Poly(β-benzyl L-aspartate) was prepared by polymerization of the recrystallized N-carboxy anhydride in 2% solution in freshly redistilled dichloromethane with sodium methoxide (0.005 equiv) as initiator and isolated by precipitation with petroleum ether (40–60°), followed by repeated washing with ethanol, mol wt (osmotic pressure in chloroform) 22,000.

Physical Measurements. Optical rotatory dispersion measurements were made on a Bellingham and Stanley Pepol spectropolarimeter with a 4-cm cell or on a Bendix-Ericsson Polarmatic 62 recording polarimeter, with a 1-mm cell. Nuclear magnetic resonance spectra were recorded on a Perkin-Elmer R10 60-MHz nmr spectrometer fitted with a Digico Digiac computer for averaging accumulated spectra. Infrared spectra were recorded, at ambient temperature, on a Hilger and Watts Infrascan H900 recording spectrophotometer.

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Poly(L-proline) II Ring Conformations Determined by Nuclear Magnetic Resonance

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ABSTRACT: From a computer simulation of the 220-MHz nmr spectrum of poly(L-proline) II, ring vicinal couplings have been obtained. These couplings are related to ring rotation angles φ, χ_i, i = 1–4, using a Karplus type function. Owing to uncertainties in couplings involving the approximately equivalent γ protons, the Karplus analysis indicates that a number of ring conformations are consistent with the nmr data; however, it is found that the ring conformation proposed for poly(L-proline) II in the solid state is inconsistent with the couplings determined from the simulation. From the nmr analysis and previously reported energy calculations, it is proposed that in form II the proline ring rapidly interconverts between two equally populated conformations, (C^+) and (C^-). C_γ is exo in (C^+) and endo in (C^-), being *ca.* 0.5 Å out of the plane of the remaining four atoms in each conformation.

The solid-state conformations of poly(L-proline) I and II have been determined by X-ray analysis.[1-3] It has been established[4,5] that in solvents such as water and acetic acid the polypeptide backbone structure is similar to that found in the solid state for form II; that is, $\omega = 0°$ (trans peptide bonds),[6] $\psi \approx 300°$ (trans′ C_α—C=O bonds), and $\varphi \approx 120°$ (as required to form the pyrrolidine ring). The form II ring conformation obtained from the X-ray analysis[2] has N, C_α, C_γ, and C_δ nearly coplanar, with C_β-exo[7] *ca.* 0.4 Å out of this plane (*i.e.*, $\chi_1 \approx 25°$). Since intermolecular (interchain) interactions present in the solid lattice are absent in solution, the ring conformation in solution may be different from that in the solid state. Calculations[8] suggest that in solution, where intramolecular interactions are expected to predominate, two puckered conformations may be present, one having C_γ exo and the other with C_γ endo.

High-resolution nmr has been widely applied[9] to the study of molecular conformation in solution. Recently, the Hyp ring conformation of cyclo(L-Pro-L-Pro-L-Hyp) benzoate and the Pro ring conformations of cyclo(tri-L-prolyl) have been deduced[10] from a Karplus type analysis[11-13] of vicinal couplings obtained from direct measurement and computer simulation of 220-MHz nmr spectra of these compounds. In this paper a similar type of analysis is made of the 220-MHz nmr spectrum of poly(L-proline) II in D_2O. Ring conformations are proposed on the basis of the nmr analysis and energy calculations.[8]

Experimental Section

Materials. The synthesis and characterization of the low-molecular-weight (DP ≈ 25) sample of poly(L-proline) used in this report have appeared in the literature.[14,15] D_2O ("100.0%") was purchased from Diaprep, Inc. and *tert*-butyl alcohol-d_1 was obtained from Merck Sharp and Dohme.

Methods. Nmr spectra were obtained using a Varian HR-220 spectrometer, and homonuclear spin decoupling was accomplished using a General Radio 1107-A audio oscillator. The *tert*-butyl resonance at τ 8.77 (relative to DSS in D_2O) of *tert*-butyl alcohol-d_1 was used as internal reference. The poly(L-proline) II solution was prepared by first equilibrating a form I sample (7 mg) in D_2O (2 ml). This solution was lyophylized, the sample dissolved in 100.0% D_2O (0.4 ml), and the spectrum obtained at 20°. It was not possible to reduce the line widths by raising the sample temperature, since the polymer precipitates at elevated temperatures ($T \gtrsim 60°$). A sharp methyl singlet[15] at τ 6.27 and the HDO side bands have been edited from the spectrum shown.

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

(2) V. Sasisekharan, *Acta Crystallogr.*, **12**, 897 (1959).

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

(4) J. P. Carver and E. R. Blout in "Treatise on Collagen," Vol. I, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1967, p 441, and references therein.

(5) L. Mandelkern in "Biological Macromolecules," Vol. I, G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, p 675, and references therein.

(6) For explanation of conventions used in dihedral angle nomenclature: J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Némethy, G. N. Ramachandran, and H. A. Scheraga, *Biopolymers*, **4**, 121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966).

(7) An out-of-plane ring atom is endo if it lies on the same side of ring plane as the carbonyl carbon. An exo ring atom lies on the opposite side of the ring.

(8) G. N. Ramachandran, A. V. Lakshmenarayanan, R. Balasubramanian, and G. Tegoni, *Biochim. Biophys. Acta*, **221**, 165 (1970).

(9) H. Booth, *Progr. Nucl. Magn. Resonance Spectrosc.*, **5**, 149 (1969), and references therein.

(10) C. M. Deber, D. A. Torchia, and E. R. Blout, *J. Amer. Chem. Soc.*, in press.

(11) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); **33**, 1842 (1960).

(12) M. Karplus, *J. Amer. Chem. Soc.*, **85**, 2870 (1963).

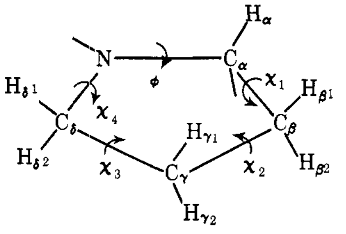
(13) M. Barfield and M. Karplus, *ibid.*, **91**, 1 (1969).

(14) C. M. Deber, F. A. Bovey, J. P. Carver, and E. R. Blout, *ibid.*, **92**, 6191 (1970).

(15) D. A. Torchia and F. A. Bovey, *Macromolecules*, **4**, 246 (1971).

Results and Discussion

(1) Computer Simulation of the Poly(L-proline) II Spectrum. The diagram (I) of a planar proline ring ($\chi_i = 0°$, i = 1–4; $\varphi = 120°$) shows the designations of ring atoms and rotation angles used in this report.[6] The 220-MHz nmr spectrum of poly(L-proline) (DP $\sim$ 25) equilibrated in D_2O is shown in Figure 1a. Since the molecular weight (*ca.* 2500) of the polymer is low, the line widths (*ca.* 5 Hz) are small enough so that the fine structure (due to spin–spin splittings) of the resonances is seen. On the other hand, the spectrum shows that the polymer length is sufficient so that, to a good approximation, all proline rings are equivalent.



I

In order to calculate the spectrum of this complex spin system, seven chemical shifts, three geminal couplings, and ten vicinal couplings are required as input data for the machine program. Four of the ring protons, H_α, the two H_δ's, and an H_β give distinct resonances at *ca.* τ 5.3, 6.2, 6.4, and 7.7, respectively. The second H_β at τ 8.1 partially overlaps the approximately equivalent H_γ protons located at τ 8.0. These assignments were checked by spin decoupling. Initially, the three geminal couplings were taken as −12.0 Hz on the basis of published data for five- and six-membered rings,[9,16] and the two $J_{\alpha,\beta}$ vicinal couplings were estimated to be 8.0 and 5.0 Hz from the fine structure of the α-proton resonance. Since the γ protons have about the same chemical shift, the calculated spectrum is sensitive to the sum, but not to the difference, of the two vicinal couplings between each β or δ proton and the two γ protons; the value of the difference of any pair of these couplings (*i.e.*, $J_{\beta^i\gamma^1} - J_{\beta^i\gamma^2}$ or $J_{\gamma^1\delta^i} - J_{\gamma^2\delta^i}$; i = 1 or 2) can be varied from 0 to ±5 Hz without significantly affecting the simulation.[17] The fine structure of the γ resonances (approximately symmetric, with 6.5-Hz splittings) indicates that the sum of couplings of each β or δ proton to the two γ protons is about 13 Hz, and initially all $J_{\beta\gamma}$ and $J_{\gamma\delta}$ were taken to be 6.5 Hz. These initial values of chemical shifts and coupling constants were varied and the ranges of vicinal couplings which gave satisfactory simulations were determined. These are summarized in Table I. The chemical shifts and couplings used to generate a satisfactory simulation, Figure 1b, are summarized in the chart at the top of the figure. While the experimental and calculated spectra are almost identical, we emphasize that equally good simulations are obtained using γ-proton vicinal couplings within the ranges given in Table I.

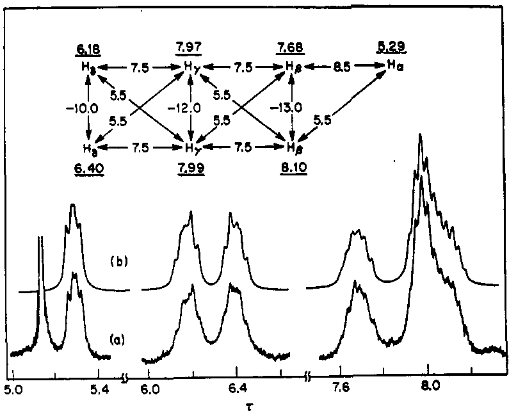


Figure 1. Comparison of 220-MHz spectra of proline ring protons of poly(L-proline) II: (a) experimental spectrum in D_2O, HDO resonance at τ 5.15, (b) computer-simulated spectrum. At top is a schematic summary of the coupling constants (Hz) and chemical shifts (underlined) used in the simulation; line width assumed in simulation, 5.0 Hz. Chemical shifts are in τ units downfield from DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate).

(2) Poly(L-proline) II Ring Conformations. The vicinal couplings in Table I can be related to the dihedral angles, φ_d, between the proline ring protons by the Karplus equation.[11–13]

$$
\begin{aligned}
J_{\mathrm{H,H}} &= A\ \cos^2\varphi_d + B \qquad 0° \leq \varphi_d \leq\ 90° \\
J_{\mathrm{H,H}} &= A'\cos^2\varphi_d + B \qquad 90° \leq \varphi_d \leq 180°
\end{aligned}
\tag{1}
$$

Experimental work indicates that a set of coefficients which applies to proline ring couplings is

$$A = 8.5\ \mathrm{Hz} \qquad A' = 10.5\ \mathrm{Hz} \qquad B = 1.4\ \mathrm{Hz} \tag{2}$$

These coefficients result from an analysis of cyclo(tri-L-prolyl) ring couplings[10] and agree closely with coefficients proposed from an analysis of ring vicinal coupling data for six-membered rings.[16]

While the vicinal couplings obtained from the simulation of the measured spectrum do not specify a unique ring conformation, they do limit the types of acceptable conformations. For instance, the planar conformation ($\varphi = 120°$, $\chi_i = 0°$, i = 1–4) is excluded since, using eq 1 and 2, one calculates $J_{\alpha\beta^1}/J_{\alpha\beta^2}$ = 2.5, a result which deviates by over 65% from the experimental values of 1.5 or 0.7 obtained from Table I. Individual nonplanar conformations, in which the ring is markedly puckered (at a single atom) or twisted (with atoms i and $i + 2$ on opposite sides of the plane of the three other ring atoms) are also prohibited. Consider, for example, a conformation puckered at C_γ (φ =

TABLE I
VICINAL COUPLINGS WHICH GIVE SATISFACTORY SIMULATIONS OF THE POLY(L-PROLINE) II SPECTRUM

Vicinal coupling	Mean value, Hz	Acceptable range, Hz
$\begin{Bmatrix} J_{\alpha\beta^1} \\ J_{\alpha\beta^2} \end{Bmatrix}$ or $\begin{Bmatrix} J_{\alpha\beta^2} \\ J_{\alpha\beta^1} \end{Bmatrix}$	8.5 5.5	8.0–9.0 5.0–6.0
$J_{\beta^1\gamma^1} + J_{\beta^1\gamma^2}$	13.0	12.0–14.0
$J_{\beta^2\gamma^1} + J_{\beta^2\gamma^2}$	13.0	12.0–14.0
$J_{\gamma^1\delta^1} + J_{\gamma^2\delta^1}$	13.0	11.5–14.5
$J_{\gamma^1\delta^2} + J_{\gamma^2\delta^2}$	13.0	11.5–14.5
$J_{\beta^1\gamma^1} - J_{\beta^1\gamma^2}$	0.0	−5.0–5.0
$J_{\beta^2\gamma^1} - J_{\beta^2\gamma^2}$	0.0	−5.0–5.0
$J_{\gamma^1\delta^1} - J_{\gamma^2\delta^1}$	0.0	−5.0–5.0
$J_{\gamma^1\delta^2} - J_{\gamma^2\delta^2}$	0.0	−5.0–5.0

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(16) A. A. Bothner-By, *Advan. Magn. Resonance*, **1**, 149 (1965).

(17) This result can be understood by considering an ABX spectrum in which we designate the chemical shifts (in frequency units) by ν_A, ν_B, and ν_X and couplings by J_{AB}, J_{AX}, and J_{BX}. In general, the ABX spectrum will depend upon all six parameters; however, if $|\nu_A - \nu_B \pm (J_{AX} - J_{BX})/2| \lesssim |J_{AB}/3|$, then, provided $\nu_A + \nu_B$, $J_{AX} + J_{BX}$, and ν_X are held constant, the frequencies and intensities of all *major* lines in the spectrum are independent, to within ±3%, of the values assumed by $\nu_A - \nu_B$, $J_{AX} - J_{BX}$, and J_{AB}.[18] While the spectrum under consideration is more complex than ABX, any one of two H_δ's or H_β's can be approximately regarded as the X part of an ABX system in which the H_γ's are the AB part, with the X resonance further split by (*ca.* first order) couplings to the other ring protons.

(18) This result follows from the standard expressions used to calculate ABX line positions and intensities. For a thorough discussion of ABX spectra, see F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, N. Y., 1969, pp 105–113.

$120°$, $-\chi_1 = \chi_4 = 15°$, $\chi_2 = -\chi_3 = 25°$). From eq 1 and 2 and the φ_d corresponding to this conformation, one finds $J_{\beta^1\gamma^1} + J_{\beta^1\gamma^2} = J_{\gamma^1\delta^1} + J_{\gamma^2\delta^1} = 10$ Hz and $J_{\beta^2\gamma^1} + J_{\beta^2\gamma^2} = J_{\gamma^1\delta^2} + J_{\gamma^2\delta^2} = 17$ Hz, which are well outside the limits given in Table I. In similar fashion, one can exclude every individual puckered or twisted conformation in which φ or χ_i, $i = 1$–4, deviates by more than *ca.* 15° from the planar ring values of these angles.[19] This result is of interest, since it shows that the ring conformations proposed for poly(L-proline) in the solid state (C_β puckered, with $\chi_1 = -\chi_2 \approx 25°$) and for cyclo(tri-L-prolyl) in solution ($\chi_1 \approx 30°$) are incompatible with the couplings in Table I. These results are a consequence of the differences between intermolecular interactions in solution and the solid state[20] and the unusual backbone structure of cyclo(tri-L-prolyl).[10,21,22]

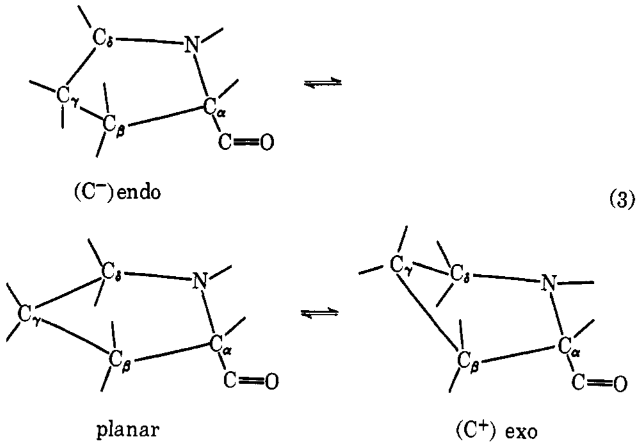
The only (single) conformations which are compatible with the nmr data have C_α slightly above (exo) or C_β slightly below (endo) the plane of the remaining four atoms (*i.e.*, $100° \lesssim \varphi \lesssim 120°$, $\chi_1 \approx -10°$, $-10° \lesssim \chi_2, \chi_3, \chi_4 \lesssim 10°$). Some calculated couplings corresponding to such conformations fall *ca.* 1–2 Hz outside those in Table I. However, coefficients which are slightly different from those in eq 2 (*e.g.*, $A = 8.0$ Hz, $A' = 9.6$ Hz, $B = 0.9$ Hz) yield calculated couplings in agreement with those in Table I.

The above analysis shows that individual conformations which have large puckerings cannot account for the nmr data. However, if such conformations are in rapid equilibrium, so that their J values average, the resultant couplings are in good agreement with those in Table I. For example, the averaged calculated couplings corresponding to the two C_γ puckered conformations

$$(C^+) \equiv (+\chi_1 = -\chi_4 = -25°, \chi_2 = -\chi_3 = 35°, \quad \varphi = 120°)\ (C_\gamma\ \text{exo})$$

$$(C^-) \equiv (+\chi_1 = -\chi_4 = +25°, \chi_2 = -\chi_3 = -35°, \quad \varphi = 120°)\ (C_\gamma\ \text{endo})$$

fall within the ranges given in Table I and agree (to within ±0.5 Hz) with those in the chart in Figure 1 (top).[23] The calculated average couplings which correspond to rapid interconversion of conformations puckered at other ring atoms or of twist conformations (one ring atom above and one below the plane of the other three) are also within the ranges given in Table I.[24] Intermolecular energy calculations[8] show that two structures, similar to (C+) and (C−), are the approximately isoenergetic lowest energy proline ring conformations.[25] On the basis of these results and the nmr analysis, it is proposed that in solution the proline rings of poly(L-proline) II rapidly interconvert, *via* the planar conformation, between two equally populated C_γ puckered conformations, similar to the (C+) and (C−) conformations shown in eq 3.[26] Assuming a torsional barrier of *ca.* 1 kcal per pair of eclipsed hydrogens, the planar conformation is no more than about 5 kcal above (C+) and (C−). Owing to the low barrier, the interconversion of the conformations is so rapid that the average of the chemical shifts and couplings corresponding to the two conformations is observed.[29]



(3)

Conclusions

A Karplus type analysis indicates that a number of proline ring conformations are consistent with vicinal couplings determined by computer simulation of the 220-MHz nmr spectrum of poly(L-proline) II. From the nmr analysis and energy calculations[8] it is suggested that in solution the form II proline ring rapidly interconverts between two conformations puckered at C_γ.

Acknowledgments. I am grateful to Dr. Charles M. Deber and Dr. Elkan R. Blout of the Harvard University Medical School for their gift of the poly(L-proline) sample used in this work, to Mr. R. L. Kornegay of Bell Telephone Laboratories for providing the computer program used to simulate the spectrum, and to Dr. Frank A. Bovey of Bell Telephone Laboratories for helpful discussions.

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(19) The exclusion of the planar and individual puckered or twisted conformations does not depend upon the particular choice of coefficients made in eq 2. It is found that if the coefficients are allowed to assume any values in the ranges $6.0 \leq A \leq 16.0$ Hz, $A \leq A' \leq 1.5A$, and $-0.5 \leq B \leq 2.5$ Hz, the calculated couplings fall outside the experimentally allowed ranges given in Table I.

(20) The X-ray study reveals that, in the solid state, poly(L-proline) II ring conformations puckered at C_γ have several unacceptably close *intermolecular* contacts. The solid-state ring conformation proposed (C_β exo) is stabilized by an *intermolecular* C_γ—H···O═C hydrogen bond.[2]

(21) C. M. Venkatachalam, *Biochim. Biophys. Acta*, **168**, 397 (1968).

(22) C. M. Deber, A. Scatturin, V. M. Vaidya, and E. R. Blout in "Peptides: Chemistry and Biochemistry," B. Weinstein and S. Lande, Ed., Marcel Dekker, New York, N. Y., 1970, pp 163–173.

(23) Satisfactory simulations are obtained only when the larger $J_{\alpha\beta}$ (8.5 Hz) is assigned to the H_β resonance at τ 7.68. This resonance is assigned to H_{β_1}, since couplings of $J_{\alpha\beta_1} \approx 8.5$ Hz and $J_{\alpha\beta_2} \approx 5.5$ Hz are calculated from eq 1 and 2 applied to every conformation compatible with the data in Table I. Acceptable simulations are not effected by interchanging chemical shifts of the two H_γ's or two H_δ's; therefore, individual assignments of these protons cannot be made.

(24) The number of conformations consistent with the nmr data could be substantially reduced if the H_γ couplings were known more accurately. These couplings can be obtained by deuteration of H_{γ_1} since it is seen from Figure 1 that an approximately first-order spectrum results, allowing individual H_{γ_2} vicinal couplings to be determined. Such results and the sum of H_γ couplings in Table I would yield all ring vicinal couplings.

(25) A survey of accurately determined (by X-rays) proline ring structures in crystalline proline oligomers indicates that C_γ puckered rings (endo and exo) are the predominate solid-state conformations provided they are not prohibited by close *intermolecular* contacts. For a thorough discussion, see R. Balasubramanian, A. V. Lakshminaranan, M. N. Sabesan, G. Tegoni, K. Venkatesan, and G. N. Ramachandran, *Int. J. Protein Res.*, **2**, 303 (1960).

(26) Preliminary results[27] obtained from an X-ray study of *S*-thiozolidine-4-carboxylic acid show that the thiozolidine ring is puckered at the γ position with S_γ exo. The $J_{\alpha\beta}$ values (7–7.5 Hz and 5–5.5 Hz) obtained from the spectrum[28] of poly[(*S*)-thiozolidine-4-carboxylic acid] and eq 1 and 2 suggest that in solution the ring of this poly(L-proline) II analog rapidly flips between S_γ endo ($\chi_1 \approx 30°$) and S_γ exo ($\chi_1 \approx -30°$) conformations.

(27) M. Goodman, G. C.-C. Niu, and K. C. Su, *J. Amer. Chem. Soc.*, **92**, 5219 (1970).

(28) M. Goodman, G. C.-C. Niu, and K. C. Su, *ibid.*, **92**, 5220 (1970).

(29) No attempt was made at a complete analysis of the 220-MHz spectrum of poly(L-proline) I for a number of reasons (low solubility of form I, δ resonance overlap, time-dependent spectrum which contains resonances due to form II segments of the isomerizing polymer[16]). The form I α resonance (a just-resolved doublet)[15] indicates that $J_{\alpha\beta_1} \approx 8$–10 Hz and $J_{\alpha\beta_2} \approx 0$–3 Hz. These couplings, incompatible with those in Table I, suggest that a single ring conformation is present in which $\chi_1 \approx 10$–30°. This conclusion must be regarded as tentative until the form I spectrum is completely analyzed (which should be possible with a suitably deuterated ring).