A Symmetrical Model for the Self-association of Xanthines in Aqueous Solution

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The self-association of caffeine, 7-ethyl-, 7-propyl-, and 7-butyl-theophylline, theophylline, 1,3-diethylxanthine, and 3-isobutyl-1-methylxanthine in aqueous solution was explored in this study. Detailed analyses of all the proton shifts in $CDCl_3$ and D_2O solutions were undertaken, measuring both concentration- and temperature-dependence. Operative factors determining the chemical shift values include solvent-structuring effects, polarizability capacities, and hydrogen bonding involving water bridges. Evidence is given to support a symmetric mode of association induced by hydrophobic interaction and hydrophilic hydration between contiguous groups. The chemical shifts of theophylline and two of its analogues were measured in D_2O, CD_3OD, and $CDCl_3$ solutions. Only in CD_3OD did the shifts show different trends which were interpreted on the basis of the solvation capacity of CD_3OD and steric hindrance to the specific solvation.

The non-micellar pattern of self-association of common xanthine derivatives is well known.[1] It is considered to be partly due to the rigidity of the molecules and partly to the presence of hydrophilic nitrogens[2,3] such as those of the imidazole ring. The association has been studied in aqueous and non-aqueous solvents by a variety of methods,[4–10] and n.m.r. spectroscopy has provided useful information about the mode of association;[5,6] a number of models have been proposed to account for the results observed with certain associating xanthine and purine systems.[5,6,8–11] Inverted plane-to-plane stacking has been proposed for caffeine association in aqueous solutions,[5,6] an arrangement which may be termed non-symmetric (N.S.) (see Figure 1). This has been criticized by Fritsche[12] and Kan *et al.*[13] on the basis of n.m.r. evidence; they proposed instead an orthogonal model to account for the data. In none of the models proposed hitherto have the energetics of the association been considered in terms of hydrophobic interactions or involvement of water bridges in stabilizing aggregate structure,[14,15] nor have their implications with regard to the observed negative enthalpy and entropy of association been investigated.[16,17] Neither the non-symmetric nor the orthogonal model are stabilized by hydrophobic bonding or water-bridging, since the appropriate positions are not strategically situated in such dimers.

An alternative symmetric (S) model, also shown in Figure 1, is considered in the present paper. With this model, the stacking alignment provides contiguity of suitable groups enabling maximization of hydrophobic and hydrophilic energy contributions to the association. It is supported by important evidence from high-resolution n.m.r. studies on concentration and temperature effects in two solvents, and also the n.m.r. changes in a sequence of compounds including caffeine (**1**) and 7-ethyl- (**2**), -propyl- (**3**), and -butyl-theophylline (**4**), together with theophylline (**5**) and its analogues 1,3-diethylxanthine (**6**) and 3-isobutyl-1-methylxanthine (**7**).

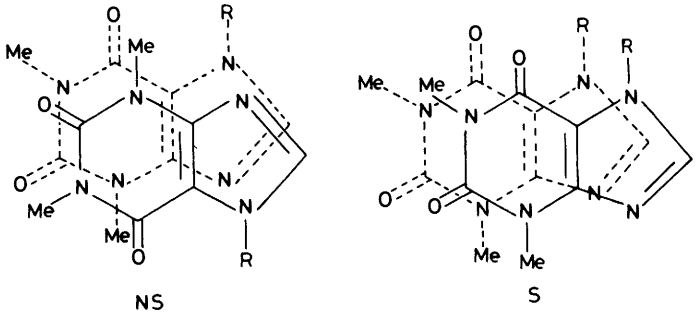
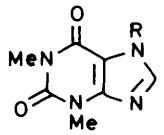
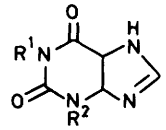


Figure 1. Overlap modes of 1,3,7-trialkylated xanthines (NS non-symmetric, S symmetric)



(1) R = Me
(2) R = Et
(3) R = Pr
(4) R = Bu
(5) R = H



(6) $R^1 = R^2 = Et$
(7) $R^1 = Me$, $R^2 = Bu^i$

Experimental

N.m.r. spectra were obtained using a 300 MHz spectrometer (Bruker, W.H. 300) with an automatic recorder. 2,2,3,3-Tetradeuterio-3-(trimethylsilyl)propionic acid sodium salt (Merck AG Darmstadt, West Germany) in deuterium oxide and tetramethylsilane in deuteriochloroform were the internal standards.

Theophylline and caffeine were anhydrous and of B.P. Standard, 3-isobutyl-1-methylxanthine was purchased from Aldrich Chemical Company, and pure 1,3-diethylxanthine was provided by Prof. F. Bergmann. Reagents were of pure grade: ethyl bromide, propanol, 2-ethoxyethanol, and red phosphorus from Merck, iodine from B.D.H. Chemicals, and butyl bromide from Riedel-De-Haen.

T.l.c. and preparative t.l.c. were carried out on aluminium oxide (GF 254, Stahl, Merck) plates; the developing solvent was chloroform. Column chromatography was performed on a 2:1 mixture of aluminium oxide (Hopkins and Williams, Ltd. Comag, 100—250 mesh alkaline) and sodium carbonate (Frutarom, Haifa).

Preparation of Ethyl- and Butyl-theophylline (**2**) *and* (**4**). These two compounds were prepared by a modification of Schmidt's method.[18] Theophylline was converted into its potassium salt by the action of potassium hydroxide in methanol. The dry salt was treated with a slight excess of the

alkyl halide at 80 °C using 2-ethoxyethanol as solvent (5 ml per 200 mg theophylline used). The reaction was followed by t.l.c., the fluorescing material being viewed under u.v. light, and was stopped when the starting material practically disappeared. The solvent was removed under reduced pressure. The dry material was chromatographed on aluminium oxide–sodium carbonate mixture and eluted using chloroform–carbon tetrachloride (1:1). After evaporation of the solvents a readily crystallized pure substance was obtained in each case.

Preparation of Propyltheophylline (**3**).—Propanol was converted into the corresponding iodide by a known procedure[19] using red phosphorus and iodine. The alkyl iodide was treated, as described above, with the potassium salt of theophylline.

Propyltheophylline (**3**) was isolated and purified from the dry mixture as above by column chromatography (aluminium oxide–sodium carbonate 2:1), m.p. 101—102 °C.

Results

The chemical shifts in $CDCl_3$, and D_2O for caffeine (**1**) and the other 7-*N*-substituted xanthines (**2**)—(**4**) are presented in Tables 1 and 2. Values listed are at corresponding concentrations (10 mM) and temperatures (20 °C) in both solvents. In $CDCl_3$ the same chemical shifts were obtained over the full measurable range (10—50 or 100 mM, according to solubility) and also from room temperature to 57 °C. Solvent displacements of the shifts are presented in Table 3. Table 4 lists shifts measured at different concentrations in D_2O. The peaks were non-composite and very sharp and the protons were identified on the basis of Alexander and Maienthal's assignments.[20] From these data, the following generalizations may be made. (1) The chemical shift values for all the compounds in $CDCl_3$ are both temperature- and concentration-independent, whereas in D_2O some are concentration dependent. (2) The major effect is that of solvent change on the aromatic 8-H protons which undergo a large paramagnetic shift (0.34—0.44 p.p.m. downfield) upon transfer from a non-aqueous environment (Table 3). This is accompanied by a small but significant upfield shift (0.05—0.08) for the pyrimidine ring methyl protons and, in most cases, also for the 7-N_α-methyl or methylene protons.

The chemical shifts of theophylline (**5**) in $CDCl_3$ (Table 1) all lie substantially downfield with respect to the 7-substituted xanthines; for 8-H the shifts are about 0.34 p.p.m., for 1-Me 0.09 p.p.m., and for 3-Me, 0.08 p.p.m. These differences probably reflect in part the absence of the 7-methyl electron-releasing groups, since a parallel displacement occurs in $CDCl_3$ for the pair *N*-methylimidazole and imidazole.[21]

In contrast, the chemical shift values for (**5**) in D_2O are closely similar to those for the 7-substituted theophyllines (Table 2). This implies that the aqueous association is not fundamentally changed by the presence of the 7-NH group in (**5**) but follows a similar stacking pattern.

At the same time, the associated structure of (**5**) in D_2O has to account for the following phenomena. (1) The paramagnetic 8-H shift between $CDCl_3$ and D_2O is much less than in the 7-substituted xanthines (Table 3). (2) With an increase in the concentration in D_2O, both the 8-H and methyl singlets move upfield in (**5**) (Table 4), whereas in the 7-substituted compounds, while the methyl protons shift similarly, the 8-H remains unchanged (in our work; others have found small changes). The pyrimidine methyl proton shifts are in fact more pronounced in (**5**), as has been previously observed.[5] (3) Temperature increase shifts the 8-H signal downfield in (**5**) but upfield in caffeine and its analogues (Table 5), whereas the methyl signals of all the compounds move downfield, those of (**5**) being the most temperature-sensitive. Caffeine and some of the xanthines exhibit peak-splitting, absent in theophylline (**5**) (Table 5). (4) In an equimolecular mixture of theophylline (**5**) and caffeine (**1**) in D_2O, the 8-H of both compounds is significantly though

Table 1. ^1H N.m.r. data in $CDCl_3$ for 7-*N*-substituted theophyllines

7-N group	1-Me[a]	3-Me[a]	7α-Me	7β-Me	7γ-Me	7δ-Me	8-H
Me	3.41	3.60	4.00[a]				7.51
Et	3.42	3.60	4.36	1.52[a]			7.56
Pr	3.42	3.60	4.26	1.92	0.95[a]		7.54
Bu	3.42	3.59	4.29	1.86	1.35	0.96[a]	7.54
H	3.50	3.67					7.85

[a] Methyl group values.

Table 2. ^1H N.m.r. data in D_2O for 7-*N*-substituted theophyllines

7-N group	1-Me[a]	3-Me[a]	7α-Me	7β-Me	7γ-Me	7δ-Me	8-H
Me	3.34	3.52	3.95[a]				7.90
Et	3.35	3.53	4.33	1.46[a]			7.99
Pr	3.35	3.53	4.27	1.83	0.86[a]		7.98
Bu	3.35	3.53	4.30	1.82	1.29	0.90[a]	7.98
H	3.36	3.55					8.01

[a] Methyl group values.

Table 3. Solvent displacements $\Delta(CDCl_3–D_2O)$ of n.m.r. shifts at 10 mM (+ = upfield)

7-N group	1-Me	3-Me	7α-Me	7β-Me	7γ-Me	7δ-Me	8-H
Me	+0.07	+0.08	+0.05				−0.39
Et	+0.07	+0.07	+0.03	+0.06			−0.43
Pr	+0.07	+0.07	−0.01	+0.09	+0.09		−0.44
Bu	+0.07	+0.06	−0.01	+0.04	+0.06	+0.06	−0.44
H	+0.14	+0.12					−0.16

Table 4. Concentration dependence of ^1H n.m.r. data in D_2O for limiting maximum concentration differences*

7-N group	1-Me	3-Me	7α-Me	7β-Me	7γ-Me	7δ-Me	8-H
Me[a]	0.08	0.09	0.04				0
Et[a]	0.07	0.09	0.05	0			0
Pr[a]	0.07	0.08	0.06	0	0		0
Bu[b]	(0.01)	(0.01)	(0.01)	0	0	0	0
H[c]	0.08	0.11					0.05
H[d]	0.06	0.08					0.04

* $c_1 - c_2$ values: [a] Δ 10 mM—100 mM; [b] Δ 5 mM—10 mM (solubility limit); [c] Δ 2 mM—50 mM (solubility limit); [d] Δ 11 mM—50 mM (solubility limit). All values are upfield at the higher concentration.

Table 5. Temperature effects (10—80 °C) on n.m.r. shifts in D_2O[a] (+ = upfield at higher temperature)

7-N group	1-Me	3-Me	7α-Me	7β-Me	8-H
Me[1,2]	−0.03	−0.04	−0.02		+0.02
Et	−0.04	−0.05	−0.03	−0.03	+0.01
Pr[1,2]	−0.03	−0.03	−0.02	−0.02	+0.02
Bu	−0.03	−0.03	−0.02	−0.02	+0.02
H	−0.05	−0.08			−0.04

[a] γ and δ shifts were difficult to measure. Peak splitting or its absence is a most significant feature of the temperature effect. Peak splitting occurs at (1) 80 °C, (2) 50 °C.

Table 6. Comparison of chemical shifts in D_2O of caffeine (1) and theophylline (5) alone and in mixtures containing 50 mM of each[a]

	1-Me	3-Me	7-Me	8-H
50 mM (5)	3.30	3.47		7.97
Mixture	3.25	3.42		7.95
50 mM (1)	3.28	3.45	3.91	7.89
Mixture	3.23	3.39	3.88	7.86

[a] The assignment of signals was made by taking a slight excess of each component in turn.

unequally displaced upfield with respect to the initial values of the separate entities (Table 6).

Discussion

Association in the symmetrical mode would be expected to lead to upfield shifts of the pyrimidine methyl protons as a consequence of the anisotropic effect of the carbonyl groups, provided the methyl protons of each molecule of the pair lie in the shielding zone of the carbonyl group of its partner,[22] which models show to be probable. This is not necessarily the case in the non-symmetrical model in which the methyl groups may be in the deshielding zones of the carbonyl groups of the adjacent molecule.

Self-association in water on concentration increase is expected to be accompanied by a large 8-H upfield shift due to the imidazole ring anisotropy.[13] Its absence indicates that either the 8-H protons lie at a distance which is near the limit of the anisotropic effect, possibly due to lateral displacement of the 8-H positions in the dimer (as in the orthogonal model proposed by Fritsche[12] and by Kan *et al.*[13]) or that another effect counterbalances this expected diamagnetic change, if the monomers are in fact aligned in a parallel orientation in the dimer or stack. The latter symmetry would be favoured by the existence of water bridges between corresponding polar groups in each paired molecule, for example at the 6-CO and the unsubstituted 9-N, which is known to be one of the sites of strong hydration.[23] The involvement of water bridges or water acting as a 'cement' in a co-operative closed resonance circuit system is a strong stabilizing factor.[14,15] This should decrease the electron charge-densities in the imidazole rings and change the ring current of the system, the effect being to counterbalance the diamagnetic effect of the anisotropy.

In terms of the energy factors, hydrophobic interaction of the 1-, 3-, and 7-N alkyl substituents, supported by water-bridging at the 6-CO and the 9-N positions (with consequent delocalization of charges in the aromatic imidazole ring), and to a lesser degree water-bridging at other contiguous polar groups in the pyrimidine ring (where much less delocalization is possible), would provide the necessary negative free energy change on association in the S-model, since the large favourable enthalpy and positive entropy of hydrophobic hydration would counterbalance the negative entropy of association. The importance of induced polarizability in purine interactions has been stressed[24] and this factor may have to be added to the above energy contribution, bearing in mind however that calculations of molecular orbital energy and polarizability have not taken into consideration the important influence of hydration and water-bridging.

Significantly, the upfield solvent shift of the 7-N methyl protons in caffeine contrasts with that of its 9-N-substituted analogue isocaffeine, in which the 9-N methyl protons are at lower field in D_2O than in $CDCl_3$ solutions.[25]

This can be interpreted in terms of the shielding effect

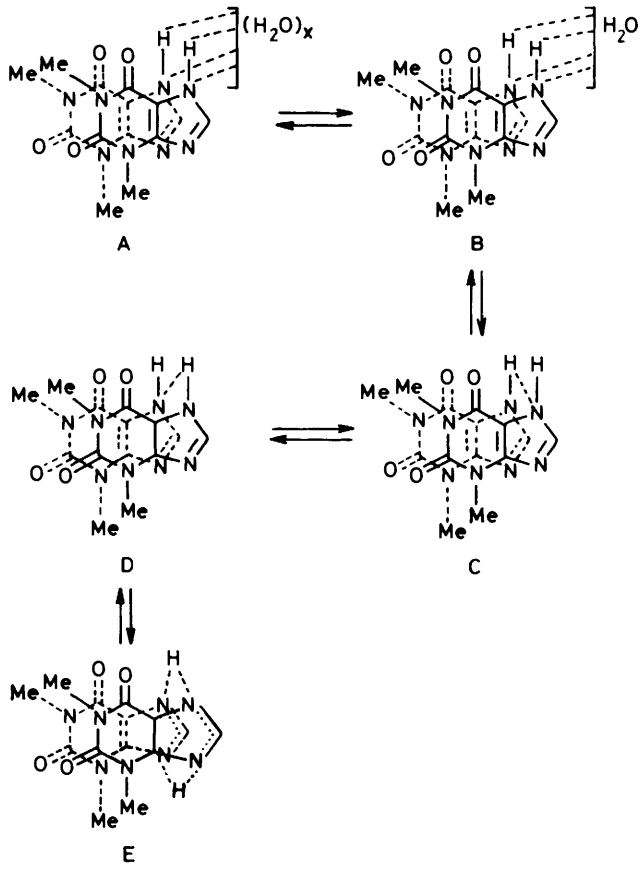


Figure 2. Structure of hydrated and non-hydrated dimers in theophylline (5)

experienced in caffeine on the 7-N substituent protons of the S-dimer in aqueous solution, which is absent in isocaffeine owing to the non-proximity of the C-6 carbonyl group to the 9-N methyl group.

Monomer and Dimer Solvation in D_2O.—In seeking structural causes for the discrepancies in behaviour between theophylline (5) and its 7-substituted derivatives, consideration must first be given to the expected differences in hydration arising from the presence of the acidic proton (pK_a 8.5) at 7-NH in (5) in place of alkyl groups, both in the monomer and on aggregation.

Lowered hydrophobicity and the probability of H-bonding of the 7-N protons to the solvent O atom gives this region a polar profile, with electron enrichment of the imidazole ring and the expectation of an upfield shift at 8-H in going from $CDCl_3$ to D_2O. The 9-N solvation, and that at 6-CO (which is conjugated to the imidazole ring), remain similar in (5) and in the 7-alkyl-substituted compounds, and a strong downfield shift at 8-H is expected in going from $CDCl_3$ to D_2O.

The opposite effects of these solvations on the chemical shifts are thought to be responsible for the 8-H displacement observed during the association of (5) and the $\Delta\delta$ solvent difference between (1) and (5).

Two possible modes in which such an effect could operate are proposed:

(*a*) *The A-B mode.* In this, the solvated monomers develop new intermolecular water-bridging between contiguous 7-NH or 7-N positions by elimination of water molecules, which is responsible for the additional shifts at 8-H. (See Figure 2.)

(*b*) *The C-D-E mode.* Envisaged here is direct H-bonding between 7-NH and 7-N without the involvement of water (C,D, Figure 2). Elimination of water molecules in the transformation to C may itself cause an upfield shift. However, no further chemical shifts will result from internal H-bonding in C, due to the neutralization of opposite charges developed in donor and acceptor N atoms. Furthermore, co-operative solvent involvement could lead to proton transfer from 7-N to 9-N, forming species E or some hydrated modification. In E, the orbital involving the 9-N–8-C double bond changes its character over the stabilized dimer as a whole, partial electron transfer occurring to the 7-N–8-C bond. The molecule hence acquires some of the character of the analogous isocaffeine, where the double bond lies at 7-N–8-C. The important implication is that the 8-H proton of isocaffeine resonates in D_2O and $CDCl_3$ at higher fields[25] than that of caffeine and theophylline in which extended π-conjugation lowers the electron density in the imidazole ring, so that form E would also be expected to show such an upfield shift.

Operation of the scheme presented in Figure 2 in the forward direction on concentration-induced association of (**5**) and the reverse direction on temperature-induced dissociation and separation would account for the respective diamagnetic and paramagnetic 8-H shifts of approximately equal value observed in these two processes, implying similar though not necessarily identical progressive reversibility in the effects of concentration and temperature (see Tables 4 and 5).

In theophylline (**5**), the opposing chemical shift trends at 8-H are unequal in the isothermal concentration-induced association because, in addition to the compensating ring anisotropy and water-bridging at 9-N and 6-CO, there is also the further 7-NH bridging (see Figure 2) which leads to the prevailing upfield effect. Allowing for some temperature-induced desolvation at 9-N and 6-CO similar to that in the 7-alkyl derivatives, the resulting small upfield shift is more than neutralized in (**5**) by the extra desolvation at 7-NH causing a downfield shift, so that the net temperature effect is downfield. Consequently, the basic overall trends in chemical shifts on association in (**5**) appear to be parallel in the concentration and temperature-induced processes.

Considering now the contrasting behaviour of (**5**) and the 7-alkyltheophyllines at 8-H, there must in the latter be almost exact compensation between the upfield anisotropic effect and the downfield water-bridging effect on concentration increase at room temperature. However, there is evidently imperfect compensation in the 7-alkyltheophyllines on temperature increase, and this most probably results from an additional small upfield desolvation component which may be attributed to some weakening of the H-bonding, limited in these compounds mainly to proton donation at 9-N and 6-CO, which does not however change the remainder of the associated structure (see Tables 4 and 5).

On the other hand, reversiblity of the concentration-temperature effects is still observed in the 1- and 3-methyl shifts of all the compounds and the 7-methyl or -methylene shifts of the 7-alkyltheophyllines *i.e.*, diamagnetic on concentration increase, paramagnetic on temperature increase. This behaviour implies a much less pronounced contribution to the chemical shift at these sites by hydration.

The proposed model also accounts for the upfield 8-H shift of both compounds in the equimolar caffeine–theophylline mixture (Table 6). Since this shift is in the same direction as that occurring in (**5**) alone with concentration increase, theophylline evidently associates with the other xanthine and relays to it a similar effect. This could be accounted for by disruption of the specific and non-specific H-bonds around the caffeine (**1**) 7-*N*-methyl position on close approach of the water-bound 7-NH of theophylline (**5**), with the formation of new H-bonds effecting a diamagnetic shift in caffeine. This mechanism could involve the elimination of a water molecule bonded *via* its proton to 7-N electrons in (**1**), and replaced by a 7-NH bond from (**5**) to the same position in (**1**), with denser packing of the stack and a slight reduction in the aromatic character of the imidazole ring, resulting in more localization and less coplanarity at the 7-*N*-methyl. Formation of a mixed dimer species analogous to E by proton transfer from solvent to 9-N and H-bonding from the 7-NH of (**5**) to the 7-N lone pair of (**1**) cannot be disregarded, but its formation seems much less favourable energetically.

Table 7. Chemical shift values of theophylline (**5**), 1,3-diethylxanthine (**6**), and 3-isobutyl-1-methylxanthine (**7**) in D_2O, CD_3OD, and $CDCl_3$

Solvent	(5)	(6)	(7)
D_2O	1-Me 3.36	1α 4.03	1-Me 3.36
		1β 1.21	
	3-Me 3.55	3α 4.13	3-CH_2 3.88
		3β 1.31	CH 2.15
			CH_3 0.92
	8-H 8.02	8-H 8.02	8-H 8.00
CD_3OD	1-Me 3.36	1α 4.07	1-Me 3.34
		1β 1.22	
	3-Me 3.56	3α 4.16	3-CH_2 3.89
		3β 1.32	CH 2.23
			CH_3 0.93
	8-H 7.92	8-H 7.95	8-H 7.85
$CDCl_3$	1-Me 3.50	1α 4.18	1-Me 3.50
		1β 1.31	
	3-Me 3.67	3α 4.25	3-CH_2 4.01
		3β 1.39	CH 2.32
			CH_3 0.98
	8-H 7.85	8-H 7.84	8-H 7.85

In view of the evidence for a stacking association of theophylline (**5**) *via* its hydrophobic groups (*i.e.*, the diamagnetic methyl proton concentration shifts, Table 4) some theophylline analogues containing larger alkyl groups were explored. The n.m.r. spectra of 1,3-diethylxanthine (**6**) and 3-isobutyl-1-methylxanthine (**7**) were studied in $CDCl_3$, D_2O, and CD_3OD solutions. In the two protic solvents an increase in hydrophobic interaction and changes in the degree of specific solvation in the vicinity of the bulky alkyl groups could occur, which could influence the mode and extent of association in these solvents, although probably not in $CDCl_3$. The data (Table 7) show that equivalent protons have closely similar shifts in D_2O and $CDCl_3$ whereas some significant differences at 8-H occur in CD_3OD.

The essential role of water bridging in D_2O association is shown up by the chemical shifts of (**6**) and (**7**) and the anomaly in CD_3OD. The latter solvent lacks the ability of water to form short bridges between stacked molecules, particularly when proton donation by the solvent to both participating stacking molecules is required, as may be the case at 9-N and 6-CO. Again, with the increase in steric demands of bulkier alkyl groups at 1-N and 3-N as in (**6**) and (**7**), access of CD_3OD to bridging positions, particularly those mentioned, is less free.

Among the possible consequences on association are the following: (1) prevention or reduction of stack formation; (2) stack formation with absence of solvent-bridging analogous to that in D_2O; (3) stack formation with alternative solvophobic effects. These represent stages between maximum association and non-association.

The chemical shift values in D_2O (Table 7) are all very similar for 8-H and also for the corresponding 1-Me of (**5**) and (**7**), and the same holds for $CDCl_3$, suggesting similar behaviour of all three compounds in both these solvents. In D_2O then, solvation and bridging evidently occur in (**6**) and (**7**) together with

hydrophobic bonding of the bulky alkyl groups, as shown by their diamagnetic shifts, upfield compared with the data in $CDCl_3$, an observation explained by the proposed symmetrical mode of stacking.

Coming now to the CD_3OD values, the alkyl-proton shifts in (**5**) scarcely differ from the D_2O shifts, whereas the 8-H shift is upfield. Since association of all three compounds is unlikely to be stronger than in D_2O but rather the contrary, the 8-H change is evidently due to alterations in solvation and/or bridging, reflecting either weaker proton donation at 9-N and 6-CO or stronger proton acceptance at 7-NH.

The noticeable features of (**6**) in CD_3OD are the downfield shifts of all 1- and 3-protons compared with the values in D_2O, particularly the α-protons. This probably reflects the reluctance of (**6**) to associate in methanol, but the larger downfield 8-H shift compared with (**5**) in the same solvent suggests retention of solvation capacity by the monomer in the alcohol.

In (**7**), on the other hand, the trend of the 1-*N*-methyl protons is opposite to that of the 1α-protons of (**6**) with the 8-H also shifted upfield to the $CDCl_3$ value. This may be accounted for by two possibilities. The first is that solvation is hindered at 9-N and 2-CO by the voluminous isobutyl group, leading to upfield 1-methyl and 8-H shifts. The appreciable methine group downfield shift (0.08 p.p.m.) could then be the result of its being in the deshielding zone of the carbonyl group, reflecting its proximity to the 2-CO position.

The second possibility arises from increased solvophobic association in (**7**), as compared with (**6**) and (**5**), with greatly diminished solvation in CD_3OD at positions subject to steric inhibition. Desolvation at 9-N would account for the large 8-H upfield shift and at 2-CO for the 1-methyl upfield shift, though these effects could conceivably occur in the monomers as well. Thus the analogous (**6**) and (**7**) exemplify the variations (1)—(3) in suppressed association behaviour, though without extended studies the specific effects cannot be more clearly identified.

In conclusion, we would say that the hydrophobic effect plays an important role in the plane-to-plane stacking of xanthine molecules and functions co-operatively with hydrophilic hydration and possible polarizability effects.

The unique temperature- and concentration-dependence and the solvent effects on the chemical shifts in the xanthine compounds studied support the symmetrical model suggested in the present work and are not readily explicable on the basis of previous non-symmetrical models.

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