small in each case, but a slight preference for the gauche–gauche rotamer is indicated.[32]

(32) NOTE ADDED IN PROOF. After submission of this manuscript an X-ray study of crystalline α-ψ appeared (D. C. Rohrer and M. Sundaralingam, *J. Amer. Chem. Soc.*, **92**, 4950 (1970)). It demonstrated that in the solid state the ribose ring of α-ψ is puckered exo C-2′, the base has the anti conformation, and the exocyclic hydroxymethyl group is

gauche–trans (II in Figure 3). Since the properties of the nucleoside in a crystal are strongly dependent on interactions with neighboring molecules, we believe the solution data to be more relevant to the conformation of a nucleoside in a biological system.

Conformational Properties of Poly-L-proline Form II in Dilute Solution[1]

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Abstract: The intrinsic viscosity of poly-L-proline has been studied as a function of molecular weight and temperature in five commonly used solvents: water, trifluoroethanol, acetic acid, propionic acid, and benzyl alcohol. The molecular weight range covered was 4400–99,000. The second virial coefficient of the high molecular weight sample has been determined as a function of temperature in four solvents. The $[\eta]$ are higher in the organic solvents than in water, but the log $[\eta]$ *vs.* log M_W plots are of the same shape in all solvents. The characteristic ratio is 14 in water and 18–20 in the organic solvents at 30°, and $d \ln \langle r^2 \rangle_0/dT$ is negative. The theoretical rotational potential function obtained by Hopfinger and Walton for L-prolyl-L-prolyl-L-prolyl-L-proline correctly predicts the characteristic ratio at 30° but predicts the wrong sign for $d \ln \langle r^2 \rangle_0/dT$. The conformational and hydrodynamic properties of poly-L-proline and cellulose derivatives have many features in common. The occurrence of cis–trans isomerization of the peptide bond is suggested in concentrated aqueous calcium chloride solutions.

In the solid state poly-L-proline has been observed to form an ordered structure in which the chain conformation is a right-handed helix with *cis*-peptide bonds (form I)[3] or a left-handed helix with *trans*-peptide bonds (form II).[4,5] In dilute solution poly-L-proline can also exist with all peptide bonds in either the cis or trans conformation.[6–14] These two forms can be reversibly interconverted by appropriate changes in solvent composition.[9,10,14] A less extended form of poly-L-proline exists in concentrated solutions of several salts.[6–8,15–17]

Theoretical conformational studies agree that there are severe steric restraints to rotation about the carbonyl carbon–α carbon bond, ψ, in both forms.[18–23] From their theoretical rotational potential function, Schimmel and Flory[19] predicted a limiting characteristic ratio of 116 for high molecular weight poly-L-proline with *trans*-peptide bonds. The other authors did not calculate the characteristic ratios predicted from their theoretical rotational potential functions. Upon carrying out the requisite calculations we have found that the predicted limiting characteristic ratios vary by more than an order of magnitude among the different investigators. All of these studies predict energy minima which yield reasonable agreement with the observed ordered structure of poly-L-proline form II.[4,5] Prediction of ordered structures only tests the minimum in the theoretical rotational potential function. The characteristic ratio is dependent upon both the location of the minimum and upon the shape of the rotational potential function and is a more exacting experimental test of the validity of the theoretical work. The measurement of the characteristic ratio of polypeptides with –CH_2R side chains,[24] which was

(1) This work was supported by a Grant from the Division of Biology and Medicine, Atomic Energy Commission.

(2) Recipient of Public Health Service Postdoctoral Fellowship 5-FO2-GM-41,377-02 from the National Institute of General Medical Sciences.

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Table I. Molecular Weight of Poly-L-proline Samples Studied

Sample	$10^{-3} M_N{}^d$	$10^{-3} M_W{}^e$	M_W/M_N	Supplier
SCC[a]	3.8 ± 0.3	4.4 ± 0.3	1.2 ± 0.2	Sigma Chemical Co.
L2[a]	7.0 ± 0.5	10.9 ± 0.7	1.6 ± 0.2	M. H. Liberman
MAN[b]	7.4 ± 0.5	9.2 ± 0.5	1.2 ± 0.2	Mann Research Corp.
MIL[b]	15.2 ± 0.6	16.3 ± 1.0	1.1 ± 0.1	Miles Lab.
GSC	15.6 ± 0.6			Gallard–Schlesinger
SCCA[c]	40 ± 3	97 ± 6	2.5 ± 0.3	Sigma Chemical Co.
SCCB[c]	53 ± 3	99 ± 6	1.9 ± 0.2	Sigma Chemical Co.

[a] Reference 27. [b] Reference 17. [c] The supplier claimed molecular weights of 200,000 and 330,000 for SCCA and SCCB, respectively, based on viscosity measurements. The absolute molecular weights listed reemphasize the previous observation[17] that molecular weights quoted for poly-L-proline by commercial suppliers are often inaccurate. [d] Number average. [e] Weight average.

important in evaluating the validity of the conformational maps for polypeptides of the poly-L-alanine type, illustrates this point.[25,26]

The hydrodynamic and thermodynamic behavior of poly-L-proline has been investigated as a function of molecular weight, temperature, and solvent in order to distinguish which of the theoretical studies best accounts for the observed properties of poly-L-proline, to explain the solvent effect on $[\eta]$, and to take advantage of the recent availability of a high molecular weight sample of poly-L-proline.

Experimental Section

Materials. The poly-L-proline samples, together with their sources and molecular weights, are listed in Table I. The molecular weights of SCC, L2, MAN, and MIL have already been reported.[17,27] All polymers were supplied as form II except MIL and GSC, which were converted from form I to form II by heating for 5.5 hr at 100° in glacial acetic acid.[6] The data sheets furnished by the suppliers of MIL and GSC indicate that these samples are identical. Prior to use all polymer samples were dialyzed against distilled water and recovered by lyophilization. Solutions were prepared by weight from polymer dried under vacuum using a Dry Ice–butoxyethanol trap. Organic solvents were reagent grade and were used without further purification. Calcium chloride solutions were prepared as previously described.[17]

Osmometry. Osmotic pressures were measured using a Mechrolab 503 high-speed membrane osmometer equipped with a variable-temperature controller. Extensive osmometry was carried out with SCCB, the sample of highest molecular weight. S&S B-20 membranes were used with water; gel cellophane 600W membranes were used with acetic acid, propionic acid, and trifluoroethanol; and both gel cellophane 600W and deacetylated acetyl-cellulose S&S 08 membranes were used in benzyl alcohol. The membranes were obtained from ArRo Laboratories, Inc., and were conditioned as recommended by the supplier. Response times were long in benzyl alcohol, and absorption occurred, as shown by a substantial increase in the base line following the introduction of poly-L-proline solution. These problems were not encountered in the other solvents.

The concentration dependence of the osmotic pressure, π, is given by eq 1, where the A_i are virial coefficients, with $A_1 = 1/M_N$.[28]

$$\frac{\pi}{c} = RT(A_1 + A_2c + A_3c^2 + \ldots)$$ (1)

Analysis of the data from all experiments with SCCB except those in benzyl alcohol, and ignoring the contribution due to A_3, lead to M_N of $(56 \pm 11) \times 10^3$, with the highest M_N being obtained when A_2 was also high. The contribution from A_3 can be included in the analysis by using eq 2, which is obtained from eq 1 with $A_3 = M_N A_2{}^2/4$.[29] When analyzed in this manner, the M_N cluster around

53 $\times$ 10³, and the results at all temperatures fall within the range $(55 \pm 7) \times 10^3$. The $(1/M_N)^{1/2}$ obtained from all experiments using SCCB were averaged and A_2 was determined from the best fit to the data according to eq 2 using this average $(1/M_N)^{1/2}$.

$$(\pi/c)^{1/2} = (RT/M_N)^{1/2}(1 + A_2M_Nc/2)$$ (2)

The M_N of SCCA and GSC were determined in water using an S&S B-20 membrane.

Ultracentrifugation. Weight-average molecular weights were obtained by sedimentation equilibrium in a Beckman Model E analytical ultracentrifuge, equipped with interference optics, utilizing eq 3.[30,31] Here $M_W{}^{App}$ is the weight-average molecular weight

$$\frac{1}{M_W{}^{App}} = \frac{1}{M_W}[1 + A_2M_W(c_T + c_B) +$$
$$0.75A_3M_W{}^2(c_T + c_B)^2]$$ (3)

evaluated from the slope of the logarithm of the fringe number vs. the square of the displacement from the center of rotation, assuming an ideal solution,[32] and c_T and c_B are the equilibrium concentrations at the top and bottom of the cell, respectively. In the derivation of eq 3 it has been assumed that A_2 and A_3 are independent of the molecular weight distribution in the ultracentrifuge cell. Approximating A_3 by $A_2{}^2M_W/3$[30] leads to eq 4, which was used for the determination of M_W for SCCA and SCCB.

$$\left(\frac{1}{M_W{}^{App}}\right)^{1/2} = \left(\frac{1}{M_W}\right)^{1/2}\left[1 + \frac{A_2M_W(c_T + c_B)}{2}\right]$$ (4)

Viscosities. The $[\eta]$ of SCC, L2, MAN, and MIL in water at 30° are taken from ref 17. All other $[\eta]$ were determined as had been done for these samples. The viscosity of SCCB in trifluoroethanol was tested for shear dependence using a Cannon-Ubbelohde four-bulb viscometer. No shear dependence was observed.

Computations. Characteristic ratios were calculated using a Control Data Corporation 6400 computer. The evaluation of a transformation matrix from a rotational potential function and the calculation of the characteristic ratio were accomplished using computer programs written to carry out the calculations as described by Flory.[33]

Results

Intrinsic Viscosities. The data for the poly-L-proline samples in water, trifluoroethanol, benzyl alcohol, acetic acid, and propionic acid at 30° are presented as log $[\eta]$ vs. log M_W in Figure 1. The points for the organic solvents overlap extensively, and the results represent two curves, one for the organic solvents and one for water. Comparison with Figure 2, in which the data for the organic solvents have been displaced vertically so that the results for MIL are coincident for all solvents, shows that the effect of variation of solvent class can be almost completely

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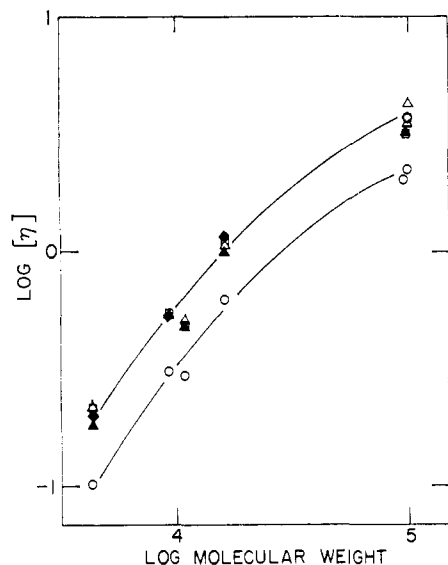(29) See ref 28, p 533.
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Figure 1. Log [η] $vs.$ log M_W for poly-L-proline at 30° in water (O), trifluoroethanol (△), benzyl alcohol (▲), acetic acid (□), and propionic acid (◆). The points for the organic solvents overlap extensively. The units of [η] are deciliters/gram.

accounted for in this manner. The dependence of [η] on M_W is of nearly the same form in all five solvents, even though the [η] themselves vary by as much as a factor of two for a given sample in going from water to an organic solvent.

Results for many polymers obey the empirical relationship given by eq 5, where K and a are constants.[34] Figures 1 and 2 clearly do not show a linear

$$[\eta] = KM^a \qquad (5)$$

dependence of log [η] on log M_W. The dashed line drawn through the data for the four lowest molecular weight samples in Figure 2 has a slope of 1.35, which is within the range previously found for these samples in water.[17] The slope decreases, however, as M_W increases and has already become less than one. Due to the molecular weight heterogeneity of samples SCCA and SCCB, the viscosity-average molecular weight of these samples may be slightly different from M_W,[35] leading to a small uncertainty in the slope at the high molecular weights.

The [η] of SCCB is greatly reduced in concentrated aqueous calcium chloride. The change in [η] with calcium chloride is qualitatively similar to that reported for MIL.[17] However, for this high molecular weight sample approximately a sixfold decrease in [η] is observed as it varies from 2.21 dl/g in water to 0.35 dl/g in 4.8 M calcium chloride at 30.

The temperature coefficients of [η] for several poly-L-proline samples in water, trifluoroethanol, and propionic acid at 5 and 30° and in benzyl alcohol at 30 and 70° are given in Table II. Ciferri and Orofino[16] report [η] at 25 and 55° in water for a sample similar to MIL. We calculate from their data that d ln [η]/dT is −6.6 × 10⁻³ deg⁻¹, which compares favorably with the results reported here.

The temperature coefficient is negative, and the absolute magnitude of the temperature coefficient
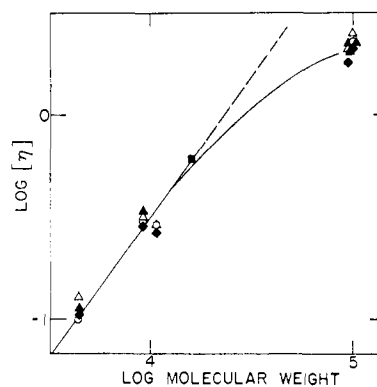
(34) See ref 28, p 310.
(35) See ref 28, p 313.

Figure 2. Data from Figure 1 with the organic solvents shifted vertically in order to bring the points for MIL into coincidence with the result obtained for MIL in water. There is considerable overlap in the data points.
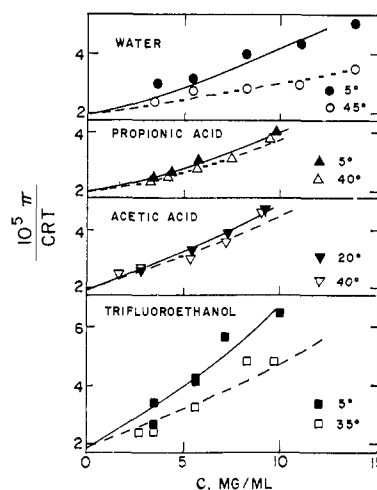

Figure 3. Reduced osmotic pressure of SCCB as a function of concentration at the highest and lowest temperatures studied in each solvent. The lines are those calculated using eq 2.

increases with increasing molecular weight. The temperature coefficient for SCCB is smaller in propionic acid and benzyl alcohol than in water and trifluoroethanol. The temperature coefficients are quantitatively similar to those reported for various cellulose derivatives.[36]

Table II. Temperature Coefficients of the Intrinsic Viscosity of Poly-L-proline

| | 10³ d ln [η]/dT, deg⁻¹ | | | |
Solvent	SCC	MAN	MIL	SCCB
Water	−4 ± 2		−6 ± 1	−8 ± 1
Trifluoroethanol	0 ± 2	−2 ± 1	−5 ± 1	−10 ± 1
Propionic acid				−4 ± 1
Benzyl alcohol				−2 ± 1

Virial Coefficients. Figure 3 shows the osmometry data, reported as π/cRT $vs.$ c, for SCCB at the highest and lowest temperatures in water, trifluoroethanol, acetic acid, and propionic acid. The lines are those calculated from eq 2 as described in the Experimental Section. Approximately the same A_2 was obtained

(36) P. J. Flory, O. K. Spurr, Jr., and D. K. Carpenter, $J. Polym. Sci.$, **27**, 231 (1958).
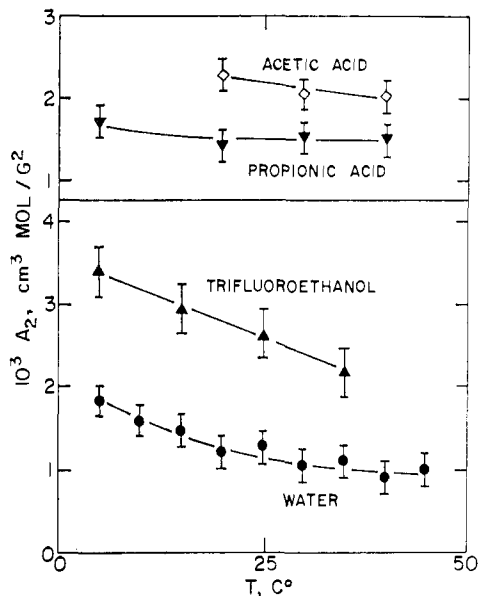
Figure 4. A_2 for poly-L-proline SCCB.

Figure 5. Predicted characteristic ratio for poly-L-proline according to the rotational potential functions of De Santis, et al. (DSGLR),[18] Schimmel and Flory (SF),[19] Hopfinger and Walton (HW),[20] and Madison and Schellman (MS).[22,23]

with SCCB in water by osmometry using eq 2 (1.1 ± 0.2) × 10^{-3} cm^{3}mol/g^2, and by sedimentation equilibrium using eq 4 (0.85 ± 0.1) × 10^{-3} cm^{3}mol/g^2.

The ability to obtain virial coefficients for poly-L-proline in water deteriorates at low molecular weight for reasons other than the onset of permeation through the membrane. It is well known that poly-L-proline heat precipitates in water at elevated temperature.[6,11,16,27] The rate of heat precipitation has been shown to be faster for L2 (M_W = 10,900) than for SCC (M_W = 4400).[27] Extension of these studies shows that the rate of heat precipitation increases as M_W increases up to 16,300. At higher molecular weight there is a dramatic change, since SCCA (M_W = 97,000) precipitates slower than does SCC (M_W = 4400). A 1% solution of SCCB (M_W = 99,000) in water exhibits a faint turbidity after 11 hr at 45°, so precipitation eventually occurs. However, for this molecular weight, osmotic equilibrium can be attained prior to aggregation, and reliable osmometry with SCCB in water at 45° can be attained.

In contrast, a 1% solution of GSC (M_N = 15,600) in water at 40° develops visual turbidity within 20 min, and the concomitant aggregation influences the π obtained. A reasonably accurate M_N for GSC could be obtained at temperatures as high as 30°, and A_2 was well defined at temperatures of 5–10°. At 35 and 40°, however, there was considerable scatter in the data. Forced extrapolation to the same intercept as found for GSC at 5° would yield an A_2 which is decidedly negative. Alternatively the data might be extrapolated to a smaller intercept, signifying an increase in M_N. It is clear that careful attention must be paid to the effects of aggregation when studying poly-L-proline samples of low to moderate molecular weight in water.

The A_2 of SCCB determined by osmometry in water, trifluoroethanol, acetic acid, and propionic acid are presented as a function of temperature in Figure 4. The A_2 are highest in trifluoroethanol and lowest in water. There is little temperature dependence of the A_2 in the acidic solvents in the range studied. The A_2
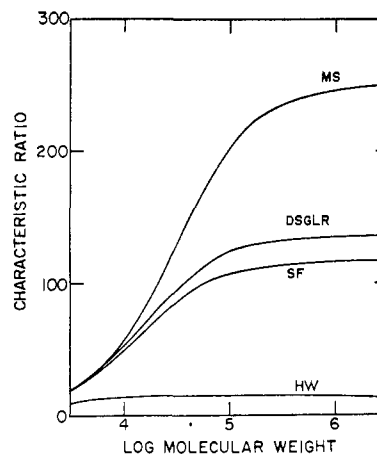
decrease with increasing temperature in water and trifluoroethanol.

Discussion

In describing the conformational properties of a disordered isolated chain, the key quantity of interest is the characteristic ratio.[37] For a polypeptide this is defined as $\langle r^2 \rangle_0/n_p l_p^2$, where $\langle r^2 \rangle_0$ is the unperturbed mean-square end-to-end distance, n_p is the number of peptide bonds, and l_p is the distance between adjacent α carbon atoms, which is 3.8 Å for planar trans-peptide bonds.[24] Theoretically, the characteristic ratio becomes independent of molecular weight at sufficiently high molecular weight, and the expected value can be calculated in a straightforward manner from the conformational map.[33]

Conformational maps for poly-L-proline have been calculated by several groups of investigators.[18−23] At least three different structures have been used for the proline residue. De Santis, et al.,[18] took their proline residue coordinates from the structure determined by Cowan and McGavin[4] for poly-L-proline form II in the solid state, thus setting the rotational angle at the α carbon–nitrogen bond, ϕ, at 104°. Schimmel and Flory,[19] Hopfinger and Walton,[20] and Holzwarth and Chandrasekaran[21] took the proline residue from the poly-L-proline form II of Sasisekharan.[5] Hopfinger and Walton[20] do not specifically state ϕ, but Schimmel and Flory[19] and Holzwarth and Chandrasekaran[21] indicate that it is set at 102°. Madison and Schellman[22,23] took the proline residue from the structure of L-leucyl-L-prolylglycine.[38] According to Madison and Schellman[39] the "standard value" of ϕ is 112°. However, according to Leung and Marsh,[38] ϕ is about 120°. Furthermore, Leung and Marsh[38] found two different structures for the proline residue, corresponding to different puckering at the γ carbon. It appears that Madison and Schellman[22,23] used a planar pyrrolidine ring. The effect of puckering of the pyrrolidine ring was considered by Hopfinger and Walton.[20] They calculated the rotational potential function for the

(37) See ref 33, Chapter II.
(38) Y. C. Leung and R. E. Marsh, Acta Crystallogr., 11, 17 (1958).
(39) V. Madison and J. Schellman, Biopolymers, 9, 569 (1970).

Table III. Expansion Coefficients and Characteristic Ratios for SCCB

Solvent	Expansion coefficient		Characteristic ratio	
	5°	30°	5°	30°
Water	1.11 ± 0.02	1.085 ± 0.025	14.8 ± 1.0	13.7 ± 0.9
Trifluoroethanol	1.105 ± 0.015	1.10 ± 0.02	22.8 ± 1.2	19.8 ± 1.3
Acetic acid		1.095 ± 0.015		18.8 ± 1.0
Propionic acid	1.065 ± 0.015	1.065 ± 0.015	21.1 ± 1.2	19.8 ± 1.2

four possible combinations of pucker in L-prolyl-L-proline and used for L-prolyl-L-prolyl-L-prolyl-L-proline that combination of puckering which permitted the greatest freedom of rotation in L-prolyl-L-proline.

The characteristic ratio has been calculated by a consideration of van der Waals repulsions and London attractions in adjacent L-proline residues by Schimmel and Flory,[19,40] with the results presented as curve SF in Figure 5. While the symptotic limit is attained only at molecular weights higher than those studied here, the characteristic ratio of SCCB would be within 8% of the limiting value. Schimmel and Flory[19] also pointed out that relatively small changes in their rotational potential function would lead to large variations in the predicted characteristic ratio.

The other groups[18,20-23] calculated rotational potential functions, but did not give the predicted characteristic ratios. We have calculated the characteristic ratios predicted by three of these rotational potential functions. Consideration of steric repulsions in poly-L-proline, with all ψ varied in concert, leads to curve DSGLR in Figure 5,[18] which is similar to SF. A rotational potential function for the poly-L-proline form II octamer has been calculated by Holzwarth and Chandrasekaran[21] with the inclusion of electrostatic interactions. These authors do not present their rotational potential function in a form which permits the prediction of a characteristic ratio, but they state that their rotational potential function is similar to that found by De Santis, et al.[18] Hence it can be assumed that they would predict a characteristic ratio similar to curves DSGLR and SF in Figure 5.

Calculation of the characteristic ratio of poly-L-proline can be carried out from Madison and Schellman's[22,23] rotational potential function for N-acetyl-L-proline-N,N-dimethylamide with a trans-peptide bond. They considered steric repulsions, London attractions, electrostatic interactions, and torsional potentials. The result, presented as curve MS in Figure 5, is predicted to be higher than that of any of the other groups.

Rotational potential functions based on the same types of interactions considered by Madison and Schellman,[22,23] plus a hydrogen-bonding function, have been presented for the poly-L-proline dimer, trimer, and tetramer.[20] All ψ were varied in concert. Calculations of the characteristic ratio from the rotational potential function for the tetramer yield curve HW in Figure 5. As mentioned earlier, this is based on the combination of pyrrolidine ring puckering which permits the greatest freedom of rotation in the

dimer. The characteristic ratio is predicted to be much lower, and SCCB would be in the limiting region.

All of the theoretical rotational potential functions have minima close to the angles observed for poly-L-proline form II in the solid state[4,5] and are in reasonable agreement with experiment in this regard. However, the predicted characteristic ratios vary by more than an order of magnitude, emphasizing the utility of the characteristic ratio in assessing the validity of a rotational potential function.

The characteristic ratio can be calculated in a standard manner, from the data presented here, using eq 6, 7, and 8.[24,41,42] The intrinsic viscosity in a θ solvent is

$$\frac{\langle r^2 \rangle_0}{n_p l_p^2} = \left(\frac{[\eta]_\theta}{\Phi M_v^{1/2}}\right)^{2/3} \frac{M_0}{l_p^2} \tag{6}$$

$$[\eta]_\theta = [\eta]/\alpha^3 \tag{7}$$

$$\frac{A_2 M}{[\eta]} = \frac{2^{5/2} \pi N_0 ln}{3^3 \Phi}\left[1 + \frac{\pi^{1/2}}{2}(\alpha^2 - 1)\right] \tag{8}$$

$[\eta]_\theta$, Φ is a universal constant, M_v is the viscosity average molecular weight, M_0 is the molecular weight of the L-proline residue, and α is the expansion coefficient. Similar procedures have previously been used to determine experimental characteristic ratios of polypeptides from measurements in good solvents.[24,43,44] In accordance with these previous studies, Φ was set equal to 0.0021 with [η] in deciliters/gram and distances in ångström. Any uncertainty in Φ is negligible compared to the diversity in the predicted characteristic ratios. The characteristic ratio depends upon $M_v^{1/3}$, and therefore an approximate value for M_v will suffice. Since M_W/M_N is nearly two for SCCB and since $M_v = (2M_W + M_N)/3$ for the most probable distribution in a θ solvent,[35] which is the condition of relevance here, this relationship was used to estimate M_v.

The calculated results for the expansion coefficient α and characteristic ratio for the high molecular weight sample are presented in Table III. The expansion coefficients are found to be very close to unity. At 30° the characteristic ratios fall into two classes, with a characteristic ratio of 14 in water and 18-20 in the organic solvents. The experimental characteristic ratios at 30° are close to the characteristic ratio of 15 predicted by the rotational potential function for the poly-L-proline tetramer.[20] They are less than those predicted by De Santis, et al.,[18] Schimmel and Flory,[19] and Holzwarth and Chandrasekaran[21] by a factor of 6-10, and less than the prediction from the rotational poten-

(40) Utilization of the transformation matrix in ref 19, which contains two significant figures, yields a limiting characteristic ratio nearly 10% lower than the value of 116 reported by Schimmel and Flory. When the transformation matrix is evaluated from their rotational potential function and three significant figures retained, the limiting characteristic ratio is 116, in agreement with their result.

(41) P. J. Flory, J. Chem. Phys., 17, 303 (1949).
(42) T. A. Orofino and P. J. Flory, ibid., 26, 1067 (1957).
(43) W. G. Miller, D. A. Brant, and P. J. Flory, J. Mol. Biol., 23, 67 (1967).
(44) S. Lapanje and C. Tanford, J. Amer. Chem. Soc., 89, 5030 (1967).

tial function of Madison and Schellman[22,23] by more than an order of magnitude.[45]

The calculation of the experimental characteristic ratio by means of eq 6–8 inherently assumes a Gaussian distribution of chain elements for the highest molecular weight poly-L-proline. This assumption would not be strictly justified if the treatment of De Santis, et al.,[18] Schimmel and Flory,[19] Holzwarth and Chandrasekaran,[21] or Madison and Schellman[22,23] were correct. It is, therefore, important to inquire whether the low characteristic ratio that has been deduced can be attributed to the misuse of eq 6–8. From Figure 5 it is seen that a poly-L-proline sample with $M = 1 \times 10^6$ would be in the asymptotic region of the SF and DSGLR curves, and nearly so for curve MS. The utilization of eq 6–8 would thus be clearly justified in this case. From eq 6 it is found that these characteristic ratios at $M = 1 \times 10^6$ require that $[\eta]_\theta$ be about 150 dl/g (SF), 190 dl/g (DSGLR), and 470 dl/g (MS). If α is in the range 1.06–1.10, eq 7 requires that the observed $[\eta]$ would have to be in the range 180–200 dl/g (SF), 230–250 dl/g (DSGLR), and 560–630 dl/g (MS). However, a linear extrapolation of the experimental results for MlL, SCCA, and SCCB in Figure 1 predicts $[\eta]$ to be no higher than 25 dl/g for $M_W = 1 \times 10^6$. We must conclude, therefore, that the characteristic ratio is substantially lower than is predicted by curves SF, DSGLR, and MS, so that the treatment of the experimental results given above is justified.

The theoretical rotational potential function for the poly-L-proline tetramer of Hopfinger and Walton[20] presents a single region of low conformational energy, surrounded by steep sides, in which two minima occur. After converting the definition of ψ to the standard convention,[46] and arbitrarily using the width where the computed energy is 2 kcal above the minimum, the rotational potential function is centered at $\psi = 0°$ with a width of 90°. The absolute minimum is at about 35° with another minimum of only slightly higher energy at about 330°. The latter is close to the ψ observed for poly-L-proline form II in the solid state.[4,5]

Schimmel and Flory[19] noted that the characteristic ratio would be very sensitive to any inadequacies in the theoretical rotational potential function. Therefore, they also presented the anticipated characteristic ratio as a function of the width of a square-well potential centered at $\psi = 310°$. The characteristic ratio was predicted to be about 20 for a width of 110°.

There are several possible causes which might contribute to the observed characteristic ratio being lower than the predictions from the conformational maps of De Santis, et al.,[18] Schimmel and Flory,[19] Holzwarth and Chandrasekaran,[21] and Madison and Schellman.[22,23] One of these is the possibility of puckering of the pyrrolidine ring. Hopfinger and Walton[20] showed that puckering does affect the conformational energy map of the L-proline dimer. The low character-

istic ratio predicted by their rotational potential function for the L-proline tetramer arises at least in part because they chose that combination of puckering which allows the greatest freedom of rotation in the dimer. Another source of conformational freedom, which was not included in any of the theoretical studies, is the possible variation in ϕ. The X-ray studies of poly-L-proline form II[4,5] and L-leucyl-L-prolylglycine[38] in the solid state show that this angle can vary from at least 102 to 120°. This might affect the predicted characteristic ratio if ϕ in solution (a) has a single fixed value different from those previously used, or (b) is not fixed but attains a range of values which could cover at least 102–120°. Another possible cause could arise from rotations about the peptide bond ω.[47] Madison and Schellman[22,23] calculated the conformational energy for rotation about ω when ψ is fixed at the angle corresponding to the minimum conformational energy. They found that ω could be rotated about $\pm 20°$ from planarity before the energy rose to 2 kcal above that found at the minimum. This effect has not been incorporated in any of the theoretical rotational potential functions.[48]

We note that the characteristic ratio is significantly lower in water than in the organic solvents. A solvent effect on the characteristic ratio has also been deduced in cellulose derivatives.[36] The theoretical characteristic ratio of cellulose is extremely sensitive to small changes in the angle at the bridge oxygen atom.[49] The suggestion[36] that solvents may modify the rotational potential function in cellulose is therefore reasonable. The characteristic ratio of poly-L-proline is also extremely sensitive to small modifications in the rotational potential function.[19] In terms of a square-well rotational potential function centered at $\psi = 310°$, a change in width from 110 to 130° would account for the solvent effect on the characteristic ratio. It has been suggested that specific solvation by water stabilizes helical poly-L-proline.[13,50] The experimental results confirm that water affects the conformational properties of poly-L-proline, but instead of stabilizing the extended helical form, a less extended structure is indicated in water than in the organic solvents.

The temperature coefficient of the unperturbed dimensions, customarily reported as d ln $\langle r^2 \rangle_0/dT$, is sensitive to the details of the rotational potential function.[51] The observed d ln $\langle r^2 \rangle_0/dT$ calculated from the characteristic ratios at 5 and 30° in Table II are given in the second column of Table IV. For most chain molecules d ln $\langle r^2 \rangle_0/dT$ is usually in the range $\pm 1 \times 10^{-3}$ deg^{-1}.[52] While the uncertainty in the observed temperature coefficients is admittedly substantial, it appears that they are more strongly negative than the usual results, particularly in trifluoroethanol.

(45) J. P. Carver and E. R. Blout (in "Treatise on Collagen," G. N. Ramachandran, Ed., Vol. 1, Academic, New York, N. Y., 1967, p 411) estimated $\langle r^2 \rangle_0{}^{1/2}$ from the $[\eta]$ of relatively low molecular weight poly-L-proline samples by ignoring nonideality. Their results correspond to characteristic ratios of about 10 and 14 in water and acetic acid, respectively, and are in qualitative agreement with those reported here for high molecular weight poly-L-proline taking account of nonideality.

(46) J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, Biopolymers, 4, 130 (1966); J. Biol. Chem., 241, 1004 (1966); J. Mol. Biol., 15, 339 (1966).

(47) G. N. Ramachandran and V. Sasisekharan, Advan. Protein Chem., 23, 283 (1968).

(48) We cannot calculate the effect of this rotation about ω on the characteristic ratio predicted by Madison and Schellman[22,23] because they only present the conformational energy as a function of ω at a single ψ, while the conformational energy at all attainable ω and ψ would be required for the calculation.

(49) N. Yathindra and V. S. R. Rao, Biopolymers, 9, 783 (1970).

(50) W. F. Harrington and P. H. von Hippel, Arch. Biochem. Biophys., 92, 100 (1961).

(51) See ref 33, p 39.

(52) See ref 33, p 45.

The temperature coefficient of the unperturbed dimensions predicted by the theoretical rotational potential functions can be calculated by assuming that these functions are independent of temperature in the range 5–30°. The results of this calculation are presented in the last column of Table IV.[53] The

Table IV. Temperature Coefficients of the Unperturbed Dimensions of Poly-L-proline

Solvent	Obsd[a] $10^3 d \ln \langle r^2 \rangle_0 / dT$, deg^{-1}	Ref	Predicted[b] $10^3 d \ln \langle r^2 \rangle_0 / dT$, deg^{-1}
Water	-3 ± 4	c	-6
Trifluoroethanol	-6 ± 4	d	-2.5
Propionic acid	-3 ± 4	e	$+1$
		f	-5

[a] Based on the experimental characteristic ratios at 5 and 30°. [b] Assuming the rotational potential function is independent of temperature. [c] Reference 18. [d] Reference 19. [e] Reference 20. [f] References 22 and 23.

predictions from the work of De Santis, et al.,[18] Schimmel and Flory,[19] and Madison and Schellman[22,23] are in agreement with experiment, even though the characteristic ratios at 30° are greatly overestimated. The rotational potential function of Hopfinger and Walton, which comes closest to predicting the observed characteristic ratio at 30°, predicts a positive temperature coefficient which is in disagreement with experiment. The positive temperature coefficient arises because the absolute minimum in the rotational potential function of the poly-L-proline tetramer is at $\psi \sim 30°$, while another minimum of only slightly higher energy occurs at ψ 330°. The latter leads to a more extended conformation.[47] Increasing population of the area near $\psi = 330°$ as the temperature increases will cause this rotational potential function to predict a positive temperature coefficient of the unperturbed dimensions.

The temperature coefficients of $[\eta]$ for most polymers are generally small at temperatures substantially above T_θ, while near T_θ they are large and positive.[54] In contrast, the temperature coefficients of $[\eta]$ for SCCB in Table II are large and negative, and are almost quantitatively similar to the results obtained with cellulose derivatives.[36,55] Equations 6 and 7 show that $[\eta]$ depends upon $\langle r^2 \rangle_0^{3/2}$. The large negative temperature coefficients of $[\eta]$ of SCCB are due to the large negative temperature coefficients of $\langle r^2 \rangle_0$ in Table IV. The α in Table III are nearly independent of temperature and have little effect on the temperature coefficient of $[\eta]$.

As had been found in the study of low molecular weight poly-L-proline,[17] $[\eta]$ for SCCA and SCCB decreases markedly in aqueous calcium chloride. In 4.8 M calcium chloride for SCCB is less than one-sixth of $[\eta]$ in pure water. When combined with the results presented previously,[17] the slope of log $[\eta]$ vs. log M_W is about 0.56 in 4.8 M calcium chloride for M_W from 4400

to 99,000. At low M_W there is a slight curvature of the same type, but of smaller extent, as observed in water. This indicates essentially a classical log $[\eta]$–log M_W relationship in this solvent system.

The characteristic ratio of SCCB in 4.8 M calcium chloride cannot be directly evaluated due to the difficulty in obtaining a reliable value for A_2 in this strongly interacting mixed solvent. Since concentrated aqueous calcium chloride is an excellent solvent for synthetic polypeptides,[56] it is reasonable to assume that A_2 must be positive. Equation 8 then requires that α be greater than unity, which in turn requires that $[\eta]_\theta$ is less than the observed $[\eta]$. Utilization of the observed $[\eta]$ in eq 6 allows the calculation of an upper limit of 4.6 for the characteristic ratio of poly-L-proline in 4.8 M calcium chloride at 30°. There is, therefore, a large reduction from the characteristic ratio observed in water. The characteristic ratio in 4.8 M calcium chloride is substantially less than the characteristic ratio of 9.0 $\pm$ 0.5 determined for four homopolypeptides with $-CH_2R$ side chains,[24] and at most only slightly greater than the characteristic ratio of 2.6 $\pm$ 0.5 determined experimentally in water for the sequential copolypeptide (Pro-Gly)$_x$.[57] It is difficult to explain how such a small $\langle r^2 \rangle_0$ could occur in poly-L-proline if the peptide bonds are all required to maintain their planar trans conformation. However, if calcium chloride interacts with the peptide group in such a manner as to increase the rotational freedom about the peptide bond,[15,58] or to promote cis–trans isomerization,[7,17,58] a low $\langle r^2 \rangle_0$ might be attained.

A very qualitative estimate of the effect of the random introduction of cis-peptide bonds into the poly-L-proline chain can be obtained by using a rotational potential function for the trans-proline residue which reproduces the observed characteristic ratio in water and by requiring ψ in the cis-proline residue to be at the energy minimum.[59] The cis-peptide bond was assigned the geometry of the standard cis-peptide unit of Ramachandran and Sasisekharan[47] and ϕ and ψ for the cis-proline were fixed at 104 and 345°, respectively.[18] Calculations were carried out using both the rotational potential function for the poly-L-proline tetramer[20] and a square-well at $\Phi = 102°$ and $\psi = 310 \pm 65°$ for the trans-proline residue. The characteristic ratios for poly-L-proline with various portions of cis-peptide units randomly distributed throughout the chain were calculated as described by Flory.[33] In order to facilitate comparison with the observed $[\eta]$, the characteristic ratios were converted to $[\eta]_\theta$ using eq 7 with $\Phi = 0.0021$ and taking account of the differences in l_p for cis- and trans-peptide bonds. The $[\eta]_\theta$ were very sensitive to the presence of cis-peptide bonds. In the limit of high molecular weight, $[\eta]_\theta$ could be reduced to half the value obtained with all peptide bonds in the trans conformation if 5–10% of the peptide bonds were converted at random to the cis conformation. This very qualitative calculation shows that peptide bond isomerization is a very reasonable mechanism for the effect of calcium chloride.

(53) The temperature coefficients in the last column of Table IV are subject to some uncertainty due to difficulty in accurately reading the conformational energy as a function of ψ from the published figures. However, this could not lead to a temperature coefficient with the wrong sign.

(54) See ref 28, p 622.

(55) L. Mandelkern and P. J. Flory, J. Amer. Chem. Soc., 74, 2517 (1952).

(56) D. R. Robinson and W. P. Jencks, ibid., 87, 2470 (1965).

(57) W. L. Mattice and L. Mandelkern, Biochemistry, in press.

(58) L. Mandelkern, J. C. Halpin, A. F. Diorio, and A. S. Posner, J. Amer. Chem. Soc., 84, 1383 (1962).

(59) We wish to thank Professor P. J. Flory for suggesting this type of calculation.

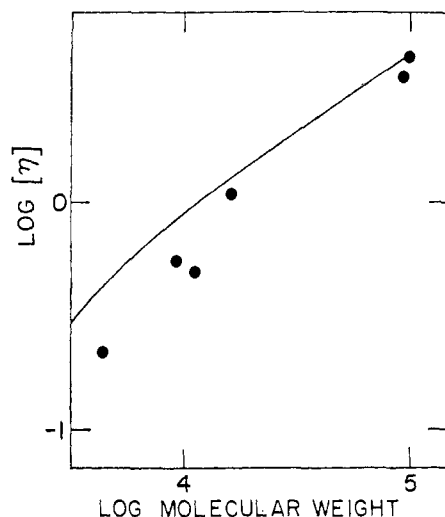Figure 6. Comparison of the observed $[\eta]$ in trifluoroethanol at 30° (●) to the $[\eta]$ calculated from eq 9 as described in the text (————).

Figure 7. Ratio of $\langle r^2_0 \rangle^{1/2}$ calculated from a square-well rotational potential function, located at $\phi = 102°$ and $\psi = 310 \pm 55°$, to the end-to-end distance of the completely extended chain with $\phi, \psi = 0°, 0°$ (————) and the ratio of the end-to-end distance for the ϕ,ψ of poly-L-proline form II in the solid state[4,5] to the end-to-end distance of the completely extended chain (— — — — —).

If the behavior predicted by eq 5 were obtained over the complete molecular weight range studied, the data in Figures 1 and 2 would describe a straight line. This equation frequently fails to predict the behavior of polymers at low molecular weight. The slope often decreases upon going to very low molecular weight for ordinary flexible polymers.[60] Less flexible polymers may exhibit curvature in the other direction at low molecular weight. Using DP for the degree of polymerization, cellulose obeys the relationship $[\eta] = 0.0177 DP^{0.92}$ at 20° in 50% sulfuric acid down to DP 150.[61] This corresponds to the DP of poly-L-proline MIL. The slope increases at lower molecular weight with a maximum slope of 1.3 at DP 15 being estimated.[61] Hunt, *et al.*,[62] suggested that the slope of 1.01 for log $[\eta]$ *vs.* log M_W observed with cellulose trinitrate of $M_W = (41-573) \times 10^3$ might decrease in the next decade of M_W. Poly-L-proline samples SCCA and SCCB are of sufficiently high molecular weight so that this change in slope can be observed.

In order to understand the causes of the curvature and high slope at low M_W in Figures 1 and 2, it is helpful to combine and rearrange eq 6 and 7 to give eq 9. The factors which could contribute to a high slope

$$[\eta] = \Phi\left(\frac{l_p^2 \langle r^2 \rangle_0}{M_0 n_p l_p^2}\right)^{3/2} M_v^{1/2} \alpha^3 \qquad (9)$$

in eq 5 have been discussed with reference to cellulose trinitrate.[62] These are (a) a positive $d\alpha/dM_v$, (b) a positive $d(\langle r^2 \rangle_0 / n_p l_p^2)/dM_v$ caused by the characteristic ratio not having attained its limiting value, and (c) a positive $d\Phi/dM_v$ caused by Φ not having attained its limiting value. The last factor could be due either to a hydrodynamic effect reflecting increasing permeation at the lower molecular weight or to deviations of the polymer configuration from random flight statistics and spherical symmetry which are required for the theory.[62]

The effect of $d\alpha/dM_v$ will be negligible for poly-L-proline because α must be unity for the monomer and is

only 1.1 at $M_W = 99,000$. The effect of $d(\langle r^2 \rangle_0 / n_p l_p^2)/dM_v$ can be estimated by the molecular weight dependence of the characteristic ratio calculated from a square-well rotational potential function at $\Phi = 102°$ and $\psi = 310 \pm 55°$, which satisfactorily reproduces the experimental characteristic ratio for SCCB in organic solvents at 30°. In Figure 6 the observed $[\eta]$ in trifluoroethanol are compared with those calculated from eq 9 using $\Phi = 0.0021$ and the characteristic ratios just described, and assuming that α varies linearly with molecular weight, being unity for the monomer and 1.1 for SCCB. It is readily apparent that molecular weight dependence of the characteristic ratio and α cannot account for the observed behavior. In order to account for the observed $[\eta]$ of the sample with $M_W = 4400$, Φ would have to be about half the value used for the sample with $M_W = 99,000$.[63]

The ratio of $\langle r^2 \rangle_0^{1/2}$, obtained from the characteristic ratios described in the preceding paragraph, to the end-to-end distance for the completely extended chain ($\Phi, \psi = 0°, 0°$) is shown as the solid line in Figure 7. The length of the fully extended chain was taken as 3.63 Å per residue.[47] For SCCB $\langle r^2 \rangle_0^{1/2}$ is only one-sixth that of the completely extended chain, indicative of a high degree of coiling. However, for the low molecular weight samples MAN and SCC it is 45 and 58%, respectively. Deviations from Gaussian statistics at these low molecular weights might cause the reduction in Φ. A similar effect has been suggested to be operative in cellulose trinitrate.[62]

It is instructive at this point to summarize the similarities in the properties of poly-L-proline and various cellulose derivatives in dilute solution. (a) Log $[\eta]$ *vs.* log M_W shows an increase in slope at low M_W.[61] (b) The temperature coefficient of $[\eta]$ is strongly negative.[36,55] (c) The temperature coefficient of the char-

(60) U. Bianchi and A. Peterlin, *J. Polym. Sci., Part A-2*, **6**, 1759 (1968).

(61) H. Vink, *Makromol. Chem.*, **94**, 15 (1966).

(62) M. L. Hunt, S. Newman, H. A. Scheraga, and P. J. Flory, *J. Phys. Chem.*, **60**, 748 (1956).
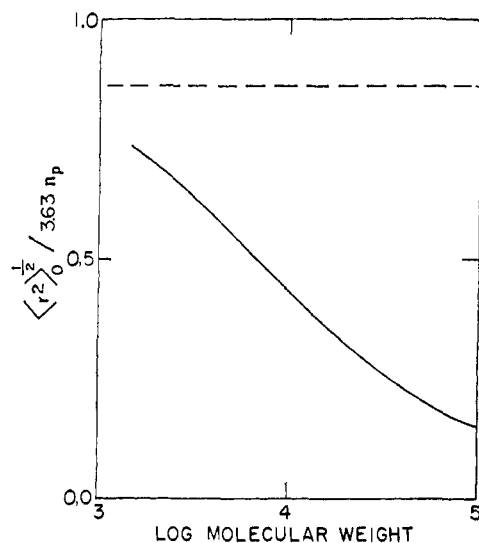
(63) A similar conclusion can be reached by comparing the $[\eta]$ calculated using the characteristic ratios predicted by the rotational potential function for the poly-L-proline tetramer[20] to the observed $[\eta]$ in water.

acteristic ratio is strongly negative.[55] (d) The characteristic ratio is solvent dependent.[36] (e) The characteristic ratios are higher than those found for most polymers.[62] (f) The α calculated in the conventional manner are close to unity.[62] It is reasonable that the explanations for the hydrodynamic behavior would be similar.

In a previous study[17] of poly-L-proline of M_W up to 16,300, the hydrodynamics were interpreted as reflecting the conformation of a somewhat flexible rod based on the a priori assumption of the Schimmel–

Flory conformational map,[19] which leads to such structures at low molecular weight. Based on the studies of the high molecular weight polymer we now find from Figure 7 SCC has an $\langle r^2 \rangle_0^{1/2}$ which is 58% that of the completely extended chain and 68% that of a rigid helix with the Φ and ψ determined for poly-L-proline form II in the solid state.[4,5] The coiling in this case is of such a low degree that spherical symmetry would not be attained. This offers an alternate, more consistent interpretation of hydrodynamic properties with molecular weight.

Communications to the Editor

Azomethine Ylide from Dimethyl 1-(p-Methoxyphenyl)aziridine-2,3(cis)-dicarboxylate. Kinetics of the Thermal Ring Opening

Sir:

The cis–trans isomeric aziridines 1 on heating establish equilibria with small concentrations of the azomethine ylides 2 by conrotatory ring opening as had been shown earlier.[1] The cyclic and the ring-opened structures are isoelectronic with cyclopropyl anions and allyl anions, respectively. The azomethine ylides 2 are 1,3 dipoles; trans-2 combines stereospecifically even with weak dipolarophiles, while in the case of the less reactive cis-2 the isomerization to trans-2 competes with the 1,3-dipolar cycloaddition except for dipolarophiles of highest activity (see Scheme I).[2]

Scheme I

Ar = $C_6H_4OCH_3$-p
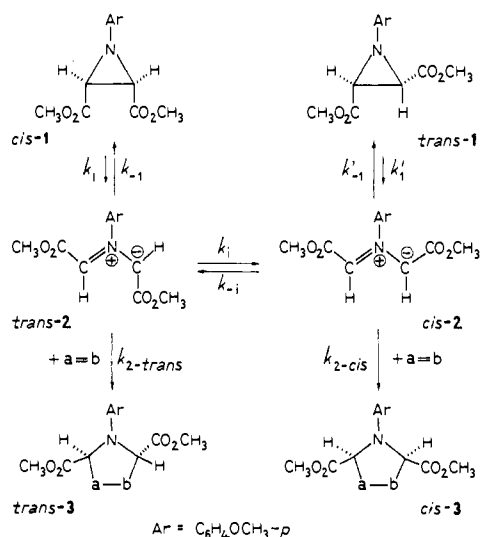
Dilatometric measurements of the reaction of cis- and trans-1 with an increasing excess of tetracyanoethylene revealed that this very active dipolarophile does not enter into the rate equation.[3] The fast cycloaddition

(1) R. Huisgen, W. Scheer, and H. Huber, J. Amer. Chem. Soc., 89, 1753 (1967).
(2) R. Huisgen, W. Scheer, H. Mäder, and E. Brunn, Angew. Chem., Int. Ed. Engl., 8, 604 (1969).
(3) R. Huisgen, W. Scheer, and H. Mäder, ibid., 8, 602 (1969).

is preceded by the rate-determining electrocyclic ring opening with the rate constants k_1 or k_1', respectively, while the ring closures with the constants k_{-1} and k_{-1}' as well as the isomerization with k_i and k_{-i} are completely suppressed.

The blue color of the solution of 1 and TCNE in ethyl acetate is due to a charge-transfer complex which suggests an alternative explanation for the dilatometric rate constants being independent of the concentration of TCNE. If the association constants of the CT complexes of cis- and trans-1 with TCNE are sufficiently high, the rate of a one-step reaction of 1 and TCNE should become virtually independent of a further excess of TCNE. However, on the basis of this one-step model the stereospecific formation of trans-3 from cis-1 and of cis-3 from trans-1 would hardly be conceivable.

This alternative interpretation can now be discarded. No charge-transfer interaction can be detected between cis- or trans-1 and diethyl fumarate by uv spectrophotometry. Nevertheless, dilatometric rate measurements[4] in ethyl acetate at 119° furnish first-order constants (k_d) for cis-1 which are independent of the concentration of fumaric ester and are identical with the ones obtained with TCNE (Table I). Thus, k_d must be identical with k_1.

On treating cis-1 with less active dipolarophiles, the recyclization trans-2 → cis-1 can compete with the cycloaddition. Still $k_{2\text{-trans}}[D]$ (where [D] is the dipolarophile concentration) is large compared with the isomerization constant k_i; k_i can, therefore, be ignored. The cycloadditions are thus stereospecific. Using the symbols of the formula scheme, steady-state treatment with respect to trans-2 leads to eq 1 for the dilatometric

$$k_d = \frac{k_1 k_{2\text{-trans}}[D]}{k_{-1} + k_{2\text{-trans}}[D]} \qquad (1)$$

rate constant k_d. This can be transformed into eq 2 of a straight line.

$$k_d = k_1 - \frac{k_{-1}k_d}{k_{2\text{-trans}}[D]} \qquad (2)$$

(4) Method and apparatus: R. Huisgen, H. Seidl, and I. Brüning, Chem. Ber., 102, 1102 (1969).