Two Trans and Two Cis Isomers in Caprylolactam in Mixed Solvent of Methanol and Water

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In ^{13}C NMR spectra, four signals of two trans and two cis conformers in caprylolactam were found in a mixed solvent of water and methanol. NMR line broadenings and coalescent phenomena in two conformers occurred at higher temperatures, giving high activation free energy ($\Delta G^{\ddagger}$) for the isomerizations between two trans and cis conformers. $\Delta G^{\ddagger}$ are greatly influenced by the mixed ratio between water and methanol.

A peptide bond is a main unit in structures of proteins and peptides and the structure of the peptide bond (its conformation, coplanality etc.) in each amino acid residue is a important factor in the structural analysis of proteins and peptides. Until now, the conformation of amides and proteins was said in general to be trans type,[1] but cis conformer coexists with trans conformer in some formyl amides.[1,2] In cyclic amide, lactam, it can be expected that cis conformer exist in a small ring lactam and trans conformer in very large ring lactam. About caprylolactam, which has a nine membered ring, there are a few papers, reporting the coexistence of the cis and trans conformers in organic solvents by NMR spectroscopic analyses.[3–6] X ray analyses were also made in the crystal structure of caprylolactam.[7,8]

In this papers, we report on the conformations of caprylolactam in various kinds of solvents by using NMR spectroscopic methods and found two cis and two trans conformers in it. The results of NMR studies of the coexistence of the four conformers, their stability, the activation parameters of the isomerization, and the solvent effects were presented in this papers.

NMR spectra measurements were recorded by a JEOL ECP-800 (800.08 MHz for ^{1}H nucleus), and a JEOL ECP-500 (499.23 MHz for ^{1}H nucleus and 125.53 MHz for ^{13}C nucleus) spectrometers. The chemical shifts were determined in ppm unit from the outer standerd TMS solution.

^{13}C NMR spectra of caprylolactom were measured in various solvents. The solvents were changed from methylene chloride, chloroform, acetone, acetic acid, methanol, and water. ^{13}C NMR spectra were also measured changing the temperatures (-80–88 °C). In all these neat solvents, only two peaks were detected for trans and cis conformers in caprylolactam, which were referred by the previous papers.[2–6]

Furthermore, we tried mixed solvents of deuterated methanol (CD_3OD) and deuterium oxide (D_2O) to get more detailed information about the conformers in caprylolactam. In the mixed solvent of CD_3OD–D_2O (vol ratio: 4:1), four peaks for the carbonyl carbon in caprylolactam were found in ^{13}C NMR spectra at 283 K (Figure 1) in contrast of two peaks in above described solvents. These signals were assigned for two peaks at the lower-field to cis conformer and two at the higher-field to trans conformer. The population ratio of cis and trans isomer is ca.7:5. The chemical shift differences ($\Delta\delta$) between the paired signals are 7.9 Hz for the cis carbonyl carbon and 7.2 Hz for the trans one, respectively (Table 1). Other carbon signals besides the carbonyl group show similar splittings for cis and trans conformers.

In the ^{1}H NMR spectrum, the relative integral intensities of the amide proton peaks were 0.021 and 0.025 (proton at 2 position as 1.0) for cis and trans respectively, showing that the amide groups were almost completely deuteriated in this sample.

When the measurement temperature was raised, each paired peaks became broader and the coalescent phenomena were found in several paired peaks. The temperature-dependent ^{13}C spectra of signals of carbonyl and *cis*-C2 carbons were shown in Figures 1 and 2. It is obvious from Figures 1 and 2 that these paired peaks coalesced at higher temperatures. These facts strongly indicate that there are two trans conformers and two cis conformers in caprylolactam in the mixed solvent of methanol and water, which are tentatively named as *trans* 1, *trans* 2, *cis* 1, and *cis* 2 (Figure 1). The ^{13}C signal assignments for *cis* 1, *cis* 2, *trans* 1, and *trans* 2 were systematically made, because

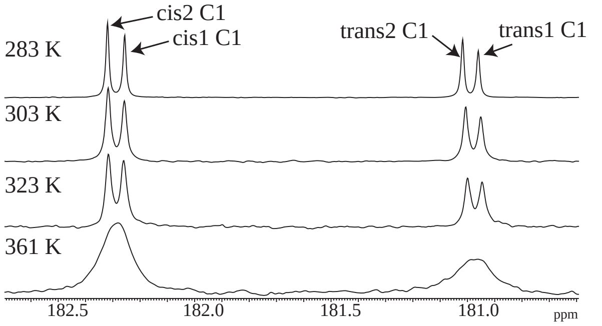

Figure 1. Temperature-dependent ^{13}C NMR spectra of carbonyl carbons of caprylolactam in CD_3OD–D_2O solvent.

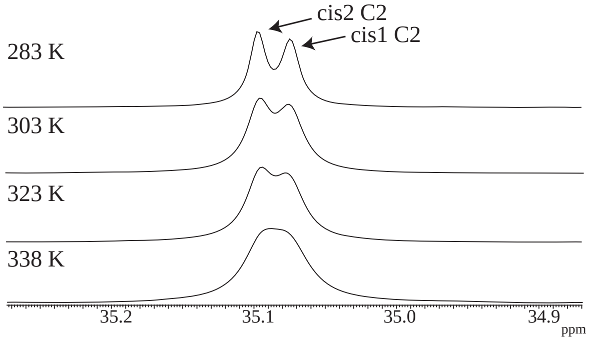

Figure 2. Temperature-dependent ^{13}C NMR spectra of C2 carbons of two cis conformers.

Table 1. ^{13}C NMR chemical shifts of caprylolactam at 283 K in CD_3OD–D_2O (4:1) solvent and the chemical shift differences between the two paired cis and trans conformers

	C1	C2	C3	C4	C5	C6	C7	C8
cis 1 (δ = ppm)	182.25	35.08	27.07	27.56	30.58	25.93	32.03	45.98
cis 2	182.31	35.10	27.07	27.56	30.58	25.93	32.03	45.85
$\Delta\delta$ (Hz)	7.9	2.60	0.00	0.00	0.00	0.00	0.00	16.30
trans 1 (δ = ppm)	180.96	40.06	28.80	27.26	32.08	26.85	31.15	42.74
trans 2	181.02	40.11	28.83	27.30	32.12	26.85	31.19	42.84
$\Delta\delta$ (Hz)	7.2	7.00	2.90	5.50	4.60	0.00	6.50	12.50

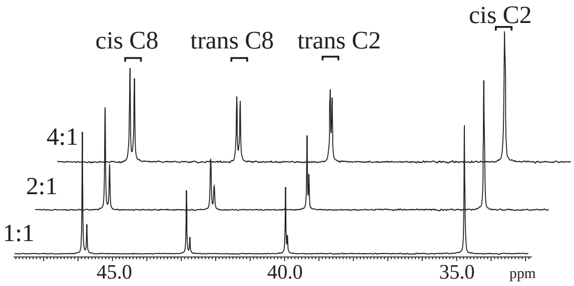

Figure 3. Difference of ^{13}C NMR spectra when volume ratio of solvent (CD_3OD:D_2O) varies from 1:1 to 4:1 at 298 K.

it was found that the population ratio *cis* 1 and *cis* 2, and between *trans* 1 and *trans* 2 are largely dependent on ratio of methanol and water in the mixed solution (Figure 3). From Table 1, it is obvious that in cis conformer, the only signals of the carbons of C1 (carbonyl), C2, and C8 split for two conformers, *cis* 1, and *cis* 2 and that in contrast, in trans conformers, all carbon signals expect C6 clearly split. These results indicate that the structures in *cis* 1 and *cis* 2 conformers differ around only the peptide bond and that other local structures around C3–C7 carbons are very similar between two cis conformers or fluctuate and are averaged in the dynamic behaviors. In contrast to this, the structures of all carbons expect C6 in *trans* 1 and *trans* 2 conformers differ greatly in almost all part of the nine membered lactam ring. This contrast in the ring structures in the four cis and trans conformers probably occurs due to the more severely rigid structure in trans conformer than cis one.

In order to get the information of the isomerizations of four cis and trans conformers, ^{13}C NMR spectra were measured changing the temperature (10–88 °C in the mixed solvent of methanol and water (4:1) (see Figures 1 and 2). Since the populations between the two conformers of cis and trans isomers are in comparable order, the activation free energy ($\Delta G^{\ddagger}$) can be obtained from the coalescence temperature (Tc) method in several carbon signal regions. The results are summarized as follows;

$\Delta G^{\ddagger} = 80.5$ kJ/mol for the isomerization between *cis* 1 and *cis* 2.

$\Delta G^{\ddagger} = 80.7$ kJ/mol for the isomerization between *trans* 1 and *trans* 2.

Similar temperature-dependent ^{13}C measurements in the mixed solvent of methanol and water were made, changing the mixing ratio between two solvents (Figure 3). In the mixed solutions, the population of the *cis* 1 and *trans* 1 decreased along with the decrease of the methanol fraction. This large difference in the population ratio between solvents with different CD_3OD:D_2O ratios causes large difficulty to analyses the NMR line shapes and we cannot determine the activation parameters for the isomerizations at the present time.

In the ^{13}C NMR analyses of caprylolactam in methanol aqueous solvent with the addition of 1 N hydrochloride, almost similar type of temperature-dependent ^{13}C spectra were observed as like as those in methanol–water (4:1) solvent.

Interestingly, in the acidic methanol–water solution, the line-broadenings and the coalescence phenomena in the paired signals occurred at considerably lower temperatures than in the case of methanol–water (4:1) solvent. Since the population ratios between *cis* 1 and *cis* 2 and between *trans* 1 and *trans* 2 in this case are almost equal, the activation free energy ($\Delta G^{\ddagger}$) were determined as 71.5 and 71.3 kJ/mol for the isomerizations of trans and cis conformers, respectively. The $\Delta G^{\ddagger}$ values are remarkably lower than in the neutral mixed solution. The obtained lower $\Delta G^{\ddagger}$ value can probably be explained by the large influence of the protonation on the carbonyl group in the peptide bond of caprylolactam in the MeOH–HCl aqueous solution.

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