Molecular Conformations of 4-Aminomethyl-1-cyclohexanecarboxylic Acids in Aqueous Solution

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(Received December 4, 1980)

The structures of *cis*- and *trans*-4-aminomethyl-1-cyclohexanecarboxylic acids were studied by proton and carbon-13 NMR, and by semiempirical molecular orbital methods (CNDO/2 and MNDO). Both isomers exist in zwitter ionic forms in aqueous solution. It was found that the most stable conformations for them are all staggered forms: the diequatorial conformer in the *trans* form and the axial carboxyl group in the *cis* form. These preferred conformers are similar to those in crystalline states. In these conformers the deformation is very small in the cyclohexane ring of the *trans* form. The atomic distance between the nitrogen of amino group and the carbon of carboxyl group in the preferred conformer of *trans* form is 0.65 nm and that in *cis* form is 0.55 nm. This distance of 0.65 nm will play an important role in the antifibrinolytic effect.

There are two geometrical isomers, *i.e.*, *trans* and *cis* isomers, in 4-aminomethyl-1-cyclohexanecarboxylic acid. The *trans* one (TAMCHA) is used as an orally active antifibrinolytic drug and its modified compounds are synthetic protease inhibitors,[1] while the biological activity of the *cis* one (CAMCHA) is quite weak.[2]

According to the study of Mangyo[3] on the relationship between the structures of various ω-amino carboxylic acids and their biological activities, their antifibrinolytic activities depend on the atomic distances between the nitrogen of amino group and the carbon of carboxyl group, and on the isoelectric point.

Conformations of the *trans* isomer[4] and hydrogen halide salts of both isomers[5] were studied by crystallography; however, the biologically active conformation in aqueous solution was not expected to be similar to that in the solid state. In this paper we report the conformations of both isomers in aqueous solutions determined by NMR; we seek some clues to the difference in their biological activities.

Experimental

Samples and Reagents. All reagents and solvents used in this research were analytical grade and were used without further purification. TAMCHA and CAMCHA were prepared from ethyl *p*-(acetamidomethyl)benzoate.[6] The nitrates of Eu(III), Pr(III), Nd(III), Gd(III), and La(III) with purity more than 99.9% were purchased from Wako Chemical Co. They were used as NMR shift and/or relaxation reagents.

NMR Measurements. The proton NMR spectra of TAMCHA and CAMCHA were observed on a Varian EM-360A (60 MHz) spectrometer using a frequency-sweep mode and a JEOL FX-100 spectrometer operating at 99.6 MHz using Fourier transform (FT) mode. Their carbon-13 NMR spectra were observed on a JEOL-PS-100 spectrometer linked with a PFT-100 FT system at 25.14 MHz and a JEOL FX-100 spectrometer operating at 25.05 MHz using FT mode. The probe temperature was kept at 30 °C. Sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP-d_4) was used as an external reference for determinations of proton and carbon-13 chemical shifts. Data were accumulated in an attached computer using 1000 Hz and 5000 Hz sweep widths for proton and carbon-13, respectively, in 8192 points (resolution: 0.2 Hz for proton and 1.2 Hz for carbon-13).

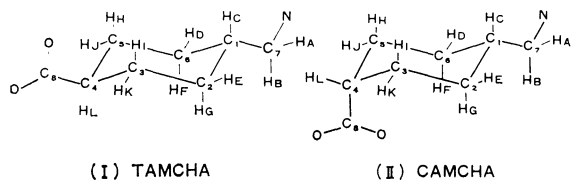
The lanthanoid induced shifts were observed with TAMCHA in D_2O solution by a successive dilution method.[7,8] The initial concentration of TAMCHA was 0.05 M (1 M=1 mol dm^{-3}) for the measurements with Eu(III), Pr(III), Nd(III), and La(III); the molar ratio (ρ) of each lanthanoid ion to the substrate was kept at a fixed value (1.0). The perturbed proton relaxation times were obtained from Gd(III) solution.[9,10] Small amounts of Gd(III) nitrate solution were added successively to the 0.20 M solution of TAMCHA. The concentration of Gd(III) was varied from 5.81 to 118.50 μM at intervals of 5—20 μM; 2-methyl-2-propanol (*ca.* 20 mM) was used as an internal standard. Proton spin-lattice relaxation times (^{1}H-T_1) were measured by the inversion-recovery method using a standard 180 °-*t*-90 ° pulse sequence, where t is the time in seconds between the 180 ° and 90 ° pulses. The 90 ° pulse recycle times were chosen to be at least five times the longest ^{1}H-T_1 time to be measured. Samples for ^{1}H-T_1 measurement were thoroughly deoxygenated with nitrogen in order to prevent any paramagnetic effect of oxygen molecules on T_1 values.

Data Analysis and MO Calculations. The computer programs "NMR-LAOCN-4A"[11] and "FINITE"[12] were applied to analyze the proton NMR chemical shifts in TAMCHA. The electronic densities and the total energies of TAMCHA and CAMCHA were calculated by CNDO/2[13] and MNDO.[14]

Results and Discussion

Assignments. Structures and notations of each atom of TAMCHA and CAMCHA are shown in Fig. 1. 100-MHz proton NMR spectra of both isomers are shown in Fig. 2. Their carbon-13 NMR titration curves are shown in Fig. 3.

Most carbon signals of both isomers were easily assigned by the aid of the off-resonance proton decoupling technique. Carbon pairs (C_2,C_6) and (C_3,C_5) in the spectrum of CAMCHA were assigned, as shown in Fig. 3, from the consideration of the gamma effect.[15]



(I) TAMCHA (II) CAMCHA

Fig. 1. Structures and notation of each atom of TAMCHA (I) and CAMCHA (II).

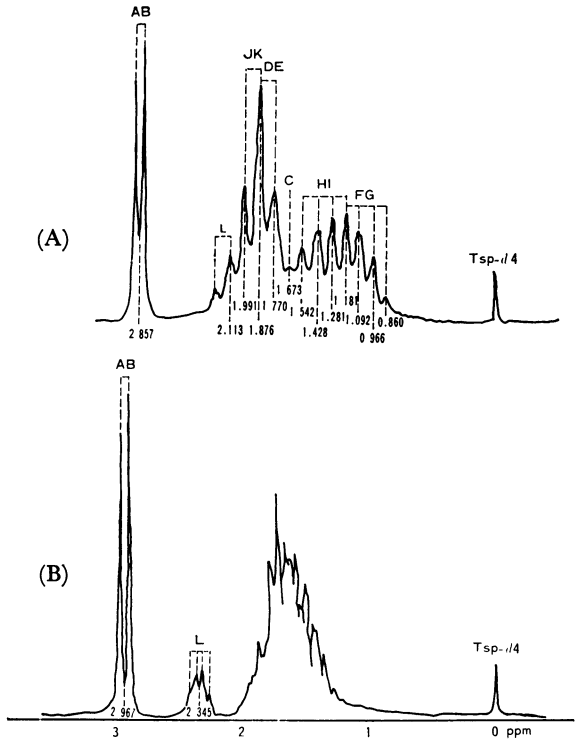


Fig. 2. The 100 MHz-proton FT NMR spectra of AMCHA at 30 °C.
(A): TAMCHA in D_2O (1%(W/V), at pH 7.40),
(B): CAMCHA in D_2O (1%(W/V), at pH 7.20).

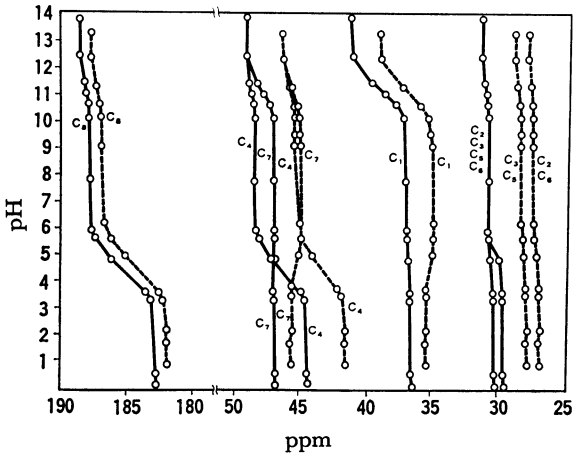


Fig. 3. The pH dependence of the carbon-13 chemical shifts of AMCHA in 15% (W/V) D_2O solution at 30 °C.
——: TAMCHA, ----: CAMCHA.

In the proton NMR spectra of TAMCHA and CAMCHA, the H_A and H_B protons were observed at 2.85 and 2.96 ppm, respectively; they showed a doublet structure due to spin coupling with the H_C proton. The triplet-like signal at 2.11 ppm in TAMCHA and the quartet-like signal at 2.34 ppm in CAMCHA were assigned to the H_L proton because of the selectively proton decoupled carbon-13 NMR spectra and the titration curves of proton NMR shown in Fig. 4. The H_L proton signal of CAMCHA appeared at lower field (by 0.1—0.2 ppm) than that of TAMCHA. An anisotropic effect[16] of the carbon-carbon bond suggest

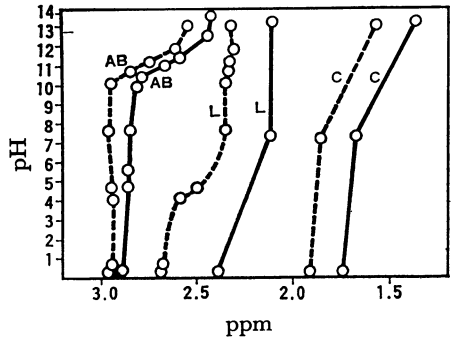


Fig. 4. The pH dependence of the proton chemical shifts of AMCHA in 1% (W/V) D_2O solution at 30 °C.
——: TAMCHA, ----: CAMCHA.
See Fig. 1 about AB, L, C.

that the H_L proton is equatorial and the carboxyl group is axial in CAMCHA.

The signal at 1.67 ppm in TAMCHA was assigned to the H_C proton by the homo-decoupling technique. Other protons of TAMCHA were assigned, as shown in Fig. 2, because of the lanthanoid-induced shift, which produced a fine signal separation while keeping the original spectral pattern.

In the case of CAMCHA, the multiplicity of the H_L proton signals varied on the addition of shift reagent. This trend would suggest some conformational change (such as chair-chair inversion). The assignments of other proton signals were difficult at this stage.

Spin Coupling Constants of TAMCHA. Observed vicinal spin coupling constants are listed in Table 1. $^3J_{AB,C}$ is the representative of $^3J_{A,C}$ and $^3J_{B,C}$ because there is no chemical shift difference between H_A and H_B. Similar notations hereafter are used with similar meanings. Five values among them, *i.e.*, $^3J_{AB,C}$, $^3J_{DE,HI}$, $^3J_{FG,HI}$, $^3J_{HI,L}$, and $^3J_{JK,L}$ were estimated from the 100 MHz proton NMR of TAMCHA to be approximately 6.7, 2.0, 11.0, 11.5, and 3—4 Hz, respectively. The homo-decoupling technique reduced the Pr(III) shifted spectrum to a five-spin system. Refined values of $^3J_{HI,L}$ and $^3J_{JK,L}$ were obtained as 12.04 and 2.49 Hz, respectively, by a computer simulation.[11]

Cyclohexane Ring Conformation of TAMCHA. To clarify whether the observed vicinal coupling constants correspond to those of the chair form, the finite perturbation theory[12] (INDO approximation) was applied. Simple model compounds which are supposed to include some parts of the chair form and of the boat form of the TAMCHA molecule were chosen and calculated. Results are shown in Table 2, in which J_g, J_t, J_c, and J_s denote vicinal coupling constants between two protons in *gauche*, *trans*, *cis*, and *skew* positions, respectively. With α-amino acids, the experimental value of J_g was 2.60 Hz and that of J_t was 13.56 Hz;[17] similar values were also obtained in calculation.

Qualitative results suggest that the observed values were those of the chair form.

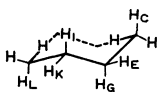
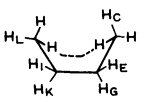
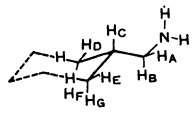
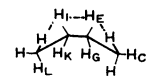
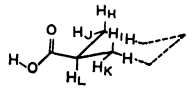
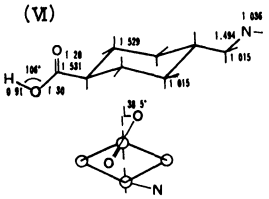
Aminomethyl Group Conformation. Three conformers around the C_1–C_7 bond were possible in the aminomethyl group of TAMCHA and CAMCHA. The Newman projections of the possible conformers are

TABLE 1. THE OBSERVED VICINAL SPIN COUPLING CONSTANTS OF TAMCHA IN AQUEOUS SOLUTION AT 30 °C AND pH 7.40

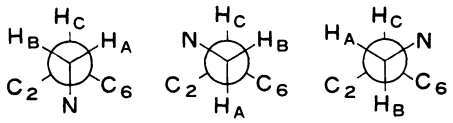
Proton[a]	Dihedral angle[b] Chair form	Dihedral angle[b] Boat form	Notation[c]	Obsd value / Hz
C A and/or B			$^3J_{AB,C}$	6.7
C D and/or E	g	g g	$^3J_{C,DE}$	
C F and/or G	t	t g	$^3J_{C,FG}$	
D and/or E H and/or I	g	c c	$^3J_{DE,HI}$	2.0
D and/or E J and/or K	g	s s	$^3J_{DE,JK}$	
F and/or G H and/or I	t	s s	$^3J_{FG,HI}$	11.0
F and/or G J and/or K	g	c c	$^3J_{FG,JK}$	
L H and/or I	t	g t	$^3J_{HI,L}$	11.5(12.0$_4$[d])
L J and/or K	g	g g	$^3J_{JK,L}$	3—4 (2.4$_9$[d])

a) See Fig. 1. b) g, t, c, and s denote *gauche*, *trans*, *cis*, and *skew*, respectively. c) See text. d) Refined value obtained by a computer simulation.[11]

TABLE 2. THE CALCULATED[a] VICINAL COUPLING CONSTANTS OF THE MODEL COMPOUNDS OF TAMCHA

	Calcd value/Hz		Calcd value/Hz
(I) 	$^3J_{I,E}=2.2_2$ (J_g)[b]	(IV) 	$^3J_{I,E}=9.7_5$ (J_c)
	$^3J_{I,G}=13.5_4$ (J_t)		$^3J_{I,G}=4.4_1$ (J_s)
	$^3J_{I,L}=13.8_6$ (J_t)		$^3J_{I,L}=2.1_1$ (J_g)
	$^3J_{K,E}=2.3_3$ (J_g)		$^3J_{K,E}=4.4_1$ (J_s)
	$^3J_{K,G}=2.2_2$ (J_g)		$^3J_{K,G}=9.7_5$ (J_c)
	$^3J_{K,L}=2.4_6$(J_g)		$^3J_{K,L}=2.1_1$ (J_g)
(II) 	$^3J_{A,C}=2.3_2$ (J_g)	(V) 	$^3J_{I,E}=9.7_5$ (J_c)
	$^3J_{B,C}=13.8_4$ (J_t)		$^3J_{I,G}=4.4_1$ (J_s)
	$^3J_{C,D}=2.3_8$ (J_g)		$^3J_{I,L}=13.9_8$ (J_t)
	$^3J_{C,E}=2.3_4$ (J_g)		$^3J_{K,E}=4.4_1$ (J_s)
	$^3J_{C,F}=13.6_7$ (J_t)		$^3J_{K,G}=9.7_5$ (J_c)
	$^3J_{C,G}=13.7_2$ (J_t)		$^3J_{K,L}=2.7_5$ (J_g)
(III) 	$^3J_{L,H}=14.3_0$ (J_t)	(VI) 	
	$^3J_{L,I}=13.6_7$ (J_t)		
	$^3J_{L,J}=2.3_2$ (J_g)		
	$^3J_{L,K}=2.1_6$ (J_g)		

a) Calculations were carried out by the finite perturbation theory[12] (INDO approximation). The geometrical parameters for calculations are shown in (VI). The bond angles were assumed as a tetrahedral about sp^3 atom and 120.0° about sp^2 atom. b) J_g, J_t, J_c and J_s denote vicinal coupling constants between two protons in *gauche*, *trans*, *cis*, and *skew* positions, respectively.



(a) (b) (c)

Fig. 5. The possible conformers about the C_1–C_7 bond of AMCHA.

shown in Fig. 5. In solutions TAMCHA and CAMCHA exist as a mixture of these rapidly interconverting rotational isomers. By assuming that J_t, the coupling constant between two hydrogens in the *trans* position with respect to each other, and J_g, the coupling constant between *gauche* hydrogens, are constant for all three isomers, observed vicinal coupling constants $J_{C,A}$ and $J_{C,B}$ are given by

$$J_{C,A} = P_a J_g + P_b J_t + P_c J_g \quad (1)$$

$$J_{C,B} = P_a J_g + P_b J_g + P_c J_t \quad (2)$$

$$P_a + P_b + P_c = 1, \quad (3)$$

where P_a, P_b, and P_c are the populations of the conformers a, b, and c. Strictly speaking, P_b would not be equal to P_c because of a small amount of rotation in the carboxyl group around the C_4–C_8 bond. But because spin-spin coupling is induced through this bond and the carboxyl group is far from the C_1–C_7 bond, P_b can be assumed to be equal to P_c. The fact that only one J value was observed in the present study suggested that $J_{C,A}$ could be equal to $J_{C,B}$ (A_2X spin system assumption). P_a, P_b, and P_c were calculated from Eqs. 1, 2, and 3 with the values J_t(13.84 Hz) and J_g(2.32 Hz) obtained from the model compound (II). P_a and P_b (equal to P_c) are estimated to be 0.240 and 0.380 in TAMCHA

(J_{obsd} was 6.7 Hz); the values in CAMCHA are 0.170 and 0.415 (J_{obsd} was 7.1 Hz). The following equilibrium would be described by either an equilibrium constant K or a free energy ΔG:

$$P_b(=P_c) \rightleftharpoons P_a;\ K = P_a/P_b;\ \Delta G = -RT\ln K.$$

The values of K are 0.632 and 0.410, and the ΔG values (at 30 °C) are 1.159 and 2.248 kJ mol^{-1} in TAMCHA and CAMCHA, respectively.

Table 3. The observed relaxation and shift ratios for TAMCHA

^1H[a]	Relaxation ratio	Shift ratio		
	Gd(III)	Eu(III)	Nd(III)	Pr(III)
L	1.00	1.00	1.00	1.00
JK	0.89	0.69	0.59	0.61
HI	0.54	0.40	0.54	0.59
C	0.21	0.19	0.13	—[b]
DE	0.18	0.24	0.25	—[b]
FG	0.17	0.29	0.32	—[b]
AB	0.01	0.12	0.15	0.15

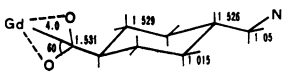
a) See Fig. 1. b) Observation was difficult due to the overlapping of other signals.

Relative Relaxation Rate and Relative Induced Shift. To confirm the above results, NMR observations were carried out with additions of shift and/or relaxation reagents. The relative values of the relaxation rate in the presence of Gd(III) are listed in Table 3 with respect to relaxation rate of H_L. Values of paramagnetically induced shift by Eu(III), Nd(III), and Pr(III) are also listed in Table 3, where the shift of H_L is chosen as a reference. These were values obtained after the subtraction of complex formation shifts, which were determined by an addition of diamagnetic La(III). The relative induced shift depends on the inter-atomic vector between the lanthanoid ion and the i-th nucleus (r_i^{-3}) and the angle between this vector and the principal axes of symmetry of the complex,[18] while the relative relaxation rate is approximately proportional to the (r_i^{-6}) term.[9,10,19] Roughly speaking, the conformation obtained from the shift and/or relaxation reagent experiments is consistent with the above results. There remain small discrepancies between them. The calculated atomic distance between Gd(III) and ^1H based on the geometry,[8,20] shown in the footnote in Table 4,

Table 4. The calculated atomic distance between Gd(III) and ^1H

^1H	L	H,I,J,K	F,G	C	D,E	A	B
r_{Ln-^1H}/nm[a]	0.595	0.607	0.801	0.810	0.826	0.997	1.018

a) The geometrical parameters for calculation are given below. As shown in Table 3, the ratios of shifts of different nuclear resonances were found to be independent of the lanthanoid ion; the lanthanoid ion was assumed to be located on the bisector of the O–C–O angle.[8,20]



are listed in Table 4. The observed relative relaxation rate and the calculated values of r_i suggest that a small deformation was produced in the cyclohexane ring, especially at the bond angles of C_2–C_3–C_4 and C_4–C_5–C_6 or the bond angle of C_8–C_4–H_L.

The small deformation in the cyclohexane ring could also be illustrated by the R-value.[21] The relationship between the torsional angle (ψ) and the R-value in a ring compound is given by

$$R = J_{trans}/J_{cis} \quad (4)$$

$$\cos\psi = (3/(2+4R))^{1/2}, \quad (5)$$

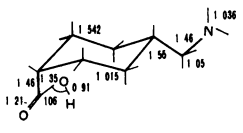
where J_{trans} and J_{cis} are the vicinal coupling constants between two protons in *trans* and *cis* positions, respectively. The R-value of C_3–C_4 bond (or C_4–C_5 bond) of TAMCHA was found to be 4.83 from the refined values listed in Table 1, and the torsional angle about C_3–C_4 bond (or C_4–C_5 bond) was calculated to be 68 degrees from Eq. 5.

Protonation. Lowering of pH values caused the downfield shifts of all proton signals and the upfield shifts of all carbon signals (except the signals of C_1 and C_7). These shifts were observed at pK_1 corresponding to protonation of carboxyl group; they might be accounted for by the changes of the net charges which are produced by the protonation of amino group.[22] Each carbon-hydrogen bond is polarized to C^-–H^+ structure by this protonation and the electron on the hydrogen atom is transmitted onto the positively charged nitrogen atom through the carbon skeleton.

Table 5. Total electron densities of each atom in AMCHA calculated by the CNDO/2 method

	TAMCHA		CAMCHA[a]	
	Neutral	Cation	Neutral	Cation
C_1	3.9777	3.9833	3.9822	3.9848
C_2	3.9961	3.9876*[b]	3.9995	3.9912*
C_3	3.9830	3.9831	3.9790	3.9790
C_4	4.0288	4.0315	4.0311	4.0318
C_5	3.9807	3.9767*	3.9881	3.9827*
C_6	3.9928	3.9967	4.0021	4.0068
C_7	3.9111	3.9246	3.9071	3.9355
C_8	3.6042	3.5984*	3.5988	3.6065
H_A	1.0262	0.9484	1.0291	0.9487
H_B	1.0087	0.9447	1.0095	0.9447
H_C	1.0004	0.9828	1.0001	0.9827
H_D	1.0000	1.0247*	0.9990	1.0223*
H_E	1.0048	0.9899	1.0017	0.9863
H_F	0.9978	0.9843	0.9828	0.9687
H_G	0.9991	0.9845	0.9944	0.9803
H_H	0.9946	0.9842	1.0021	0.9910
H_I	0.9893	0.9779	0.9985	0.9860
H_J	1.0056	0.9821	1.0028	0.9785
H_K	1.0036	0.9764	0.9966	0.9698
H_L	0.9860	0.9767	0.9790	0.9547

a) The geometrical parameters of CAMCHA for calculation were as follows. b) See text.



The downfield shifts of the proton signals are due to the decreases in the total charge densities on the protons and the upfield shifts of the carbon signals are the increases in the total charge densities on the carbons. To estimate quantitatively these polarization effects, the electron densities were calculated by the CNDO/2 method; the results are shown in Table 5. The difference of the net charge on each atom between the neutral molecules and the cationic ones give the qualitative explanation for the protonation shifts, except for the few atoms marked with asterisks.

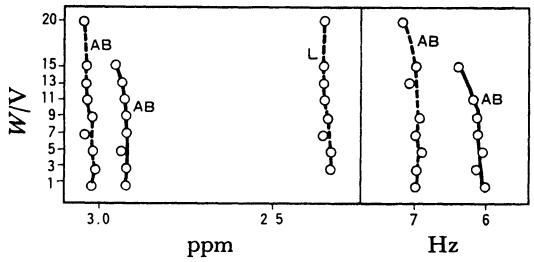


Fig. 6. The concentration dependence of the proton chemical shifts (left) and the proton coupling constants (right) of AMCHA in D_2O solution at 30 °C and 60 MHz.
——: TAMCHA, ----: CAMCHA.
See Fig. 1 about AB, L.

Association. The dependences of the chemical shifts and the coupling constants on the concentration are shown in Fig. 6. A strong hydrogen bond is formed between the solute and the solvent D_2O,[23] and no association of the solute molecules occurred in solutions with the concentration below *ca.* 9% (*ca.* 5.73 mM). The titration curves of carbon-13 chemical shifts (Fig. 3) were drawn at the concentration of 15%, where the association can take place. Small shifts were observed with C_4 and C_8 of TAMCHA and CAMCHA at pK_2 (the protonation of amino group) and the C_1 and C_7 chemical shifts of CAMCHA showed some anormaly in the acidic region.

Semiempirical Molecular Orbital Calculations. To estimate the most stable conformation of TAMCHA and CAMCHA, the conformational energies were calculated by the semiempirical molecular orbital method. The changes of the conformational energies which were caused by the rotations of the aminomethyl group and the carboxyl group were calculated by the CNDO/2 method, based on the assumption that the cyclohexane ring conformations were chair forms free from strain. The calculation was carried out by changing the rotational angle around the C_4–C_8 bond at 30-degree intervals, keeping the N atom *trans* to the position of C_2 to find out the smallest conformational energy value; then the rotational angle around the C_1–C_7 bond was changed at 30- or 60-degree intervals, keeping the carboxyl group fixed. The results are shown in Fig. 7 where the minimum of energy is put to zero as a standard. The curves of the electronic energies were related smoothly with the change of rotational angles, while abnormal changes were observed in the curves of the total energies, in which the contributions of the

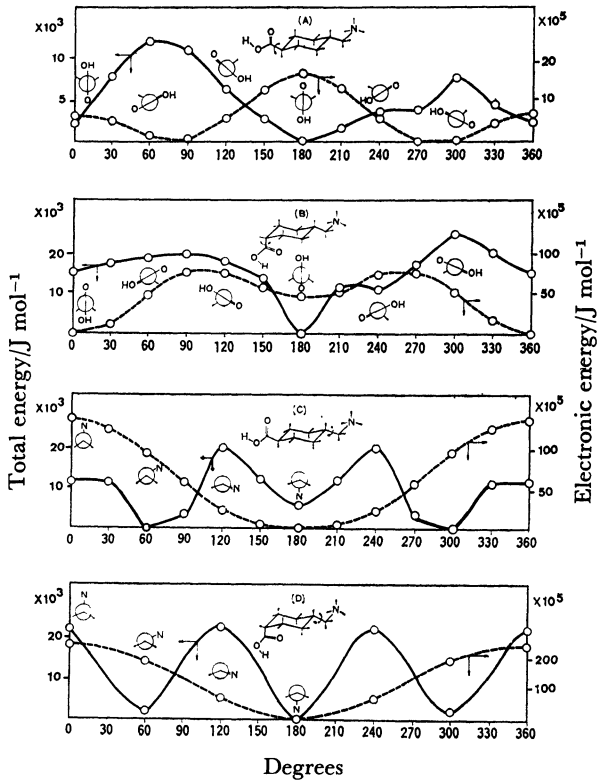


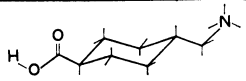
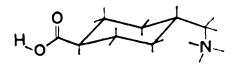
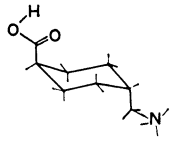
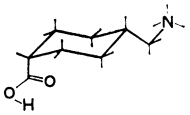
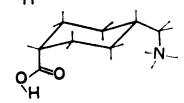
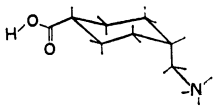
Fig. 7. Relative electronic energies and total energies on the rotational angle about (A) C_4–C_8 bond of TAMCHA, (B) C_4–C_8 bond of CAMCHA, (C) C_1–C_7 bond of TAMCHA, and (D) C_1–C_7 bond of CAMCHA varied respectively and calculated by the CNDO/2 method.
——: Total energies, ----: Electronic energies.

core-core repulsion energies to the electronic energies are taken into account. The CNDO/2 method is inadequate to estimate the energy differences among the conformers.[24] As shown in Table 6, the conformer (IV) was more unstable than that (V) in the calculation of CAMCHA, but this result is inconsistent with the experimental result of the proton NMR. The energies of the conformers were, therefore, calculated by the MNDO method[25,26] at the energy minimum points of the CNDO/2 method. The results are, in this calculation, consistent with proton NMR results.

The energy difference between the diaxial and the diequatorial conformers was also calculated. The results showed the conformer (III) was less stable than (I) by 130.42 kJ mol^{-1} (Table 6), and no equilibrium among them was expected. In the case of CAMCHA, the chair-chair inversion conformer (VI) was more stable than (IV) by 44.617 kJ mol^{-1}. The most probable conformer (IV) would be changed to the conformer (VI) by an addition of the shift reagent. This seems to correspond to the change of the spin-coupling pattern in the proton NMR spectrum due to an addition of the shift reagent.

The Atomic Distance (r_{N-C}) between the Nitrogen of Amino Group and the Carbon of Carboxyl Group. The r_{N-C} was a function of the rotation around the C_1–C_7 bond (aminomethyl group rotation). Mangyo related the

Table 6. The calculated relative conformational energies of AMCHA by the CNDO/2 and MNDO methods, and the atomic distances between N and C_8 (r_{N-C_8})

Conformer	Relative total energy/kJ mol^{-1}			r_{N-C_8} / nm
	CNDO/2	MNDO	NMR[a)]	
(I) 	0	0	0	0.658
(II) 	+5.759	+22.782	+1.159	0.610
(III) 	+1.821	+130.42	—	0.556
(IV) 	0	0	0	0.555
(V) 	−2.269	+3.587	+2.248	0.437
(VI) 	+22.271	−44.617	—	0.551

a) Our proton NMR results.

antifibrinolytic activities of ω-amino carboxylic acids with the distances of r_{N-C}, and concluded that the distance of 0.65 nm is the most active, from the comparison between molecular models with all staggered form and with diequatorial conformation.[3)]

This paper has shown that the most stable conformer of TAMCHA in aqueous solution is the conformer (I) in which the deformation is very small in the cyclohexane ring, that it is a similar conformer to that in the crystal, and that the distance of r_{N-C} is 0.65 nm. CAMCHA exists in aqueous solution as the conformer (IV) in which r_{N-C} is 0.55 nm and does not agree with that value. 6-Aminohexanoic acid is another antifibrinolytic drug and the r_{N-C} of the active conformation is also thought to be the same value of conformer (I). The antifibrinolytic drug is said to interact with plasminogen or plasmine and the distance r_{N-C} of these drugs will play an important role in the antifibrinolytic effect.

The authors wish to express their thanks to Messrs. Atsushi Tomonaga and Hiroshi Chuman of this laboratory for their helpful discussions.

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