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CONFORMATIONAL STUDIES OF CYCLO-GLY-PRO(S) BY X-RAY DIFFRACTION AND PROTON MAGNETIC RESONANCE

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Abstract—The X-ray crystal structure and the PMR spectrum in ^2H_2O and DMSO-d_6 of cyclo-glycyl-4-thiaprolyl has been determined. In the crystal, the diketopiperazine ring of the molecule adopts a boat conformation and the thiazolidine ring an envelope conformation, very similar to the analogous compound cyclo-glycyl-prolyl. Comparison of proton-proton dihedral angles derived from the crystal structure and from vicinal coupling constants in solution indicates that the conformation of cyclo-glycyl-4-thiaprolyl is nearly the same in crystal and in solution in the limits of applied methods.

INTRODUCTION

Semi-empirical Karplus-type curves are now widely used to determine from proton-proton spin coupling constants the dihedral angles around the N–C_α[3-5] and C_α–C_β[6] bonds of peptides. With this method ambiguities often occur since a coupling constant defines more than one dihedral angle. Furthermore not all of the conformation-defining angles can be obtained by this method. These problems have necessitated recent studies of the conformational dependence of vicinal coupling constants J_{NH},[7-9] J_{CH},[9,10] J_{CN}[11] and J_{CC}.[12] The most direct way to establish these relationships is to study the corresponding NMR spectra of molecules for which the geometry has been determined by X-ray crystallography, with the assumption that the crystalline and solvated conformations are equivalent. Cyclic molecules like the diketopiperazine cyclo-glycyl-prolyl are most suitable for these studies since the two rings greatly limit the number of conformational contributions.

Here we report a study by X-ray crystallography and PMR of the conformation of cyclo - glycyl - 4 - thiaprolyl (cyclo - Gly - Pro(S)), an analog of cyclo - Gly - Pro in which the ν-methylene group has been substituted by a sulphur. This molecule reveals a straightforward PMR spectrum which determines how close the conformation found in the crystalline state is confirmed by the values of the vicinal proton–proton coupling constants found in solution. Furthermore this molecule has been selectively enriched in different positions in ^{13}C so that the results presented here will establish as a next step relationships between dihedral angles and vicinal proton–carbon and carbon–carbon coupling constants.

EXPERIMENTAL

Thiazolidine 4-carboxylic acid was synthesized by acid-catalyzed condensation of formaldehyde with L-cysteine.[13] Coupling with glycine and cyclisation to the diketopiperazine cyclo-Gly-Pro(S) was carried out as previously described for the analog compound cyclo-Gly-Pro.[14]

NMR studies. PMR spectra were recorded at 250 MHz on a CAMECA TSN 250 spectrometer in the frequency sweep mode. t-Butanol and TMS served as internal references in ^2H_2O and DMSO-d_6 solution, respectively. Spectra were analyzed by the LAOCN$_3$ program.[15]

X-ray studies. Few single crystals were obtained from a concentrated aqueous solution of the compound upon standing at 4°.

Examinations of preliminary rotation and Weissenberg photographs revealed the crystals to be of orthorhombic symmetry, space group $P2_12_12_1$, with one molecule in the asymmetrical unit. For determination of lattice constants and data collection, the crystals were rather too big, but after unsuccessful attempts to reduce their volume, we decided to use the last one as it was. Its volume was about $0.8 \times 0.6 \times 0.4$ mm^3 and it was mounted along its longest edge (a axis). The cell parameters are $a = 5.622 \pm 0.002$ Å, $b = 12.217 \pm 0.004$ Å, $c = 10.318 \pm 0.004$ Å. X-ray diffraction intensities were recorded on a CAD-3 Enraf-Nonius automatic diffractometer in the θ-2θ scan mode up to $\theta = 66°$ employing Ni-filtered CuK_α radiation. Two standard reflections were measured each 40 reflections and showed no systematic deviations.

From the 747 independent reflections of one octant, 679 were considered as observed with $I \geq 3\sigma(I)$, where $\sigma(I)$ is the standard deviation based on counting statistics. The intensities I were corrected for the Lorentz-polarization factor, but not for absorption. A Wilson plot gave a general temperature factor B of 3.65 Å.^{2}

The structure was solved by direct methods using the MULTAN program.[16] The eleven highest peaks on the E map corresponded to the atomic positions of the non-H atoms. After least-squares refinement of the structural parameters, a difference map has been computed and has led to the coordinates of all H atoms except the one bound to the nitrogen N1. The isotropic thermal factor of each hydrogen atom was that of the atom to which it is attached.

For refinement calculations, a local version of ORFLS program[17] was used, which minimizes the function $w(|F_o| - |F_c|)^2$. The scattering factors were those of the "*International Tables for X-Ray Crystallography*".[18] During the last refinement cycles, the anisotropic thermal parameters were introduced for "heavy" atoms, the B factors of hydrogen atoms were unvariable and each structure factor was weighted by the weighting scheme of Cruickshank.[19] The final conventional factor R is 0.079.

RESULTS AND DISCUSSION

Crystal structure. The fractional atomic coordinates, the thermal parameters and their standard deviations are given in Tables 1 and 2. The list of structure factors can be obtained from the authors. Bond lengths and bond angles

Table 1. Fractional coordinates and thermal parameters of the non-hydrogen atoms (the estimated standard deviations are given in parentheses and refer to the last decimal places)

Atom	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}	B
S	−0.0492(4)	0.7481(2)	0.7271(2)	0.0304(8)	0.0073(2)	0.0069(2)	−0.0066(3)	0.0007(3)	−0.0003(2)	2.78
N_1	0.5902(13)	1.0043(5)	0.8466(5)	0.0289(28)	0.0061(5)	0.0049(6)	−0.0011(10)	−0.0011(11)	−0.0001(4)	2.34
N_2	0.3070(10)	0.8804(5)	0.6882(5)	0.0231(20)	0.0053(4)	0.0040(5)	−0.0022(9)	0.0011(10)	−0.0008(4)	1.95
O_1	0.4205(11)	0.9229(5)	1.0202(5)	0.0334(22)	0.0079(5)	0.0045(5)	0.0006(9)	−0.0027(10)	0.0014(4)	2.71
O_2	0.5373(10)	0.9219(5)	0.5158(5)	0.0292(20)	0.0062(4)	0.0048(4)	−0.0012(8)	0.0023(9)	−0.0005(4)	2.36
C_1	0.0831(17)	0.8116(7)	0.8658(7)	0.0338(31)	0.0061(5)	0.0054(6)	−0.0042(12)	0.0014(15)	0.0008(6)	2.55
C_2	0.2151(12)	0.9106(5)	0.8166(6)	0.0226(24)	0.0043(4)	0.0035(5)	0.0005(9)	0.0004(11)	−0.0003(4)	1.73
C_3	0.4181(13)	0.9462(6)	0.9037(6)	0.0215(24)	0.0049(5)	0.0050(6)	−0.0004(10)	−0.0016(11)	−0.0003(5)	2.14
C_4	0.5828(15)	1.0302(6)	0.7083(6)	0.0261(26)	0.0060(5)	0.0046(6)	−0.0035(11)	−0.0006(12)	−0.000(5)	2.23
C_5	0.4785(12)	0.9383(5)	0.6300(6)	0.0209(25)	0.0039(5)	0.0048(6)	−0.0004(9)	0.0010(12)	−0.0001(5)	1.75
C_6	0.1741(18)	0.7970(8)	0.6204(8)	0.0428(38)	0.0074(7)	0.0062(7)	−0.0076(15)	0.0001(17)	−0.0020(6)	3.12

are shown in Fig. 1. No large deviations can be detected compared with the crystal structures of other diketopiperazines[20-27] and thiazolidine 4-carboxylic acid[28,29] except for the bond length between C6 and H6'.

As for cyclo-Gly-Pro[30] and cyclo-Leu-Pro,[20] the diketopiperazine ring is not planar. It may best be described by two planes, each of them being constituted by a peptide unit. The first one includes the atoms C2, C3, O1, N1, C4 with an average deviation of 0.002 Å (the maximum being 0.004 Å); the amide bond lying in it is essentially planar. For the other, the atoms C2, N2, C5, O2, C4 reveal an average deviation of 0.04 Å from the least squares plane (the max being 0.06 Å) and the amide bond is slightly twisted ($\omega = 9.2°$). This distortion of the amide bond is slightly larger than in cyclo-Gly-Pro ($\omega = 7.2°$).[30] The intersection of the two planes occurs along the line joining C2 and C4 with a dihedral angle of 143°.

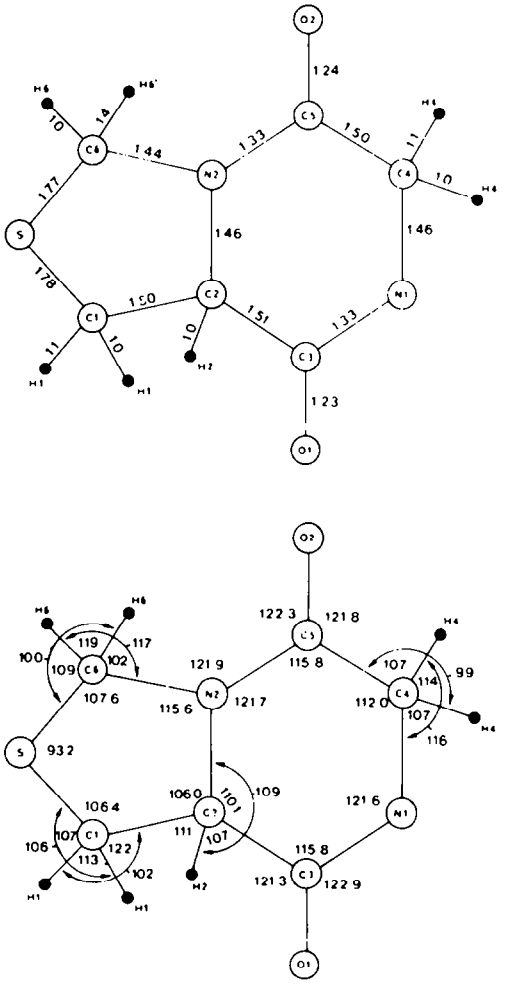

Fig. 1. Bond lengths and bond angles in cyclo-Gly-Pro(S). Statistical errors are 0.01 Å for bonds between non-hydrogen atoms and 0.1 Å for bonds between carbons and hydrogens. For the angles, the statistical errors are 0.5° (three non-hydrogen atoms), 5° (two non-hydrogen atoms and one hydrogen), and 6–7° (one non-hydrogen atom and two hydrogens).

Table 2. Fractional coordinates of hydrogen atoms (the estimated standard deviations are given in parentheses and refer to the last decimal places)

Atom	x/a	y/b	z/c	B
H1	−0.067(18)	0.850(7)	0.923(8)	2.55
H1'	0.166(16)	0.750(7)	0.915(8)	2.55
H2	0.102(17)	0.976(7)	0.807(8)	1.73
H4	0.487(17)	1.105(7)	0.681(8)	2.23
H4'	0.750(19)	1.027(8)	0.676(8)	2.23
H6	0.268(17)	0.731(8)	0.584(9)	3.12
H6'	0.033(19)	0.860(8)	0.533(9)	3.12

The thiazolidine ring assumes an envelope conformation: C2, N2, C6 and S form a plane with a mean square deviation of 0.02 Å (the maximum being 0.05 Å) and C1 projects 0.53 Å out of this plane *syn* to O1. This conformation is also very similar to that of the corresponding pyrrolidine ring in cyclo-Gly-Pro in which Cl was found to be 0.55 Å out of the plane of the four other atoms.[30] Thus only little influence of the small bond angle C1-S-C6 on the puckering of the 5-membered ring of the molecule studied can be detected.

In fact, the atoms S, C6, C2, N2, C5, O2, C4 are nearly in the same plane. The distance of S and C6 from the second least squares plane is only 0.14 Å and 0.06 Å. The stereochemistry of the molecule is summarized in Fig. 2 which shows a projection of the molecule through this plane.

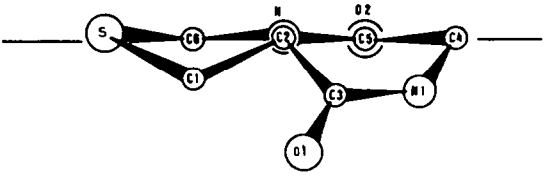

Fig. 2. Projection of cyclo-Gly-Pro(S) in the plane containing the largest number of atoms.

Some proton-proton dihedral angles have been calculated to be compared with the NMR results. They are given in Table 3 and will be discussed in next paragraph. Since the proton bound to N1 was not found in our crystal structure analysis, its position was derived from known bond geometry with the assumption that it lies in the same plane as C2, C3, O1, N1, C4, e.g. maintaining planarity of the amide bond.

The arrangement of the molecules in the crystal is assumed to be mainly determined by Van der Waals contacts. The shortest Intermolecular distances are shown in Fig. 3. The particular distance of 2.87 Å between the atoms N1 and O2 of two different molecules is indicative of a hydrogen bond.

Table 3. Proton-proton dihedral angles of cyclo-Gly-Pro(S)

	$\sigma \simeq 8°$ ^2H_2O solution	$\sigma \simeq 8°$ DMSO-d_6 solution	Crystal structure	
Θ(H1, H2)	39°[a]	37°[a]	27	$\sigma \simeq 6°$
Θ(H1', H2)	151°[a]	143°[a]	154	
Θ(H4, N1H)	—	90°[b]	92.0	$\sigma \simeq 5°$
Θ(H4', N1H)	—	33°[b]	36.0	

[a] Calculated from vicinal coupling constants by the relationship of Kopple *et al.*[6]

[b] Calculated from vicinal coupling constants by the relationship of Cung *et al.*[5]

NMR spectrum analysis. Figure 4 shows the PMR spectrum of cyclo-Gly-Pro(S) in ^2H_2O solution at 250 MHz. Signal assignments indicated in the figure were achieved by homonuclear decoupling and confirmed by spectrum simulation. Chemical shifts and coupling constants so obtained are listed in Table 4. Interestingly a large number of long-range couplings exists, indicating the molecule is relatively rigid in solution.

In DMSO-d_6 solution the line-widths of the resonances are larger and the long-range couplings no longer resolved. However a vicinal coupling of 3.9 Hz between the glycyl proton H4 and the non-exchanged, N1-bound proton is seen. The coupling of this to the proton H4' of the glycine residue is not resolved (the coupling constant should be close to zero, since no sharpening of this glycyl resonance can be observed upon decoupling the amide proton). From these vicinal coupling constants the corresponding dihedral angles have been calculated applying the relationship of Cung *et al.*[5] and are shown in Table 3. They are in good agreement with those derived from the crystallographic calculations. We thus conclude that the conformation of the diketopiperazine ring is the same in

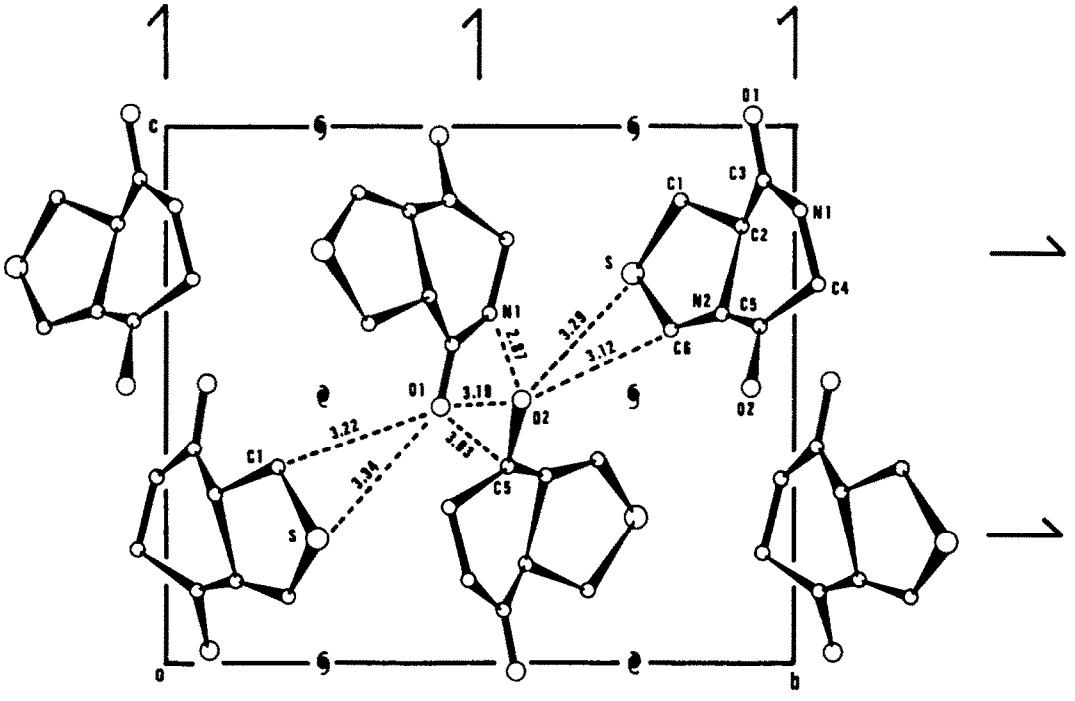

Fig. 3. Arrangement of cyclo-Gly-Pro(S) molecules in the crystal.

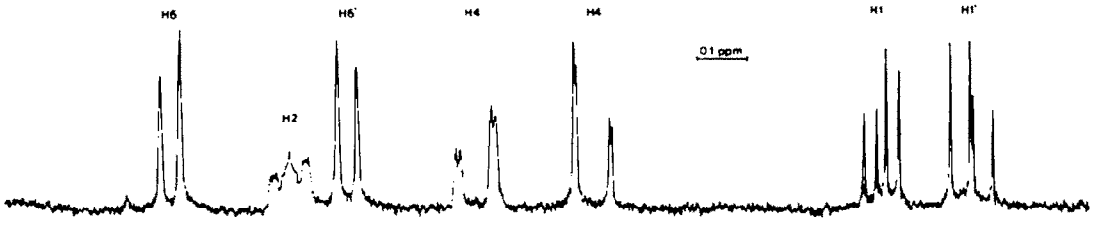

Fig. 4. 250 MHz proton magnetic resonance spectrum of 0.13 M cyclo-Gly-Pro(S) in ^2H_2O. The spectrum recorded at pH 8.3 is independent of pH.

Table 4. Proton magnetic resonance chemical shifts and coupling constants of cyclo-Gly-Pro(S) in ^2H_2O solution[a]

δ, ppm[b]	J, Hz
$\delta_{H1} = 3.404$	$^2J_{H1H1'} = -11.3$
$\delta_{H1'} = 3.229$	$^2J_{H4H4'} = -17.8$
$\delta_{H2} = 4.594$	$^2J_{H6H6'} = -9.8$
$\delta_{H4} = 4.216$	$^3J_{H1H2} = 6.2$
$\delta_{H4'} = 3.992$	$^3J_{H1'H2} = 10.1$
$\delta_{H6} = 4.833$	$^5J_{H2H4} = 2.4$
$\delta_{H6'} = 4.480$	$^5J_{H2H4'} = 1.0$
	$^5J_{H4H6} = 0.8$
	$^5J_{H4H6'} = 0.8$

[a] Concentration was 0.13 M at pD 8.3.
[b] Chemical shifts were determined relative to the resonance of t-butanol (1.236 ppm downfield from DSS) and are given in ppm downfield from DSS.

solution and crystal. Similar boat conformations in solution of the DKP ring were also derived from the coupling constants $J_{HC_\alpha NH}$ for the related compounds cyclo-Gly-Pro[31] and cyclo-Leu-Pro.[32]

The vicinal coupling constants $^3J_{H1H2}$ are 6.2 Hz and 6.4 Hz in ^2H_2O and DMSO-d_6 solution, respectively. We have calculated the corresponding dihedral angle Θ(H1, H2) to be between 39° and 37°, using the correlation of Kopple *et al.*[6] The coupling constant $^3J_{H1'H2}$ is 10.1 Hz in ^2H_2O and 8.8 Hz in DMSO-d_6; corresponding dihedral angles Θ(H1',H2) are 151° and 143°, respectively. This difference is not likely to reflect conformational differences of the thiazolidine ring in ^2H_2O and DMSO-d_6 since in this case the angle Θ(H1,H2) should also be affected. Therefore we believe it results from the different solvation properties of the two solvents influencing the electronic structure of the molecule and thus the coupling constants.

The dihedral angle Θ(H1, H2) derived from the coupling constants in solution is about 10° higher than found in the crystalline form, whereas Θ(H1',H2) has about the same value in ^2H_2O but is about 10° lower in DMSO-d_6 (Table 3). These deviations are not likely to reflect conformational differences of the thiazolidine ring between crystal and solution, since a change of the envelope conformation of the ring should lead to deviations of the two dihedral angles between the protons in the same and not in opposite directions as observed. They may simply originate from the inaccuracy in the determination of the proton-proton dihedral angles from the crystal structure, since the position of these atoms exhibits a rather large incertitude. This is supported by the comparison of the thiaprolyl side chain angle χ_1 (N2, C2, C1, S) from the X-ray structure (−34°) with that calculated from the dihedral proton-proton angles Θ(H1, H2) and Θ(H1', H2) derived from the coupling constants (−39° and −31°), assuming tetrahedral geometry at the carbons C1 and C2. On the other hand also the correlation between coupling constants and dihedral angles applied may not be entirely appropriate for the kind of molecule studied since the introduction of the sulphur atom and/or ring strain in the molecule may cause changes in bond lengths, bond angles and hybridization. The influence of these factors on the values of vicinal coupling constants generally introduces an incertitude on the dihedral angles of 10° (as has been discussed by Kopple *et al.*[6]).

CONCLUSION

It can be said that the conformation of cyclo-Gly-Pro(S) in crystal and solution are the same in the limits of the methods applied. Therefore, this molecule will be a good model to use its crystal structure reported here to establish the relationships between dihedral angles and vicinal coupling constants $^3J_{CH}$ and $^3J_{CC}$.[33]

On the other hand the similarity of the structures of cyclo-Gly-Pro[30] and cyclo-Gly-Pro(S) suggests that the conformational characteristics of the thiazolidine ring and the pyrrolidine ring of proline are similar. So the observation of different thiazolidine ring conformations in the cis- and trans-isomers of the linear dipeptide glycyl-thiazolidine 4-carboxylic acid[34] may be indicative of similar conformational differences in the pyrrolidine ring of the analogous dipeptide glycyl-proline.

Addendum. After completion of this manuscript, von Dreele[35] published a more detailed crystal structure determination of cyclo-Gly-Pro. From these results it turned out that it is isomorphous with cyclo-Gly-Pro(S). However, von Dreele[35] did not mention the corresponding inter-molecular hydrogen bond found in the crystals of cyclo-Gly-Pro(S) between atoms N1 and O2.

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