tal error, T_1's for the α-methylene carbons did not differ from each other or from the β carbon, at a given isomer composition. (The values for T_1 are given in Table III.) The average T_1 of 530 msec for a 85.5% trans polymer is close to values reported for similar carbons in high molecular weight polymers.[31,32,41,42] An increase in T_1 to 1.29 sec for the high cis polymer is noteworthy and possibly reflects an increase in segmental motion.[50] Until we do a more detailed study of the effect of isomer composition on T_1, we do not feel it is advisable to comment further on the increase in T_1. However, as mentioned in the Discussion section, the data were obtained under instrument conditions that allowed for the longer value in T_1. Consequently, the analytical results in Table II are reliable. Sample A was reanalyzed using only an acquisition time of 2.8 sec (the 12.2-sec delay time was eliminated). The same analysis for trans content was obtained, and the analysis time was reduced from 1.2 hr to 14 min.

We indicated that in Figures 1 and 2, the trans α and cis α resonances are each actually composed of two resonances, and the β resonances, three resonances. We suggest that each of the three major kinds of methylene carbons may also reflect linkage of cis and trans monomers. Figure 3 shows how the appearance of the spectrum changes at a higher cis concentration. The interpretation of the spectra in terms of sequences of configurational linkages is not needed for the analytical determination of cis and trans configuration. However, we do point out that there is no resonance due to cis–trans linkages intermediate in chemical shift to the trans at 33.1 ppm and cis at 27.7 ppm. Such an intermediate peak was expected for cis–trans linkages in polybutadiene.[29] If such a peak had been present in polypentenamer the analytical problem would have been considerably more complicated. An interpretation of the ^{13}C spectra of polypentenamers in terms of configurational sequence distribution will be the topic of a later report.[44]

The simplicity and internal consistency of these data demonstrate that polypentenamer is the very regular, repeating structure, $(-CH{=}CHCH_2CH_2CH_2-)_n$. There is no apparent isomerization during polymerization forming sequences having two and four methylene groups between olefinic carbons. If $-CH{=}CHCH_2CH_2CH{=}CH-$ were present, we would observe resonances analogous to polybutadiene at 27.7 ppm for cis α-CH_2 carbons or at 33.1 ppm for trans α-CH_2 carbons. These were not detected. If a significant amount of $-CH{=}CHCH_2CH_2CH_2CH_2CH{=}CH-$ were present, we would not have observed the consistency in the relationship, (cis α + trans α)/2β = 1, as a function of composition. The consistency observed for this relationship precludes a significant contribution from the sequence of four methylene groups between a pair of olefinic carbons.

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Influence of Conformational Isomers on the Circular Dichroism of Poly(L-prolylglycine)[1]

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ABSTRACT: The circular dichroism of poly(L-prolylglycine), which has previously been shown to be in a statistical conformation, has been measured in 2:1 (v/v) ethylene glycol–water over the temperature range 50 to −132°. Only negative circular dichroism is observed in the spectral range covered. The minimum near 202 nm changes from −6.0 cm^2/mmol at 50° to −9.3 cm^2/mmol at −132°, and an isosbestic point is observed near 213 nm. There is a one-to-one correlation between the circular dichroism at the minimum and the area of the two resonances observed for the α proton of the L-prolyl residue and the amide proton of the glycyl residue in the 220-MHz pmr obtained by Torchia. Consequently the same configurational change must be responsible for the effects seen in the proton nmr and CD. These effects have previously been attributed to cis–trans isomerization about the glycyl-L-prolyl peptide bond. However, the possibility that isomerization may also occur about the C^α—C′ bond in the L-prolyl residue cannot be eliminated.

The statistical conformation of a polypeptide chain is determined by its conformational energy map[3-5] and the concomitant statistical mechanical averaging.[4,6] The usual considerations of torsional potentials, attractive and repulsive nonbonded interactions, and dipolar interactions, which determine the conformational energy as a function of rotational angle, are influenced by the side group on the α-carbon atom and the solvent medium. Isomerization about the peptide bond will, in addition, also affect the conformational energy and thus the average chain structure. We can, therefore, expect that different polypeptide chains, or the same chain in different thermodynamic environments, despite being in statistical conformations and displaying the properties expected for disoriented chain molecules, will reflect different populations of allowed rotational states characteristic of the single bonds. A randomly coiled polypeptide chain can thus re-

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sult from a variety of local chain structures. For example, the average dimensions of polypeptides, as manifested by the characteristic ratio, are dependent on the nature of the side group[3,7-9] and in the case of poly(L-proline) on the solvent medium.[8]

The circular dichroism of dilute polypeptide solutions reflects interactions that extend primarily over a small number of repeating units. It is, therefore, not necessarily expected that there will be a universal spectrum representing all randomly coiled polypeptide chains. A great deal of confusion has been engendered because of this circumstance. In certain cases, based solely on the observed circular dichroism spectra, new ordered polypeptide structures have been assigned.[10-14] It is important, therefore, that the types of spectra that are associated with disordered chains be established and identified with the responsible features of the local chain structure. This procedure requires that the chain structure be established by methods other than optical measurements. In the present paper we continue previous work along these lines,[15,16] focusing attention on the sequential copolypeptide poly(L-prolylglycine), whose characteristic ratio has already been determined.[17] The proton nmr spectra of the same sample of this sequential copolypeptide has been obtained in several solvents from 0 to 80°,[18] and its ^{13}C nmr spectra has been obtained at 32°.[19]

Experimental Section

The polypeptide studied here is the same as was described in previous work.[17,20] The number- and weight-average amino acid residues are 104 ± 5 and 171 ± 4, respectively, for this sample. The intrinsic viscosity in water at 30° is 0.15 dl/g. The characteristic ratio was found to be 2.28 ± 0.29 in water at 30°. The circular dichroism spectra were determined using a Durrum-Jasco ORD/UV-5 recording spectropolarimeter. Temperatures below ambient were achieved by means of a heat exchanger (AC-110) supplied by Air Products and Chemicals, Allentown, Pa. Temperatures above ambient were obtained by circulating water from a constant-temperature bath through the jacketed cell. Temperatures were measured by means of a copper-constantin thermocouple and were maintained constant to within ±1.0°. Calibration of the instrument was accomplished with *d*-10-camphorsulfonic acid.[21] The light paths varied from 0.10 to 2.0 mm and the polymer concentration was usually about 1 mg/ml. The circular dichroism is reported as $\Delta\epsilon$ (cm^2/mmol) per peptide bond.

Because of the nearly 200° temperature range encompassed by these studies, it was necessary to correct for the change in polymer concentration. This was accomplished by determining the thermal expansion coefficient of the solvent, which was found to be constant over the range 50 to −132°. The polymer concentration increased by 10.8% from room temperature to −132° and decreased by about 1.5% from room temperature to 50°, the highest temperature of measurement. This correction, although slightly smaller than that proposed by Brown, Carver, and Blout,[22] is significant and cannot be neglected.

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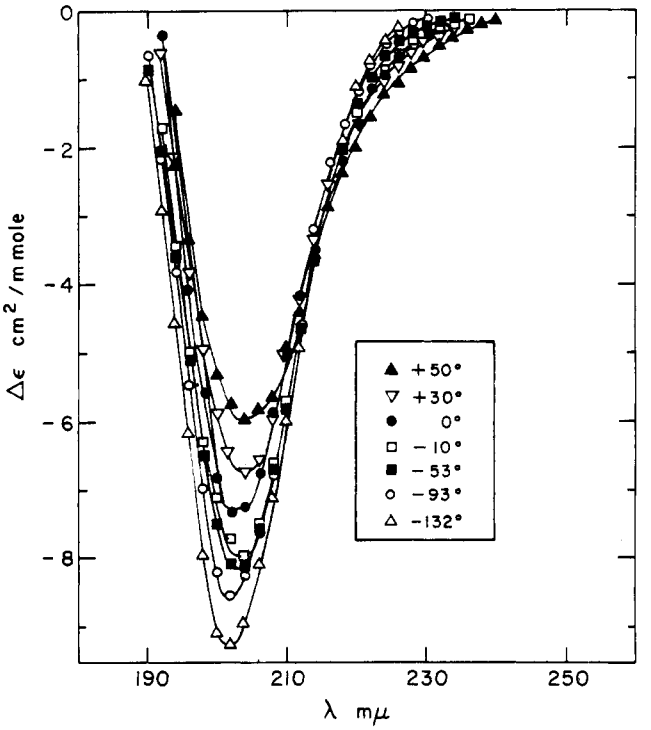

Figure 1. Circular dichroism spectra of the sequential copolypeptide poly(L-prolylglycine) in 2:1 (v/v) ethylene glycol-water at the indicated temperatures.

Results and Discussion

The CD for poly(L-prolylglycine) in an ethylene glycol-water mixture (2:1, v/v) is shown in Figure 1 over the temperature range 50 to −132°. At temperatures where comparison can be made, the spectra are very similar to those previously found for this polypeptide in either pure water or in trifluoroethanol.[20] This polypeptide exhibits only negative circular dichroism, over the range 190–250 nm, at all temperatures. A minimum is observed at 202–204 nm, and the corrected $\Delta\epsilon$ at the minima increase from −6.0 to −9.3 cm^2 per mmol as the temperature is lowered. The spectra are all similar at wavelengths below the minima. While there is a common cross-over point at about 213 nm, the spectra at the different temperatures show small but discernible differences at higher wavelengths. The presence of an isosbestic point in the circular dichroism spectra suggests a two-state analysis, which is also the conclusion obtained from the proton nmr spectra.[13]

In order to interpret these spectra it is necessary to describe the chain structure as determined by other methods. The structure of the repeating unit, in the sequence glycyl-L-prolyl, is shown in Figure 2. The characteristic ratio of this polypeptide in water at 30° indicates that the chain is in a statistical conformation under these conditions.[17] The high-resolution proton nmr spectra display two resonances of unequal areas for the glycine peptide protons and L-proline α protons.[18] Two resonances of unequal area are also observed in the ^{13}C nmr for the C^β and C^γ atoms.[19] These spectral features can be interpreted as resulting from the presence of two conformational isomers of the glycyl-L-prolyl unit whose interconversion is slow on the nmr time scale.

The two conformational isomers were interpreted by Torchia[18,19] as resulting from the random distribution of *cis*- and *trans*-glycyl-L-prolyl peptide bonds due to 180° rotations about ω_{Gly},[23-25] as illustrated in Figure 2. The

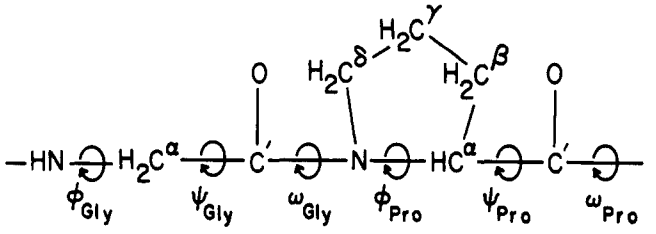

Figure 2. The structure of the glycyl-L-prolyl unit using the convention described in ref 23.

relative concentrations of the isomers depend on the solvent and temperature. In water at 22° the ratio of trans to cis isomers was found to be 4:1.[18] The temperature dependence of the relative area of the two resonances, over the range 0–80°, suggests a simple two-state analysis. This analysis yields ΔS of about 1.3 cal/(mol deg) and ΔH of about 1.2 kcal/mol for the entropy and enthalpy changes upon rotation from ω_{Gly} = 0–180° in an isolated glycyl-L-prolyl peptide bond.[18] The experimental uncertainty of *ca.* 10%[18] suggests an uncertainty of about ±0.7 kcal/mol for ΔH and ±2.3 cal/(mol deg) for ΔS. Therefore the experimental measurements do not allow for an unequivocal assessment of the sign of ΔS. Recent conformational energy calculations indicate a substantial decrease in configurational entropy when the glygyl-L-prolyl peptide bond changes from the trans to the cis conformation.[26]

An alternative assignment for the two isomers is suggested by two calculations of conformational energy maps for the L-prolylglycyl unit with all peptide bonds in the trans conformation.[25,27] One calculation employed a rigid pyrrolidine ring with the Leung and Marsh geometry,[25,28] while the other used a flexible pyrrolidine ring.[27] These conformational energy maps exhibit two minima, separated by high-energy barriers, for rotation about ψ_{Pro}. Evaluation of the Boltzmann factors at 10° intervals for ϕ_{Pro} and ψ_{Pro}, using a temperature of 22°, indicates that 24–40% of the L-prolyl residues would have ψ_{Pro} in the minimum near 130°, and the remainder would populate the minimum near 350°. The relative population is reasonably close to the 4:1 ratio of the resonances observed in the proton nmr of poly(L-prolylglycine) at 22° in water.[18]

It has recently been suggested that the high-energy barriers between ψ_{Pro} = 130° and ψ_{Pro} = 350° can be reduced substantially if certain alterations are made in the molecular geometry.[29] The energy barriers are reduced to less than 10 kcal/mol of the calculation is performed using a rigid pyrrolidine ring with the same geometry as is found in poly(L-proline II) in the solid state.[30] Certain of the bond angles in the main chain are altered by 3 or 6° and a hydrogen bonding interaction is included.[29] It was concluded that population of these two states would not lead to separate nuclear magnetic resonances.[29]

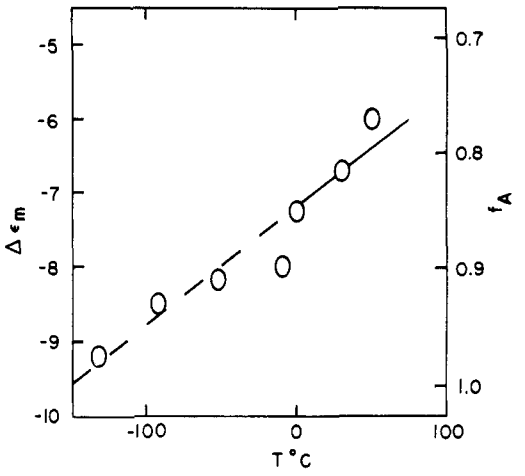

Figure 3. The minimum value of $\Delta\epsilon$, open circles, and fraction isomer A, f_A, represented by the straight line, as a function of temperature for poly(L-prolylglycine) in ethylene glycol-water mixture.

For the sake of generality we shall designate the two isomers as A and B, respectively, with A being the prevalent isomer at 22° in water. By applying the two-state theory, the fraction of residues in the A conformation, f_A, can be expressed as

$$f_A = (1 + \exp(-\Delta G/RT))^{-1} \quad (1)$$

Utilizing the parameters obtained from the proton nm experiments,[18] the calculated values of f_A are given as a function of temperature in Figure 3. The solid line represents the actual temperature of the proton nmr experiment. The dashed line represents an extension to lower temperatures calculated by eq 1. Also plotted in the figure is the minimum value of $\Delta\epsilon$ for each temperature. The f_A and the minimum value of $\Delta\epsilon$ vary with temperature in essentially the same manner. The one-to-one correlation that is found between these two quantities is shown in Figure 4. The relative population of the A and B isomers changes from 78:22 at 50° to 98:2 at −132°. Based on previous experience,[15] in order to obtain a more quantitative comparison, resolved spectra rather than the observed spectra should be used. Although only negative circular dichroism is found, the spectra cannot be consistently represented by either a single Gaussian band or a combination of two such bands. A more detailed comparison must therefore await a more quantitative theory for circular dichroism.

The spectra of Figure 1 and the plot of Figure 4 are easily extrapolated to a chain which is entirely in the A conformation. It can be concluded that such a chain will exhibit only negative circular dichroism. The analysis of the nmr spectra by Torchia[18,19] as well as an analysis of isomerization about ψ_{Pro} show that a chain entirely in the A conformation will have all of its peptide bonds trans. It does not follow, however, that all polypeptide chains which are in a statistical conformation and which contain only trans peptide bonds, will exhibit this type of circular dichroism. It has been recently shown that poly(L-proline),[31] poly(L-glutamic acid),[9] and poly(N^5-ω-hydroxyethyl-L-glutamine)[9] have quite different spectra when in statistical conformation as compared with that for poly(L-prolylglycine). The aforementioned polypeptides display a definite positive contribution in their CD spectra at 30°.

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(24) Calculations of the effect of randomly introducing cis peptide bonds in an all trans chain indicates that the characteristic ratio is not sensitive to the presence of cis peptide bonds in a chain rich in glycine. The glycine content is also the primary factor in determining the characteristic ratios of of random[7,25] and sequential[17] copolypeptides rich in glycine when all of the peptide bonds are in the trans conformation.

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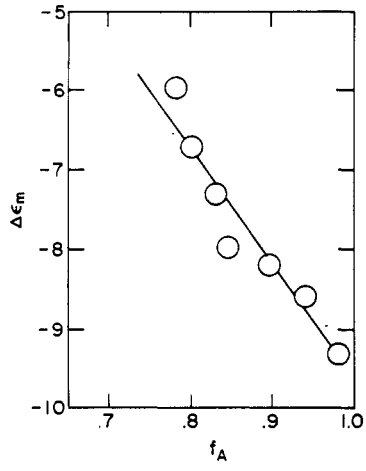

Figure 4. Plot of the minimum value of $\Delta\epsilon$ *vs.* f_A for poly(L-prolylglycine) in the ethylene glycol-water mixture.

Unionized low molecular weight derivatives of L-alanine also exhibit a positive circular dichroism in the range 208–218 nm in water at 15°.[32,33] These distinctly different spectra, for chains in statistical conformation, must reflect differences in the population of accessible rotational states among the polypeptides.

The random introduction of the B conformation systematically changes the magnitude of the circular dichroism. At the highest temperature reported 22% of the B conformation is present but the major characteristics of the spectrum remain unaltered.

Proton and ^{13}C nmr spectra of small peptides are important in attempting to characterize the B isomer. Two sets of resonances are observed with Ac-Pro,[19,34] Ac-4Hyp,[19] and Ac-Pro-OMe.[35]

Since conformational energy calculations[33,35,36] have shown that the substitution of an ester for an amide markedly lowers the energy barrier for rotation about ψ, these resonances can be assigned with reasonable certainty to isomerization about the amide bond. A similar chemical shift pattern is observed[35] for the acetyl methyl protons and L-proline α proton in Ac-Pro-OMe, Ac-Pro-NH_2, and Ac-Pro-NHMe. Since the two sets of resonances in Ac-Pro-OMe are assigned to isomerization about the Ac–Pro bond, it is tempting to make a similar assignment in Ac-Pro-NH_2 and Ac-Pro-NHMe. This assignment implies that no new resonances can be observed due to rotation about ψ_{Pro} in Ac-Pro-NH_2 and Ac-Pro-NHMe. Either the isomerization about ψ_{Pro} is fast on the nmr time scale, or the additional resonances produced by slow rotation about ψ_{Pro} in Ac-Pro-NH_2 and Ac-Pro-NHMe cannot be separated from the other resonances in the nmr spectrum. The short-range interactions which hinder rotation about ψ_{Pro} in poly(L-prolylglycine) containing peptide bonds in the trans conformation are present in Ac-Pro-NH_2 and Ac-Pro-NHMe.

The presence of only one set of resonances in Pro-Gly and Pro-NH_2 shows that isomerization about ψ_{Pro} is rapid on the nmr time scale in these compounds.[19] However, arguments based on Pro-Gly and Pro-NH_2, in which the pyrrolidine ring contains a tetrahedral nitrogen atom, are not necessarily pertinent to poly(L-prolylglycine), in which the pyrrolidine ring contains a planar nitrogen atom.[37]

The effect of the isomerization on the chemical shift of C_{Pro}^{α} and C_{Pro}^{δ} is about an order of magnitude smaller in poly(L-prolylglycine) and in oligopeptides than it is in Ac-Pro.[19] In some respect Ac-Pro is not an appropriate model compound for the interpretation of the nmr spectrum of poly(L-prolylglycine). A possible explanation is that isomerization occurs about the peptide bond in both cases, but that replacement of the acetyl group by an amino acid residue alters the magnetic environment of the peptide bond. Another possibility is that conformational effects, due to the steric interaction of residue $i - 1$ with residues $i + 1$ and $i + 2$ when $\omega_i = 180°$,[26] may be responsible for the different nmr spectra.

The above considerations do, however, lend weight to the interpretation that the two sets of resonances in poly(L-prolylglycine) reflect isomerization about ω_{Gly}. It does not follow, however, that conformational alteration occurs only about ω_{Gly}. Steric interactions are severe in poly(L-prolylglycine) when $\omega_{Gly} = 180°$, and extend well beyond neighboring residues. Rotations about ω_{Gly} and ψ_{Pro} are interdependent in poly(L-prolylglycine),[26] and it may be an oversimplification to characterize the two conformational isomers by a single dihedral angle. A more complete description might require the specification of two or more dihedral angles, as, for example, isomer A with ω_{Gly}, $\psi_{Pro} \cong 0°$, 350° and isomer B with ω_{Gly}, $\psi_{Pro} \cong 180°$, 130°.[26]

Many other polypeptides, under appropriate conditions, display CD spectra which exhibit only negative circular dichroism with a single minimum near 200 nm. Poly(glycylglycyl-L-prolylglycine) exhibits this type of CD under all conditions studied,[20,38] and the proton[18] and ^{13}C[19] nmr spectra indicate the presence of 17% of the B isomer near room temperature in aqueous solution. Many low molecular weight derivatives of L-alanine,[32,33] sequential copolypeptides,[20,22,39-42] homopolypeptides,[16] and collagen[11] exhibit this type of spectrum at sufficiently high temperature. Similar spectra can be generated from poly(L-proline),[15] and also from uncharged oligopeptides[32,33] and polypeptides[16] containing $-CH_2R$ side chains, by the isothermal addition of certain salts.

The strong similarity of the circular dichroism generated in the many cases cited makes it tempting to search for a common explanation. Isomerization about the peptide bond has been suggested to occur in those cases where the polypeptide contains L-proline residues.[18,19,43,44] This suggestion does not appear to be as attractive a possibility for molecules which contain only L-alanine residues or for derivatives of L-glutamine. Isomerization about ψ, from a region near 350° to a region near 130°, is plausible in the sequential copolypeptides and in the homopolypeptides with $-CH_2R$ side chains. Rotational states in the vicinity of $\psi = 130°$ must also be considered in the case of poly(L-proline) and its aqueous salt solutions. Conformational energy maps which correctly predict the dimensional

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properties of poly(L-proline) exhibit two minima for rotation about ψ.[45] The effect of calcium chloride on the intrinsic viscosity of poly(L-proline)[8,15] can be accounted for if 20% or more of the L-prolyl residues have ψ near 130° in concentrated calcium chloride solution.[46] The ir spectrum of poly(L-proline) in aqueous calcium chloride is also ambiguous concerning isomerization about ψ or ω.[47,48] If the salt effect on poly(L-proline) is due to isomerization about ψ the ^{13}C nmr chemical shifts would have to be similar for isomerization about ψ and about ω.[44] Inspection of molecular models does not provide any basis for ruling out such a coincidence. In summary, it is clear that at present an unequivocal description of the structure of the B isomer cannot be made from the evidence in hand.

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Evidence for β-Turn Analogs in Proline Peptides in the Solid State. An Infrared Study

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ABSTRACT: The solid-state infrared spectra of a group of proline-containing di- and tripeptide carboxylic acids and esters are interpreted in terms of peptide secondary structure. When an L-Pro-D-Pro, L-Pro-Gly, or Gly-L-Pro sequence occurs in the positions nearest the C-terminal acid, the peptides are proposed to fold into 10-membered hydrogen-bonded structures analogous to β turns. Experimental evidence for this folding is obtained from observation of consistent shifts of appropriate carbonyl bands due to hydrogen-bond formation. Confirmation of assignments of amide I bands in *t*-Boc-Gly-L-Pro-OBz and *t*-Boc-Gly-L-Pro-OH is obtained through a comparison of the infrared spectra of natural abundance samples of these peptides with samples enriched 60% with ^{13}C at the Gly carbonyl carbon atom. Additional structures deduced from solid-state infrared spectra are proposed for peptides containing L-Pro-L-Pro, D-Pro-D-Pro, or L-Pro-Sar sequences nearest the C-terminal acid. Infrared spectra in chloroform and dioxane solutions indicated the absence of any shifted bands which could be correlated with specific intra- or intermolecular structures.

The folding of a peptide chain into a β turn (also called β bend, or hairpin bend), producing a hydrogen bond between the first and fourth residues of the chain, and causing a 180° reversal in chain direction, has been recognized as an essential feature of protein secondary structure.[1-3] Experimental observations of β turns have come largely from X-ray crystallographic determinations of peptide[4a,b,5] and protein structure,[6] as well as from nuclear magnetic resonance studies of solution conformations of a variety of naturally occurring[7-9] and synthetic[10-13] cyclic peptides.

The present report provides evidence from solid-state infrared spectra for the formation in linear peptide carboxylic acids of structures analogous to β turns. These structures are observed when the sequence Gly-L-Pro, L-Pro-Gly, or L-Pro-D-Pro occurs in the two residues nearest the C terminus of the peptide chain.

Results

A study was made of the carbonyl regions of the infrared spectra of the peptide acids listed in Table I, and their corresponding benzyl esters. These regions (1600–1800 cm^{-1}) include bands attributable to ester, carboxylic acid, urethane, and peptide amide I functional groups. All acids were studied as solids in KBr disks; some (noncrystalline) esters were examined as smears between NaCl plates.

In the absence of specific interactions, these multifunctional compounds would have carbonyl frequencies the same as those observed for corresponding monofunctional compounds. Such "normal" frequencies are observed for peptide benzyl esters. The ester carbonyl position in each case was 1740–1745, appropriate for a benzyl ester carbonyl in simple organic esters.[14] The band for the *tert*-butyloxycarbonyl group (*t*-Boc) occurred at 1685–1695, in the region previously noted for secondary and tertiary urethanes (carbamates).[14] The peptide amide I bands[15] oc-

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