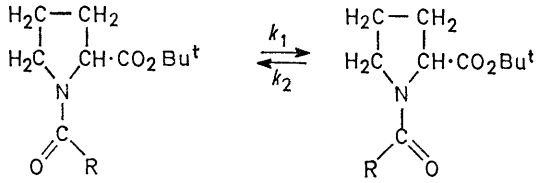
Evaluation of the Equilibrium and Activation Parameters for the Interconversion of the Conformational Isomers of Some *N*-Acylprolines by Nuclear Magnetic Resonance Spectroscopy [1]

By **Hernani L. Maia, Keith G. Orrell,** and **H. N. Rydon,*** Department of Chemistry, The University, Exeter EX4 4QD

Study of the ^{1}H n.m.r. spectra of six *N*-acyl-L-prolines (I)—(VI) over a range of temperatures has enabled the equilibrium and activation parameters for the interconversion of the *cis*- and *trans*-conformers to be evaluated. The energy barrier for the *cis–trans*-interconversion in (I), involving a urethane bond, is much lower than that for the other compounds, involving peptide bonds; the significance of this difference for the well known resistance of urethanes to racemisation in peptide synthesis is discussed.

It is generally accepted, following Pauling *et al.*,[2] that peptide bonds in acyclic peptides of α-amino-acids have the planar *trans*-conformation. This is not so for peptides of imino-acids, in which the interconversion of the *cis*- and *trans*-conformers is sufficiently slow for their independent existence to be detected by n.m.r. spectroscopy; such rotamers have been detected in this way in peptides of proline,[3] *N*-methylalanine,[4] and sarcosine.[5] Variable temperature studies enable the equilibrium and activation parameters for the interconversion of the conformers to be evaluated and this has been done for peptides of *N*-methylalanine [6] and for derivatives of *N*-acetylpyrrolidine;[7] we now report similar studies in the biologically important proline series.

The ^{1}H n.m.r. spectra, in deuteriochloroform at room temperature, of the six *N*-acyl-L-proline t-butyl esters (I)—(VI) show by their general complexity, and especially by the presence of two well-defined signals for the



(I) R = $PhCH_2O$ (II) R = $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH(CH_2OBu^t)$
(III) R = $Cl^- H_3\overset{+}{N}CH_2$ (IV) R = $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH(CH_2Ph){\cdot}$
(V) R = $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2$
(VI) R = $NH_2CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2$

t-butyl ester protons, the presence of the *cis*- and *trans*-rotamers, (a) and (b) respectively; these 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 the other five compounds studied.

In all but one instance, the chemical shift differences between the two signals given by the t-butyl ester protons were within the range 0.6—2.4 Hz in both deuteriochloroform and hexadeuteriodimethyl sulphoxide; compound (IV) was exceptional, the separation of the two signals being 12.5 Hz in the former solvent but undetectably small in the latter. It is not possible to assign signals unambiguously to the individual rotamers; as in our preliminary communication,[1] we assign, in every case, the larger signal to the *trans*-conformer (b) on the basis of general considerations, since this would be expected to be the more stable, and on the basis of the results of Madison and Schellman [3c] and Liberek *et al.*[5] In four of the compounds studied, *viz.* (I), (III), (V), and (VI), the larger, *trans*-signal was observed at higher applied field; the converse was true for the other two. The reason for this difference is not clear; it may be due to the presence of bulky, and chiral, side-chains in (II) and (IV).

The 220 MHz spectrum of the urethane (I) in deuteriochloroform at −12 and −45 °C showed the signal for the benzyl methylene protons to be made up of two overlapping AB quartets of about equal size, thus confirming the presence of two rotamers; the magnetic non-equivalence of this pair of protons in both rotamers no doubt results from the presence of an asymmetric carbon atom in the L-proline residue. Garner and Watkins [3a] observed two signals (a singlet and an AB quartet) for the corresponding protons in the corresponding *p*-nitrophenyl ester.

The relative abundances of the rotamers were determined by measurement of the relative areas of the two t-butyl ester signals in expanded spectra at temperatures below the coalescence temperature using a Dupont curve analyser generating Lorentzian curves. Apart from some scatter in the values, the relative abundances (mole fractions) of the rotamers were virtually invariant over the temperature range studied and average values were therefore used to calculate the equilibrium constants (see Table). In some cases there was a very slight increase in the relative abundance of the *trans*-rotamer at temperatures *ca.* 100 °C below the coalescence temperature

[1] Preliminary communication, H. L. Maia, K. G. Orrell, and H. N. Rydon, *Chem. Comm.*, 1971, 1209.
[2] L. Pauling, R. B. Corey, and H. R. Branson, *Proc. Nat. Acad. Sci. U.S.A.*, 1951, **37**, 205.
[3] (*a*) R. Garner and W. B. Watkins, *Chem. Comm.*, 1969, 386; (*b*) C. M. Deber, F. A. Bovey, J. E. Carver, and E. R. Blout, *J. Amer. Chem. Soc.*, 1970, **92**, 6191; (*c*) V. Madison and J. Schellman, *Biopolymers*, 1970, **9**, 511; (*d*) H. Okabayashi and T. Isemura, *Bull. Chem. Soc. Japan*, 1970, **43**, 359; (*e*) W. A. Thomas and M. K. Williams, *J.C.S. Chem. Comm.*, 1972, 788.
[4] M. Goodman and N. S. Choi, Peptides: Proceedings Ninth European Peptide Symposium, 1968, p. 1.
[5] B. Liberek, K. Steporowska, and E. Jereczek, *Chem. and Ind.*, 1970, 1263.
[6] S. L. Portnova, V. F. Bystrov, T. A. Balashova, V. T. Ivanov, and Yu. A. Ovchinnikov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 825; M. Goodman, F. Chen, and C.-Y. Lee, *J. Amer. Chem. Soc.*, 1974, **96**, 1479.
[7] C. H. Bushweller, J. W. O'Neil, M. H. Halford, and F. H. Bissett, *J. Amer. Chem. Soc.*, 1971, **94**, 1471.

which was unimportant. Since the equilibrium constant, K, is temperature invariant, $\Delta H^{\ominus}$ for the interconversion must be zero and this value was used in calculating the other thermodynamic parameters, which are incorporated in the Table.

Evaluation of the activation parameters requires the evaluation of the mean life-time, τ, for the rotamers and hence of the rate constant, k ($= k_1 + k_2$), for their interconversion in both directions at various temperatures. This was done by the curve-fitting procedure described below.

The natural line-widths and the internal chemical shifts for the t-butyl ester signals in the slow exchange limit were obtained by study of their temperature dependence. At temperatures >30 °C below coalescence appreciable broadening of the signals was observed, accompanied by increased separation. It was thought that this might be due to some other, faster inversion process, but low temperature studies at 220 MHz provided no further evidence for this. This broadening complicated the determination of the natural line-widths and internal chemical shifts and the difficulty could only be overcome by treating these as variable parameters and successively refining the curve-fitting procedure;[8] the sensitivity of calculated kinetic parameters to input line-width has been noted by others.[9] In most cases a natural line-width of 0.6 Hz (T_2 0.53 s) was found to be optimal.

The curve-fitting procedure involved computation of line shapes for the t-butyl ester signals for a range of mean life-times, τ, using a computer program [10] based on the equations of Gutowsky and Holm,[11] and fitting these to the experimental curves. Although the spectra were observed over a wide range of temperature (ambient to *ca.* 120 °C), curve-fitting was restricted to a narrower, more sensitive range (*ca.* 60 °C) around the coalescence temperature. The line shapes were described in terms of four parameters, *vis.* line-widths at half-height (used above the coalescence temperature), line-width at half-height of the stronger signal (used below the coalescence temperature), minimum to maximum ratio, and separation of peak maxima. This enabled four relationships between $\log_{10} 1/\tau$ and $1/T$ to be determined; for ideal fit all should be identical straight lines. In the course of the work it was found that the most sensitive of these relationships was that based on the separation of peak maxima and the compatibility of this relationship with the other three was therefore adopted as the criterion for a good fit. Although we recognise the advantage of complete line-shape fitting methods,[9] we believe that the method we have used minimises any differences between experimental and computed line shapes; any residual errors are small compared with those associated with non-linearity of the spectrometer field-sweeps. Once a satisfactory fit had been achieved, an Arrhenius plot of $\log_{10}k$ against $1/T$ was made. In all cases the plots were good straight lines, the slopes and intercepts of which were determined by the method of least squares.

The activation parameters for the conversion of the *cis*- into the *trans*-rotamer of compounds (I)—(VI), determined in this way, are given in the Table; they are very similar to those found by other workers [6] for *N*-methylalanine peptides, except that the entropy of activation for the urethane (I) is negative. The errors quoted are based on least square fittings; those for $\Delta H^{\ddagger}$ are primarily a measure of the accuracy of the curve-fitting and the consequent linearity of the Arrhenius plot; those for $\Delta G^{\ddagger}$ and $\Delta S^{\ddagger}$ are largely dependent on the accuracy of the temperature measurements, estimated to be ±1 °C for the 60 MHz and ±1.5 °C for the 100 MHz instrument.

The enthalpies of activation for the interconversion processes involving rotation about a peptide bond, *viz.* compounds (II)—(VI), all lie within the narrow range 85.8—92.7 kJ mol^{-1}, in good agreement with the values (84—96 kJ mol^{-1}) obtained by other methods [12] for the interconversion of individual *cis*- and *trans*-proline residues in the much slower isomerisation of polyproline-I. In contrast, $\Delta H^{\ddagger}$ for the process involving rotation about the urethane bond in (I) is much less (60—67 kJ mol^{-1}). Since rotamer interconversion involves overcoming the electron delocalisation which results in the planarity of the N–CO bond, this difference between peptide and urethane implies that the degree of delocalisation, and

Equilibrium and activation parameters [a] for the interconversion of the *cis*- and *trans*-rotamers of some *N*-acyl-L-proline t-butyl esters at 298 K

Compound	f_{trans}	K	E_A/kJ mol^{-1}	$\log_{10}A$	$\Delta G_c^{\ddagger}$/kJ mol^{-1}	$\Delta G_t^{\ddagger}$/kJ mol^{-1}	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S_c^{\ddagger}$/J K^{-1}mol^{-1}	$\Delta S_t^{\ddagger}$/J K^{-1}mol^{-1}
(I) [b]	0.56	1.27	68.9 ± 0.2	12.4 ± 0.03	+72.3 ± 0.1	+72.9 ± 0.1	+66.4 ± 0.2	−20.0 ± 0.8	−22.0 ± 0.8
(I) [c]	0.58	1.38	62.8 ± 0.2	11.5 ± 0.03	+72.8 ± 0.1	+73.6 ± 0.1	+60.3 ± 0.2	−41.9 ± 1.2	−44.6 ± 1.2
(II) [b]	0.63	1.70	90.4 ± 0.4	14.8 ± 0.06	+80.3 ± 0.4	+81.6 ± 0.4	+87.9 ± 0.4	+25.4 ± 2.4	+21.0 ± 2.4
(III) [b]	0.72	2.57	88.5 ± 0.4	14.0 ± 0.05	+80.6 ± 0.3	+82.9 ± 0.3	+86.0 ± 0.4	+18.0 ± 2.0	+11.1 ± 2.0
(IV) [b]	0.77	3.35	95.2 ± 1.0	15.0 ± 0.10	+82.0 ± 0.4	+85.0 ± 0.4	+92.7 ± 1.0	+35.8 ± 4.0	+25.7 ± 4.0
(V) [b]	0.70	2.33	92.7 ± 1.0	15.1 ± 0.10	+78.7 ± 0.4	+80.8 ± 0.4	+90.2 ± 1.0	+38.7 ± 4.0	+28.8 ± 4.0
(VI) [b]	0.74	2.85	88.3 ± 0.4	14.4 ± 0.10	+78.2 ± 0.4	+80.8 ± 0.4	+85.8 ± 1.0	+25.3 ± 2.8	+16.6 ± 2.8

[a] The Arrhenius parameters are for the conversion of *cis* to *trans*. The subscripts to $\Delta G^{\ddagger}$ and $\Delta S^{\ddagger}$ denote the rotamer being converted into the transition state; since $\Delta H^{\ominus}$ is zero $\Delta H^{\ddagger}$ is the same for both rotamers. [b] In $CDCl_3$. [c] In [$^2H_6$]DMSO.

[8] I. O. Sutherland, *Ann. Reports NMR Spectroscopy*, 1971, **4**, 106.

[9] S. van der Werf and J. B. F. V. Engberts, *Rec. Trav. chim.*, 1971, **90**, 663.

[10] T. Nakagawa, *Bull. Chem. Soc. Japan*, 1966, **39**, 1006.

[11] H. S. Gutowsky and C. H. Holm, *J. Chem. Phys.*, 1956, **25**, 1228.

[12] A. R. Downie and A. A. Randall, *Trans. Faraday Soc.*, 1959, **55**, 2132; I. Steinberg, W. F. Harrington, A. Berger, M. Sela, and E. Katchalski, *J. Amer. Chem. Soc.*, 1960, **82**, 5263; D. A. Torchia and F. A. Bovey, *Macromolecules*, 1971, **4**, 246.

hence the contribution of the charge-separated structure $^{+}N{=}CRO^{-}$, is less in the latter than in the former. The resulting lesser rigidity, and lesser charge, of the urethane as compared with the peptides could also underly the marked differences in the entropies of activation, which are negative for the urethane (I) and positive for the peptides (II)—(VI).

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 [13] have ascribed this difference to the lesser nucleophilicity of the carbonyl oxygen, and greater double-bond character of the carbonyl group, in urethanes compared with peptides. Our results provide some quantitative support for this view.

EXPERIMENTAL

Materials.—*N*-Benzyloxycarbonyl-L-proline t-butyl ester, (I) was prepared by the method of Anderson and Callahan [14] and had m.p. 45—46°, $[\alpha]_D^{22}$ −56.3° (*c* 2.2 in EtOH) (lit.,[14] 44—45°, $[\alpha]_D^{25}$ −52.5°).

N-Benzyloxycarbonyl-O-t-butyl-L-seryl-L-proline t-butyl ester (II). Dicyclohexylcarbodi-imide (2.06 g, 10 mmol) was added with stirring to *N*-benzyloxycarbonyl-*O*-t-butyl-L-serine [15] (2.95 g, 10 mmol) in acetonitrile (50 ml) at −10°. After 10 min L-proline t-butyl ester hydrochloride [15] (2.07 g, 10 mmol) was added, followed at once by triethylamine (1.38 ml; 10 mmol). After 1 h at −10° and 22 h at room temperature, the mixture was kept for 2 h at −5°, filtered, and the solid washed twice with ether. The filtrate and washings were evaporated to dryness and the residue taken up in ethyl acetate and re-evaporated. The residue was dissolved in ethyl acetate (50 ml) and the solution kept at −10° for 1.5 h. A little insoluble material was removed by filtration and the filtrate washed successively with M-hydrochloric acid, water, saturated sodium hydrogen carbonate, and saturated brine, dried, and evaporated. The last traces of dicyclohexylurea were removed by dissolution in acetone and filtration after 18 h at −10°. Evaporation, dissolution in light petroleum (b.p. 40—60°), and re-evaporation (three times in all) followed by drying in high vacuum to constant weight, gave the *peptide* as an oil (4.04 g, 90%), $[\alpha]_D^{23}$ −52.5° (*c* 2.0 in MeOH), which could neither be crystallised nor distilled. The compound was chromatographically homogeneous (t.l.c. in two solvent systems) and gave the expected i.r. and n.m.r. spectra.

Glycyl-L-proline t-butyl ester hydrochloride (III). *N*-Benzyloxycarbonylglycine (20.9 g, 100 mmol) and L-proline t-butyl ester hydrochloride [15] (20.7 g, 100 mmol) were suspended in ethyl acetate (200 ml) and cooled to 0°; dicyclohexylcarbodi-imide (20.6 g, 100 mmol) and triethylamine (13.8 ml, 100 mmol) were then added and the mixture kept for 20 h at room temperature. Working up essentially as described for (II) gave *N*-benzyloxycarbonylglycyl-L-proline t-butyl ester (18.8 g, 97%) as a chromatographically homogeneous, but uncrystallisable oil, $[\alpha]_D^{23}$ −79.3° (*c* 2.0 in MeOH). Hydrogenation of this in ethanol (250 ml) over 10% palladised charcoal (3.6 g) was complete in 4 h. The reaction mixture was filtered and the filtrate evaporated. The residue was taken up in anhydrous ether (250 ml), filtered, and treated with 4.5M-hydrogen chloride in ether (24 ml). Evaporation, followed by recrystallisation from ethanol–ether gave the *hydrochloride* (22.8 g, 87%), m.p. 157.5—158° (decomp.), raised to 162—162.5° by further recrystallisation, $[\alpha]_D^{23}$ −96.8° (*c* 2.0 in MeOH) (Found: N, 10.6. $C_{11}H_{21}ClN_2O_3$ requires N, 10.6%).

N-Benzyloxycarbonyl-L-phenylalanyl-L-proline t-butyl ester (IV). Ethyl chloroformate (0.65 g, 6 mmol) was added at −10° to a solution of *N*-benzyloxycarbonyl-L-phenylalanine (1.79 g, 6 mmol) and triethylamine (0.83 ml, 6 mmol) in chloroform (12 ml). After 10 min at 0°, a mixture prepared from L-proline t-butyl ester hydrochloride (1.24 g, 6 mmol) and triethylamine (0.83 ml, 6 mmol) was added. The mixture was stirred overnight at room temperature, washed successively with M-hydrochloric acid, water, saturated sodium hydrogen carbonate, and water, dried, and evaporated. The residue was stirred for 4 h with light petroleum (b.p. 40—60°) (25 ml) and the solution decanted from the gummy residue. Evaporation, followed by drying in high vacuum, gave the *peptide* as an uncrystallisable oil (2.0 g, 74%), which was chromatographically homogeneous and gave the expected i.r. and n.m.r. spectra.

N-Benzyloxycarbonyldiglycyl-L-proline t-butyl ester (V). *N*-Benzyloxycarbonylglycine (5.2 g, 25 mmol) and glycyl-L-proline t-butyl ester (6.6 g, 25 mmol) were coupled by means of dicyclohexylcarbodi-imide (5.2 g, 25 mmol) in ethyl acetate (50 ml), essentially as described under (III) above. Two recrystallisations from ethyl acetate–light petroleum (b.p. 40—60°), removal of residual dicyclohexylurea by precipitation from hot ethanol (25 ml) with water (25 ml), and a third recrystallisation from ethyl acetate–light petroleum gave the *peptide* (4.3 g, 41%), m.p. 89—91°, $[\alpha]_D^{23}$ −72.5° (*c* 2.0 in MeOH) (Found: C, 54.5; H, 7.2; N, 10.3. $C_{21}H_{29}N_3O_6$ requires C, 60.1; H, 7.0; N, 10.0%).

Diglycyl-L-proline t-butyl ester (VI). Hydrogenation of the protected peptide (V) in ethanol, as described under (III) above, and recrystallisation from ethanol gave the *peptide*, m.p. 109—110°, $[\alpha]_D^{23}$ −103° (*c* 1.0 in MeOH) (Found: C, 54.5; H, 8.5; N, 14.9. $C_{13}H_{23}N_3O_4$ requires C, 54.7; H, 8.1; N, 14.7%).

Spectra.—All compounds were investigated as *ca.* 30% solutions in the solvents indicated in the Table. N.m.r. spectra were obtained either at 60 MHz using a Perkin-Elmer R10 spectrometer or at 100 MHz using a JEOL MH-100 spectrometer. Both instruments were fitted with standard variable temperature accessories. Tetramethylsilane was used both as internal reference and as a field homogeneity check at elevated temperatures. Spectra were recorded under optimum stability conditions. Several spectra were obtained at each temperature in order to average out small field–frequency shifts and changes in instrument resolution.

Theoretical line shapes were obtained using a modified version of the program SHAPE FUNCTION, kindly supplied by the n.m.r. program library, Harwell. Computations were performed on an ICL-450 computer.

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[13] H. Determann, J. Heuer, P. Pfaender, and M. L. Reinerts, *Annalen*, 1966, **694**, 190; H. Determann, Peptides: Proceedings Eighth European Peptide Symposium, 1966, p. 73.

[14] G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1960, **82**, 3359.

[15] E. Schröder, *Annalen*, 1963, **670**, 127.