Tetrahedron Vol. 34, pp. 553 to 556. Pergamon Press 1978. Printed in Great Britain

SYNTHESIS OF ^{13}C-LABELLED [S]-PROLINE AND ITS CONFORMATIONAL ANALYSIS BY NUCLEAR MAGNETIC RESONANCE

François Piriou, Karl Lintner, Hung Lam-Thanh, Flavio Toma and Serge Fermandjian*
Service de Biochimie, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, B.P. No. 2, 91190 Gif-sur-Yvette, France

(*Received* 15 *July* 1977; *accepted for publication* 22 *August* 1977)

Abstract—In order to analyze completely the ring conformation of [S]-Proline (L-thiazolidine-4-carboxylic acid) this compound has been synthesized with its C_δ atom enriched at 90% in ^{13}C. The ^{1}H–^{1}H, ^{13}C–^{1}H, ^{13}C–^{13}C vicinal coupling constants measured at several pH values describe well the geometry of the thiazolidine ring. The results suggest that the ring has an average planar conformation only in a narrow range of pH close to the pK of the amine group. Above and below this pH the ring tends to adopt preferentially the S_γ *endo* and S_γ *exo* puckered conformations respectively. It is concluded that a good correlation exists between the value of the dihedral angle ϕ and the character S_γ *endo* or S_γ *exo* of the ring.

In a previous report we showed that the thiazolidine and the pyrrolidine rings behave identically in many physicochemical aspects.[1] This has been emphasized recently by the fact that [S]-proline (L-thiazolidine-4-carboxylic acid) replacing Proline in natural compounds such as thyrotropin releasing hormone leads to only weak changes in their biological properties.[2] These results indicate that the residue [S]-Pro provides an excellent means to investigate the conformations of a variety of proline containing peptides since the former presents a substantially less complicated ^{1}H NMR spectrum. For instance in order to analyze the 7 spin system of proline completely a computer is necessary which is not a trivial task,[3] whereas for [S]-Pro only the ^{1}H–^{1}H vicinal coupling constants $H–C_\alpha–C_\beta–H_A$ and $H–C_\alpha–C_\beta–H_B$, which furnish a simple and directly analyzable spin system, are available. Unfortunately these two couplings are insufficient to analyze in detail the thiazolidine ring conformation. We now report that the selective ^{13}C enrichment of C_δ (C_2 atom in thiazolidine) is useful in resolving fully the problem of the [S]-proline geometry by way of the vicinal couplings ^{1}H–^{1}H, ^{13}C–^{1}H and ^{13}C–^{13}C.[4]

EXPERIMENTAL

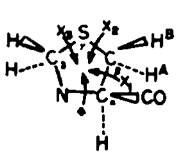
C_2 labelled L-thiazolidine-4-carboxylic acid ($^{13}C_\delta$-[S]-proline) was synthesized by condensation of 90% ^{13}C enriched formaldehyde with L-cysteine hydrochloride[5] and was examined by ^{1}H NMR in 0.15 M soln in D_2O as a function of "pH".

The ^{13}C NMR spectra were recorded in the Fourier transform mode on a Varian CFT-20 spectrometer equipped with a 16K computer. The ^{13}C–^{13}C coupling constants (notably $^3J_{^{13}C\delta-^{13}C\delta}$) were measured with a "resolution" of 0.122 Hz/point (500 Hz spectral width, 8.192 sec. acquisition time) by observing the non-enriched C=O carbon signal.

The majority of the ^{1}H NMR spectra were recorded in the C.W. mode on a Cameca spectrometer operating at 250 MHz. The ^{1}H chemical shifts are given downfield from the signal of TSP–d_4 used as internal reference. The ^{1}H–^{1}H and ^{1}H–^{13}C coupling constants were obtained with ±0.1 Hz precision. As the measurements of the vicinal $^3J_{^{13}C\delta-^1H\alpha}$, $^3J_{^{13}C\delta-^1H\beta A}$ and $^3J_{^{13}C\delta-^1H\beta B}$ coupling constants were carried out on the ^{1}H signals, there arose no ambiguity of their assignment and respective values in contrast to the ^{13}C spectra where these measurements (whenever they were feasible) were effected on the only $^{13}C_\delta$.

RESULTS AND DISCUSSION

The ^{1}H NMR spectra of L-thiazolidine-4-carboxylic acid, unlabelled and specifically ^{13}C labelled in position C_δ, obtained at pH 5.90, are shown in Fig. 1. Proceeding from high field to low field one finds successively the $H_\beta{}^B$ (*cis* with respect to the carboxyl group), $H_\beta{}^A$, H_α and H'_δ, H_δ:



The $H_\beta{}^A$, $H_\beta{}^B$ protons (spectrum A) form the AB part (3.2–3.4 ppm) of an ABX system ($X = H_\alpha$) from which the values of $^3J_{HC_\alpha-CH_\beta{}^A}$ and $^3J_{HC_\alpha-CH_\beta{}^B}$ are easily extracted. The two δ protons give an AB type spectrum (4.3–4.4 ppm) which partly overlaps with the H_α multiplet.

90% ^{13}C enrichment at C_δ (spectrum B) produces a splitting of the H_δ spectrum into two AB quadruplets as a consequence of $^1J_{^{13}C\delta-^1H}$ and $^1J_{^{13}C\delta-^1H'}$ (159 Hz and 160 Hz at this pH), whereas the AB quadruplet of the residual 10% $^{12}C_\delta H_2$ is still visible in the spectrum.

Simultaneously, each line of the $H_\beta{}^A$, $H_\beta{}^B$ and H_α multiplets is split into a doublet as a consequence of the vicinal couplings with $^{13}C_\delta$. The values of $^3J_{^1H-^{13}C}$ are easily read on the ^{1}H spectrum and, as in the case of $^3J_{^1H-^1H}$, can be systematically followed against the pH.

Differentiation of the β^A and β^B protons is of importance to appreciate the χ^1 and χ^2 angles and has been effected through comparison with Proline and by taking into account principally the following criteria:

(1) The amplitude of the variation of their respective coupling with the H_α proton during pH titration, in relation to the maximally allowed puckering for the 5-membered heterocycle.

(2) The X-rays data on this compound,[6–8] its N-t-Butyloxy-derivative[9] and its glycine-diketopiperazine, for which the ^{1}H NMR data are available.[1]

The coupling constants which can be discerned in both spectra (two ^{1}H–^{1}H and ^{1}H–^{13}C vicinal couplings) have

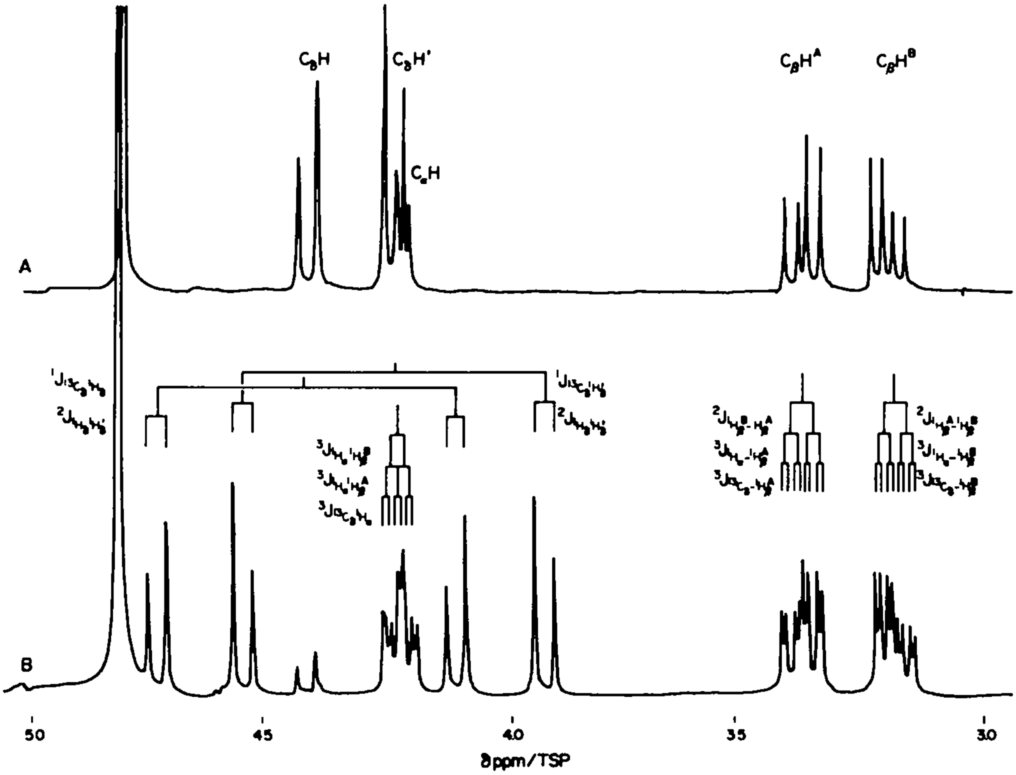


Fig. 1. 250 MHz ^1H NMR spectra of L-thiazolidine-4-carboxylic acid (A) and 90% $^{13}C_\delta$ L-thiazolidine-4-carboxylic acid (B) obtained in D_2O solution at pH 5.90.

been measured between pH 1 and pH 11.3 and give rise to the curves of Fig. 2. They all suggest important conformational modifications of the ring, particularly during the ionization of the amine group with its p*K* around 6.3. The values of $^3J_{^1H-^1H}$ and $^3J_{^{13}C-^1H}$ as well as those of $^3J_{^{13}C-^{13}C}$ determined from the ^{13}C spectra at the most characteristic pH values are summarized in Table 1 together with the main geometrical features we derived from them. We note in particular that at pH 6.10 where the two species zwitterion and anion are about in equal amount the couplings $^{13}C_\delta\text{–}H_\beta{}^A$ and $^{13}C_\delta\text{–}H_\beta{}^B$ have the same value which shows that on average the angles $H^B\text{–}\widehat{C_\beta\text{–}S}\text{–}C_\delta$ and $H^A\text{–}\widehat{C_\beta\text{–}S}\text{–}C_\delta$ are equivalent and are 120° on the NMR time scale. Since the $^{13}C_\delta\text{–}{}^1H_\alpha$ coupling is identical to the preceding one, the angle $C_\delta\text{–}\widehat{N\text{—}C_\alpha}\text{–}H$ is also 120°; this signifies that the ring has a mean conformation of planarity at pH 6.10.

Accordingly the $^3J_{^{13}C0\text{-}^{13}C\delta}$ coupling constant found reflects by itself a mean angle $C{=}O\text{–}\widehat{C_\alpha\text{—}N}\text{–}C_\delta$ of 120° in perfect agreement with the value obtained for such an angle from the theoretical curve proposed by Barfield *et al.*[10] for butanoic acid. It matches equally well with the experimental values determined from a series of carboxylic compounds by Miller *et al.*[11] The same is true for the $^{13}C\text{–}^1H$ coupling constants which agree with those proposed by several authors[4,12,13] for angles of 120°. On the other hand, the coupling constant $H^A\text{–}C_\beta$ $C_\alpha\text{–}H$ furnishes a mean dihedral angle of 30° according to the curve by Kopple.[14] For this value to be in accordance with the preceding data showing that there is not preferential puckering of the ring, we must assume that X_1 alternatively adopts the values of +30° and −30°, S_γ moving symmetrically with respect to the mean plane of

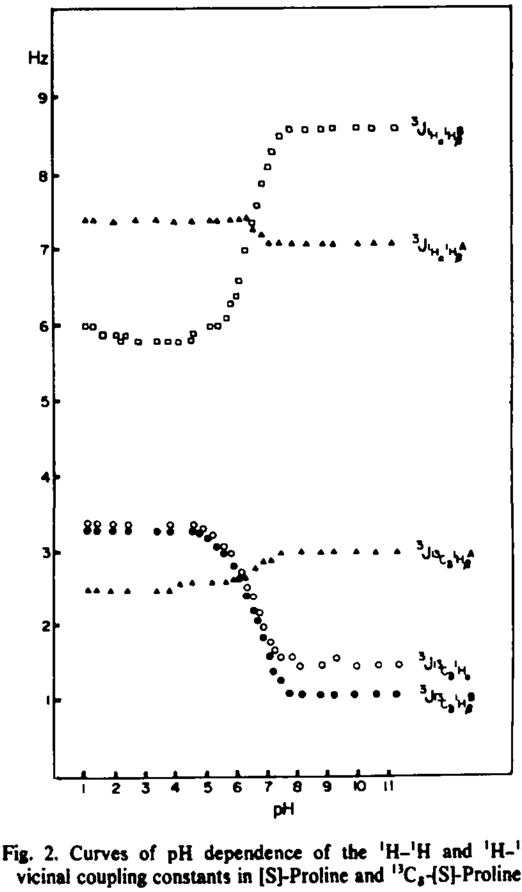


Fig. 2. Curves of pH dependence of the $^1H\text{–}^1H$ and $^1H\text{–}^{13}C$ vicinal coupling constants in [S]-Proline and $^{13}C_\delta$-[S]-Proline.

Table 1. [S]-Proline: NMR and geometrical parameters

pH	^3J H_α–$H_\beta{}^A$ (Hz)	$H\widehat{C_\alpha C_\beta}H^A$ (a)	^3J H_α–$H_\beta{}^B$ (Hz)	$H\widehat{C_\alpha C_\beta}H^B$ (b)	X_1 (d)	^3J $^{13}C_\delta$–H_α (Hz)	$C_\delta\widehat{NC_\alpha}H$ (b)	^3J $^{13}C_0$–C_δ (Hz)	$C_\delta\widehat{NC_\alpha}C_0$ (c)	ϕ	^3J $^{13}C_\delta$–$H_\beta{}^A$ (Hz)	$C_\delta\widehat{SC_\beta}H^A$ (b)	^3J $^{13}C_\delta$–$H_\beta{}^B$ (Hz)	$C_\delta\widehat{SC_\beta}H^B$ (b)	X_2 (d)	X_3 (d)	mean ring conformation
1.3	7.4	30°	6.0	130°	Pn	3.4	130°	1.4	105°	−70° −75°	2.5	115°	3.3	130°	Np	Pn	S_γ *endo*
6.1	7.4	30°	6.6	135°	PN	2.7	120°	2.4	125°	−60° −55°	2.7	120°	2.7	120°	PN	PN	planar
8.3	7.1	35°	8.6	145°	Np	1.5	105°	4.75	150°	−45° −30°	3.0	125°	1.1	110°	Pn	Np	S_γ *exo*

(a) values from Kopple *et al.*[14] rounded to the nearest 5°.
(b) values derived from the propane and 2-fluoropropane curves proposed by Wasylishen and Shaeffer[13] rounded to the nearest 5°.
(c) values derived from the butanoic acid curve proposed by Barfield *et al.*[10] rounded to the nearest 5°.
(d) P and p designate large and small fractions respectively, of the corresponding positive dihedral angle, N and n denote large and small fractions respectively of the corresponding negative dihedral angle.

the ring. This assumption is supported by the second $^3J_{H^B\text{-}C_\beta C_\alpha\text{-}H}$ coupling constant the value of which corresponds neither to 150° (e.g. 120° + 30°) nor to 90° (e.g. 120° − 30°) but rather approaches their average. Thus, all these elements show that at pH 6.10 the thiazolidine ring fluctuates rapidly between two extreme and equally probable conformations which might correspond to the S_γ *endo* ($X_1 = +30°$) and S_γ *exo* ($X_1 = -30°$) forms (S_γ *endo* and S_γ *exo* are relative to the orientation *syn* and *anti* of the S atom in respect of the carboxyl group). Lastly, it is obvious that in this situation which only prevails in a very narrow pH range near the amine pK value, the dihedral ϕ possesses a mean value of −60° concording with the proposed averaged planar conformation of the ring.

At pH 1.35 the $^{13}C_\delta$–$H_\beta{}^A$ coupling is now smaller than the $^{13}C_\delta$–$H_\beta{}^B$ which shows that on the average the two corresponding angles $C_\delta\text{-}\widehat{S\text{—}C_\beta}\text{-}H^A$ and $C_\delta\text{-}\widehat{S\text{—}C_\beta}\text{-}H^B$ deviate from 120°, the former being smaller than the latter. In much the same way the $^3J_{^{13}C\delta\text{-}H\alpha}$ and $^3J_{^{13}C0\text{-}^{13}C\delta}$ values which have increased and decreased respectively suggest a slight opening up of the dihedral angle ϕ ($> -60°$). These variations show that we no longer have the ideal situation found at pH 6.10 where all the atoms C_α, N, C_δ, S and C_β of the ring are, on the average, coplanar. In agreement, the ^1H-^1H coupling constants, too, show that now the ring has on the average a mean puckered conformation. Notably $^3J_{H^B\text{-}C_\beta C_\alpha\text{-}H}$ is smaller than at pH 6.10 which suggests that either the average life time of the S_γ *endo* form is larger than the one of the S_γ *exo*, or rather that at conformational equilibrium these two forms (equally populated) are no longer characterized by the same absolute values of X_1 (±30°). Since at the same time $^3J_{H^A\text{-}C_\beta C_\alpha\text{-}H}$ remains constant, the change of the puckering in each conformation is such that the average value of X_1 is maintained close to |30°|. Accordingly it might be assumed that the absolute value of X_1 increases in S_γ *endo* while it decreases simultaneously in S_γ *exo* within such limits, however, as to keep $^3J_{H^A\text{-}C_\beta C_\alpha\text{-}H}$ constant whereas $^3J_{H^B\text{-}C_\beta C_\alpha\text{-}H}$ changes; c.f. the shape of the curve given by Kopple *et al.*[14] in the regions between 20° and 40° and 120° and 150°.

At pH 8.30 the $^{13}C_\delta$–$H_\beta{}^B$ coupling constant is now smaller than $^{13}C_\delta$–$H_\beta{}^A$ in contrast to what had been found at acid pH. This indicates a very distinct closing of the angle $C_\delta\widehat{S\text{—}C_\beta}H^B$. The increase of the $^{13}C_0$—$^{13}C_\delta$ coupling and the consecutive decrease of $^3J_{^{13}C\delta\text{-}H\alpha}$ on the other hand show that the dihedral angle ϕ has narrowed appreciably; it must now adopt a value quite below −60°. From these data it appears that we confront a situation totally opposed to the one found at acidic pH, as the observed ring conformation is now rather of the S_γ *exo* type. Again this is confirmed by the new value of the $^3J_{H^B\text{-}C_\beta C_\alpha\text{-}H}$ coupling constant, suggesting an increase in the mean angle $H\text{-}\widehat{C_\alpha\text{—}C_\beta}\text{-}H^B$.

CONCLUSION

In agreement with the ^1H-^1H, the ^{13}C-^1H and ^{13}C-^{13}C coupling constants are parameters that can be utilized in structure determination with a certain confidence. Even for [*S*]-proline which contains several electronegative atoms, they lead to conformationally reasonable values of the dihedral angles, particularly in reference to radiocrystallographic data. It is interesting to underline the existence of a relationship between the relative apparent proportions of S_γ *endo* and S_γ *exo* forms and the value of the ϕ angle on which depends the strain in the rest of the molecule. We have seen for instance that with a mean angle of $\phi = -60°$ the S_γ *endo* and S_γ *exo* forms are equally probable, very likely because they correspond to symmetrical deformations of S_γ with respect to the plane of the ring. Since the most important conformational modifications closely follow the titration of the nitrogen group, it is certain that the geometry of the nitrogen plays an essential role in this mechanism, especially in influencing the value of the ϕ angle.

REFERENCES

[1] W. Haar, S. Fermandjian, F. Robert, O. Lefebvre-Soubeyran and S. Savrda, *Tetrahedron* 32, 2811–2815 (1976).

[2] C. G. Wilson, C. Gilon, B. Donzel and M. Goodman, *Biopolymers* 15, 2317–2322 (1976).

[3] F. A. Bovey, *J. Polymer Sci.: Macromolecular Reviews* 9, 1–81 (1974).

[4] V. F. Bystrov, *Progress in NMR Spectroscopy*, Vol. 10, pp. 41–81. Pergamon Press, Oxford (1976).

[5] S. Ratner and H. T. Clarke, *J. Am. Chem. Soc.* 59, 200–206 (1937).

[6] M. Goodman, G. C. C. Niu and K. C. Su, *Ibid.* 92, 5219–5220 (1970).

[7] M. Goodman, V. Chen, E. Benedetti, C. Pedone and P. Corradini, *Biopolymers* 11, 1779–1787 (1972).

[8] J. Loscalzo, R. G. Kallen and D. Voet, *Arch. Biochem. Biophys.* 157, 426–430 (1973).

[9] F. Robert, *Acta Cryst.* B32, 2367–2369 (1976).

[10] M. Barfield, I. Burfitt and D. Doddrell, *J. Am. Chem. Soc.* 97, 2631–2634 (1975).

[11] J. L. Marshall and D. E. Miller, *Ibid.* 95, 8305–8308 (1973).

[12] R. U. Lemieux, *Ann. N. Y. Acad. Sci.* 222, 915–934 (1973).

[13] R. Wasylishen and T. Shaeffer, *Can. J. Chem.* 51, 961–973 (1973).

[14] K. D. Kopple, G. R. Wiley and R. Tauke, *Biopolymers* 12, 627–636 (1973).