fraction of insoluble material was observed to increase as the degree of bromination increased.

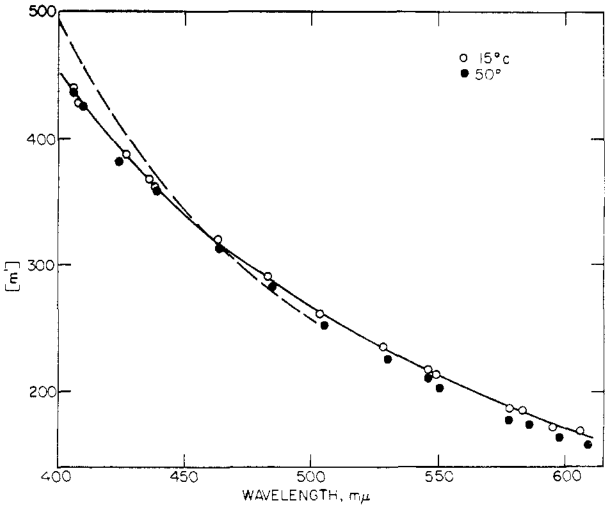


Figure 11. Residue rotation *vs.* wavelength for poly-L-tyrosine T-14 in quinoline at 15 and 50°. The dashed line is calculated from the data of Fasman, *et al.*[7]

Optical Rotatory Dispersion. The residue rotation [*m'*] of poly-L-tyrosine in quinoline is shown as a function of wavelength in Figure 11. The rotatory dispersion is seen to be essentially unaffected by temperature in the interval 15–50°, as is consistent with our conclusion that the molecule is a rigid helix and that the average conformation of the side chains does not vary in this interval. The dashed line in Figure 11 shows the rotatory dispersion in aqueous 0.2 *M* NaCl, pH 11.2, calculated from the dispersion parameters reported by Fasman, *et al.*[7] The near coincidence with our own data suggests that the sense of the helix is the same in the aqueous medium as in quinoline. Our assignment of helix sense thus conflicts with that made by Fasman, *et al.*, who have concluded that the helix is right handed from the observed sign of the rotation at 238 mμ.

Discussion

Perhaps the most interesting of the conclusions reached in this work is that regarding the sense of the helix. Few assumptions beyond well-established stereochemical principles were required to reach this conclusion. However, we must ask whether the observed effect of bromination (Figure 10) can be explained in any way other than our assignment of helix sense.

The fact that the molecular relaxation time shows little or no change when up to half of the tyrosine groups are brominated argues strongly against the possibility that solute aggregation or flexibility of the helix could explain the behavior in Figure 10. The absence of concentration dependence of the dipole moments in samples BT-3 and BT-4 is consistent with the assumption of unassociated solute molecules. Likewise, the concentration dependence of viscosity is, for poly-L-tyrosine, in the range expected for unassociated solute (Huggins constant = 0.51 for sample T-6).

Since Buckingham's equations were derived under the assumption that specific solute–solvent associations could be neglected, one might ask whether the effect of bromination is merely to change the solvation of poly-L-tyrosine by quinoline in such a way as to decrease the "apparent" dipole moment. The evidence weighs against such a possibility. The relative constancy of the dipole moment with temperature is not consistent with a hypothesis that loosely bound polar molecules contribute significantly to the measured dipole moment. Moreover, the agreement of μ_r for poly-L-tyrosine with the expected value suggests that the effect of solvation is at most a small perturbation on the apparent moment. The effect of bromine substitution on solvation can then reasonably be regarded as a perturbation of second order.

The fact remains that the observed $\langle\mu_z\rangle$ for 3-bromotyrosine is in the range predicted for a left-handed helix and is beyond that for the opposite sense, giving due consideration to the experimental errors. The interpretation that the helix is in fact left handed is the only reasonable explanation that has presented itself.

Our conclusions have particular bearing on the interpretation of the optical rotation and circular dichroism behavior of poly-L-tyrosine. It has been observed that the Cotton effect in the vicinity of the $n-\pi^*$ transition of the peptide group near 225 mμ is of negative sign,[7,8] as is also found for myoglobin in solution.[5,6] While Fasman, *et al.*,[7] have concluded that the helix is right handed in poly-L-tyrosine in this basis, Beychok and Fasman[8] have pointed out that the side-chain group has a transition at 225 mμ which shows a significant Cotton effect superimposed upon and opposite in sign to that of the $n-\pi^*$ transition. This circumstance raises two questions. (1) Can the sign of the $n-\pi^*$ Cotton effect be determined unambiguously? (2) If one knows the sign of the $n-\pi^*$ Cotton effect, is this necessarily related to the sign observed in myoglobin? The latter question arises because of the existence of a nearly coincident side-chain transition whose interaction with the $n-\pi^*$ transition could, in principle, have a large effect on the rotational strength of the latter.[49]

Pao, Longworth, and Kornegay[16] have attempted to find theoretical answers to these or related questions for poly-L-tyrosine. They have concluded that the side-chain transition at 225 mμ should have a negligible effect on the rotation in the vicinity of the $n-\pi^*$ Cotton effect. Unfortunately, this does not help us to resolve the dilemma. However, their conclusion is not consistent with the observation that the apparent rotational strength of the $n-\pi^*$ transition in poly-L-tyrosine is only about one-third of that for poly-L-glutamic acid in its helical state.[8] One may therefore suspect that the approximations used in the theory are not adequate.

We conclude that our finding of a left-handed helix sense for poly-L-tyrosine creates a need for a reexamination of the approximations used in the theory of optical rotatory dispersion, at least as applied to this material.

We emphasize also, in closing, that our study has not revealed any *cause* for the particular preference of helix sense found in poly-L-tyrosine. The free energy difference which produces this preference must be much greater than kT per molecule, but may still be a small energy per residue. Thus the responsible interactions may prove difficult to identify.

Acknowledgments. This investigation was supported by grants from the Research Corporation, the National Institute of Arthritis and Metabolic Diseases (A-4376 and A-5355), and the National Institute of General Medical Sciences (GM-10882 and GM-13684). We are grateful to Dr. A. D. Buckingham for a helpful discussion of certain aspects of this work, and to Dr. Morton L. Wallach and Mr. R. C. Miller for their careful molecular weight determination on sample T-19.

---

(49) I. Tinoco, Jr., *Advan. Chem. Phys.*, 4, 113 (1962).