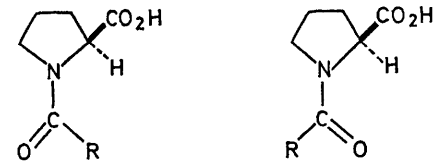
^{13}C Nuclear Magnetic Resonance Spectroscopy and *cis/trans* Isomerism in Dipeptides containing Proline

By W. A. THOMAS* and M. KEVIN WILLIAMS
(*Department of Chemistry, University College of Swansea, Singleton Park, Swansea* SA2 8PP)

Summary ^{13}C N.m.r. spectroscopy is shown to be a valuable technique for determining the isomerism about the amide bond in simple dipeptides.

ALTHOUGH *cis/trans* isomerism about the amide bond involving the nitrogen of proline is a well-recognised phenomenon, it is only recently that extensive studies of proline derivatives[1,2] have revealed that the *cis*-form (Ia) is far more prevalent than thought previously. As the ^1H spectra of even the simplest dipeptide, glycylproline, has been misinterpreted at 60 MHz,[3,4] and is still complex at 220 MHz, we decided to turn to ^{13}C n.m.r. spectroscopy in order to examine and compare the isomerism in aqueous solution of glycyl-, alanyl-, and valyl-proline, respectively, using *N*-acetylproline as a model. Previous measurements of ^{13}C chemical shifts of acylated proline derivatives did not report isomerism about the amide bond.[5,6]



cis (Ia) *trans* (Ib)

these dipeptides has apparently a negligible effect on the *cis/trans* ratio, nor does the nature of the preceding residue significantly affect the chemical shifts of the proline carbon atoms; (ii) the chemical shift difference between the α-carbon atoms in the two forms is remarkably small, as is the corresponding shift between the carbons, yet the β- and γ-carbons in the two forms are well separated. Perhaps conformational changes in the proline ring in the two forms account for these differences.

TABLE

		Proline ring carbons					Other carbons				CON	Approx. % *trans*
		C_α	C_β	C_γ	C_δ	CO_2^-	C_α	C_β	C_γ			
Proline[a]		61·6[c]	29·7	24·4	46·5	174·6	—	—	—		—	—
N-Ac-proline[b] ..	*cis*	62·78	34·19	27·61	49·19	177·12	25·78[e]	—	—		172·05	71
	trans	61·48	32·36	27·61	50·59	176·79	25·35[e]	—	—		171·72	
Gly-Pro	*cis*	64·61	34·52	25·24	50·27	181·21	43·36	—	—		168·70	61
	trans	64·94	32·47	27·18	49·62	182·08	43·58	—	—		167·95	
Ala-Pro	*cis*	64·94	34·40	25·13	[50·35[d]	181·00	[50·35[d]	18·45	—		172·37	57
	trans		32·25	27·61	51·24]	181·75	51·24]	18·23	—		171·51	
Val-Pro	*cis*	65·04	34·19	25·03	50·05	181·00	59·87	32·04	21·03[f]	19·52	171·18	59
	trans		31·71	27·51	50·70	181·43	60·30	32·58		18·78	170·43	

[a] From ref. 6. [b] $(CD_3)_2SO$ solution: all other compounds measured in aqueous solution. [c] In p.p.m. downfield from external Me_4Si. [d] Overlapping of four carbons precludes assignment. [e] CH_3CO. [f] The valyl methyl carbons are diastereotopic, but only three out of four peaks are observed.

A comparison of the proton noise-decoupled, 22·63 MHz ^{13}C chemical shifts, assigned by analogy with other proline derivatives is shown in the Table. The presence of two forms in equilibrium is clearly seen, the ratio of one form to the other being *ca.* 60:40 in each case (since integrals from pulsed Fourier spectra are not accurate, estimates of the true ratios are based on 220 MHz ^1H spectra). Assignments of *cis*- and *trans*-forms are based on analogy with *N*-acetylproline, which is known to prefer the *trans*-form in all solvents.[1,7]

From the results some remarkable points emerge; (i) the bulk of the side chain in the amino-acid preceding proline in

The relative ease of assignment of the isomeric forms of proline derivatives in ^{13}C spectra suggests that this technique will be valuable in the conformational analysis of more complex peptides containing proline or other cyclic imino-acids, or *N*-methylated amino-acids where the same considerations apply.

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