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^{13}C-^{15}N SPIN-SPIN COUPLING CONSTANTS OF AMIDES: H-BONDING EFFECTS IN A CYCLOHEXAPEPTIDE[1]

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The effect of intramolecular H-bonding and solvent acidity on the magnitude of one-bond ^{13}C-^{15}N spin-spin coupling constants has been examined using specifically ^{15}N-labeled cyclo-(Gly-L-Pro-Gly)$_2$ and related linear peptides.

In the study of the conformational behaviour of peptides in solution using ^{13}C NMR spectroscopy,[1,2,3] a rewarding approach has been the search for key segments such as intramolecular hydrogen bonds which lock the peptide backbone in a particular conformation and can occur in linear (e.g. elastin[2d]) or cyclic peptides (e.g. gramicidin S,[2d] alumichrome[2g]). Such a feature is an arrangement of four amino-acid units with an intramolecular 4 → 1 hydrogen bond resulting in a reversal of chain direction,[4] which has variously been referred to as a β-turn, β-loop, hair-pin bend or U-turn (Fig. 1). Many temperature and solvent titration studies have demonstrated the effect of intramolecular H-bonding on the chemical shift of ^{1}H,[5] ^{13}C,[2c-h] or ^{15}N,[6] resonances in a peptide group. Using specifically ^{15}N-labeled cyclo-(Gly-L-Pro-Gly)$_2$ (1) (Fig. 2) we have examined the effect of this type of intramolecular H-bonding on $^{1}J_{CN}$ amide coupling constants

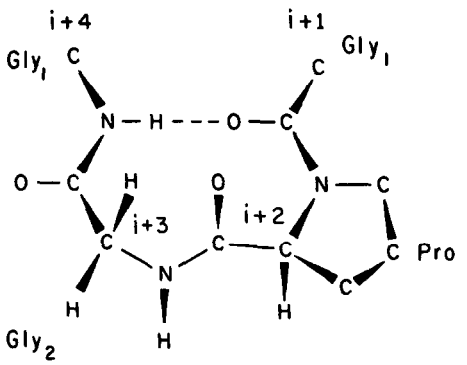

Fig. 1. Type II β turn with L-proline and glycine in consecutive corner positions. The corresponding amino acid residues in cyclo-(Gly-L-Pro-Gly)$_2$ are shown and represent one half of the proposed symmetrical conformation (see ref. 8).

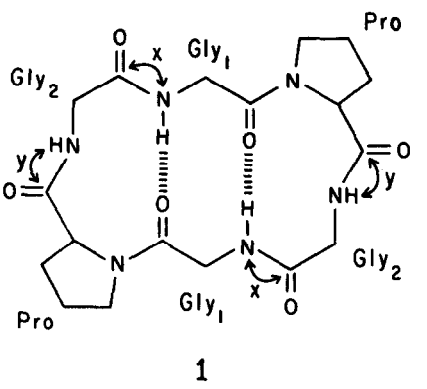

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Fig. 2. Structure of cyclo (Gly-L-Pro-Gly)$_2$ showing intramolecular H-bonds and the ^{13}C-^{15}N couplings measured: x with [^{15}N-Gly$_1$]-labeling, y with [^{15}N-Gly$_2$]-labeling.

[1]NRCC No. 17526.

The peptide (1) is a useful model which has been examined by ^{1}H and ^{13}C NMR spectroscopy[7,8], both at natural isotopic abundance and using specifically deuterated and ^{13}C-enriched samples[8,9]. The results established that it adopts an extremely stable conformation, with a C_2 axis of symmetry, two transannular H-bonds and two type II β-turns in which the glycine preceeding proline is intramolecularly H-bonded and the glycine residue following proline is not.[8] A recent X-ray study[10] on related cyclic hexapeptides indicates that H-bonding should be efficient and that each amide group is approximately planar with normal bond angles and distances.

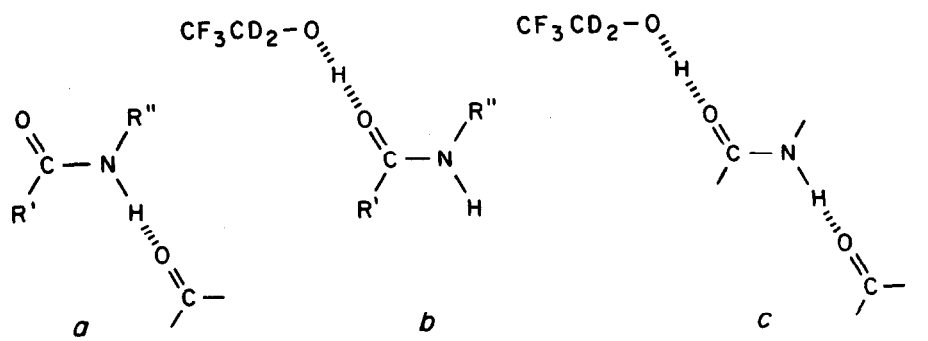
Table 1: $^{1}J_{^{13}C^{15}N}$ Amide Coupling Constants (Hz) of ^{15}N-Labeled Peptides in $CDCl_3$, DMSO, TFE and TFA[a]

PEPTIDE	Coupling Constant (Hz)			
	$CDCl_3$	DMSO	TFE	TFA
[^{15}N-Gly$_1$]cyclo-(Gly-L-Pro-Gly)$_2$	-[b]	15.90[c] *16.25[c]	17.7	18.6
[^{15}N-Gly$_2$]cyclo-(Gly-L-Pro-Gly)$_2$	-[b]	14.76[c] *14.79[c]	15.7	16.5
[^{15}N-Gly$_2$]Boc-Gly-L-Pro-Gly-OBz	14.3	14.7	16.3	-[b]
[^{15}N-Gly$_2$]Boc-Gly-Gly-OMe	15.2	14.6	16.3	-[b]

a Spectra were recorded with Varian Associates XL-100/15 and FT-80A (figures in brackets) Fourier transform NMR spectrometers under the following conditions (except for those under c below): Frequency 25.16 MHz (20 MHz) spectral bandwidth 5120 Hz (4132 Hz) aquisition time 1.6s or 3.2s (1.98s) 16384 or 32768 (16384) data points, separation of data points 0.6 Hz or 0.32 Hz (0.5 Hz) but estimated errors ± 0.3 Hz or ± 0.15 Hz (± .25 Hz) due to extrapolation to peak centres between data points, flip angle 40° (25°) pulse length 20 μsec (3 μsec) broadband decoupling of ^{1}H at 100.06 MHz (80 MHz) by 0 to 180° phase modulation at 200 Hz of decoupling field, $\gamma H_2/2\pi$ ca. 3800 Hz, (bandwidth 2 KHz, $\gamma H_2/2\pi$ ca. 4KHz) internal lock to ^{2}H, reference to internal $(CH_3)_4Si$, temperature 29°C, 5 mm diameter sample tube, solvent volume ca. 0.4 ml, sample amount ca. 9 mg for cyclic peptides, ca. 50 mg for linear peptides.

b Not recorded

c Recorded on XL-100 spectrometer with acquisition time 16s, spectral width 500 Hz, flip angle 78°. Value denoted by * is for deuterated amide. Error ± 0.03 Hz.



Scheme I

Both [^{15}N- Gly_1]- and [^{15}N- Gly_2]cyclo-(Gly-L-Pro-Gly)$_2$ were synthesised by established procedures[11] from [^{15}N]glycine (95 atom % ^{15}N). The ^{13}C NMR spectrum of [^{15}N- Gly_1]cyclo-(Gly-L-Pro-Gly)$_2$ in DMSO was identical with that already reported[8,9], except that the resonance assigned to the amide carbonyl carbon of Gly_2 now appeared as a doublet ($^1J_{CN}$= 15.90 Hz, Table 1, coupling x in Fig. 2) due to spin-spin coupling with ^{15}N. (The resonance for C_α of Gly_1, was masked by solvent peaks). The magnitude of this coupling constant is larger than the usual values for amides and other labeled peptides[12], including [^{15}N- Gly_2]cyclo-(Gly-L-Pro-Gly)$_2$ (coupling y in Fig. 2) and related linear peptides (Table 1) which do not form any intramolecular H-bonds although they may form intermolecular H-bonds with the solvent. Thus, the anomalously high value observed in the [^{15}N-Gly_1]-labeled cyclic peptide is not merely a consequence of the primary structure.

To confirm that this larger coupling constant is a consequence of intramolecular H-bonding, both the [^{15}N-Gly_1] and [^{15}N-Gly_2]-labeled peptides were exchanged in 1/1 $^1H_2O/^2H_2O$ and the couplings remeasured to high accuracy in DMSO. Substitution of the amide ^{1}H with ^{2}H should increase H-bond enthalpy by ca. 6%.[15] If formation of an H-bond is responsible for the change in $^1J_{CN}$ from 14.76 Hz to 15.90 Hz, strengthening the H-bond by deuterium substitution should further increase $^1J_{CN}$. An increase of 0.35 ± 0.06 Hz was found for the intramolecularly H-bonded amide ([^{15}N Gly_1]-labeled, Table 1) whereas the non-H-bonded amide ([^{15}N Gly_2]-labeled) showed negligible change (0.03 ± 0.06 Hz). The signals for the deuterated amides had slight isotope chemical shifts, enabling couplings for both protonated and deuterated amides to be measured in a single experiment.

According to Schulman and Venanzi[13] the carbon-nitrogen spin-spin coupling of amides is dominated by the Fermi contact mechanism, hence the magnitude of coupling should be proportional to the percent s character of the orbitals as proposed by Binsch and co-workers.[14] This increase in the magnitude of amide coupling is most plausibly accounted for by an increase in the hybridisation of the amide bond through a transfer of electron charge density from the nitrogen to the carbonyl as depicted in the H-bonded state a (Scheme I). A similar explanation has been invoked by Llinas and co-workers[2g,h] to account for increased shielding of an amide carbonyl in which the NH group is involved in H-bonding.

The $^1J_{CN}$ values for amide couplings were essentially similar in $CDCl_3$ and DMSO (Table 1), but increased upon protonation of the amide group.[16] Consequently protonation of the solvent-exposed carbonyl in the coupled amide of [^{15}N-Gly_1]cyclo-(Gly-L-Pro-Gly)$_2$, represented by H-bonded state c (Scheme 1), should result in an even larger $^1J_{CN}$ value through increased polarisation of the amide. In fact the coupling increased to a value of 17.7 Hz (TFE) and 18.6 Hz (TFA) as the acidity of the solvent increased (Table 1). A similar effect was observed with the non-H-bonded amides, [^{15}N-Gly_2]cyclo-(Gly-L-Pro-Gly)$_2$ and the two model peptides (all represented by H-bonded state b: Scheme 1), though the high values observed with the H-bonded amide were not reached. It appears that these solvent-induced increases are independent of the intramolecular H-bonded state of the amide, but it is important to note that in each case examined here the amide carbonyl group is exposed. The solvent-dependence may differ for an amide containing an intramolecularly H-bonded carbonyl (e.g. [^{15}N-L-Pro]cyclo-(Gly-Pro-Gly)$_2$). Furthermore it is likely that coupling will be larger in protonated trans amides than in cis amides.[16]

The spectral data and isotope exchange measurements show that the $^{1}J_{CN}$ coupling between the i + 4 amide nitrogen and the i + 3 carbonyl carbon is high in the β turn of this cyclic peptide as a consequence of intramolecular H-bonding, and that this coupling is further increased by protonation of solvent-exposed carbonyls. More studies are necessary to establish whether or not these effects are general for trans amides having strong intramolecular H-bonds; if so they may prove useful in conformational studies of peptides from organisms cultured on ^{15}N-enriched media.

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References

(1) Abbreviations: NMR, nuclear magnetic resonance; ppm, parts per million; DMSO, dimethyl sulfoxide or dimethyl -d_6-sulfoxide; TFE, trifluoroethanol or trifluorethanol-d_2; TFA, trifluoroacetic acid; BOC, t-butyloxycarbonyl.

(2) Many studies on peptide structure and conformation employ ^{13}C NMR spectroscopy in conjunction with other techniques, however, some pertinent references are: (a) K. Wüthrich, A. Tun-Kyi and R. Schwyzer, FEBS Letters 25 104 (1972); (b) C. Grathwohl., A. Tun-Kyi, A. Bundi, R. Schwyzer and K. Wüthrich, Helv. Chim. Acta., 58, 415 (1975); (c) D. W. Urry, L. W. Mitchell and T. Ohnishi, Biochemistry, 13, 4083 (1974); (d) D. W. Urry, Res. Dev., 25, 18 (1974); (e) D. W. Urry and M. M. Long, Crit. Rev. Biochem., 4, 1 (1976); (f) M. Llinás, D. M. Wilson, M. P. Klein and J. B. Neilands, J. Mol. Biol., 104, 853 (1976); (g) M. Llinás, D. M. Wilson and J. B. Neilands, J. Am. Chem. Soc., 99, 3631 (1977); (h) M. Llinás, D. M. Wilson and M. P. Klein, J. Am. Chem. Soc., 99, 6846 (1977).

(3) For a recent review on ^{13}C NMR of peptides and proteins see; O. W. Howorth and D. M. J. Lilley, Prog. N.M.R. Spec., 12, 1 (1978).

(4) (a) C. M. Venkatachalam, Biopolymers, 6, 1425 (1968); (b) R. Chandrasekharan, A. V. Kalshminarayanan, U. V. Pandya and G. N. Ramachandran, Biochim. Biophys. Acta., 303, 14 (1973).

(5) (a) K. D. Kopple, M. Ohnishi and A. Go, J. Am. Chem. Soc., 91, 4264 (1969); (b) K. D. Kopple, M. Ohnishi and A. Go, Biochemistry, 8, 4087 (1969). (c) K. D. Kopple, A. Go, R. H. Logan, Jr., J. Savrda, J. Am. Chem. Soc., 94, 973 (1972); (d) D. W. Urry and M. Ohnishi in Spectroscopic Approaches to Biomolecular Conformation ed. D. W. Urry, Amer. Med. Ass. Press, Chicago, pp. 263-300 (1970); (e) T. P. Pitner and D. W. Urry, J. Am. Chem. Soc., 95, 1399 (1972); (f) M. Llinás and M. P. Klein, J. Am. Chem. Soc., 97, 4731 (1975); (g) A. Ballardin, A. J. Fischman, W. A. Gibbons, J. Roy, I. L. Schwartz, C. W. Smith, R. Walter and H. R. Wyssbrod, Biochemistry, 17, 4443 (1978).

(6) (a) M. Llinás, W. J. Horsley and M. P. Klein, J. Am. Chem. Soc., 98, 7554 (1976); (b) Md. Abu Khaled, D. W. Urry, H. Sugano, M. Miyoshi and N. Izumiya, Biochemistry, 17, 2490 (1978).

(7) R. Schwyzer and U. Ludescher, Helv. Chim. Acta., 52, 2033 (1969).

(8) L. G. Pease, C. M. Deber and E. R. Blout, J. Am. Chem. Soc., 95, 258 (1973).

(9) R. Schwyzer, Ch. Grathwohl, J. P. Meraldi, A. Tun-Kyi, R. Vogel and K. Wüthrich, Helv. Chim. Acta, 55, 2545 (1972).

(10) M. B. Hossain and D. van der Helm, J. Am. Chem. Soc., 100, 5191 (1978).

(11) These ^{15}N-labeled cyclic hexapeptides were synthesized via cyclodimerisation of the appropriately labeled tripeptide H-Gly-L-Pro-Gly-p-nitrophenyl ester hydrochloride in dilute solutions of pyridine: see R. Schwyzer, J. P. Carrion, B. Gorrup, H. Nolting and A. Tun-Kyi, Helv. Chim. Acta., 47, 441 (1964).

(12) V. F. Bystrov, Prog. N.M.R. Spectroscopy, 10, 41 (1976).

(13) (a) J. M. Schulman and T. Venanzi, J. Am. Chem. Soc., 98, 4701 (1976); (b) J. M. Schulman and T. Venanzi, J. Am. Chem. Soc., 98, 6739 (1976).

(14) G. Binsch, J. B. Lambert, B. W. Roberts and J. D. Roberts, J. Am. Chem. Soc., 86, 5564 (1964).

(15) C. N. R. Rao. J.C.S. Faraday I, 71, 980 (1975).

(16) S. Berger, Tetrahedron, 34, 3133 (1978).

(17) A. Severge, F. Juttner, E. Breitmaier and G. Jung, Biochim., Biophys., Acta., 437, 289 (1976).

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