Evaluation of the Thermodynamic Parameters for the Interconversion of the Conformational Isomers of Two *N*-Acyl-prolines by Nuclear Magnetic Resonance Spectroscopy

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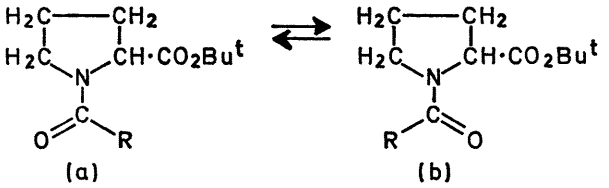
Summary Study of the ^{1}H n.m.r. spectra of the *N*-acyl-prolines (I) and (II) in deuteriochloroform over a range of temperature has enabled the thermodynamic parameters for the interconversion of such conformers by rotation about the N–CO bond to be evaluated for the first time.

IT is generally accepted, following Pauling, Corey, and Branson,[1] that peptide bonds in acyclic peptides of α-amino-acids have the planar *trans*-conformation. This is not so for imino-acids, in which the interconversion of the *cis*- and *trans*-rotamers is sufficiently slow for their independent existence to be detected by n.m.r. spectroscopy. In the past two years such rotamers have been detected in this way in peptides of proline,[2] *N*-methylalanine[3] and sarcosine,[4] while very recently the thermodynamic parameters for the interconversion of the rotamers of *N*-methylalanine peptides have been determined.[5] We now report a similar determination of the thermodynamic parameters for rotamer interconversion in the biologically important proline series.

The ^{1}H n.m.r. spectra of *N*-benzyloxycarbonyl-L-proline t-butyl ester (I) and *N*-benzyloxycarbonyl-*O*-t-butyl-L-seryl-L-proline t-butyl ester (II) in deuteriochloroform show by their complexity the presence of the *cis*- and *trans*-rotamers, (*a*) and (*b*), which undergo interconversion, at a rate slow on the n.m.r. time scale, by rotation about the N–CO bond, which is of the urethane type in (I) and of the peptide type in (II). The signals given by the t-butyl ester group in these compounds (τ 8·62 for I and 8·57 for II) are intense and sharp, with negligible spin–spin splitting; the proportions and rates of interconversion of the rotamers can be accurately determined by observing the change of line shape of these signals with tempertaure. The coalescence temperatures were 45° for (I) and 67·5° for (II).



[I; R = Ph·CH$_2$O–; II; R = Ph·CH$_2$·O·CO·NH·CH(CH$_2$OBu^t)–]

The procedure used involved computing line shapes for the t-butyl signals for a range of proton mean life-times, using a computer programme[6] based on the equations of Gutowsky and Holm,[7] and fitting these to the experimental curves obtained between 25 and 90°. The line shapes were described in terms of four parameters, *viz.* line width at half height (above the coalescence temperature), line width at half height for the stronger signal (below the coalescence temperature), peak to valley ratio, and chemical shift difference. The sensitivity of these parameters to changes in line shape varied appreciably and the curve fitting procedure made due allowance for this. This procedure is free from the assumptions made in other, more approximate, methods[8] and we believe that the thermodynamic parameters given below are among the most accurate so far reported for the interconversion of rotamers in amides and peptides.

The fraction of the most abundant rotamer (0·56 for I; 0·63 for II) remained constant, within experimental error, over the temperature range investigated; the more abundant rotamer is assigned the *trans*-conformation (b) on the basis of general considerations and the results of Madison and Schellman[2c] and Liberek *et al.*[4] The Arrhenius plots for the rates of interconversion of the rotamers were good straight lines, the slopes and intercepts of which were determined by the method of least squares.

The thermodynamic parameters so obtained for the conversion of the *cis*- into the *trans*-rotamers of (I) and (II)

TABLE

Equilibrium constants and thermodynamic parameters for conversion of cis- *into* trans *rotamers in deuteriochloroform at* 25°

	K	ΔG^0	ΔH^0	ΔS^0	$\Delta G^\ddagger$	$\Delta H^\ddagger$	$\Delta S^\ddagger$
		kcal mol^{-1}	kcal mol^{-1}	cal K^{-1} mol^{-1}	kcal mol^{-1}	kcal mol^{-1}	cal K^{-1} mol^{-1}
(Ia) ⇌ (Ib)	1 27	−0 14	0 00	+0 47	+17 29	+15 87	−4 77
(IIa) ⇌ (IIb) ..	1 70	−0 31	0 00	+1 05	+19·18	+21 01	+6 14

are given in the Table, they are very similar to those found by Portnova *et al* [5] for *N*-methylalanine peptides, save that the entropy of activation for the urethane (I) is negative The energy barrier (21 kcal mol^{-1}) we have observed in the interconversion of the rotamers for the peptide (II) is very similar to the barriers (20—23 kcal mol^{-1}) measured in other ways[9] for the interconversion of individual *cis*- and *trans*-proline residues in the very much slower isomerisation of polyproline-I

It is noteworthy that the energy barrier for the interconversion of the rotamers of the urethane (I) is 5 kcal mol^{-1} less than that for the interconversion of the rotamers of the peptide (II) Since rotamer interconversion involves overcoming the delocalisation which results in the planarity of the amide bond this implies that the degree of delocalisation, and hence the contribution of the charge-separated structure $^{+}N{=}CRO^{-}$, is less in the urethane than in the peptide It is a well known fact, of great importance in peptide synthesis, that urethanes form oxazolones much less readily than do peptides Determann and his colleagues[10] have ascribed this to the lesser nucleophilicity of the carbon oxygen, and greater double-bond character of the carbonyl group, in urethanes than in peptides, our results give quantitative support for this view

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