one of which is perturbed, and the cumulative effect in the molecule are principally resonance terms, E.

The p-band shifts have been shown[24,28] to be principally accounted for by E. The difference between the reduction potential shifts and the p-band shifts results in an essentially pure I term. The latter is in agreement with a simple inductive treatment of ionization potentials on methyl substitution.[29] Therefore the charge-transfer bands should be amenable to consideration using only the I term.

The plot of ΔE_π vs. Σc_{ir}^2 in Fig. 1 shows scatter similar to that[4] for the AH–TCNE E_π vs. x_1. The least squares fit of the points in Fig. 1 to a straight line also passes near the origin. Therefore, a single parameter, h, was evaluated. The slope, t, of Fig. 1 is seen from eq. 15 to equal $h\beta$ since $k = 1$. The coulomb integral used for the x_{1AH} vs. E_π proportionality was -3.06 e.v.[4] The value for the inductive perturbation parameter is therefore

$$h = -0.21$$

(28) C. A. Coulson, *Proc. Phys. Soc.* (London), **A65**, 933 (1952); H. C. Longuet-Higgins and R. G. Sowden, *J. Chem. Soc.*, 1404 (1952).

(29) A. Streitwieser, Jr., *J. Phys. Chem.*, **66**, 368 (1962).

The negative sign of this coefficient is required by the earlier qualitative treatment and Fig. 1. This value is in excellent agreement with other recent treatments ($h = -0.21$ from reduction potentials).[18]

The MO coefficients x_{1Me} for the h.o.o. may be determined directly from eq. 2. This is in agreement with the inclusion of methyl aromatics in the ionization potential, charge-transfer relationship.[7,8] The x_{1Me} values in Tables I and II are inversely related to the coulomb perturbation coefficients, eq. 2 and 15. The perturbation model used for determination of h is, however, not explicit in the x_{1Me} values.

The perturbation parameter determined may be used to calculate directly the x_{1Me} for AH compounds using the Hückel MO method. Calculations for comparison with the semiempirical h.o.o. coefficients are currently in progress.

Evidence for a Cooperative Intramolecular Transition in Poly-L-proline

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The specific rotation of poly-L-proline was measured in solvent mixtures of glacial acetic acid and 1-propanol. After proper allowance for time-dependent mutarotations, an abrupt decrease in the equilibrium levorotatory power of these solutions was observed to occur (with increasing alcoholic content) in the solvent composition interval 50–60% alcohol. It was also shown that a corresponding crystallographic transition occurs among equilibrated poly-L-proline samples removed from solution in the vicinity of this narrow solvent composition interval, this transition also being accompanied by the appearance of several infrared absorption bands. These results offer additional verification for the previously reported occurrence of the reversible intramolecular interconversion of two crystallographically distinct forms of poly-L-proline. The present study demonstrates for the first time the cooperative nature of the transition.

Ever since its synthesis almost a decade ago,[2] poly-L-proline has attracted the continuing interest of several groups of investigators. In large part this interest has centered on diverse observations leading to the now widely accepted view that poly-L-proline may exist in either of two configurational forms differing markedly in their solution properties as well as in their crystallographic structures. Early evidence for such dimorphism was noted by Kurtz, Berger, and Katchalski[3] who observed that, as obtained from its polymerizing mixture, poly-L-proline is water insoluble and slightly dextrorotatory. When this form, which is designated I, is dissolved in aliphatic acids, it proceeds to mutarotate slowly (*i.e.*, for many days) to a water-soluble highly levorotatory form designated II.

Subsequent investigations have shown that forms I and II are further distinguished by differences in optical rotatory dispersion,[4,5] hydrodynamic properties,[4] and absorption spectra, both in the infrared[5,6] and in the

ultraviolet[7] regions of the spectrum. It has also been shown that the two forms are chemically indistinguishable[3] and that the back mutarotation II → I may be effected by the dilution of acid solutions of form II with large excesses of aliphatic alcohols.[6] Thus the two forms may be interconverted in a completely reversible manner, the kinetics of both the forward and back mutarotations also having been studied. In this connection, we note that in their study of the forward (I → II) mutarotation of dilute poly-L-proline in glacial acetic acid Downie and Randall[8] reported an activation energy of 22.9 kcal./mole of prolyl residue and that Steinberg, Harrington, Berger, Sela, and Katchalski[9] reported a value of 20.6 kcal./mole for the same process and 20.2 kcal./mole for the reverse mutarotation (II → I) in a solvent consisting of a 1:9 (v./v.) mixture of acetic acid and 1-propanol.

When one couples these observations with calculated values of about 21 kcal. per mole for the resonance en-

(1) Institute for Molecular Biophysics, Florida State University, Tallahassee, Fla.

(2) A. Berger, J. Kurtz, and E. Katchalski, *J. Am. Chem. Soc.*, **76**, 5552 (1954).

(3) J. Kurtz, A. Berger, and E. Katchalski, *Nature*, **178**, 1066 (1956).

(4) W. F. Harrington and M. Sela, *Biochim. Biophys. Acta*, **27**, 24 (1958).

(5) E. R. Blout and G. D. Fasman in "Recent Advances in Gelatin and Glue Research," Pergamon Press, London, 1957.

(6) I. Z. Steinberg, A. Berger, and E. Katchalski, *Biochim. Biophys. Acta*, **28**, 647 (1958).

(7) E. R. Blout and G. D. Fasman, *Biopolymers*, **1**, 1 (1963).

(8) A. R. Downie and A. A. Randall, *Trans. Faraday Soc.*, **55**, 2132 (1959).

(9) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela, and E. Katchalski, *J. Am. Chem. Soc.*, **82**, 5263 (1960).

ergy of the amide bond,[10] it becomes increasingly plausible to assume that whatever other distinctions may be found between them, forms I and II are stereoisomers whose imide linkages are either all in the *cis* configuration I or all in the *trans* configuration II. We further note that the imide linkage is one of three distinct backbone bonds per prolyl residue; the other two are (1) the nonrotatable bond of the pyrrolidine ring and (2) the sterically hindered, though rotatable, C_α—C=O bond. This latter bond is also subject to stereoisomerism but of the type encountered in moderately flexible chain molecules containing carbon–carbon single bonds. It has been suggested[6] that restrictions to rotation in this bond lead to the occurrence of two preferred conformations which have been denoted *cis'* and *trans'*.[11] It should however be noted that, depending on the extent of free rotation about this bond and upon the torsional freedom associated with the above preferred conformations, it should be possible to generate statistically disordered conformations for poly-L-proline even when the imide linkages are uniformly in *cis* or *trans* configurations.

Crystallographic studies of oriented films of poly-L-proline (II) have been reported by Cowan and McGavin[12] and Sasisekharan.[13] These authors agree that individual peptide chains of crystalline form II exist as left-handed helices of threefold rotational symmetry in which planar imide bonds are in the *trans* configuration while the C_α—C=O bonds are in the *trans* conformation.[14] This structure has been denoted *trans–trans*.[6] A very recent X-ray diffraction study by Traub and Shmueli[15] confirms earlier proposals[16] (based on rather diffuse reflections) that poly-L-proline (I) is a well-defined right-handed helix of the *cis–trans'* type. From reported unit cell dimensions as well as from examination of molecular models, it therefore is apparent that form II poly-L-proline is a more extended structure than is form I. If it is assumed that both of these helical structures are retained in solution then, as Harrington and Sela[4] have pointed out, the transition in solution of form I to form II should be accompanied by an increase in viscosity.[17] Moreover, since the two helices are of opposite sense, the helical contributions to their rotatory powers should be of opposite sign, II being the more levorotatory of the two forms. Both of these effects have been noted[4] and optical rotatory dispersion studies[7] have tended to confirm this picture of a transition between helical structures of opposite sense.

Experimental

Poly-L-proline.—All poly-L-proline used in the present study originated from a single commercial preparation (Lot B3593,

(10) L. Pauling, "The Nature of the Chemical Bond," 3rd Ed., Cornell University Press, Ithaca, N. Y., 1960, p. 281.

(11) For a discussion of the stereochemistry of the C_α—C=O, see W. F. Harrington and P. H. von Hippel in "Advances in Protein Chemistry," Vol. 16, Academic Press, Inc., New York, N. Y., 1961, p. 19.

(12) P. M. Cowan and S. McGavin, *Nature*, **176**, 501 (1955).

(13) V. Sasisekharan, *Acta Cryst.*, **12**, 897 (1959).

(14) The term *conformation* is used here to denote a disposition of atoms about a bond resulting from simple rotations. In contrast, the *cis–trans* isomerization about the imide bond requires the addition and loss of some 20 kcal./mole accompanying the sequence, *cis* planar → freely rotating → *trans* planar; here the applicable term is configuration.

(15) W. Traub and U. Shmueli, *Nature*, **198**, 1165 (1963).

(16) A. Rich, private communication; see also reference to work of Cowan and Burge (ref. 10, p. 19).

(17) This deduction is rigorously correct only if both forms behave hydrodynamically as impermeable rigid bodies.

Mann Research Laboratories, New York) with a reported weight average molecular weight of 68,000, *i.e.*, based on a reduced specific viscosity of 1.6 dl./g. at a concentration of 0.20 g./dl. in dichloroacetic acid. Verification of the reported molecular weight was deemed unnecessary for the purposes of this investigation. It was, however, ascertained from the indetectable solubility in water of form I of the sample that it contained at most a negligible fraction of low molecular weight components (D.P. < 50). Paper chromatographic analysis of the hydrolysate of a small portion of the preparation showed it to be free of residues other than L-proline.

To ensure uniformity in the poly-L-proline used as a starting material in the mutarotation experiments discussed below, all samples were treated identically upon removal from the original preparation. The procedure consisted of (1) heating a 5 mg./cc. solution of the peptide in glacial formic acid for 1 hr. at 45°, thus ensuring its complete conversion to form II; (2) passing the solution through an ultrafine sintered-glass frit to a tube in which it was cooled to the freezing point of formic acid and freeze dried until the peptide was visibly free of solvent; (3) heating overnight under vacuum (10^{-5}–10^{-6} mm.) in a vapor-jacketed drying pistol maintained at the boiling point of acetone; and (4) storing over P_2O_5 in a vacuum desiccator.

Solvents.—Glacial formic acid was purified by slowly freezing and partially remelting 98.5% formic acid. After two such cycles, about a third of the original volume was retained. *n*-Propanol of reagent grade was purified by distillation in a good column from which a constant boiling center cut (97.2° uncor.) was obtained. Glacial acetic acid of Spectrograde was used without further treatment.

Preparation of Solutions.—Solutions used in measurements of optical rotation were prepared by direct weighing of the peptide followed by dilution from burets graduated at intervals of 0.05 cc. In each case a calculated volume of glacial acetic acid was added to the peptide and the mixture shaken at room temperature until complete dissolution was observed. Only then was the required volume of 1-propanol added. Failure to adhere to this procedure resulted in excessively low rates of dissolution and, in the case of solutions consisting of 80% or more alcohol, a failure to achieve solubility after periods of several days. Uncertainties in solution concentrations (typically consisting of 25 mg. of polypeptide in 15 cc. of solvent mixture) were judged to be less than 2%.

Optical Rotation.—Measurements of optical rotation were made with a Rudolph Model 80 precision spectropolarimeter. The light source was a medium pressure mercury lamp provided with the instrument; all measurements were confined to the single wave length 546.1 mμ. All-glass, 1- and 2-dm. jacketed polarimeter tubes (Optical Cell Corp., Brentwood, Md.) were used throughout and found to be sufficiently strain free that no rotation due to window effects could be detected. Values of observed absolute rotations could be determined within 0.003°. For a 1-dm. optical path and a concentration of 0.2 g./dl., this corresponds to an uncertainty of 1.5° in specific rotation.

Comparison of specific rotations of a given substance in two solvents, 1 and 2, requires correction of the observed rotations in solvent 2 by a factor of $(n_1^2 + 2)/(n_2^2 + 2)$, where n_1 and n_2 are the refractive indices of 1 and 2, the former being the reference solvent. For mixtures of glacial acetic acid ($n = 1.3718$) and 1-propanol ($n = 1.3854$) ranging from 0 to 84.3% alcohol, application of the above factor results in a correction of, at most, 1% of the observed value of [α]. In view of other uncertainties in the measurement, this correction was accordingly deemed insignificant in the present experiments.

Infrared Spectra.—Infrared spectra of peptide samples (usually less than 1 mg.) pressed into KBr pellets were determined on a Perkin–Elmer Model 221 infrared spectrometer.

X-Ray Diffraction.—Finely ground specimens of poly-L-proline were sealed in 0.5-mm. Lindemann glass capillaries which were then positioned in a G.E. powder camera. X-Ray diffraction patterns were obtained by irradiating the sealed specimens, which were rotated continuously *in situ*, with nickel-filtered Cu radiation from a Norelco generator set at 40 kv. and 20 ma. Exposure times ranged from 6 to 16 hr.; *d*-spacings were calculated from diametral spacings on the film. It was found that such spacings could be determined as precisely with a mm. rule as with a Zeiss–Nikon visual comparator fitted with a precision screw! The relative intensities and breadths of the diffraction peaks were compared by means of a Joyce–Lobl photoelectric recording microdensitometer.

Results and Discussion

Approach to Equilibrium Values of $[\alpha]_{546}$.—As previously noted, the dissolution of poly-L-proline (I) in an aliphatic acid is followed by a time-dependent change in the specific rotation of the peptide, this process continuing until the conversion I → II is completed. Dilution of the acid solution with a large excess of an aliphatic alcohol reverses the process so that after a sufficiently large time interval form I is recovered. Removal of the peptide from solution following such a procedure therefore represents the completion of a closed thermodynamic cycle.

Both forward and back mutarotations have been studied in several solvent systems.[7,9,10] In these studies primary emphasis has been placed on the observation of transient properties of the unstable intermediate between forms I and II. The present study is concerned with the relationships between solvent composition and the *equilibrium* properties of poly-L-proline. Therefore, we have selected for more detailed examination one of the acid–alcohol systems previously reported by Steinberg, et al.,[6] namely, the system acetic acid–1-propanol.
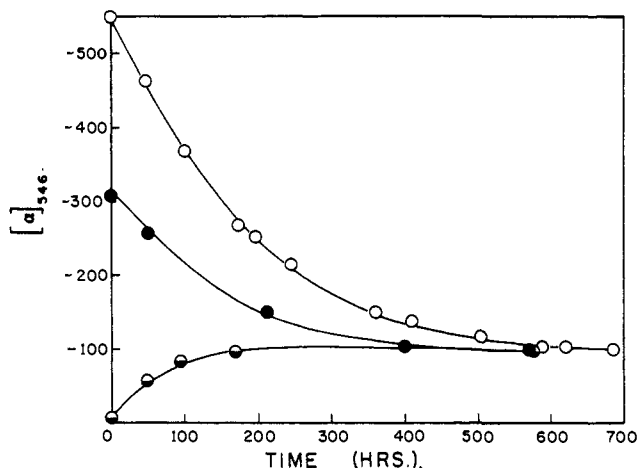

Fig. 1.—The time-dependent approach to the equilibrium values of $[\alpha]_{546}$ in 60% (v./v.) 1-propanol at room temperature for three different initial values of $[\alpha]_{546}$.

Our procedure consists of preparing dilute solutions of poly-L-proline in solvent mixtures ranging in composition from pure acetic acid to a mixture of 15.7% acetic acid–84.3% 1-propanol. At room temperature (26°), the specific rotations of these solutions were observed to change for periods ranging from 15 to 30 days after which time daily incremental changes in the observed rotations of the solutions became less than the uncertainties in the measurement. Values of $[\alpha]_{546}$ thus obtained were tentatively accepted as indicative of equilibrium conditions in the solvent mixture considered.

As further verification of the establishment of true equilibria, it was demonstrated that several of the observed limiting specific rotations could be approached along various kinetic paths. In one such experiment three specimens of poly-L-proline, previously obtained by ether precipitation of three aliquots of a solution in various stages of mutarotation, were redissolved in solvent mixtures consisting of 60% (v./v.) 1-propanol. The results of the ensuing mutarotations are shown in Fig. 1.

In another series of experiments four poly-L-proline samples with values of $[\alpha]_{546}$ ranging from −580 to +30 (measured in acetic acid solution) were placed in 50% (v./v.) 1-propanol and subjected to various heating and cooling cycles in which the temperature ranged from 15 to 90° but which all terminated at room temperature. The limiting values of $[\alpha]_{546}$ for the four solutions were in agreement to within about 1% of their mean value.

These experiments demonstrate that, when observed as a function of time, the asymptotic values of the specific rotation of poly-L-proline are independent of (a) their initial values at $t = 0$ (the time of dissolution), (b) the direction from which they mutarotate, and (c) their thermal history. They therefore may be presumed to be true equilibrium values uniquely determined at a given temperature by the solvent composition. Results of equilibration studies in ten different solvent mixtures are summarized in Table I and illustrated in Fig. 2.
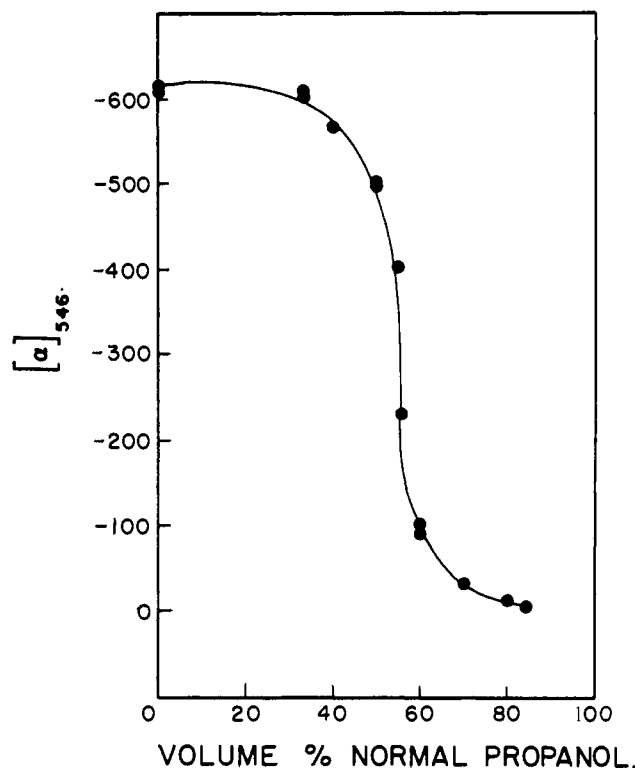

Fig. 2.—Equilibrium values of $[\alpha]_{546}$ as a function of solvent composition.

In the last column of Table I we have presented values of the cumulative fractional change in the specific optical rotation between the extremes of the solvent compositions studied. Denoting this quantity by θ, we have

$$\theta = ([\alpha] - [\alpha]_{II})/([\alpha]_I - [\alpha]_{II}) \qquad (1)$$

where $[\alpha]_{II} = -608°$ and $[\alpha]_I = -8°$, are the equilibrium values of $[\alpha]_{546}$ at 26° in 0 and 84.3% 1-propanol mixtures, respectively. Discussion of the significance of θ is reserved for a later section, but we do observe here that almost two-thirds of the variation in θ occurs in the solvent composition interval 50 to 60% 1-propanol.

Solubility, Precipitation, and Redissolution of Poly-L-proline.—In attempting to prepare poly-L-proline in

TABLE I

SPECIFIC ROTATIONS OF POLY-L-PROLINE IN MIXTURES OF
GLACIAL ACETIC ACID AND 1-PROPANOL

% 1-propanol	No. of independent determinations	$[\alpha]_{546}$	θ (eq. 1)
0	3	-608 ± 4	0
0.333	2	-606 ± 2	0.003
.400	1	-567	.068
.500	4	-496 ± 5	.187
.550	1	-400	.347
.559	1	-233	.625
.560	2	-230 ± 2	.630
.600	3	-97 ± 4	.851
.700	1	-33	.958
.800	1	-13	.992
.843	1	-8	1.0

different stages of mutarotation, it was observed that, in agreement with Steinberg, et al.,[9] certain changes in specific rotation accompanying transfer from one solvent to another were virtually instantaneous and independent of subsequent slow mutarotations to equilibrium values of $[\alpha]_{546}$. It was, for example, noted that the specific rotation of poly-L-proline (II), measured immediately after dissolution, was invariably less levorotatory in alcoholic mixtures than in glacial acetic acid. Paradoxically, the dextrorotation of poly-L-proline (I) was enhanced by transfer from the alcoholic medium in which it was equilibrated to pure glacial acetic acid. If we assume that the helices of forms I and II increase in perfection with increasing dextro- and levorotation, respectively, then it seems reasonable to conclude that acetic acid is actually a "better" solvent for form I than is one of the alcohol-rich mixtures in which the back mutarotation II → I is known to occur.

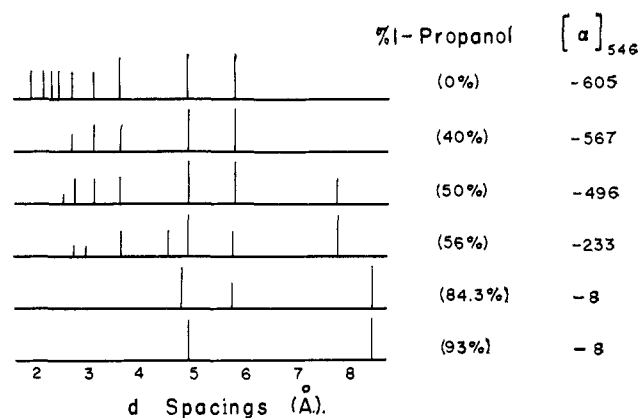

Fig. 3.—Observed d-spacings calculated from Debye–Scherrer powder patterns of poly-L-proline samples. Precipitates were obtained from solutions of the indicated alcohol content. See text for discussion of the distinction between the two lowest diagrams. Heights of the vertical lines in the diagrams are intended to signify relative diffraction intensities in a qualitative manner.

It was also noted that attempts to accelerate the rate of equilibration in alcohol-rich solutions of poly-L-proline by increasing the temperature were often frustrated by heat precipitation. This effect was absent in solutions containing less than 50% (v./v.) 1-propanol; in these, temperatures as high as 90° could be maintained indefinitely. However, solutions richer than 50% in alcohol precipitated at temperatures which decreased successively with increasing alcoholic content. In one solvent mixture consisting of 90% 1-

propanol, heat precipitation occurred at 35°. The solution was thereupon cooled to room temperature but failed to redissolve completely even after several days.

Regarding the dependence of specific rotation of poly-L-proline on temperature, it was observed that in those solutions which were homogeneous (i.e., containing less than 50% 1-propanol), re-equilibration at successively higher temperatures appeared to have minimal effects on the observed values of $[\alpha]_{546}$. For example, a solution of form II equilibrated at 25° in 40% 1-propanol was re-equilibrated for several days at each of five higher temperatures. The values of $[\alpha]_{546}$ observed were: 26° ($-567°$), 34° ($-561°$), 43° ($-557°$), 49.5° ($-542°$), 59°, ($-537°$), and 68° ($-533°$). We note that the decrease in $[\alpha]_{546}$ between 26 and 68° can be accounted for by a thermal expansion of the solvent of about 6%, this figure being consistent with reported expansion data for the solvents used. Similar results were obtained for other solutions of poly-L-proline, in some of which temperatures as high as 90° were maintained for several hours. In no instance was it possible to induce an appreciable variation in $[\alpha]_{546}$ solely as a result of a change in temperature.

X-Ray Diffraction.—We have obtained diffraction patterns and determined interplanar (d) spacings for equilibrated specimens of poly-L-proline following their removal from solution by ether precipitation. The results are summarized in Fig. 3 where we have listed all of the observed diffraction maxima of six precipitates representing different stages of mutarotation. To characterize these patterns further we show in Fig. 4 the corresponding photometric traces of the more prominent peaks in these specimens.

We note first that the spacings obtained from the form II specimen recovered from glacial acetic acid do, as expected, agree with spacings reported by other investigators. For a comparison see Table II. We

TABLE II

COMPARISON OF OBSERVED d-SPACINGS FOR POLY-L-PROLINE
(II) WITH THOSE REPORTED BY OTHER INVESTIGATORS

Sasisekharan[a]	Rich[b]	Present work
5.76	5.82	5.80
4.90	4.92	4.92
3.65	3.68	3.61
2.75	2.74	2.74
2.71		
	2.45	2.45
	2.30	2.28
2.15	2.15	2.15
	1.97	1.9
	1.80	
	1.60	

[a] See ref. 13. [b] Spacings reported here were calculated from film kindly lent to us by Prof. A. Rich.

further observe that while the more prominent form II spacings continue to appear in specimens precipitated from 40 and 50% 1-propanol solutions, they diminish in sharpness and their relative intensities are altered. In addition, a broad shoulder appearing first in the 40% pattern appears as a diffuse peak in the 50% pattern and then a sharp 7.85 Å. spacing in the 56% pattern. The latter is seen to be comparable in sharpness to the form II pattern but, while possessing several Bragg spacings in common, the two patterns are clearly dis-

tinct. In the last two patterns we see the successive obliteration of the sharp diffraction patterns discussed above. The specimen designated 93% was obtained by further dilution with 1-propanol of an equilibrated 84.3% solution. The diluted sample was allowed to stand for 2 days before it was ether-precipitated.

Before attempting to interpret these complex diffraction patterns, the work of Sasisekharan[18] should be mentioned. This author has observed that if poly-L-proline (I) is mixed as a paste with glacial acetic acid and precipitated at various times during its mutarotation from form I to form II, then at some point before completion of the mutarotation, a highly crystalline form, which he designated IA, is seen to appear. From the very recent work of Traub and Shmueli,[15] it appears, however, that, rather than being a new form intermediate between I and II, form IA is in fact a well crystallized form I. This conclusion is drawn from the observation that specimens whose specific rotations identify them as form I give the same diffraction pattern as IA provided that they are crystallized from appropriate solvents. The same specimens crystallized from their polymerizing mixtures or from alcohol-rich solvents show only broad halos and diffuse rings characteristic of poorly developed crystalline order. The spacings reported by Sasisekharan for IA and those reported by Traub and Shmueli are shown in Table III with the spacings of our 56% precipitate. Comparison of these data show rather good agreement considering the disparity in the conditions under which they were crystallized.

TABLE III

SUMMARY OF d-SPACINGS IN POLY-L-PROLINE (I); COMPARISON OF PRESENT RESULTS FOR 56% PRECIPITATE WITH RESULTS OF SASISEKHARAN[18] AND TRAUB AND SHMUELI[15]

Sasisekharan "IA"	Traub and Shmueli[a]	Present results 56% precipitate
7.83	7.84	7.78
5.76		5.75
4.96		4.92
4.42	4.49	4.37
4.10		4.04
3.90	3.92	
3.62		3.63
2.92	2.94	2.93
		2.71

[a] d-Spacings are equatorially oriented reflections; other spacings are unreported.

We conclude therefore that the abrupt transition of specific rotation occurring between compositions of 50 and 60% 1-propanol is accompanied by a corresponding crystallographic transition between forms II and I. If this conclusion is correct, then the increased diffuseness of the diffraction patterns of the 84.3 and 93% specimen (see Fig. 4) reflects the specific effect of alcohol on the crystallization process (three-dimensional ordering) rather than a decrease in isomeric order along individual peptide chains.

To test further this conclusion we have recrystallized poly-L-proline (I) ($[\alpha]_{546} = +40°$ in propionic acid) from two solvents: (a) pure glacial acetic acid and (b) an 80% 1-propanol–20% glacial acetic acid mixture. Except for solvent composition, both recrystallizations were carried out under nearly identical conditions; the solutions were of equal concentration and were precip-
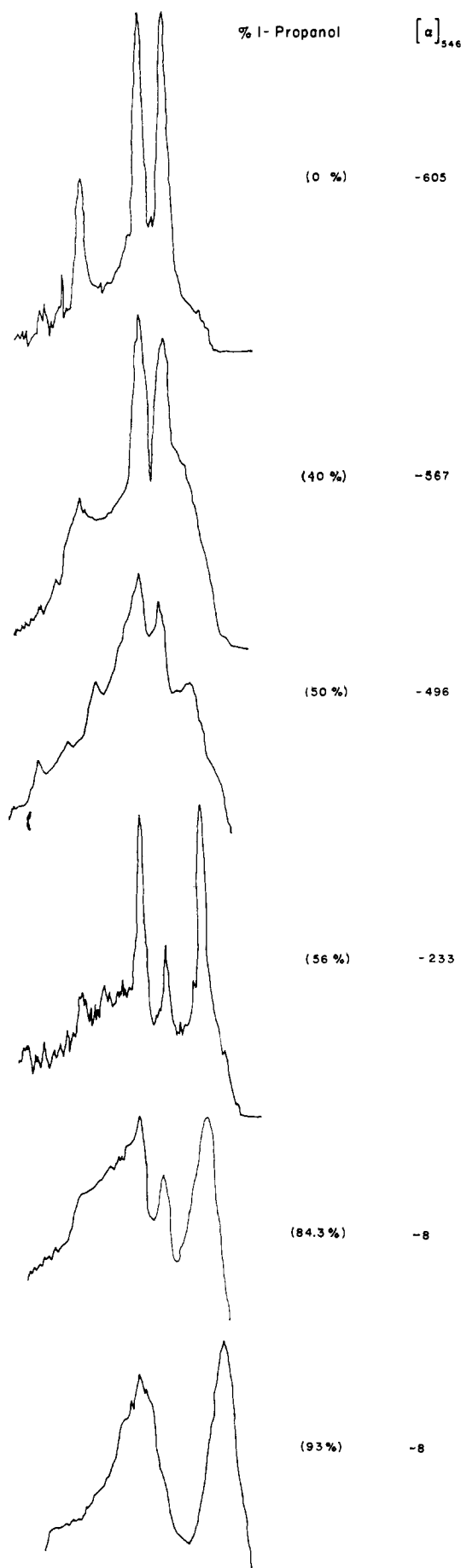
(18) V. Sasisekharan, *J. Polymer Sci.*, **47**, 373 (1960).

Fig. 4 —Photometric microdensitometer traces of the powder patterns of Fig. 3.
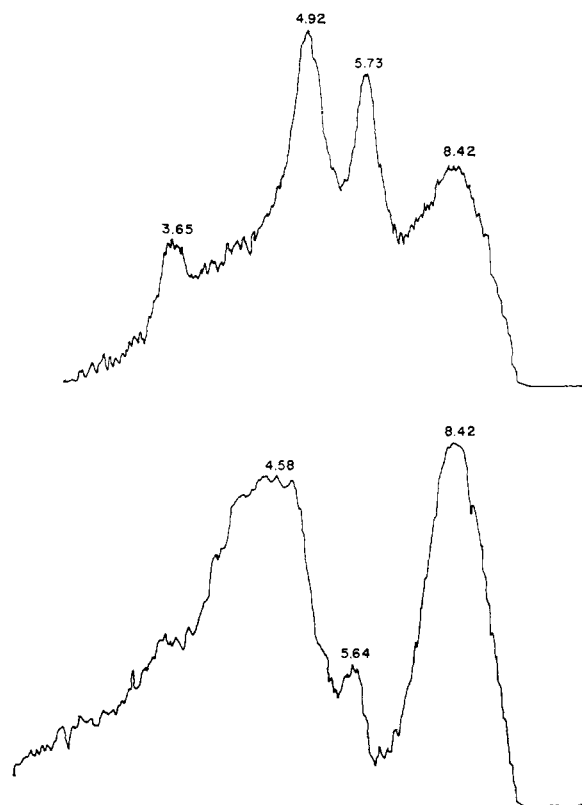
Fig. 5.—Comparison of microdensitometer traces obtained from powder patterns of poly-L-proline (I) precipitated from (a) pure glacial acetic acid (upper trace) and (b) 80% 1-propanol–20% glacial acetic acid (lower trace).

itated by the addition of equal volumes of ether immediately after dissolution, thus precluding the possibility of any appreciable amount of mutarotation prior to precipitation. A comparison of microdensitometer traces obtained from their diffraction patterns, shown in Fig. 5, indicates that the specimen crystallized from glacial acetic acid does exhibit a higher degree of crystalline order than that crystallized from the alcohol-rich medium. The aforementioned hypothesis is therefore verified.

Infrared Spectra.—Blout and Fasman have reported that poly-L-proline I and II differ in their infrared absorption spectra at wave numbers less than 1400 cm.$^{-1}$. In this region of the spectrum, poly-L-proline (I) exhibits relatively weak but clearly discernible absorption bands at 1355 and 960 cm.$^{-1}$, poly-L-proline (II) being transparent at these wave numbers.

We have observed the occurrence of these absorption bands in those specimens of poly-L-proline precipitated from equilibrated solutions containing 56% or more 1-propanol, but not in those containing 50% or less 1-propanol. While these observations are qualitative in nature, they do provide further indication of a rather abrupt structural transition during the mutarotation process.

Summary and Discussion

For reasons previously stated we accept as a basis for further discussion that forms I and II of poly-L-proline are configurationally homogeneous structures whose imide linkages are, respectively, all cis and all trans and that the transitions between them in dilute solution are governed kinetically by processes consisting of repeated cis–trans isomerizations (not entailing chain cleavage)

along the backbones of individual peptide chains. The question therefore arises as to the possible existence of an array of intermediate structures characterized by varying fractions of trans-imide linkages.

In the analysis of their data, Downie and Randall[8] have put forth arguments precluding the possibility of an all-or-none mechanism of mutarotation, i.e., consisting of an isomerization in unison of all of the prolyl residues of a single chain. The alternative to such a mechanism must therefore be viewed as a progressive disordering of sequences of contiguous prolyl residues of like configuration. This process continues until some maximal steric disorder is attained (possibly when the number of cis and trans configurations are nearly equal) after which reordering proceeds until the thermodynamically favored structure is attained.

The basic problem faced in elucidation of the mechanism of such a process is the establishment of a quantitative relationship between optical rotatory power and the microscopic structure of individual peptide chains. As an initial step toward the attainment of this goal we have equilibrated solutions of poly-L-proline in solvent mixtures whose compositions range from those in which form II is stable to those in which form I is the favored species. We have shown that, by carefully controlling the solvent composition and allowing for sufficient equilibration time, any desired value of specific rotation between that of forms I and II may be obtained, each value of [α] thus being representative of some intermediate stage in the mutarotation.

We have observed, however, that the equilibrium values of [α] undergo an abrupt change within a narrow interval of solvent composition. This suggests and is consistent with the occurrence of a cooperative transition between the two forms assuming of course that [α] measures the extent of the transition. The latter assumption was made by Steinberg, et al.,[9] who identified the fraction θ defined by eq. 1 as the fraction of cis residues in the system. If θ does in fact measure the number of isomerization reactions in an even approximately linear fashion, then the above conclusion regarding the cooperative nature of the transition is preserved.

This conclusion is furthermore supported by the results of our X-ray diffraction studies which indicate that a crystallographic transition occurs among precipitated samples of poly-L-proline equilibrated in solutions near the aforementioned narrow solvent interval. If we accept the hypothesis that in each stage of its mutarotation, poly-L-proline is characterized by an equilibrium distribution of cis- and trans-imide linkages, then it may be expected that rapid crystallization of this peptide from solutions equilibrated at different specific rotations should constitute a "freezing in" of the configurational pattern of cis and trans isomeric structures along the backbones of individual peptide chains. It therefore seems reasonable to assume that such an effect would be reflected in variations in the diffraction patterns of the precipitates following solvent removal. Ideally it should be possible to interpret the observed diffraction data in terms of the (now) known spacings of forms I and II. Unfortunately, this is not the case for the following reasons: (a) previously noted effects of alcohol on the crystallization process persist even after complete solvent removal; (b) an intense,

though broad, diffraction band at 8.4 Å. cannot be assigned to either pure structure; (c) diffraction maxima corresponding to spacings below 3.5 Å., which should be most sensitive to changes in the configurational order of single chains, are relatively weak. In their X-ray diffraction analysis of the structure of poly-L-proline (I), Traub and Shmueli[15] have identified the major equatorial reflections. This leads to the conclusion that the major nonequatorial reflections, corresponding to Bragg spacings of 4.9 and 5.7 Å., are common to both forms. This is a surprising result since the conformations of the individual chains are deduced to be so distinctly different.

In addition to the above, it seems quite likely that, owing to the inherent broadness of the transition, both forms occur on either side of the transition midpoint; i.e., the degree of "cooperativeness" is probably comparable to that observed in the order–disorder transitions of the α-helix type polypeptides.[19] If this is so, then it might be expected that in the transition region sequences of both the cis and trans types might co-exist over an observable solvent composition interval. We do however reiterate that the infrared absorption bands below 1400 cm.$^{-1}$ associated with form I cannot be observed in samples precipitated from solutions containing less than 56% alcohol and that conversely the diffraction maximum at 7.75 Å., which appears sharply in the 56% sample, appears only as a diffuse halo in the 50% sample and not at all in samples removed from solutions of lower alcoholic content. In this connection, it should also be noted that none of the precipitated samples exhibited diffraction patterns characteristic of virtually uncrystallizable molecules such as conventional (atactic) vinyl polymers. In the latter, the customary explanation for their inability to crystallize

is that their chain structures are sterically disordered. Applying this line of reasoning to the poly-L-proline precipitates, and recalling that at some point in the mutarotation the number of cis- and trans-imide bonds must be equal, it follows that the transition from an all-cis to an all-trans structure must proceed in a manner tending to favor the coordinated isomerization of cis sequences whose lengths are sufficient to crystallize. If this were not the case and the isomerization were totally uncooperative, each bond isomerizing independently of its neighbors, then at the midpoint of the transition the average sequence length (either cis or trans) would be two and the total fraction of cis- or trans-imide bonds found in isoconfigurational sequences exceeding four in length would be less than one-fifth (0.1875, to be exact).

We conclude with the observation that the cooperative configurational transition described here occurs in an isolated polymer chain devoid of intramolecular hydrogen bonding. In synthetic polypeptides and proteins of the α-helix type, the abruptness of the transition between ordered helix and disordered coil has been ascribed[20] to the large difference between the entropic increase accompanying the first break in a sequence of intact hydrogen bonds and the entropic increase per bond in propagating the break once it is started. Stated alternatively, the formation of a break in an otherwise intact α-helical sequence is less effective in imparting rotational freedom to the chain than is the breaking of an equal number of hydrogen bonds located at the ends of such sequences. There appears to be no equally appealing explanation for the cooperativeness of the transition between forms I and II of poly-L-proline. It is hoped that further research will illuminate this problem.

(19) For a summary of these observations see P. Doty, Rev. Mod. Phys., 31, 107 (1959).

(20) B. H. Zimm and J. K. Bragg, J. Chem. Phys., 31, 526 (1959).

Organic Ions in the Gas Phase. XIV.
Loss of Water from Primary Alcohols under Electron Impact

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Loss of water from the parent ion is a prominent reaction in the mass spectra of primary alcohols. Such loss has been viewed as 1,2-elimination to form an olefin ion, but growing evidence favors 1,4- over 1,2-elimination. The hope of confirming the specificity of this reaction prompted study of the mass spectra of hexanol unlabeled, -4-d_2, -5-d_2, and -6-d_3. The extra hydrogen atom lost as water comes from C-4 in 91% of the reacting molecules, and from a position closer than C-4 in the remaining 9%. No measurable hydrogen comes from C-5 or C-6. Label retentions in secondary decomposition products suggest that loss of water is accompanied or followed by making of a new bond between the carbon atoms from which H and OH are removed.

Many classes of substituted alkanes $C_nH_{2n+1}X$ characteristically split out HX under electron impact in a mass spectrometer to produce an "olefin" ion $C_nH_{2n}^+$. Such loss of H_2O is especially prominent in the spectra of primary alcohols, where it has been ascribed to 1,2-elimination via a four-membered bridged cyclic intermediate.[2,3] Postulated 1,2-elimination in

other classes of alkane derivatives[4-6] has rested in part on analogy with alcohols. Little evidence has been available until very recently on the mechanism by

ferty in "Determination of Organic Structures by Physical Methods," Vol. 2, F. C. Nachod and W. D. Phillips, Ed., Academic Press, Inc., New York, N. Y., 1962, p. 93.

(3) See also F. W. McLafferty in "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press, Inc., New York, N. Y., 1963, p. 309.

(4) F. W. McLafferty, Anal. Chem., 29, 1782 (1957).

(5) E. J. Levy and W. H. Stahl, ibid., 33, 707 (1961).

(6) F. W. McLafferty, ibid., 34, 2 (1962).

(1) (a) American Oil Co.; (b) National Research Council.

(2) (a) F. W. McLafferty, 8th Annual Meeting of ASTM Committee E-14 on Mass Spectrometry, Atlantic City, N. J., 1960; (b) F. W. McLaf-