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EVIDENCE FOR NITROGEN-INVERSION ISOMERISM OF PROLINE

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The proton NMR chemical shifts of L-Phe-L-Pro and D-Phe-L-Pro can be explained by the proline stereoisomerism due to the nitrogen inversion.

Although many studies have been reported on the proline peptide stereoisomerism,[1] none of them has explained it by the nitrogen inversion. Recently, Thomas and Williams reported the C-13 NMR spectra of aminoacylprolines and explained their isomerism by the peptide-bond rotation.[2] During the course of an NMR conformational study of proline dipeptides, it was found that the proton chemical shifts of L-Phe-L-Pro (I) and D-Phe-L-Pro (II) show irregular behavior and that it cannot be explained by the peptide-bond rotation.

The assignment of the chemical shifts of I and II was based on spin decoupling and a chemical-shift comparison with other proline dipeptides. The spectrum of proline in D_2O was assigned as shown in Fig. 1.[3] In the spectrum of I, when the signal of Phe-β was irradiated, the signals of both Phe-α at 4.4 ppm and at 4.1 ppm were decoupled; the sum of the intensities of these Phe-α signals corresponds to one proton and that of Phe-β to two protons. Irradiation of signal Pro-βγ at 1.8 ppm affected only signal Pro-αδ at 3.4 ppm, the intensity of which corresponds to three protons. Therefore, the assignment of I were as shown in Fig. 1. In the spectrum of II, by irradiation of signal Phe-α at 4.3 ppm, signal Phe-β at 3.1 ppm was decoupled. The intensity of signal Phe-α corresponds to one proton and that of signal Phe-β to two protons. When signal Pro-βγ was irradiated, signal Pro-δ at 3.3 ppm was changed, but signal Pro-α at 2.4 ppm is too close to be observed. By irradiation of signal Pro-δ, only signal Pro-βγ was affected. The intensity of signal Pro-δ corresponds to two protons, that of signal Pro-α to one, and that of signal Pro-βγ to four. As a result, the assignment of II was as shown in Fig. 1.

As shown in the Table, the Pro α-H signals of I and II appeared at a moderately high field 3.4 ppm and at a very high field 2.4 ppm, respectively. The Phe α-H of II had only one signal, but that of I showed two signals. The other proline ring protons of II showed a different signal shape from those of proline, but those of I showed a shape similar to those of proline. This fact suggests that the ratio of the spin-spin coupling constants and the chemical shift differences between the proline ring protons of II are very much different from those of proline itself.[4] Since the phenyl magnetic anisotropy could not

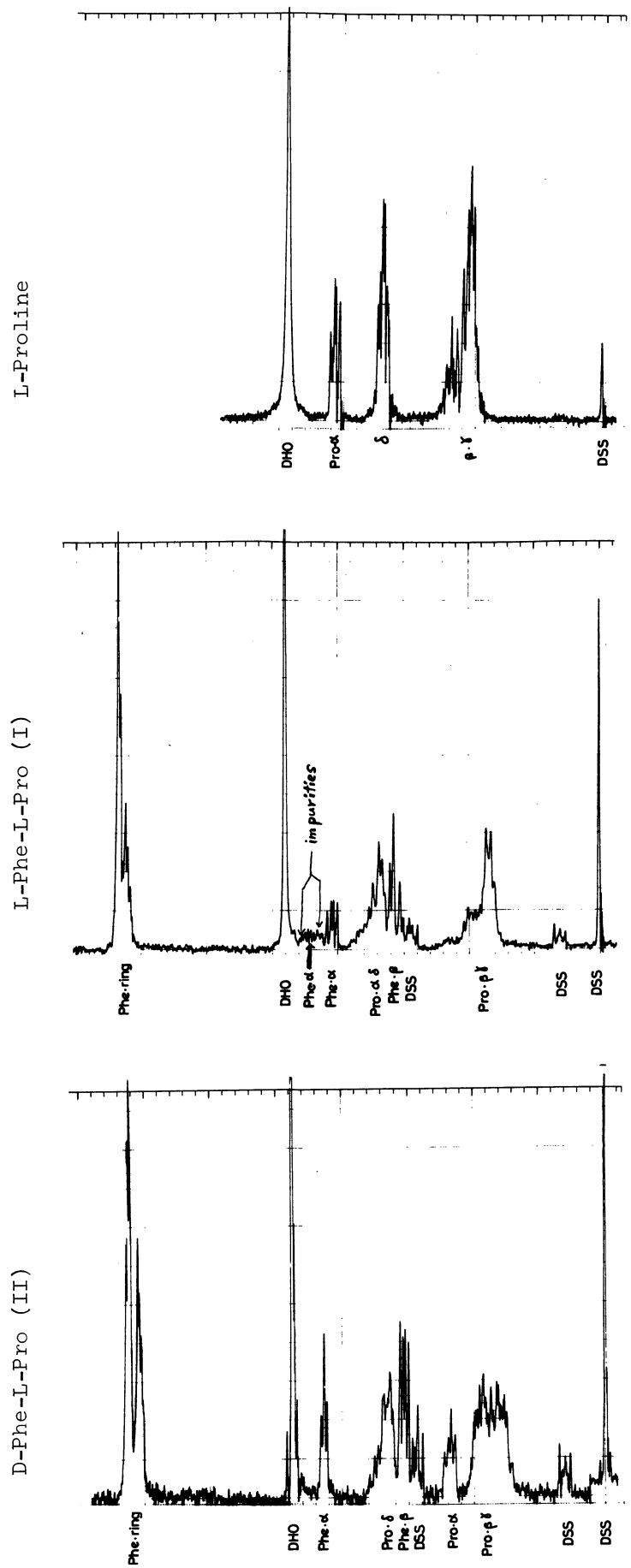
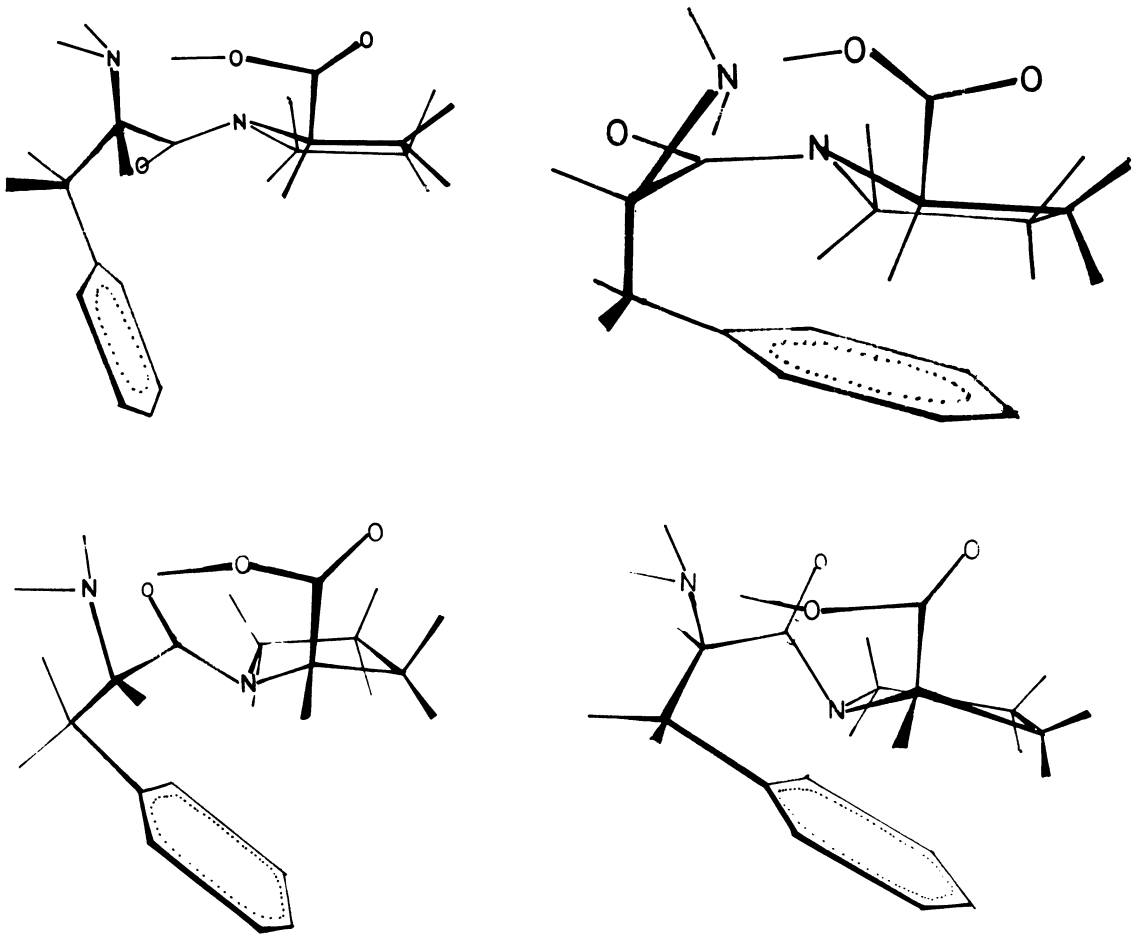


Figure 1. NMR spectra of proline, L-Phe-L-Pro (I), and D-Phe-L-Pro (II) in D_2O with DSS as an internal reference at 100 MHz.

Table. α-Proton chemical shifts of aminoacylprolines in D_2O. Ppm from internal DSS.

	Pro α-H	Aminoacyl α-H
L-Ala-L-Pro	4.34	4.01 and 4.38
L-Val-L-Pro	4.32	4.20 and 4.45
L-Phe-L-Pro	3.4	4.10 and 4.40
D-Phe-L-Pro	2.40	4.28

Figure 2. Conformations of L-Phe-L-Pro (I) (left-hand side) and D-Phe-L-Pro (II) (right-hand side).



change the spin-spin coupling constant, the chemical shift differences between the proline ring protons of II are very much different from those of proline. If we assume the hydrogen bonding or the salt bridge between Phe-NH_2 and Pro-COOH, and then, the hydrophobic interaction between the phenyl ring and the proline ring of I and II, the structures of these two peptides can be drawn as in Fig. 2. These structures can well explain the NMR spectra of I and II. The phenyl ring of I can be close not so much to the proline ring and can affect only on Pro α-H, but that of II can be very close to the proline ring, so that the Pro α-H signal is very much shifted to a high field and the chemical shift differences between the proline ring protons of II changes from those of proline. The stereochemical relationship between the phenyl ring and the proline ring is not changed so much by the nitrogen inversion as shown in Fig. 2. The Phe α-H of I is situated at the same side of Phe-NH_2 and Pro-COOH and experiences very much different environments by the nitrogen inversion, but that of II is at the other side of Phe-NH_2 and Pro-COOH and does not experience different environments by the nitrogen inversion.

If we assume the rotational isomerism around the peptide bond, and then, the hydrophobic interaction between the phenyl and proline rings, the trans rotamers of I and II have the almost same conformational relationship as II and I shown in Fig. 2 have, respectively, which are the cis rotamers. Therefore, the spectral difference between I and II cannot be accounted for.

The spectral data of C-13 NMR[2] showed that N-acetylproline has two 1.3 ppm separate peaks for Pro α-C and two 0.3 ppm separate peaks for acetyl C=O and that aminoacylprolines have two 0.3 ppm or non-separate peaks for Pro α-C and two 0.7 - 0.8 ppm separate peaks for aminoacyl C=O. The difference in these peak separations could suggest that N-acetylproline has the peptide-bond rotational isomerism and that proline peptide has the nitrogen-inversion isomerism.

This evidence for the nitrogen-inversion isomerism should be noted for the peptide conformation in aqueous solution, because such a small peptide like I or II has a distinct nitrogen-inversion isomerism, even though many research workers have ignored this isomerism and assumed very rapid nitrogen-inversion or sp^2-type flat nitrogen conjugated with the carbonyl group.

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