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Influence of Temperature and Ionization on Self-Association of Theophylline in Aqueous Solution. Studies by Proton Nuclear Magnetic Resonance Spectroscopy

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Thermodynamic parameters for self-association of theophylline in aqueous solution adjusted to ionic strength 0.20 were determined by proton nuclear magnetic resonance spectroscopy. The results obtained at pD 5.8 were as follows: $\Delta G^\circ = -0.95$ kcal/mol at 30 °C ($K = 4.8\,\text{M}^{-1}$), $\Delta H^\circ = -6.3$ kcal/mol, and $\Delta S^\circ = -18$ e.u. It was found that the driving force for self-association involves electrostatic, polarization, and dispersion interactions, and the contribution of hydrophobic bonding is small. A possible mode of self-association of theophylline is postulated. It was found that self-association of theophylline occurs more easily in the neutral state than in the ionized state.

Keywords——theophylline; self-association; ^{1}H-NMR; dimerization constant; thermodynamic parameter

It has been demonstrated by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy that theophylline molecules are associated in aqueous solution, and the mode of the association is considered to be vertical stacking.[1] The self-association constant for the dimer has been determined, though the enthalpy and entropy changes have not been determined. Therefore, we tried to determine these parameters by ^{1}H-NMR spectroscopy to clarify the mechanism of self-association. The thermodynamic parameters for self-association of caffeine[2] have been determined.

Theophylline is a weakly acidic substance releasing the proton from >NH at position 7 by ionization (pK_a, 8.64).[3] On the other hand, in acidic medium, protonation may occur due to the basicity of =N– (pK_a, 0.7)[4] at position 9. Accordingly, we investigated how the degree of self-association changes in the relation to such ionization.

Experimental

Materials——Reagent-grade theophylline supplied by Tokyo Chemical Industry Co., Ltd. was used without further purification. Theophylline solutions ranging in concentration from 1.0×10^{-3} to 4.3×10^{-2} M were used. Deuterium oxide (99.8%), deuterium chloride (20% in deuterium oxide), and sodium deuteroxide (30% in deuterium oxide) were used as received from Merck Co. Other chemicals were of reagent grade. Na_2DPO_4 and KD_2PO_4 were

obtained by treating Na_2HPO_4 and KH_2PO_4, respectively, with deuterium oxide followed by evaporation of the solvent.

^{1}H-NMR Spectra——^{1}H-NMR spectra were recorded in deuterium oxide on a Varian XL-200 (200 MHz) spectrometer with tetramethylsilane (TMS) as an external reference, because a slight interaction was observed when an internal reference, 2,2-dimethyl-2-silapentane-5-sodium sulfonate (DSS), was used in a preliminary experiment. Temperatures of measurement were 0, 15, 30, and 40 °C, each controlled to ±0.2 °C. The pD values of the solution were adjusted with NaOD, DCl, Na_2DPO_4, and KD_2PO_4, and are given as the readings of the pH meter without correction. The ionic strength of the solution was held at 0.20 by using NaCl. Bulk susceptibility corrections to chemical shifts of theophylline were examined, but were found to be unnecessary, because the volume magnetic susceptibilities of the test solutions are equal to that of the medium having no theophylline, owing to the low concentrations of theophylline used. The chemical shifts were reproducible to better than 0.003 ppm.

The conditions for Fourier transfer measurements were: spectral width, 2400 Hz; pulse width, 3.0 μs (flip angle, about 40 °); acquisition time, 3 s; number of data points, 16384; number of transients, 40—800.

Calculation——Calculations of K and $\delta_d - \delta_m$ in Eq. 4 were carried out by using the SALS program, based on the iterative least-square method on an NEC ACOS system 1000 computer at the Computer Center of Osaka University.

Results and Discussion

Thermodynamic Parameters of Self-Association

The ^{1}H-NMR spectrum of 0.003 M theophylline at pD 6.1 shows signals due to 8-H, 3-Me, and 1-Me at 7.27, 2.79, and 2.59 ppm (from external TMS), respectively, with the relative intensity of 1 : 3 : 3 at 30 °C. At 15 °C these signals are observed at 7.32, 2.81, and 2.62 ppm, respectively. At 0 °C they are observed at 7.41, 2.95, and 2.75 ppm, respectively, and at 40 °C at 7.25, 2.77, and 2.58 ppm, respectively. Figure 1 shows the dependence of the chemical shifts of these protons on concentration of theophylline at 30 and 15 °C. All the signals moved significantly upfield with an increase in concentration. This was also the case at 0 and 40 °C. The magnitude of upfield shifts was in the order 0 > 15 > 30 > 40 °C. These upfield shifts may be caused by an increase of the number of vertically stacking molecules with increase of concentration. It is known that 8-H, 1-Me, and 3-Me are located above the pyrimidine or

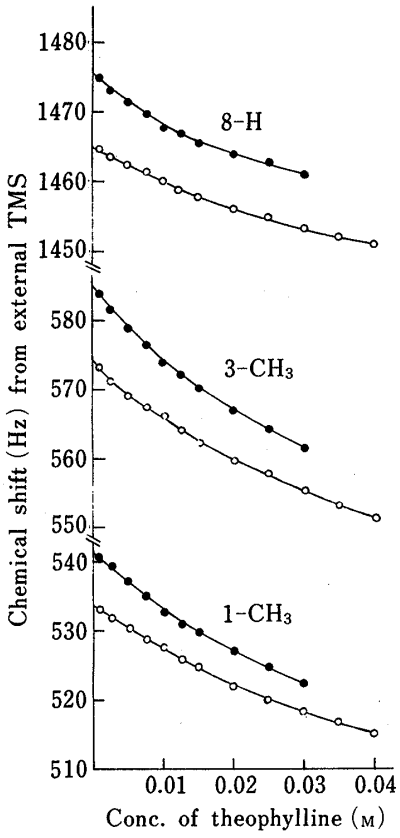

Fig. 1. Concentration Dependence of Theophylline Proton Chemical Shifts at pD 5.8
○, at 30 °C; ●, at 15 °C.

imidazole ring, and so that signals are shifted upfield by the magnetic anisotropy effects due to >C=O, >C=C<, and >C=N. Further, ring current effects of the pyrimidine and imidazole rings contribute significantly to the upfield shifts. The magnitude of the shift was in the order 3-Me > 1-Me > 8-H at all four temperatures.

The dimerization reaction may be written as follows;

$$2A \underset{}{\overset{K}{\rightleftarrows}} A_2 \tag{1}$$

where K is the dimerization constant. The chemical shift observed, δ_{obs}, is given by Eq. 2 under the condition that chemical exchange is fast (in present system, this condition was fulfilled),

$$\delta_{obs} = f_m \delta_m + f_d \delta_d \tag{2}$$

where f_m and f_d represent the fractions of monomer and dimer, and δ_m and δ_d are the chemical shifts in the respective state. Using the initial concentration $[A_0]$, Eq. 3 is obtained from Eq. 2

$$\delta_{obs} = \frac{[A_0]-2[A_0]}{[A_0]}\delta_m + \frac{2[A_0]}{[A_0]}\delta_d \tag{3}$$

Using K, Eq. 3 can be rewritten to give Eq. 4.

$$\delta_{obs} = \frac{1+4[A_0]K-(1+8[A_0]K)^{1/2}}{4[A_0]K}(\delta_d - \delta_m) + \delta_m \tag{4}$$

Because δ_m and δ_d can not be measured directly, the most probable values of K, δ_m, and δ_d were calculated by the method described in Experimental. The computed values of K and the dimer shift, $\delta_d - \delta_m$ are listed in Table I. It was assumed that theophylline associates to form only the dimer, because it has been reported that even caffeine[5] which tends to associate more readily than theophylline, associates to form only the dimer at concentrations below 0.04 M. Thermodynamic parameters were also derived by using Eqs. 5, 6, and 7

$$\Delta G^\circ = -2.303 \boldsymbol{R} T \log K \tag{5}$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{6}$$

$$\log K = -\frac{\Delta H^\circ}{2.303 \boldsymbol{R} T} + \text{constant} \tag{7}$$

where ΔG°, ΔH°, ΔS°, $\boldsymbol{R}$, and T are standard molar Gibbs free energy change, standard enthalpy change, standard entropy change, the gas constant, and absolute temperature,

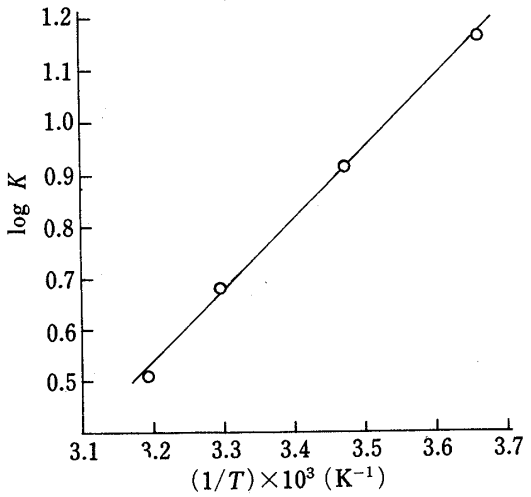

Fig. 2. The van't Hoff Plot of Data in Table I

TABLE I. Dimer Shift, Dimerization Constant, and Thermodynamic Parameters for Dimerization of Theophylline at pD 5.8[a]

Temp. (°C)	$\delta_d-\delta_m$[b] (Hz)			K[b,c] (M^{-1})				$\Delta G°$ (kcal/mol)	$\Delta H°$[e] (kcal/mol)	$\Delta S°$[e] (e.u.)
	1-CH_3	3-CH_3	8-H	1-CH_3	3-CH_3	8-H	Mean			
40	−85	−107	−64	3.4	3.2	3.1	3.2	−0.73		
30	−78	−98	−60	4.9[d]	4.8[d]	4.7[d]	4.8	−0.95		
15	−71	−88	−56	8.2	8.4	8.2	8.2	−1.21	−6.3±0.2	−17.6±0.7
0	−61	−77	−48	14.5	14.8	14.4	14.6	−1.46		

a) The ionization of theophylline does not occur at pD 5.8. *b*) Average probable error; ±8% for $\delta_d-\delta_m$ and ±6% for K. *c*) Reported values for caffeine (reference 2); $K=13\,M^{-1}$ at 25 °C, $\Delta H°=-3.4$ kcal/mol, and $\Delta S°=-6.9$ e.u. *d*) Reported values for 1-CH_3, 3-CH_3 and 8-H (reference 1) 5.9, 6.0, and 3.8 M^{-1}, respectively. It is considered that our values for 8-H are similar to those for 1-CH_3 and 3-CH_3 because the pD of theophylline solution was constant and the dissociation of >NH at position 7 by dilution was depressed. *e*) These values were determined by least-squares fitting of the plots to the van't Hoff equation.

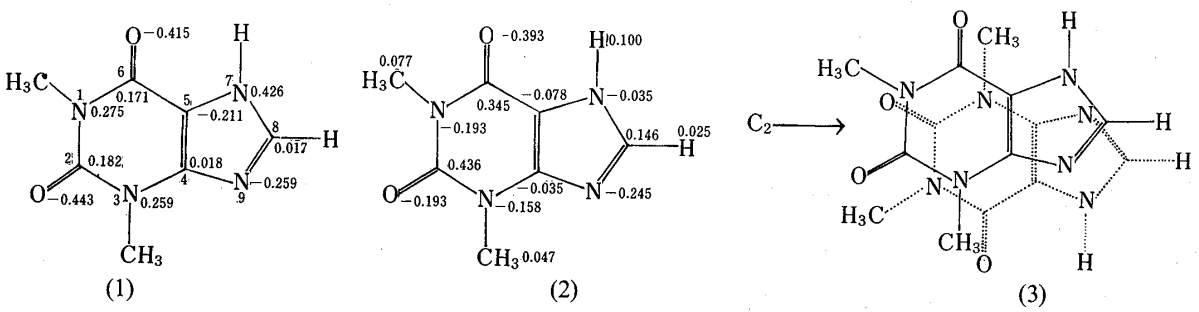

Fig. 3. π-Electron Charge Distribution (1), Total Atom Charge Distribution (2), and a Possible Mode of Association (3) for Theophylline

respectively. The van't Hoff plot of the data based on Eq. 7 is shown in Fig. 2. The thermodynamic parameters obtained are listed in Table I. The driving force for the self-association of theophylline is the enthalpy term, while the entropy term decreased. These results are similar to those obtained for purine derivatives by heat measurement and vapor pressure osmometry.[2] Judging from the ^{1}H-NMR spectrum, the driving force of vertical stacking is not hydrogen bonding but is a combination of electrostatic, polarization, and dispersion interactions. The self-association constant of theophylline was smaller than that of caffeine,[6] but the decreases in standard enthalpy and entropy changes were greater than those of caffeine (see footnotes of Table I). The greater decrease in standard enthalpy change may be due to the fact that theophylline has no methyl group at position 7 and is a less hydrophobic (more polar) molecule than caffeine. Therefore, the interactions between theophylline molecules mentioned above are stronger than those between caffeine molecules. The greater decrease in standard entropy change in the case of theophylline than in the case of caffeine is due to the fact that theophylline is a less hydrophobic molecule, and therefore the breakage of iceberg structure caused by vertical stacking is less. Namely, the contribution of hydrophobic bonding is small for vertical stacking.

We have calculated the π-electron charge distribution and also the total atom charge distribution for theophylline by the CNDO/2 method (Fig. 3). The geometry was obtained from the literature.[7] Electrostatic, polarization and dispersion forces were considered to be the driving force for vertical stacking of theophylline. It has been reported[8,9] that for distances which are not significantly larger than the dimensions of the molecules, the dipole approximation is inadequate and the monopole one, *i.e.*, considering all the negative and positive charges in the system as interacting in a single coulombic fashion, is adequate. It has

also been shown that in the case of dimers of nucleic acid bases stacked in parallel planes (vertical stacking), only the electrostatic interaction (monopole interaction) depends strongly upon the orientation of the molecules in the dimer.[10] That is, the overlapping between a position of more positive charge and one of more negative charge or between a position of less positive charge and one of less negative charge may occur. Therefore, the orientation of the molecules in the dimer may be fixed. In view of these charge distributions and the order of upfield shift of each proton signal, a possible mode of association of theophylline is postulated (Fig. 3). This can be explain qualitatively why the magnitudes of the upfield shifts are in the order 3-Me > 1-Me > 8-H; 3-Me is located above the pyrimidine and imidazole rings and above >C=O, 1-Me is located above the pyrimidine ring and above >C=O, and 8-H is located above the imidazole ring alone.

Influence of Ionization on Self-Association

The ^{1}H-NMR spectra of theophylline in deuterium oxide at pD 0.5, 8.1, 6.1, and 12.1 were measured. Chemical shift changes for 8-H, 3-Me, and 1-Me of theophylline are illustrated in Fig. 4 as a function of theophylline concentration. In acidic solution, the change of chemical shift for 8-H was very large and those for 3-Me and 1-Me were not pronounced. Figure 5 shows the changes of chemical shift with change of pD the concentrations of 0.03 and 0.001 M. As shown in Fig. 5, the signal of 8-H proton shifted upfield with increase of pD. This can be explained as follows. In acidic solution, protonation occurs at =N– at position 9 and this results in a large downfield shift of the signal of the 8-H proton adjacent to =N–. As pD

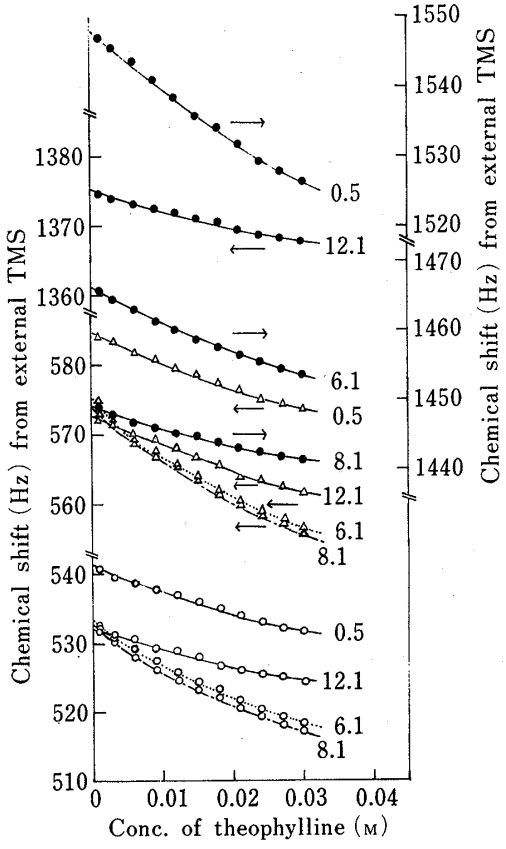

Fig. 4. Concentration Dependence of Theophylline Proton Chemical Shifts at 30 °C at Various pD Values

Numbers represent pD of the solution. ○, 1-CH_3; △, 3-CH_3; ●, 8-H.

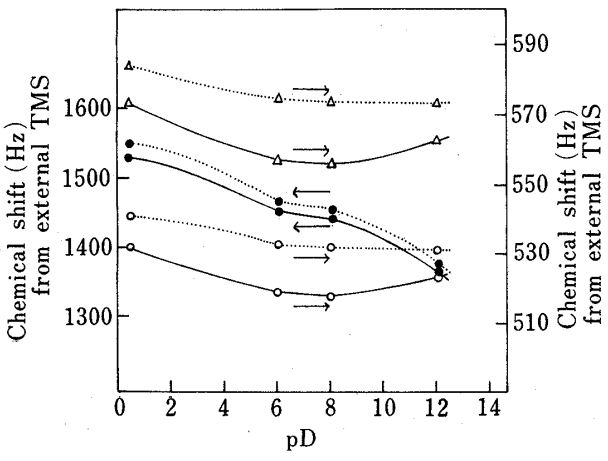

Fig. 5. Influence of pD on Theophylline Proton Chemical Shifts in 0.001 M (------) and 0.03 M (——) Concentrations at 30 °C

○, 1-CH_3; △, 3-CH_3; ●, 8-H.

TABLE II. Influence of Ionization of Self-Association

pD	Proportion of ionization[a] (%)	$\delta_d - \delta_m$[b] (Hz)			K[b] (M^{-1})		
		1-CH_3	3-CH_3	8-H	1-CH_3	3-CH_3	8-H
0.5	61 (=NH^+–)	−89	−110	−211	2.1	2.0	2.2
6.1	1 (>N^-)	−81	−101	−66	4.7	4.7	4.9
8.1	22 (>N^-)	−110	−126	−70	2.8	2.7	2.8
12.1	100 (>N^-)	−178	−214	−150	0.7	0.8	0.8

a) The value was calculated from pK_a of theophylline. *b*) Average probable error; ±9% for $\delta_d - \delta_m$ and ±8% for K.

is increased the proton is released from $=NH^+$ at position 9, resulting in an upfield shift. In the case of 0.03 M theophylline concentration, the upfield shift owing to stacking is added to the upfield shift owing to deionization. In alkaline solution, the proton is released from >NH at position 7 to give >N^-, and the signal of 8-H adjacent to >N^- is shifted upfield; this effect was so large that the downfield shift due to the dissociation of the associated molecules could not be detected. On the other hand, the signals of 3-Me and 1-Me in 0.03 M solution shifted upfield as the solution changed from acidic to weakly alkaline. These upfield shifts may be due to the increase of the number of associated molecules by stacking because the influences of protonation at $=N^-$ at position 9 and dissociation at >NH at position 7 on these signals seem to be small. The downfield shift in strongly alkaline solution is due to the dissociation of associated molecules. In a dilute solution such as 0.001 M, the upfield shifts of 3-Me and 1-Me signals with pD change were small in acidic to weakly alkaline solutions. In alkaline solution, these signals tended to shift to upfield slightly. This may be because only a few molecules are released by dissociation of associated molecules since there is little stacking, and therefore, the upfield shift caused by deionization can be seen.

The values of K and $\delta_d - \delta_m$ computed from data shown in Fig. 4 are listed in Table II. It is clear that neutral theophylline molecules tend to associate more readily than ionized molecules. It is considered that ionization weakens the self-association of theophylline because of electrostatic repulsion between molecules.

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References and Notes

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