(5) J. L. Cohen and K. A. Connors, *ibid.*, **34**, 197(1970).
(6) P. O. P. Ts'o, in "Molecular Associations in Biology," B. Pullman, Ed., Academic, New York, N. Y., 1968, p. 39, and references therein.
(7) A. L. Thakkar, L. G. Tensmeyer, R. B. Hermann, and W. L. Wilham, *Chem. Commun.*, **1970**, 524.
(8) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Amer. Chem. Soc.*, **85**, 1289(1963).
(9) F. M. Goyan and H. N. Borazan, *J. Pharm. Sci.*, **57**, 861 (1968).
(10) B. W. Bangerter and S. I. Chan, *J. Amer. Chem. Soc.*, **91**, 3910(1969).
(11) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. M. Helmkamp, *ibid.*, **86**, 4182(1964).
(12) C. D. Jardetzky and O. Jardetzky, *ibid.*, **82**, 222(1960).
(13) K. Hoogsteen, in "Molecular Associations in Biology," B. Pullman, Ed., Academic, New York, N. Y., 1968, p. 21, and references therein.
(14) D. J. Sutor, *Acta Crystallogr.*, **11**, 83(1958).
(15) *Ibid.*, **11**, 453(1958).

ARVIND L. THAKKAR
LOWELL G. TENSMEYER
WILLIAM L. WILHAM
The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, IN 46206

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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°. 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.

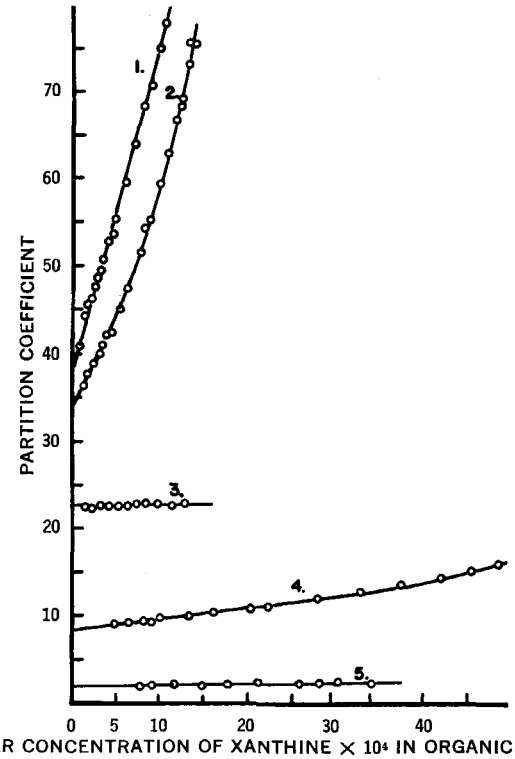

Figure 1—*A plot of the partition coefficients of a number of xanthines between water and an organic solvent at 30°. The organic solvent used for all of the studies except theophylline was isooctane. In the case of theophylline, chloroform–isooctane (90:10) was used. Key: 1, ethyltheobromine; 2, 7-ethyltheophylline; 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°) 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°. Such small amounts of associated species appear to be nearly undetectable and within experimental error.

Furthermore, in the NMR study, neither δ_M nor δ_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 δ_D values and, hence, falsely high dimer constants.

In conclusion, we believe that: (*a*) the NMR data suggest that theophylline does associate to a limited degree in water but do not permit useful quantitative estimates; and (*b*) for the most practical range of concentrations, the associative tendency of theophylline still appears to be negligible.

(1) A. Thakkar, L. G. Tensmeyer, and W. L. Wilham, *J. Pharm. Sci.*, **60**, 1267(1971).

(2) D. Guttman and T. Higuchi, *J. Amer. Pharm. Ass., Sci. Ed.*, **46**, 4(1957).

DAVID GUTTMAN*
TAKERU HIGUCHI
Department of Analytical Pharmaceutical Chemistry and Pharmaceutics
University of Kansas
Lawrence, KS 66044

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* Present address: Smith Kline & French Laboratories, Philadelphia, PA 19101

Interference in GLC Determination of Iodoamino Acids in Hydrolysis Products of Thyroid Extracts

Keyphrases ☐ Iodoamino acids—GLC analysis, thyroid extracts ☐ Thyroid extracts—GLC analysis of iodoamino acids ☐ GLC—assay, iodoamino acids

Sir:

The quantitative determination of iodoamino acids in dried thyroid extract is a problem of current interest in many laboratories. The separation and quantitative determination of standard mixtures of the iodoamino acids by GLC were reported by many authors (1–8). However, the extension of these methods to the determination of the hydrolysis products of dried thyroid extract has not been reported.

To shed light on the problems encountered in this determination, we wish to make the following report. In our work, we noticed that our standard mixture of trimethylsilyl derivatives of iodoamino acids did not yield the usual peak areas when injected onto a column that had been used for repeated injections of derivatives of hydrolysis products of dried thyroid extract. However, on injection onto a second column used previously only in the temperature-programming sequence, a return to normal peak areas was obtained (Fig. 1).

Our GLC investigation followed the method of Hansen (7), modified by substituting 1% dimethyl silicone[1] for the stationary phase coated onto a support of acid–base washed, silane-treated diatomaceous earth[2], mesh size 80/100. Three-foot glass U-shaped columns were utilized in this part of the study. A standard derivative mixture was prepared by heating 1.017 mg. of liothyronine (T_3) and 1.510 mg. of thyroxine (T_4) in 100 μl. of anhydrous pyridine and 300 μl. of *N,O*-bis(trimethylsilyl)-acetamide[3] for 2 hr. at 50°.

The first column had some carbon present at the point of injection. To determine if the carbon was interfering with the complete elution of the iodoamino acids, three columns were prepared: one containing the normal

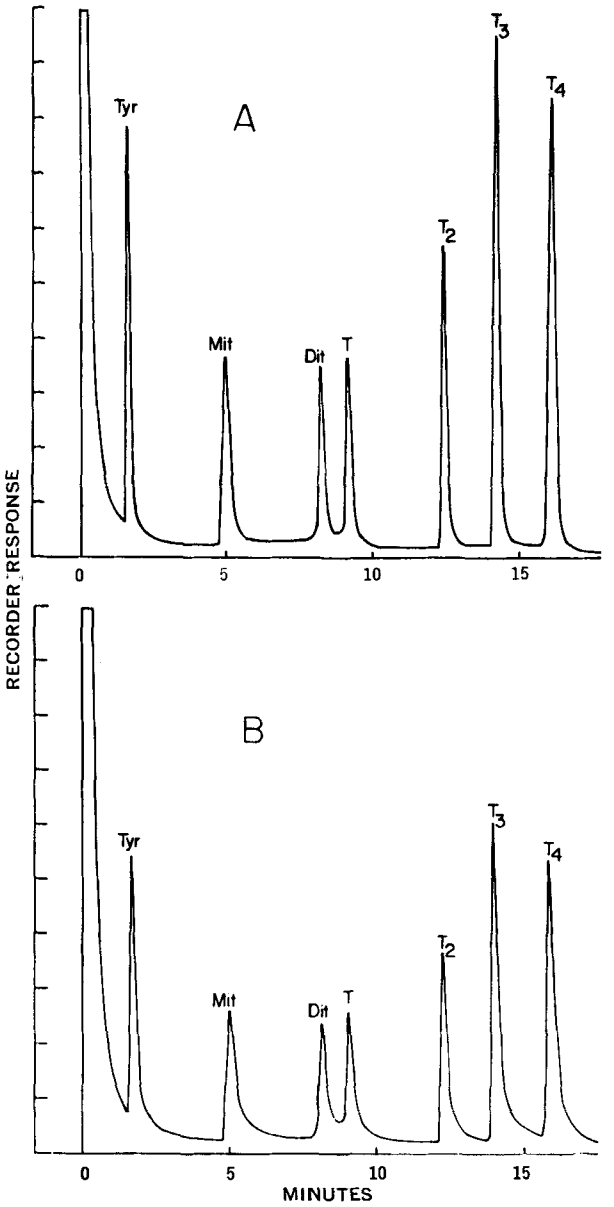

Figure 1—*GLC detection of a synthetic mixture of iodoamino acids on a new column (A) and a used column (B). Key: Tyr, tyrosine; Mit, monoiodotyrosine; Dit, diiodotyrosine; T, thyronine; T_2, diiodothyronine; T_3, liothyronine; and T_4, thyroxine. Chromatographic conditions were: temperature programmed at 165–285°, 3 min. isothermal followed by a programmed rate of 10°/min.; nitrogen carrier gas flow, 100 ml./min. at 165° (40 p.s.i.); injector temperature, 235°; detector temperature, 315°; attenuation, 9 × 10^{-10} amp.; and chart speed, 0.5 in./min.*

[1] OV-1, Applied Science Laboratories, Inc., State College, Pa.

[2] Gas-Chrom Q, Applied Science Laboratories, Inc., State College, Pa.

[3] BSA, Pierce Chemical Co., Rockford, Ill.