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## COMMUNICATIONS

### NMR Evidence for Self-Association of Theophylline in Aqueous Solution

**Keyphrases** □ Theophylline, association in aqueous solution—determination, NMR spectroscopy □ Dimers, theophylline—in aqueous solution, NMR spectroscopy □ NMR spectroscopy—determination, theophylline self-association □ Association, theophylline—aqueous solution

Sir:

In 1957, Guttman and Higuchi (1) observed that the partition coefficient of theophylline between water and an organic phase remained unaltered (at  $\sim 23$ ) over a concentration range (in the aqueous phase) of  $2.3\text{--}28 \times 10^{-3}$  M. This observation and the assumption that theophylline would exist only as the monomer in the nonaqueous phase (chloroform, 90%; isooctane, 10%) led them to conclude that: "for all practical purposes the partition coefficient of theophylline remains a constant over the concentration range studied, indicating the absence of association tendencies." In this respect, theophylline was unique; all the other xanthines examined, including several theophylline derivatives, were found to associate. The idea that theophylline does not self-associate in aqueous solution appears to have been generally accepted (2-5).

We present NMR spectroscopic evidence showing that theophylline does, in fact, associate in aqueous solution. This communication is part of a larger NMR investigation of the various association properties of xanthines.

The high-resolution 100-MHz. NMR spectrum of a 0.004 M  $D_2O$  solution of theophylline (I) has three

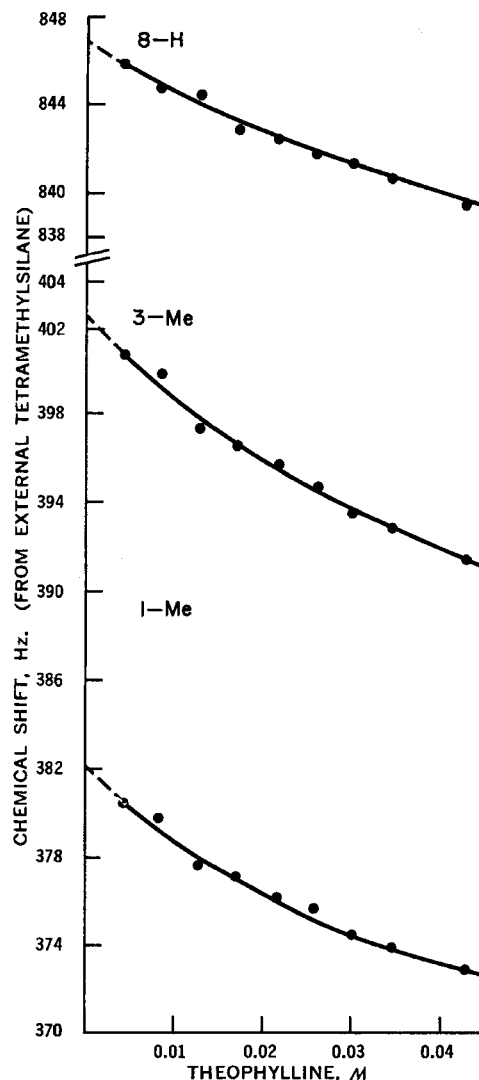
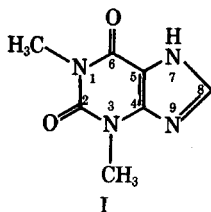
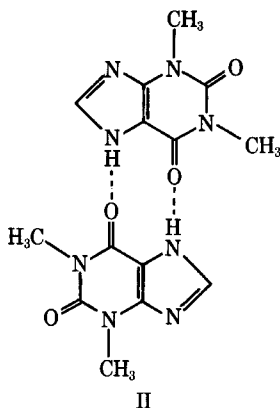


Figure 1—Concentration dependence of theophylline proton chemical shifts at 30° in  $D_2O$ .

signals of relative intensities, 1:3:3, at about 846, 401, and 380 Hz. (from external tetramethylsilane), corresponding to the proton at C-8 and to the 3- and 1-



methyl protons, respectively. Figure 1 shows the concentration dependence of chemical shifts. All the signals move significantly upfield with an increase in concentration. This is in contrast to the unchanging chemical shift to be expected in nonassociating systems. It is well established that upfield changes in chemical shift, as observed here, indicate plane to plane or vertical stacking (6). We have observed a similar chemical shift dependence for caffeine (7). Previous studies, using methods other than NMR, unequivocally showed that caffeine self-associates (1, 8, 9).

For a dimerization process, it can be shown that the observed chemical shift,  $\delta_{\text{obsd}}$ , is:

$$\delta_{\text{obsd}} = \frac{1 + 4MK - (1 + 8MK)^{1/2}}{4MK} \cdot (\delta_D - \delta_M) + \delta_M \quad (\text{Eq. 1})$$

where  $M$  is the molar concentration of theophylline in  $D_2O$ ;  $K$  is the dimerization constant in liter  $\cdot$  mole $^{-1}$ ; and  $\delta_M$  and  $\delta_D$  are chemical shifts of the monomer and dimer, respectively. Because  $\delta_M$  and  $\delta_D$  cannot be measured directly, the best values for  $K$ ,  $\delta_M$ , and  $\delta_D$  were obtained simultaneously by fitting these parameters to the experimental data<sup>1</sup>. The computed values of  $K$  and the dimer shift,  $\delta_D - \delta_M$ , are listed in Table I.

The values of  $K$  and the dimer shifts calculated by the three methods show excellent agreement for a given proton group. The best value of  $K$ , 6.0  $M^{-1}$ , for theophylline can be compared with that for caffeine, 8.6  $M^{-1}$ , also obtained by NMR (7). Both these values are smaller than the values of 11.7  $M^{-1}$  at 30° (1) and 19  $M^{-1}$  at 25° (2) obtained for caffeine by partition methods. The lower value of the dimerization constant for theophylline compared to that for caffeine can be readily understood, considering that the theophylline molecule has one methyl group less than does caffeine. The free proton at N-7 in theophylline provides one more site for hydration. Theophylline is, therefore, less hydrophobic than caffeine and self-associates to a lesser degree in water. A parallel situation is reported by Chan *et al.* (11) who found that the dimerization constant of purine, 2.1  $M^{-1}$ , is smaller than that of 6-methylpurine, 6.7  $M^{-1}$ .

<sup>1</sup> Three computer programs, based upon different methods of minimizing error functions, were used. Two of the programs minimize different error functions and calculate all three parameters. A third program, due to Bangarter and Chan (10), requires a predetermined value of  $\delta_M$ , which can be obtained from the other two programs or by visual extrapolation. Mathematical details of these methods will be presented in a subsequent paper.

**Table I**—Dimer Shifts and Dimerization Constant for Theophylline in  $D_2O$  at 30°

Protons	$\delta_D - \delta_M$ , Hz.			$K$ , $M^{-1}$		
	Method I	Method II	Method III	Method I	Method II	Method III
1-Me	34.6	34.5	34.6	5.9	5.9	5.9
3-Me	41.8	41.8	42.0	6.0	6.0	6.0
8-H	35.8	35.7	38.3	4.0	3.9	3.4

As suggested here, the upfield dimer shifts indicate vertical stacking similar to that found for caffeine (7) and for various structurally analogous purines (6, 11, 12). The experimental errors to which the values in Table I are subject and their confidence limits will be discussed in a subsequent communication. Suffice it to say here that the 8-H signal is quite broad in the very dilute solutions used in this study and, therefore, the  $K$  and dimer shifts derived from the observed chemical shifts are considerably less precise than those for the methyl signals.

It seems worthwhile to consider the reasons why partition studies (1) did not reveal the self-association of theophylline. If theophylline were to associate to the same extent in the nonaqueous phase as in the aqueous phase over the limited concentration range examined, then no detectable change in the partition coefficient would occur. Several purine derivatives with a free proton at N-7, as in theophylline, were shown (13) by NMR and IR studies to self-associate by hydrogen bonding in nonaqueous solvents such as chloroform. In the partition studies, the assumption was made that xanthenes exist only in the unassociated monomeric form in nonaqueous solutions, and it was tested only for caffeine. This assumption probably is valid for all the xanthenes with alkyl substituents at N-7 but not for theophylline. One possible way in which the free proton at N-7 in theophylline could act as a hydrogen-bond donor in intermolecular hydrogen-bond formation is shown in II.

In the crystalline state, theophylline occurs in such a hydrogen-bonded form (14) while, of course, caffeine does not (15). The stronger intermolecular bonding of theophylline in the crystalline state as compared with caffeine is reflected in the higher melting point of theophylline and its more limited solubility in water (1). We have attempted, using both IR and NMR techniques, to observe spectral changes due to hydrogen bonding of theophylline in nonaqueous solvents. The very low solubility of theophylline, however, presents practical problems; as yet, we have not obtained conclusive data.

In summary, NMR data provide evidence that theophylline self-associates to a significant degree in aqueous solution, as do caffeine and other xanthenes. The self-associated complexes of theophylline and caffeine in aqueous solution are alike in configuration and have similar association constants.

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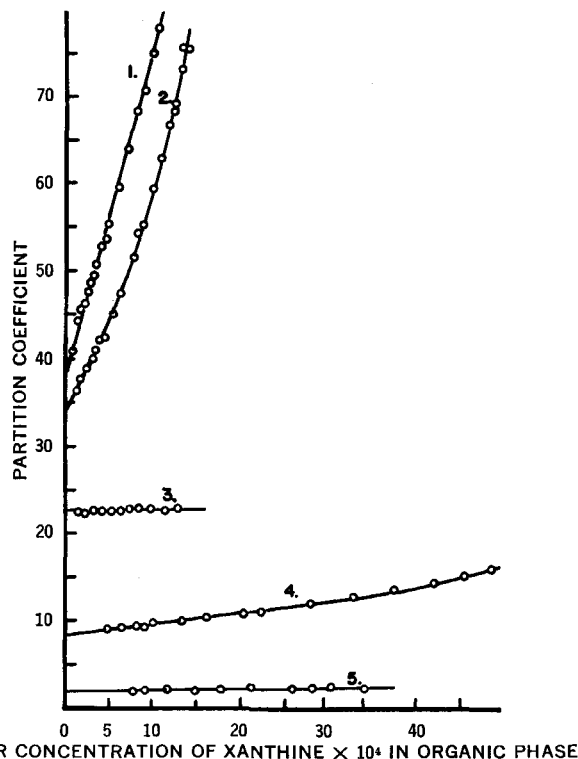
*Note Added in Proof:* While this paper was being considered for publication, S. Ng [*Mol. Pharmacol.*, **7**, 177(1971)] presented IR evidence that theophylline does indeed self-associate in nonaqueous (chloroform) solution. The association is quite strong—even stronger than the association in water—the dimerization constant being  $313 M^{-1}$  at  $25^\circ$ . The structure of the hydrogen-bonded dimer given by Ng is essentially identical to that postulated by us (II). Ng's data confirm our conclusion that our NMR data, when examined with the partition coefficient data of Guttman and Higuchi (1), point to association of theophylline in the nonaqueous phase.

## NMR Evidence for Self-Association of Theophylline in Aqueous Solution: A Response

**Keyphrases**  Theophylline, association in aqueous solution—nonquantitation  Association, theophylline—aqueous solution

Sir:

In all probability, theophylline does dimerize or associate in some fashion in aqueous solution, as suggested by Thakkar *et al.* (1). A total lack of associative tendency is quite unlikely, since it was shown conclusively that closely related derivatives of theophylline readily form not only dimers but also more highly associated species (2). This is evident in Fig. 1, reproduced from our earlier partitioning studies, the increase in distribution coefficients toward water being attributed to formation of multimolecular species in the aqueous phase.



**Figure 1**—A plot of the partition coefficients of a number of xanthines between water and an organic solvent at  $30^\circ$ . The organic solvent used for all of the studies except theophylline was isooctane. In the case of theophylline, chloroform-isooctane (90:10) was used. Key: 1, ethyltheobromine; 2, 7-ethyltheophylline; 3, theophylline; 4, 7-propyltheophylline; and 5, butyltheophylline.

There are two reasons why the associative tendency of theophylline can usually be neglected. As is evident from the NMR data of Thakkar *et al.* (1), even if all of the observed upfield shift is ascribed to dimerization, their calculated dimerization constant ( $3.4$ – $6.0$  l./mole at  $30^\circ$ ) is significantly smaller than that for caffeine ( $12$  l./mole). The other reason is that the solubility of theophylline in water is relatively low and does not permit concentration levels conducive to high degrees of association.

If we accept the NMR values, we can calculate the ratio of dimer to monomer for the average concentration of theophylline used in the study shown in Fig. 1. This ratio comes to 1 in 14 for  $K_D = 6.0$  l./mole or 1 in 23 for the lower  $K_D = 3.4$  l./mole. These values contrast with the associative tendency of caffeine, where more than two-thirds of the total caffeine is present by weight in associated forms at half-saturation in water at  $30^\circ$ . Such small amounts of associated species appear to be nearly undetectable and within experimental error.

Furthermore, in the NMR study, neither  $\delta_M$  nor  $\delta_D$  is obtained directly but is estimated along with  $K$  by simultaneous best fit to the experimental data; therefore, any slight perturbation arising from formation of higher species would produce an out of proportion error in these results. Although the exact methods of mathematical analysis employed are not provided, it would appear that contributions from tetramer formation would tend to yield too low  $\delta_D$  values and, hence, falsely high dimer constants.