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Conformation and Circular Dichroism of Several *N*-Acyl-L-prolines

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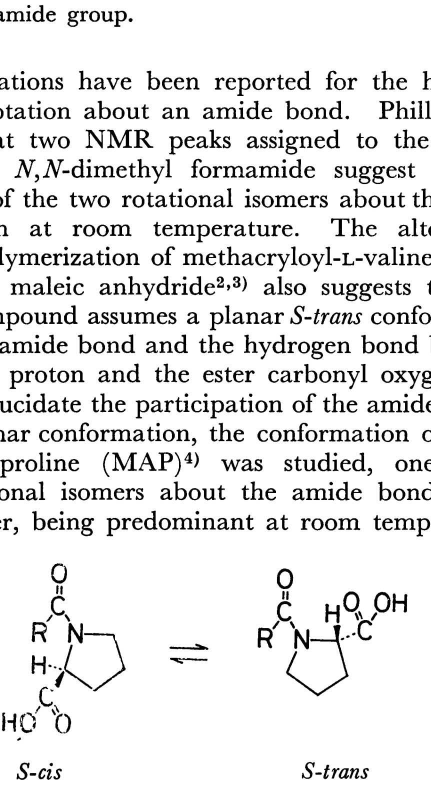
NMR and CD studies were carried out for acetyl-L-proline, isovaleroyl-L-proline, isobutyroyl-L-proline, pivaloyl-L-proline and benzoyl-L-proline. It was found that *N*-acetyl-L-proline has two rotational isomers about the amide bond, *S-trans* and *S-cis* conformers even at room temperature, and that the population of the former is greater than that of the latter except for pivaloyl-L-proline, which consists only of the *S-trans* conformer. The two isomeric *N*-acyl-L-prolines showed different CD peaks of opposite sign associated with the n→π* transition of the amide group.

Investigations have been reported for the hindered internal rotation about an amide bond. Phillips[1] reported that two NMR peaks assigned to the methyl groups of *N*,*N*-dimethyl formamide suggest the coexistence of the two rotational isomers about the amide bond even at room temperature. The alternating photocopolymerization of methacryloyl-L-valine methyl ester with maleic anhydride[2,3] also suggests that the amide compound assumes a planar *S-trans* conformation about the amide bond and the hydrogen bond between the amide proton and the ester carbonyl oxygen. In order to elucidate the participation of the amide proton in this planar conformation, the conformation of methacryloyl-L-proline (MAP)[4] was studied, one of the two rotational isomers about the amide bond, the *S-trans* isomer, being predominant at room temperature.



On the other hand, the CD spectrum of MAP shows a negative CD maximum at 227 nm., accompanied by a shoulder at about 245 nm,[4] which would be ascribed to the *S-cis* isomer which was less stable than the other one.

We have synthesized several *N*-acyl-L-prolines, acetyl-L-proline (AP), isovaleroyl-L-proline (IVP), isobutyroyl-L-proline (IBP), pivaloyl-L-proline (PP) and benzoyl-L-proline (BP) in order to investigate their CD and NMR spectra. The NMR signals assigned to the α-methine proton of L-proline moiety are given in Fig. 1. Two quartet resonances appeared except for PP. They would be ascribed to the two rotational isomers about the amide bond as in the case of MAP. PP, however, showed only one resonance peak at 4.17 ppm even at −30 °C. The Stewart model of PP showed that the *S-cis* conformation could not possibly exist and the resonance peak of the methine proton of PP would correspond to that of the *S-trans* conformer. This suggests that a resonance peak at a higher magnetic field (Fig. 1) should be assigned to the methine proton of the *S-trans* conformer, which was the more predominant of the two conformations.

The equilibrium constant ($K=[S\text{-}trans]/[S\text{-}cis]$) (Table 1) was calculated from the area ratio of the corresponding two resonance peaks (Fig. 1), and tends to increase with the bulkiness of the R group.

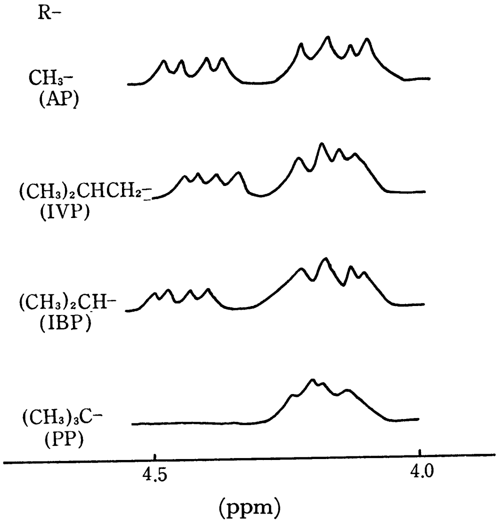


Fig. 1. NMR signal of α-methine proton of *N*-acyl-L-prolines in DMSO-d_6 at 21 °C. Lock; HMDS

Table 1. Equilibrium constant and activation free energy for the transformation between *S-cis* and *S-trans* conformers (in DMSO-d_6)

Compound	R-	$K\left(=\frac{[S\text{-}trans]}{[S\text{-}cis]}\right)$	$\Delta F^{\ddagger}_{Tc}$ (kcal/mol)	T_c (K)
AP	CH_3-	2.9	19.0±0.2	365
IVP	$\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CHCH_2-$	3.1	18.6±0.2	361
IBP	$\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH-$	3.5	18.5±0.2	359
PP	$\begin{matrix}CH_3\diagdown\\CH_3-C-\\CH_3\diagup\end{matrix}$	*a)	—	—

a) *S-trans* only

Temperature dependence of the NMR signals assigned to the α-methine proton of IVP (Fig. 2) shows coalescence between them as well as AP and IBP. The activation free energy for the transformation between the two isomers at the coalescence temperature ($\Delta F^{\ddagger}_{Tc}$) was caluclated from the NMR data in DMSO-d_6 by

$$\Delta F^{\ddagger}_{Tc} = 2.303\,RT_c \log\frac{\sqrt{2}\,\kappa k T_c}{\pi \Delta \nu h} \tag{1}$$

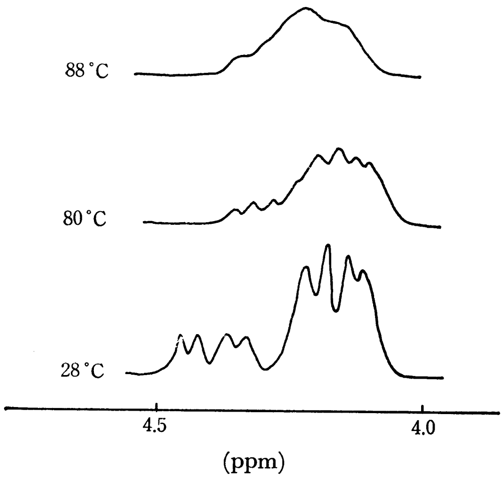


Fig. 2. NMR signal of α-methine proton of IVP in DMSO-d_6. Lock; HMDS

Table 2. Chemical shift of α-methine proton of N-acyl-L-prolines from HMDS at 21 °C

Compound	in $CDCl_3$		in DMSO-d_6	
	S-cis	*S-trans*	*S-cis*	*S-trans*
AP	4.37 ppm	4.50 ppm	4.43 ppm	4.15 ppm
IVP	4.42	4.53	4.40	4.17
IBP	4.44	4.51	4.47	4.17
PP	—	4.48	—	4.19
BP	4.22	4.67	4.27	4.37
MAP	4.52	4.53	4.43	4.19

where T_c is the coalescence temperature, $\Delta\nu$ the difference in chemical shift of the α-methine proton of the two isomers, the transmission coefficient κ being assumed to be unity. The $\Delta F^{\ddagger}_{T_c}$ values suggest that it would not be easy to separate the two isomers from each other.

The chemical shift of α-methine proton of N-acyl-L-proline was measured in DMSO-d_6 and $CDCl_3$ at 21 °C. The δ values are shown in Table 2. PP showed only one methine proton signal in both solvents at 21 °C and −30 °C, but the other N-acyl-L-prolines showed two methine proton signals in both solvents even at room temperature. The larger of the two methine proton signals was assigned to that of *S-trans* conformer. Thus a methine proton of *S-cis* conformer shows an almost equal δ value in both solvents, but the δ value of methine proton of *S-trans* conformer differed by about 0.35 ppm. It seems that the methine proton of *S-trans* conformer has a larger solvent effect than that of *S-cis* conformer, since the latter would receive a larger steric hindrance by the R group. In the case of BP, however, the methine proton signal of *S-trans* conformer appeared at a lower magnetic field than that of *S-cis* one in both solvents. This might be ascribed to the magnetic anisotropy due to the benzene ring.

The CD spectra of N-acyl-L-prolines and L-proline were measured in methanol at 25 °C (Fig. 3). The small CD absorption maximum of L-proline at 220 nm derived from the n–π* transition of the carboxyl group would contribute a negligible effect on the CD curves of N-acyl-L-prolines below 225 nm. The CD absorption of the four N-acyl-L-prolines over the spectral region from 225 to 255 nm. would be correlated to the n–π* transition of the amide group. Since the only one conformation of PP was illustrated to be the *S-trans* one by NMR data, the positive CD maximum of PP at 230 nm. would be ascribed to the n–π* transition of the amide group with *S-trans* conformation. In the case of AP, IVP and IBP which have a *S-cis* conformer together with the *S-trans* conformer, a weak negative absorption maximum appears at 235 to 250 nm. besides a positive absorption maximum around 230 nm. The negative CD maximum, therefore, would be due to the *S-cis* conformer of N-acyl-L-proline. The same CD phenomenon as this was found for poly-L-proline.[5,6] The maximum wavelength and sign of the CD maximum of the *trans* isomer was found to differ from those of the *cis* isomer.

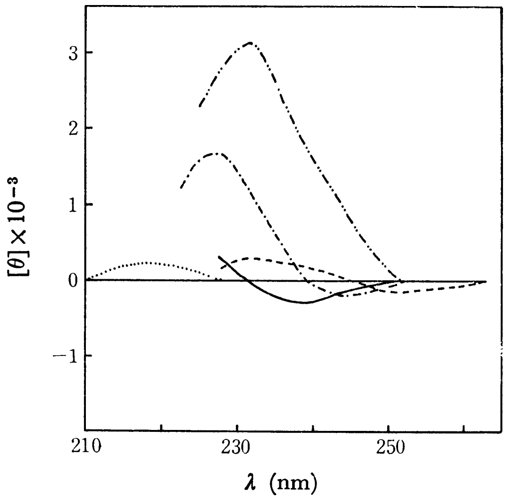


Fig. 3. The CD spectra of AP (——), IVP (----), IBP (—·—), PP (—··—) and L-proline (······) in methanol at room temperature.

The CD spectrum of MAP was measured at 25 °C in methanol.[4] MAP showed a CD curve with a different shape from those of N-acyl-L-prolines (Fig. 3).

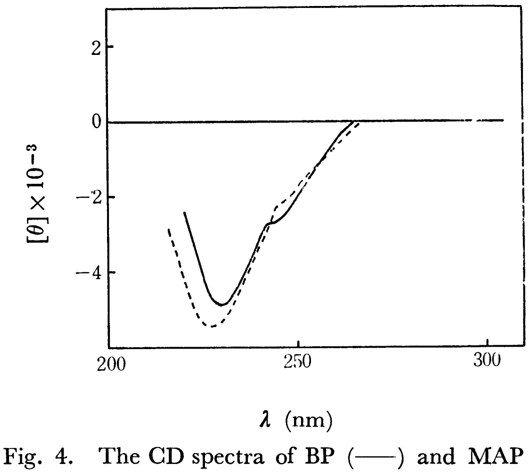


Fig. 4. The CD spectra of BP (——) and MAP (----) in methanol at room temperature.

The former showed a large negative peak centered at 228 nm with a shoulder near 245 nm, while the latter showed a positive peak with a small negative one which appeared at a higher wavelength region. The CD spectrum of BP was similar to that of MAP. Both spectra are shown in Fig. 4. The result will be explained from the fact that both BP and MAP have an α,β-unsaturated carbonyl group.[4,7] They also have two rotational isomers, *S-trans* and *S-cis* conformers as in the case of AP, IVP and IBP, all of which exhibited two CD peaks of opposite sign. Thus, it seems that the sign of the CD maximum of *S-cis* conformer of *N*-acyl-L-proline differs from that of *S-trans* conformer. A shoulder at *ca.* 245 nm appearing in the negative large CD absorption of MAP and BP, would be ascribed to the overlap of a minor positive CD absorption of the *S-cis* conformer on a major negative one of the *S-trans* conformer.

Experimental

N-Acyl-L-prolines were prepared by the reaction of the corresponding acyl chloride with L-proline according to the method of Sakota.[7] Acetyl-L-proline: mp 114.0—115.5 °C, $[\alpha]^{24}_{D}-79.1$ ($c=1$, methanol). Isovaleryol-L-proline: mp 70.2—71.9 °C, $[\alpha]^{24}_{D}-81.1$ ($c=1$, methanol). Isobutyrol-L-proline: mp 123.0—124.1 °C, $[\alpha]^{24}_{D}-86.7$ ($c=1$, methanol). Pivaloyl-L-proline: mp 128.3—129.6 °C $[\alpha]^{24}_{D}-71.9$ ($c=1$, methanol). Benzoyl-L-proline: mp 153.9—154.3 °C, $[\alpha]^{24}_{D}-90.9$ ($c=1$, methanol).

NMR spectra were recorded with a JEOL Model JNM–4H–100 spectrometer operating at 100 MHz. CD spectra were measured with a JASCO Model ORD/UV5 spectropolarimeter.

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