Self-association of Caffeine in Aqueous Solution: ^{1}H Nuclear Magnetic Resonance Study

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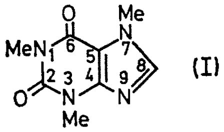
Summary A possible mode of self-association of caffeine in aqueous solution is suggested on the basis of the concentration dependence of chemical shifts.

ALTHOUGH conclusive evidence has been presented for the self-association of caffeine[1-3] and of other xanthine derivatives[1] in aqueous solutions, the mechanism and mode of



(I)

association of these medicinally important molecules have not been elucidated. We report a study of the self-association of caffeine (I) by ^{1}H n.m.r. spectroscopy, a tool which has been widely used for the study of the structurally analogous purine bases and nucleosides.[4,5]

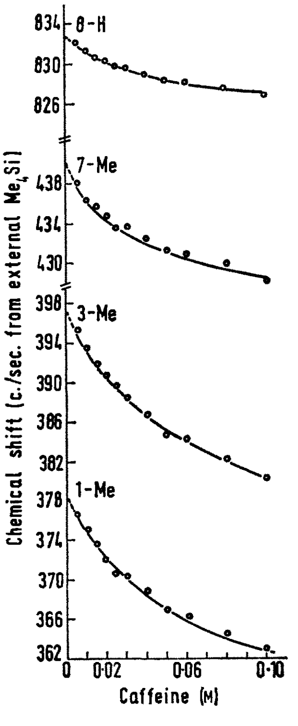


FIGURE 1. *Concentration dependence of caffeine proton chemical shifts.*

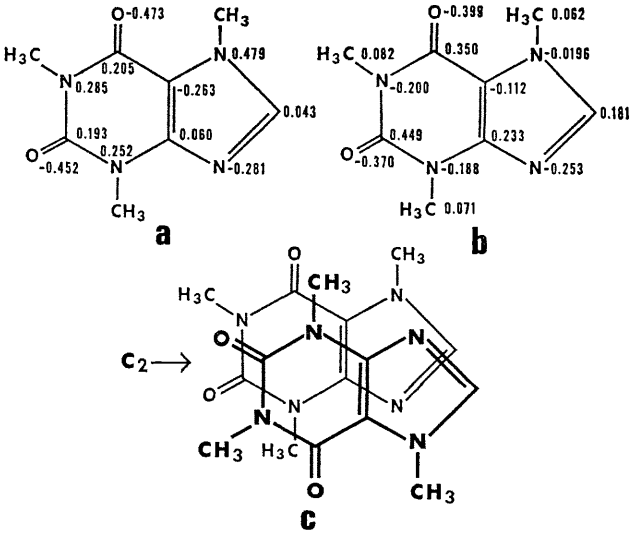


FIGURE 2. (a) *π-Electron charge distribution and* (b) *total atom charge distribution calculated by the CNDO/2 method.* (c) *A possible mode of association for caffeine.*

The high resolution 100 MHz ^{1}H n.m.r. spectrum of a 0·01 M-D$_2$O solution of caffeine has four signals of relative intensities 1 : 3 : 3 : 3 at *ca.* 831, 436, 393, and 375 c./sec. (from external Me$_4$Si). These signals have been assigned[6] to the proton at C-8 and to the 7- ,3- , and 1-methyl protons, respectively. We have confirmed the assignment of the 7-methyl signal from observation of the nuclear Overhauser effect[7] for the 8-vinyl proton upon irradiation of the methyl signal at 436 c./sec.

The ^{1}H n.m.r. spectrum of caffeine was examined over the concentration range of 0·005 to 0·1 M. Figure 1 shows the concentration dependence of chemical shifts of the various protons. The 1- and 3-methyl signals experience upfield shifts of nearly equal magnitude; signals of the 7-methyl and the C-8 protons also undergo significant upfield shifts. The chemical-shift data were treated by the method of Bangerter and Chan.[8] Based on the assumption that association is limited to dimerization, the method requires visual extrapolation of chemical shift data to zero concentration. A digital computer then performs a least-squares search for best values of the dimer shift, $\delta_{\text{dimer}} - \delta_{\text{monomer}}$, and the dimerization constant, K (see Table). The close agreement of the dimer shifts of the 1- and 3-methyl protons indicates that these protons move into similar magnetic environments, different from those of the 7-methyl and 8-H protons.

Dimer shifts and dimerization constant for caffeine in D_2O *at* 30°

Protons	$\delta_{\text{dimer}} - \delta_{\text{monomer}}$ (Hz)	K (l. mole^{-1})	
1-Me	−37·7	5·3	
3-Me	−36·9	7·4	Average K
7-Me	−21·2	12·5	8·6
8-H	−11·3	9·3	

We have calculated the π-electron charge distribution (Figure 2a) and also the total atom charge distribution (Figure 2b) for caffeine by the CNDO/2 method. Assuming a parallel stacking interaction, these charge distributions suggest that the molecules are stacked directly over one another and oriented so that the complex has a C_2 symmetry axis, as shwon in Figure 2c. Such an arrangement would place the 1- and 3-methyl groups under the shielding region associated with the carbonyl groups of the adjacent molecule with the result that the methyl protons on the pyrimidine ring will experience a larger shielding than those on the five-membered ring. The observed chemical shift behaviour is consistent with this.

We thank Prof. B. W. Bangerter (University of Illinois at Chicago Circle) and Prof. S. I. Chan (California Institute of Technology, Pasadena) for providing us with their computer program.

(*Received, February 12th,* 1970; *Com.* 208.)

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[1] D. Guttman and T. Higuchi, *J. Amer. Pharm. Assoc.* (*Sci. Edn.*), 1952, **41**, 10.

[2] P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Amer. Chem. Soc.*, 1963, **85**, 1289.

[2] F. M. Goyan and H. N. Borazan, *J. Pharm. Sci.*, 1968, **57**, 861.

[4] S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. M. Helmkamp, *J. Amer. Chem. Soc.*, 1964, **86**, 4182.

[5] C. D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.*, 1960, **82**, 222; P. O. P. Ts'o, "Molecular Associations in Biology," ed. B. Pullman, Academic Press, New York and London, 1968, p. 39, and references therein.

[6] T. G. Alexander and M. Maienthal, *J. Pharm. Sci.*, 1964, **53**, 962; R. Ottinger, G. Boulvin, J. Riesse, and G. Chiurdoglu, *Tetrahedron*, 1965, **21**, 3435; M. W. Hanna and A. Sandoval, *Biochim. Biophys. Acta*, 1968, **155**, 433.

[7] F. A. L. Anet and A. J. R. Bourn, *J. Amer. Chem. Soc.*, 1965, **87**, 5250.

[8] B. W. Bangerter and S. I. Chan, *J. Amer. Chem. Soc.*, 1969, **91**, 3910.