help in some of the calculations, and Mr. J. Vanophalvens, for technical assistance. The numerical calculations have been performed on the CDC 6400 computer at the Centre de Calcul de l'Université Libre de Bruxelles. This work was supported in part by a grant from the Fonds de la Recherche Fondamentale Collective. One of us (Marc Leclerc) is Aspirant au Fonds National de la Recherche Scientifique.

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Conformational Properties of Poly(L-azetidine-2-carboxylic acid) in Solution as Studied by Carbon-13 and Proton Nuclear Magnetic Resonance Spectroscopy

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Received July 30, 1974

ABSTRACT: The proton and ^{13}C magnetic resonance spectra of poly(L-azetidine-2-carboxylic acid) were determined in water and in formic acid. The 100-MHz ^1H nmr spectra do not give evidence of cis–trans isomerism in the two solvents. On the other hand, the 220-MHz ^1H nmr spectrum in water shows two peaks for the $^{\alpha}CH$ proton. ^{13}C nmr spectra show two separate peaks for each carbon atom, which are assigned to cis and trans isomers of the amide bond. Some model compounds have been examined to aid this assignment. The percentage of the cis isomer decreases from water to formic acid and increases upon addition of $CaCl_2$ to the water solution. High yields of high molecular weight poly(L-azetidine-2-carboxylic acid) (mol wt = 23,000) have been obtained by the polymeric self-condensation of the $(Aze)_3$ pentachlorophenyl ester trifluoroacetate.

The study of the structure of poly(L-azetidine-2-carboxylic acid) (PLAze) in solution is part of a research program intended to elucidate the conformational effects of the replacement of L-proline with L-azetidine-2-carboxylic acid (L-Aze-COOH) in polypeptide chains.

The optical properties of PLAze in solution have been discussed in a recent paper.[1]

The CD spectra in water and fluorinated alcohols have been interpreted as indicating the occurrence of disordered chain structures. This disordering could originate from cis–trans isomerization and/or increased range of accessible $^{\alpha}CC{=}O$ rotation angles.[2]

Here we report investigations of PLAze in solution by means of ^{13}C and ^1H nuclear magnetic resonance (nmr) spectroscopy.

Materials and Methods

L-Azetidine-2-carboxylic acid (Serva) was used as received; found: $[\alpha]^{22}_{589}$ −123.3° (*c* 3.6% in H_2O). *N-tert*-Butyloxycarbonyl-L-azetidine-2-carboxylic acid, *N-tert*-butyloxycarbonyl-L-azetidine-2-carboxylic acid *N*-hydroxysuccinimide ester, and L-azetidine-2-carboxylic acid pentachlorophenyl ester hydrochloride were prepared as previously described.[3] *N*-Carbobenzoxyglycine-*N*-hydroxy-

succinimide ester was purchased from Fluka. *N*-Methylmorpholine was distilled and the fraction boiling at 115.5° was used. Triethylamine was purified following the procedure described by Kovacs, *et al.*[4]

Chloroform, trifluoroacetic acid, dichloroacetic acid, and dimethyl sulfoxide were purified by distillation immediately prior to use. All other solvents were of the highest obtainable purity. The viscosity measurements were made at 25 ± 0.05° with an Ubbelhode viscosimeter, type BS/IP/MSL, size 2 (Poulten, Selfe and Lee, Wickford, England). Solvent flow time was 524.3 sec. Flow times have been automatically determined with the Schott Viscotimer (Schott, Mainz, Germany).

The elemental analyses were carried out at the Laboratorio di Microbiologia, Snamprogetti S.p.A., Monterotondo, and the Scandinavian Microanalytical Laboratory, Herlev, Denmark. Circular dichroism was measured on the Cary 60 spectropolarimeter equipped with the Model 6001 CD attachment. The concentrations have been calculated by taking into account the water content of the sample.

Melting points (uncorrected) were determined with a Tottoli apparatus and optical rotations with a Perkin-Elmer 141M automatic polarimeter in a standard 100 mm thermostated cell. Infrared spectra have been recorded with the Perkin-Elmer 325 spectrophotometer. The 25-MHz ^{13}C spectra and the 100-MHz ^{1}H spectra were obtained on a Varian XL-100-15 spectrometer equipped with the VFT-100 Fourier transform accessory. The 220-MHz ^{1}H spectrum was obtained on the Varian HR 220 MHz instrument at the Johnson Research Foundation (nmr facilities supported by NIH (Grant No. RR542)), Philadelphia, Pa. Analytical determination of compound III (see later) was performed on a Varian T-60 spectrometer. For the ^{13}C spectra, Fourier transform operation was used with an 8K 620i computer and proton decoupling was provided by a Varian Gyrocode spin decoupler. For high-temperature measurements the Varian variable temperature accessory provided the temperature control.

In aqueous and formic acid solutions dioxane was used as internal reference for the ^{13}C spectra, and the chemical shifts were converted to the TMS scale using the dioxane–TMS shift difference of 67.4 ppm. For the ^{13}C spectrum in dimethyl sulfoxide, TMS was added as internal standard. In ^{1}H nmr measurements, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as internal reference for aqueous and formic acid solutions, and TMS for chloroform solutions.

Experimental Section

Synthesis of Model Compounds. *N*-Acetyl-L-azetidine-2-carboxylic acid. This compound was prepared as described in the literature.[5] A crystalline material was obtained in 77% yield: mp 90° (lit. mp 43–45°).

Anal. Calcd for $C_6H_9NO_3$: C, 50.3; H, 6.29; N, 9.79. Found: C, 50.25; H, 6.27; N, 9.66.

***N*-Carbobenzoxyglycyl-L-azetidine-2-carboxylic Acid (I).** To a solution of 1.54 g (0.15 mol) of L-azetidine-2-carboxylic acid and 1.26 g (0.015 mol) of $NaHCO_3$ in 16 ml of water, 3.06 g (0.01 mol) of *N*-carbobenzoxyglycine-N-hydroxysuccinimide ester dissolved in 20 ml of dimethoxyethane was added. After 1 hr at room temperature with stirring, water and concentrated HCl were added to the reaction mixture to a pH of 2. The solution was then extracted with ethyl acetate. The organic layer was washed with water and brine, dried, and evaporated to give an oil which did not crystallize.[6] The oil was purified by washing with *n*-hexane and dried under vacuum at 40° overnight, and 2.3 g of the analytically pure acyldipeptide was obtained: yield 78.8%.

Anal. Calcd for $C_{14}H_{16}N_2O_5$: C, 57.5; N, 9.6; H, 5.45. Found: C, 57.0; N, 9.2; H, 5.8.

The tlc on silicagel gave a single spot after exposure to I_2 vapors, using $CHCl_3$–CH_3OH 9:1 (v/v) and 1-butanol–AcOH–H_2O 3:1:1 as eluants.

***N*-Carbobenzoxyglycyl-L-azetidine-2-carboxylic Acid Methyl Ester (II).** Compound I (2.0 g) was converted to the methyl ester as described in the literature.[7] White crystals (1.3 g) were obtained after crystallization of the oil from benzene–*n*-hexane: yield 59.3%; mp 72–74°.

Anal. Calcd for $C_{15}H_{18}N_2O_5$: C, 58.8; N, 9.1; H, 5.88. Found: C, 58.68; N, 9.02; H, 5.95.

The product appeared as a single spot on tlc in $CHCl_3$–CH_3OH and 1-butanol–AcOH–H_2O 3:1:1 after exposure to I_2 vapors.

Synthesis and Characterization of Poly(L-azetidine-2-carboxylic acid). The synthesis of poly(L-azetidine-2-carboxylic acid) has been recently described.[3] To improve the yield of the reaction and the molecular weight of the polymer we have polycondensed the pentachlorophenyl ester of $(Aze)_3$ trifluoroacetate.

***N*-*tert*-Butyloxycarbonyl-L-azetidine-2-carbonyl-L-azetidine-2-carboxylic Acid (III).** A solution of 11.94 g (0.04 mol) of the *N*-hydroxysuccinimide ester of *N*-*tert*-butyloxycarbonyl-L-azetidine-2-carboxylic acid in 80 ml of dimethoxyethane was added under stirring to a solution of 6.06 g (0.06 mol) of L-azetidine-2-carboxylic acid and 5.04 g (0.06 mol) of $NaHCO_3$ dissolved in 70 ml of water.[8] The reaction mixture was stirred for 20 hr at room temperature. The clear solution was concentrated under vacuum and acidified with concentrated HCl to pH ~2. After chilling for about 2 days, 4.47 g of crystals, mp 149–151°, was collected by filtration. The mother liquors were then extracted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, and evaporated *in vacuo* to give 3.5 g of a spongy solid. A first crop (2.6 g) of product, mp 148–152°, crystallized spontaneously after dissolving the solid in ethyl acetate and standing at room temperature for about 10 min. A second crop (0.3 g) with identical melting temperature was isolated from the mother liquors: total yield 64%; $[\alpha]^{25}_{578}$ −202.0° (*c* 1% in $CHCl_3$)

Anal. Calcd for $C_{13}H_{20}N_2O_5$: C, 54.97; N, 9.86; H, 7.04. Found: C, 54.80; N, 9.78; H, 7.1.

The product showed only one spot by tlc in several solvent systems: ir (Nujol) ν 1760, 1702, 1688, 1615 cm^{-1}; nmr ($CDCl_3$) δ 1.4 (9 H, singlet, CH_3), 1.7–2.9 (4 H, complex, $^{\beta}CH_2$), 3.6–5.3 (6 H, complex, $^{\alpha}CH$, $^{\gamma}CH_2$), 11.3 (1 H, singlet, COOH).

***N*-*tert*-Butyloxycarbonyl-L-azetidine-2-carbonyl-L-azetidine-2-carbonyl-L-azetidine-2-carboxylic Acid Pentachlorophenyl Ester (IV).** III (6.0 g, 0.021 mol) dissolved in 250 ml of $CHCl_3$ was chilled to −15° and stirred. Then 2.36 ml (0.021 mol) of *N*-methylmorpholine and 2.99 ml (0.0228 mol) of isobutyl chloroformate were pipeted in. Thirty minutes later 8.15 g (0.021 mol) of the hydrochloride of L-azetidine-2-carboxylic acid pentachlorophenyl ester were added. Finally, a solution of 2.36 ml (0.021 mol) of *N*-methylmorpholine in 20 ml of $CHCl_3$ was added dropwise.[9] The cooling bath was removed and the reaction mixture was stirred at room temperature for 20 hr. After washing with water and brine, the solution was dried over anhydrous Na_2SO_4 and evaporated to dryness. A spongy solid was obtained by maintaining under vacuum over KOH overnight. After trituration with ethyl ether, a semicrystalline powder, mp 148–151°, was recovered by filtration. A recrystallization from ethyl acetate–light petroleum gave 6.7 g of a product melting at 153–154°. A second crop (1.8 g), mp 154°, was collected from mother liquors: total yield 65%; $[\alpha]^{25}_{578}$ −135.2° (*c* 1% in $CHCl_3$); ir (Nujol) ν 1787, 1695, 1678, 1650 cm^{-1}; nmr ($CDCl_3$) δ 1.4 (9 H, singlet, CH_3), 2.2–3.1 (6 H complex, $^{\beta}CH_2$), 3.8–4.9 (8 H, complex, $^{\gamma}CH_2$, 2 $^{\alpha}CH$), 5–5.3 (1 H, quartet, $^{\alpha}CHCOOC_6Cl_5$).

Anal. Calcd for $C_{23}H_{24}N_3O_6Cl_5$: C, 44.84; N, 6.81; H, 3.9; Cl, 28.84. Found: C, 44.9; N, 6.81; H, 3.97; Cl, 28.58.

Poly(L-azetidine-2-carboxylic acid) (PLAze). Freshly distilled trifluoroacetic acid (4.97 ml, 0.065 mol) was added to a stirred solution of IV (2 g, 0.0032 mol) in $CHCl_3$ (18 ml). The reaction mixture was kept under stirring at room temperature for 4 hr. The solvent and the excess of trifluoroacetic acid were removed by bubbling with nitrogen and then by maintaining *in vacuo* at 40°. Trituration of the residual oil with ethyl ether gave the product as a powder, which was washed repeatedly with ether and dried overnight *in vacuo* over KOH (mp 138–140°). To the entire product dissolved in 23 ml of dimethyl sulfoxide, 0.91 ml (0.0065 mol) of triethylamine were added dropwise under stirring. The mixture quickly set to a viscous shakable jelly. The polymerization was allowed to proceed for 24 hr at room temperature under stirring. Triethylamine (0.91 ml) was further added dropwise to the mixture and the stirring was allowed to continue for 12 hr. On addition of the reaction mixture to a large excess of ethyl ether under vigor-

ous stirring, a powder was obtained that was washed with methylene chloride and dried over P_2O_5 *in vacuo*. Crude product (0.78 g) was recovered.

The purification of the water-soluble aliquot (95%) from low molecular weight impurities was carried by gel chromatography on Sephadex G-50 fine. Two fractions of different molecular weight were collected and lyophilized to give a total yield of 0.54 g of a fluffy white solid; yield 67%. The polymer fractions were soluble in water, trifluoroethanol, hexafluoroacetone·H_2O, and trifluoroacetic acid.

Anal. Calcd for $C_{12}H_{15}N_3O_3 \cdot 2.25(H_2O)$: C, 49.74; N, 14.5; H, 6.73. Found: C, 49.48; N, 14.2; H, 6.7.

The water content derived from elemental analysis is in agreement with the value found from hydration measurements of a sample exposed to atmospheres of increasing relative humidity.[3] The circular dichroism spectrum in water is identical with that observed with a polymer sample obtained by the polymeric self-condensation of the pentachlorophenyl and *N*-hydroxysuccinimide esters of L-azetidine-2-carboxylic acid hydrochlorides.[3] The molecular weight of the water-soluble fractions has been estimated by chromatography on Bio-Gel A 5m agarose gel (100–200 mesh) using columns equilibrated in 6M guanidinium chloride as recently described.[3] We have used as molecular weight markers ovalbumin (mol wt = 41,000) chymotrypsinogen (mol wt = 25,000), myoglobin (mol wt = 17,200), lima bean trypsin inhibitor (mol wt = 8400), and insulin B Chain (mol wt = 3400). The molecular weight obtained from the calibration plot, V_e/V_0 against log mol wt, is 23,000. (η_{sp}/c) = 0.953 in dichloroacetic acid at 25 ± 0.05° (c = 0.2%).

Results and Discussion

The polymer has been studied in pure water, in aqueous salt solutions, and in formic acid.

Figure 1 shows the 100-MHz proton (pmr) spectra of the polymer in water and in formic acid. Only one resonance is observable for the $^{\alpha}$CH main chain protons in the formic acid solution, as is also the case in the spectrum of poly(L-proline) in formic acid or water when the isomerization process from the all-cis form (form I) to the all-trans form (form II) is complete.[10–12] In the spectrum of aqueous solution the α-proton absorption is, however, slightly asymmetric, and at 220 MHz it reveals two peaks (Figure 2).

Since the appearance of one $^{\alpha}$CH resonance at 100 MHz for PLAze may have been due to the overlap of the resonances of two conformers, we examined the ^{13}C nmr spectra, in which chemical shift coincidences are more improbable.

^{13}C spectra of PLAze ring carbon atoms in water and in formic acid are shown in Figure 3. Two separate peaks appear for each ring carbon atom. The chemical shift values (given in Table I) are approximately the same in both solvents but their intensity ratios are quite different in water and formic acid. In this latter solvent the minor resonance at highest field is not resolved.

Doubled peaks in the ^{13}C nmr spectra have been previously observed for the β and γ ring carbons of poly(L-proline) in aqueous salt solutions. The doubling is attributed to cis–trans isomerism at the peptide bond.[2] This assignment was made by comparison of the polymer spectra with the spectra of a series of acyclic low molecular weight proline derivatives, from which were drawn general conclusions about the effect of cis–trans isomerism on the spectral pattern of proline ring carbons.[13]

In analogy with this procedure we have examined some model compounds containing L-Aze-COOH. These compounds are listed in Table I, where the chemical shift values for their ring carbon atoms and for those of the polymer are reported. Apart from the problem of distinguishing cis and trans absorptions, the assignments are straightforward and are based upon general data available from the ^{13}C nmr literature.[14]

In Figure 4 the ^{13}C nmr spectrum of the ring carbons of *N*-acetyl-L-azetidine-2-carboxylic acid in water is shown.

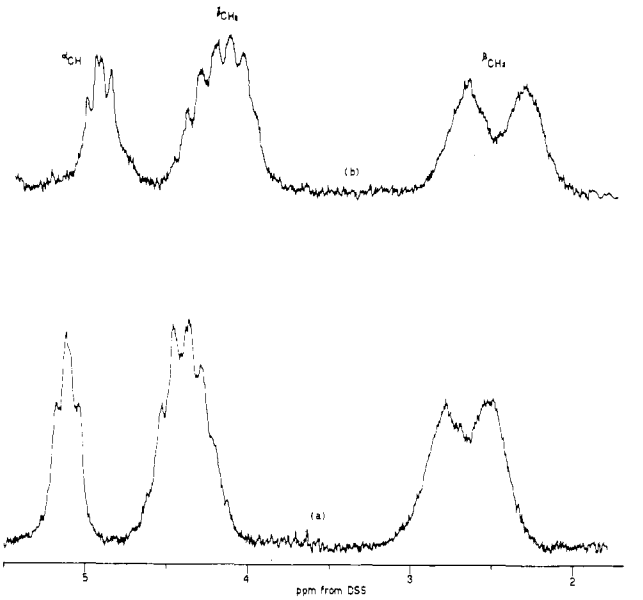

Figure 1. Pmr spectrum (100 MHz) of PLAze: (a) in D_2CO_2; (b) in D_2O 99.5%–D_2CO_2 0.5%.

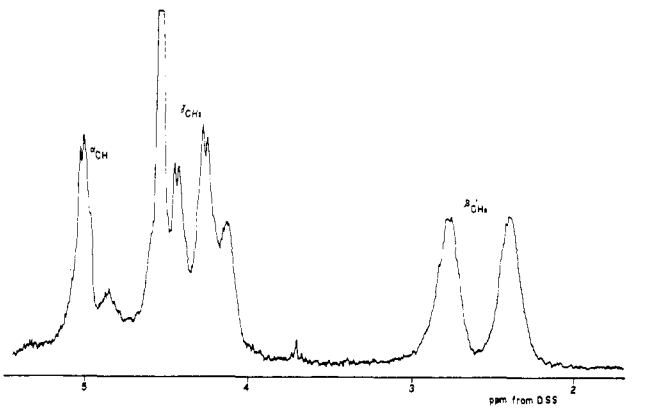

Figure 2. Pmr spectrum (220 MHz) of PLAze in D_2O at 50°; the resonance at 4.6 ppm is from HDO.

Previous proton nmr work on this compound demonstrated the coexistence of cis and trans isomers in ratios dependent on the solvent.[5] In water the trans isomer is prevailing and we can easily assign the various ^{13}C resonances on the basis of this assumption. The main spectral feature is the back-to-back pattern of the α and γ carbon peaks, which is due to the shielding effect of the carbonyl group on the syn carbons relative to those which are anti.

A similar effect in the cis and trans isomers of α and δ carbon atoms of *N*-formyl- and *N*-acetylproline has not been adopted as a general criterion for identifying cis–trans isomers in proline derivatives, because the pattern may be altered by steric effects when the acyl group on the proline nitrogen is an amino acid.[13] However, in benzyloxycarbonylglycyl-L-azetidine-2-carboxylic acid methyl ester we still observe the back-to-back pattern for α and γ carbon peaks (Figure 5). (The assumption that the trans conformer is the major species in this compound is by analogy with *N*-acetylazetidine-2-carboxylic acid. Also proline derivatives are generally known to prefer the trans form in every solvent.) The two ring carbons are more removed from the acyl group in the case of the four-membered ring of L-Aze-COOH derivatives than in proline derivatives and are less likely to be influenced by steric compression by the acyl substituent.

Table I
^{13}C Chemical Shifts[a] of the Ring Carbons of L-Azetidine-2-carboxylic Acid Derivatives

Compd	C_α Cis	C_α Trans	C_β Cis	C_β Trans	C_γ Cis	C_γ Trans	Solvent
L-Azetidine-2-carboxylic acid (zwitterion)	60.1		24.3		43.9		D_2O
N-Acetyl-L-azetidine-2-carboxylic acid	62.6	60.2	20.9	20.4	47.4	50.1	D_2O
N-Benzyloxycarbonylglycyl-L-azetidine-2-carboxylic acid methyl ester	60.5	58.9	20.2		46.1	48.1	$(CD_3)_2SO$
Poly(L-azetidine-2-carboxylic acid)	60.5	57.7	21.3	20.5	48.5	50.3	D_2O
Poly(L-azetidine-2-carboxylic acid)	61.4	58.8	21.2		49.5	51.1	D_2CO_2

[a] Relative to TMS.

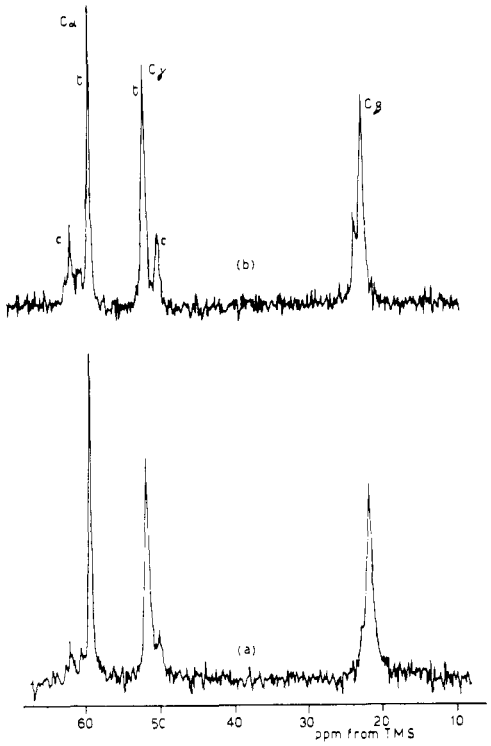

Figure 3. ^{13}C spectra (25 MHz) of the ring carbons of PLAze: (a) in D_2CO_2; (b) in D_2O.

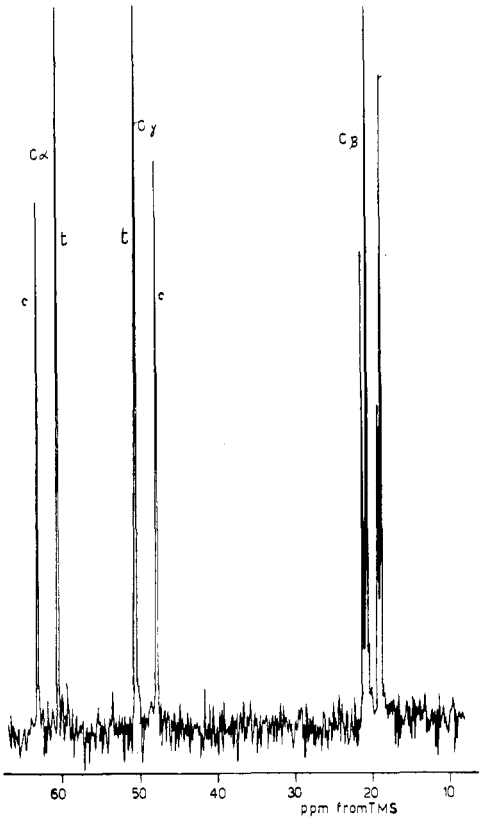

Figure 4. ^{13}C spectrum (25 MHz) of *N*-acetyl-L-azetidine-2-carboxylic acid in water. The two resonances at highest field are of CH_3 carbon, carbonyls are not shown.

These considerations and the observation of the same pattern for α and γ carbons in aqueous and acidic solutions of PLAze appeared to us as a strong indication of cis–trans isomerism at the peptide bond.

When the polymer is dissolved in 2 *M* aqueous $CaCl_2$ solution the intensity of the carbon peaks attributed to the cis isomer increases markedly relative to that of trans resonances (Figure 6). At high temperature the signals of the two forms coalesce, and for each carbon atom there is observable one peak at an intermediate position (Figure 7).

This behavior gives further support to the interpretation of the doubled ^{13}C nmr resonances as arising from the occurrence of cis and trans forms in aqueous solution.

The alternative explanation of the salt effects which has been offered for poly(L-proline) is that the added ions permit the chain structure a wider range of ψ values, with a loss of regular structure, reflected by a lowered characteristic ratio.[15-17]

This explanation would also account for the peak doubling in ^{13}C nmr spectra only if the barrier to rotation between cis′ (ψ = 120) and trans′ (ψ = 300) conformations in an all-trans chain were about 20 kcal/mol, like the cis–trans barrier.[2] A recent theoretical estimate of this barrier in an

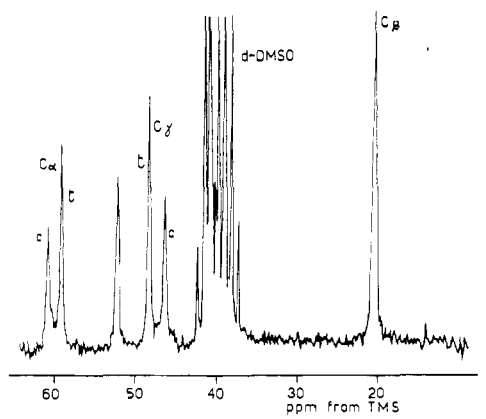

Figure 5. ^{13}C spectrum (25 MHz) of *N*-benzyloxycarbonylglycyl-L-azetidine-2-carboxylic acid methyl ester. The multiplet centered at 41 ppm is of DMSO-*d*; the resonance at 52 ppm is of methyl ester carbon; carbonyls are not shown.

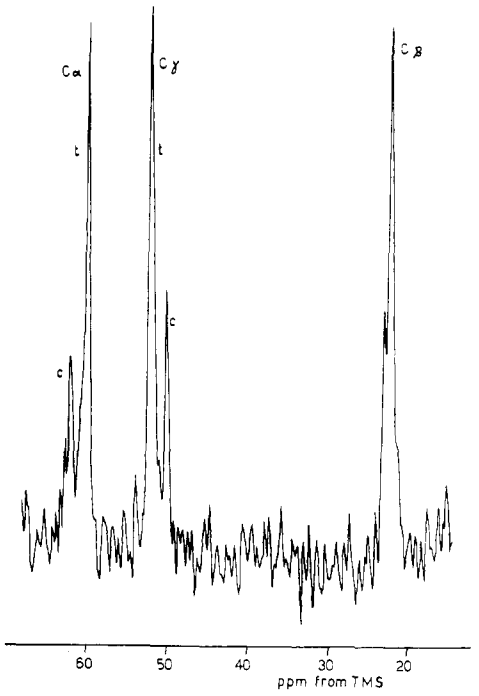

Figure 6. ^{13}C spectrum (25 MHz) of the ring carbons of PLAze in 2 *M* $CaCl_2$ in D_2O at 35°.

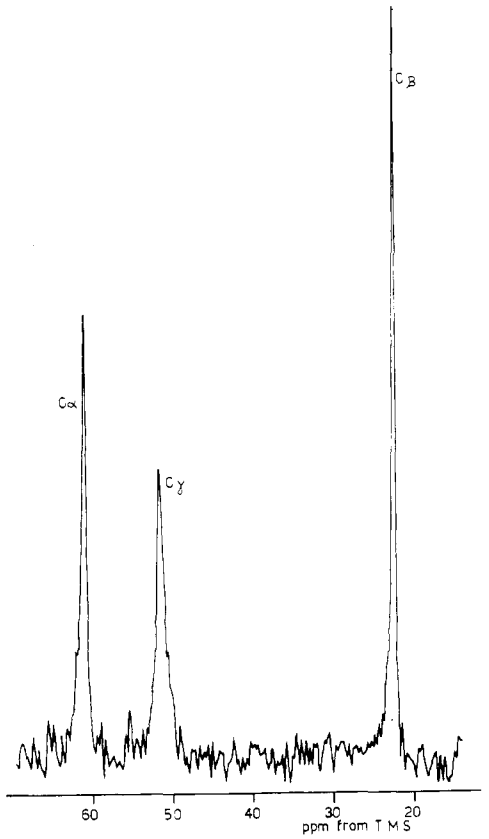

Figure 7. ^{13}C spectrum (25 MHz) of the ring carbons of PLAze in 2 *M* $CaCl_2$ in D_2O at 105°.

isolated L-proline residue gives a value of less than 10 kcal/mol, which suggests a dynamic equilibrium, rapid on the nmr time scale between conformations differing at ψ.[18] This value has to be considered only indicative for a polymer, and does not exclude by itself the possibility of separate ^{13}C resonances for cis′ and trans′ conformers. However, it adds further support to the cis–trans explanation based on the consistency in pattern and magnitude of ^{13}C shifts in PLAze and in the model compound *N*-acetyl-L-Aze-COOH, where the cis′ and trans′ conformers are absent.

Our results on poly(L-azetidine carboxylic acid) indicate, then, the occurrence of cis and trans units along the chain in pure water and formic acid, whereas for poly(L-proline) an all-trans ordered structure (form II) is stable in the same solvents. Previous pmr studies on *N*-acetyl-L-proline and on *N*-acetyl-L-azetidine-2-carboxylic acid in water have shown little difference in the cis–trans ratios of the two compounds.[19]

From this we can infer that the occurrence of cis and trans conformers in PLAze aqueous solutions is not a local residue property but a property of the polymer.

Empirical conformational calculation studies on poly(L-proline) indicated the importance of factors such as ring conformation, variations of the rotational angle Φ, and deviations from planarity of the amide bond in determining the chain structure.[20] It is then reasonable to expect different conformational behavior for PLAze in view of the considerable differences in some of these factors from poly(L-proline). The azetidine ring is planar or nearly planar and certainly more rigid than the pyrrolidine ring. Not only simple geometrical considerations but also experimental data support this view. In the crystal structure of L-Aze-COOH the ring is buckled only to the extent of 11°.[21] In Boc-L-Aze-COOH, the azetidine ring is planar.[22]

Our ^{13}C nmr data, obtained from solutions of PLAze and L-Aze-COOH derivatives in which cis–trans isomerism occurs, show that the chemical shift difference between cis and trans isomers at the β carbon is much smaller than at α and γ carbons (Table I). In contrast, the observations on proline derivatives show that the cis–trans shift difference for the β and γ carbons is greater than that for the α and δ.[23] This could suggest that whereas cis–trans isomerism about the peptide bond results in conformational changes in the pyrrolidine ring of proline, it produces only minor effects in the azetidine ring.

However, Torchia, *et al.*, have recently shown[24] that the chemical shift differences between cis and trans isomers in proline derivatives can be accounted for by the effects of syn–anti geometry of the preceding carbonyl oxygen atom.

Calculations of van der Waals potential energy were previously carried out on PLAze as a function of the internal rotation angles ψ and ω by assuming a rigid planar ring structure and keeping constant the Φ angle (64.6°).[22] They indicated two stable conformations, corresponding respectively to the cis and the trans form, with almost identical values of potential energy (calculated difference is 0.01 kcal/mol).

Previous calculations on poly(L-proline) have indicated that the trans form is more stable by 1.03 kcal/mol.[24] The absolute energy values given by conformational calculations are probably not reliable because of their strong dependence on the potential functions used. However, this result is at least in agreement with our experimental nmr observation that cis and trans isomers of PLAze occur in solvents in which only the trans form of poly(L-proline) appears. Another result provided by conformational calculations is that the helical structure in *cis*-PLAze is more extended than in *cis*-poly(L-proline). The calculated monomer repeat distance for *cis*-PLAze is 2.16 Å, while the X-ray value for cis poly(L-proline) is 1.90 Å.[25,26]

Furthermore, from inspection of molecular models it appears that in the cis helix of PLAze the orientation of carbonyl oxygens is less directed toward the interior of the chain than in *cis*-poly(L-proline), so that they are less shielded from the solvent.

These factors, *i.e.*, the more extended chain structure, the different orientation of backbone carbonyls, and the smaller size of the ring, less hydrophobic than the pyrrolidine ring, make more favorable the interactions with the solvent in *cis*-PLAze than in *cis*-poly(L-proline).

In conclusion we suggest that cis units of our polymer appear in water, whereas they do not for poly(L-proline), because they are, to a certain degree, stabilized by their capability of establishing polar interactions with this solvent.

Acknowledgment. We are indebted to Professor K. Kopple for his advice and help during the course of this work. We are also indebted to Dr. F. Podo (visiting scientist of the Istituto Superiore di Sanità at the Johnson Research Foundation, Philadelphia, Pa.) for the 220-MHz pmr measurement. The technical assistance of Mr. G. Anastasio, E. De Gregoriis, and M. Pinori is gratefully acknowledged.

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Side-Chain Interactions and Conformation in α-Helical Poly(γ-phenacyl L-glutamate). Aggregation in Dilute Solutions

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Received March 26, 1974

ABSTRACT: The behavior of α-helical poly(γ-phenacyl L-glutamate) (PPLG) has been studied by hydrodynamic, optical rotation, dielectric, and nmr measurements in different solvents. Special attention has been paid to side-chain interactions which depend on the ease of solvation of the phenacyl chromophore. Thus, in hexafluoro-2-propanol the circular dichroism spectrum is very similar to that usually observed for an α helix, and hydrogen bonding was shown to occur between the solvent and the phenacyl CO group. In other helicogenic solvents, side chain–side chain interactions occur. In dimethylformamide and pyridine they give rise at low temperature and low concentrations to an aggregation phenomenon whose molecular mechanism implies a molecular weight dependent folding of the molecules. Models for the side-chain conformation are proposed for the polymer in the solid state on the basis of infrared dichroism.

Polypeptide side chain–side chain interactions are detected in the circular dichroism (CD) spectra by the appearance of optically active bands located in the absorption region of the chromophores, although the chromophores themselves have no optically active center. These extrinsic Cotton effects are due to an asymmetrical ordering of the chromophores and to electronic interactions with the dissymmetrical field of the helix or of the asymmetrical carbon atoms. Thus, overlapping of the CD bands of the side chains and that of the peptide chromophore makes backbone conformational determination from optical rotation or CD measurements hazardous. Such side-chain interactions have been observed in particular with polymers composed of aromatic amino acids. In these cases, the chromophores are separated from the backbone by only one methylene group[2] (*e.g.*, polytyrosine, polytryptophan, and polyphenylalanine). When the nonaromatic part of the side chains becomes more important, these interactions can still be observed for some polypeptides such as polyaspartic acid nitrobenzyl esters.[3] In polyglutamic acid esters, which contain one more methylene group in the side chains, these would be expected to be more flexible and thus to give rise to less optical activity due to the side-chain chromophores. However, it was recently shown that polyglutamic acid nitrobenzyl esters in solution also display optical activity in the side-chain absorption bands.[4] Another ester of a strong chromophore (ϵ_{250} = 13300), poly(γ-phenacyl L-glutamate) (PPLG), was also shown to display in some helicogenic solvents peculiar optical properties arising from side chain–side chain interactions competing with side chain–solvent