A Study of the *cis/trans* Isomerism of *N*-Acetyl-L-proline in Aqueous Solution by ^{17}O N.M.R. Spectroscopy

Jürgen Lauterwein,* Ioannis P. Gerothanassis, and Roger N. Hunston

Institut de chimie organique de l'Université, 2,rue de la Barre, 1005 Lausanne, Switzerland

The *cis* and *trans* isomers of both the enriched carboxy and amide groups of *N*-acetyl-L-proline can be observed by ^{17}O n.m.r. spectroscopy and are shown in each case to give parallel pH titration curves.

The *cis/trans* isomerism about the amide bond in acylprolines may be important in the structure–activity relationship of proteins and small peptide hormones.[1] *N*-Acetyl-L-proline (AcProOH) and its *N*-methyl amide (AcProNHMe) have been extensively studied in various solvents by ^{1}H, ^{13}C, and ^{15}N n.m.r. spectroscopy.[2] Although there is increasing evidence that the *trans* isomer exists as an intramolecularly hydrogen-bonded C_7 conformer (γ-turn structure) in nonpolar solvents, Figure 1, its conformation in water at low pH remains uncertain.[3] It seems apparent that the detection of hydrogen bonds by ^{1}H and ^{13}C n.m.r. spectroscopy demands a very careful interpretation of the results, however, there are strong indications that ^{17}O n.m.r. chemical shifts and linewidths are sensitive to hydrogen bonding.[4]

We report here the ^{17}O n.m.r. spectra of AcProOH, enriched at either the carboxy (Ac^{17}ProOH 10 atom % ^{17}O) or amide oxygen atom (^{17}AcProOH 1 atom % ^{17}O), and demonstrate for the first time the sensitivity of ^{17}O chemical shifts to *cis/trans* isomerism. The pH titration curves show the influence of protonation of the carboxy group on the chemical shifts of both the carboxy and amide oxygen atoms in the *cis* and *trans* forms and these results are discussed in terms of the possible existence of an intramolecular hydrogen bond for the *trans* isomer.

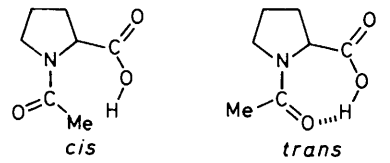

Figure 1

The high field ^{17}O n.m.r. spectrum of the carboxy group of Ac^{17}ProOH revealed the existence of two resonances throughout the whole pH range (Figure 2). At low pH the different signal intensities permitted a straightforward assignment of the two isomers, the smaller resonance corresponding to the *cis* form, approx. 20%, in good agreement with integration data from ^{1}H and ^{13}C n.m.r. spectroscopy.[2] It is of interest to note that replacement of the carboxy proton by a methyl group, as in the case of *N*-acetyl-L-proline methylester does not influence the high proportion of *trans* isomer.[7] Deprotonation of the carboxy function resulted in a high frequency shift for the carboxy oxygen atoms of both the *cis* and *trans* isomers (18.2 and 18.9 p.p.m., respectively) producing two parallel titration curves (Figure 3), and a corresponding reduction in population of the *trans* isomer. Table 1 summarizes the titration shifts and the pK_a values evaluated. These shifts are larger than those observed for free

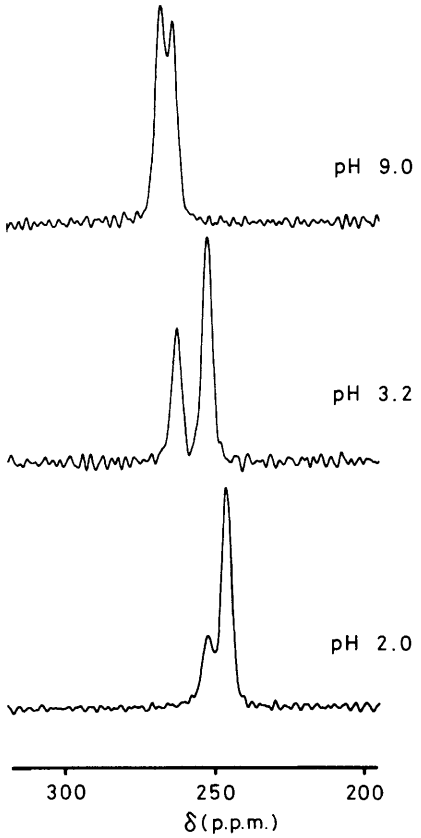

Figure 2. 48.8 MHz ^{17}O N.m.r. spectra of 0.1 M *N*-acetyl-L-proline measured on a Bruker WH 360 instrument (10 mm sample tubes, quadrature detection) at 40 °C in H_2O in the presence of 1 M NaCl and 0.0005 M ethylenediaminetetra-acetic acid (EDTA).[5] The resonances of the carboxy group are shown at three different pH values. In all cases the resonance at high frequency corresponds to the *cis* isomer. The spectra were obtained after multiplication of the free induction decay with a Gaussian-exponential function (line broadening = −300 Hz; Gaussian broadening = 0.5).[6]

Table 1. ^{17}O N.m.r. chemical shifts, titration shifts, and pK_a values of *N*-acetyl-L-proline.[a]

Resonance	Isomer	δ_1[b]/p.p.m.	δ_2[c]/p.p.m.	Δ[d]/p.p.m.	pK_a
CO_2H	*cis*	251.1	269.3	18.2	2.79±0.02
	trans	246.1	265.0	18.9	3.36±0.02
CONR	*cis*	297.2	288.9	−8.3	2.79[e]
	trans	298.4	290.3	−8.1	3.43±0.14

[a] Measured in 0.1 M solution in H_2O which contained 1 M NaCl and 0.0005 M EDTA; *t* 40 °C. The chemical shifts were obtained from nonlinear least-squares fits of one-proton titration curves[8] to the experimental data. They were measured relative to 1,4-dioxane used as external reference, + 0.2 p.p.m. relative to water. The errors for the chemical shifts were estimated to be ±0.5 p.p.m. for the carboxy resonances and ±1 p.p.m. for the amide resonances. [b] δ_1 is the chemical shift at acid pH (carboxy oxygen atoms protonated). [c] δ_2 is the chemical shift at neutral pH (carboxy oxygen atoms deprotonated). [d] Δ values are the chemical shift changes on deprotonation. Positive values indicate deshielding. [e] The pK_a value was fixed according to that of the more precise titration curve of the carboxy oxygen atom.

amino acids[8] but in close agreement with the value of 19 p.p.m. found for glycylglycine.[9] The pK_a value of the *trans* isomer was *ca.* 0.6 units larger than that of the *cis* isomer, confirming earlier results from ^{13}C n.m.r. spectroscopy.[10]

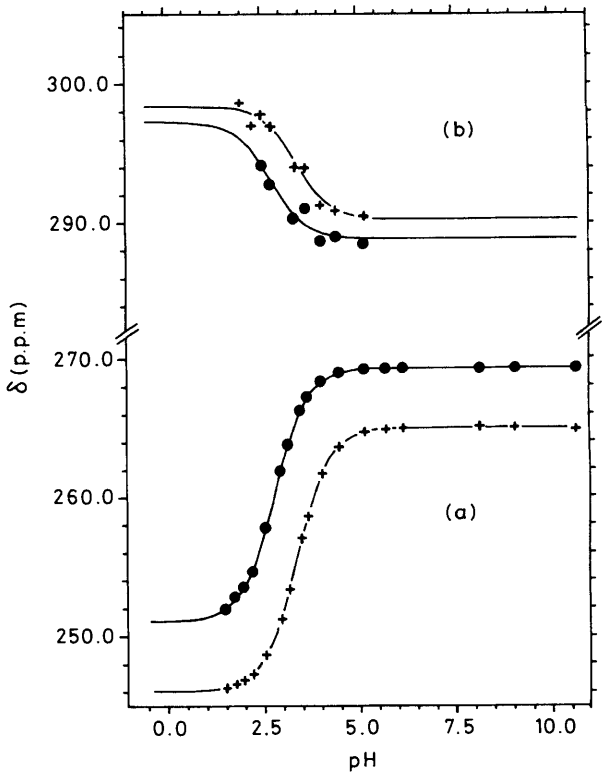

Figure 3. The ^{17}O n.m.r. titration shifts of *N*-acetyl-L-proline obtained under the conditions of Figure 2. The solid lines correspond to nonlinear least-squares fits of one-proton titration curves[8] to the experimental data: (a) resonances from the carboxy group, (b) resonances from the amide group. The resonances from the *cis* isomer are marked (●), those from the the *trans* isomer (+).

The chemical shift difference between the carboxy oxygen atom resonances in the *cis* and *trans* forms was 5.0 p.p.m. when protonated and 4.3 p.p.m. when deprotonated, a much greater sensitivity than that observed for the carbonyl carbon resonances.[10] This difference in chemical shift of the *cis* and *trans* isomers may be due to several factors, the most important being the magnetic anisotropy of the amide bond, electric field effects, and hydrogen bonding. However, on considering the distances involved[7] the magnetic anisotropic effect of the amide bond is small (< 1 p.p.m.),[11] and as no intramolecular hydrogen bonding is possible at high pH we believe that the chemical shift difference arises from the field effect of the amide dipole on the carboxy group. This effect should be significant in the *trans* isomer as the amide dipole lies in the same direction as that of the carboxy group. This would result in a shielding of the *trans* carboxy resonance throughout the whole pH range, this effect thereby offering a similar explanation for the difference in chemical shift of the two isomers at low pH. The observance of parallel titration curves therefore suggests the absence of an intramolecular hydrogen bond at low pH.

We propose that the difference in the pK_a values of the *cis* and *trans* isomers of AcProOH can also be explained by the electric field effect of the amide bond and does not need the assumption of a γ-turn structure. The unfavourable dipole interaction present in the *trans* isomer at neutral pH is expected to reduce its acidity and offers an explanation for the decrease in its population on deprotonation. The relationship between the differences in the pK_a values and that of the carboxy ^{17}O chemical shifts is indicated by the values obtained for AcProOH (ΔpK_a *ca.* 0.6, $\Delta\delta$ *ca.* 5 p.p.m.) and *N*-acetylsarcosine (ΔpK_a *ca.* 0.4, $\Delta\delta$ *ca.* 2 p.p.m.).

The ^{17}O n.m.r. spectrum of the amide oxygen atom of ^{17}AcProOH gave an asymmetric resonance throughout the pH range studied. Resolution of the overlapping *cis* and *trans* peaks could only be obtained by use of strong resolution enhancement.[6] The chemical shifts (Figure 3) were found to fall in the same region as previously reported for amides[4] and peptides.[9,12] In contrast to the behaviour of the carboxy group the amide resonances of both isomers shift to low frequency at high pH (−8.3 and −8.1 p.p.m. for *cis* and *trans*, respectively, see Table 1) in good agreement with that observed for glycylglycine (−6 p.p.m.).[9] As in the case of the carboxy group, the pH titration curves for each isomer of ^{17}AcProOH were very similar, the small difference in chemical shift being independent of the degree of carboxy group protonation. We also conclude therefore, from the analysis of the amide oxygen resonances, that the γ-turn structure does not exist in appreciable quantities in aqueous solution. We feel that ^{17}O n.m.r. spectroscopy may prove to be a powerful tool in studying secondary structure, particularly in the case of small peptides where the problems of selective enrichment and large linewidths are relatively minor.

This work was supported by a grant from the Swiss National Science Foundation.

Received, 5th December 1983; Com. 1575

References

1 J. F. Brandts, M. Brennan, and L. N. Lin, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 4178.
2 R. Deslauriers and I. C. P. Smith in 'Biological Magnetic Resonance,' eds. L. J. Berliner and J. Reuben, Plenum Press, New York, 1980, vol. 2, pp. 243–344, and references therein; R. E. London, *Int. J. Pept. Protein Res.*, 1979, **14**, 377; V. Madison and K. D. Kopple, *J. Am. Chem. Soc.*, 1980, **102**, 4855.
3 J. Feeney, G. R. Bedford, and P. W. Wessels, *FEBS Lett.*, 1974, **42**, 347; R. E. Galardy, J. R. Alger, and M. Liakopoulou-Kyriakides, *Int. J. Pept. Protein Res.*, 1982, **19**, 123.
4 J. Reuben, *J. Am. Chem. Soc.*, 1969, **91**, 5725; M. I. Burgar, T. E. St. Amour, and D. Fiat, *J. Phys. Chem.*, 1981, **85**, 502.
5 Standardized conditions used: R. Hunston, I. P. Gerothanassis, and J. Lauterwein, *Org. Magn. Reson.*, 1982, **18**, 120. EDTA was added to prevent line broadening by paramagnetic impurities: I. P. Gerothanassis, R. Hunston, and J. Lauterwein, *Helv. Chim. Acta.*, 1982, **65**, 1774.
6 G. Braoudakis, I. P. Gerothanassis, and J. Lauterwein, *J. Magn. Reson.*, 1983, **52**, 288. For a review, see: J. C. Lindon and A. G. Ferrige, in 'Progress in NMR Spectroscopy,' eds. J. W. Emsley, J. Feeney and L. H. Sutcliffe, Pergamon Press, Oxford, 1980, vol. 14, pp. 47–52.
7 C. Grathwohl and K. Wüthrich, *Biopolymers*, 1976, **15**, 2043.
8 I. P. Gerothanassis, R. Hunston, and J. Lauterwein, *Helv. Chim. Acta*, 1982, **65**, 1764.
9 C. S. Irving and A. Lapidot, *J. Chem. Soc., Chem. Commun.*, 1976, 43.
10 G. R. Bedford and P. J. Sadler, *Biochim. Biophys. Acta*, 1974, **343**, 656.
11 N. J. Clayden and R. J. P. Williams, *J. Magn. Reson.*, 1982, **49**, 383.
12 D. Fiat, M. I. Burgar, D. Dhawan, T. E. St. Amour, A. Steinschneider, and B. Valentine, *Dev. Endocrinol.*, 1981, **13**, 239.