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Studies on Molecular Interaction in Solution. I. Effects of Organic Ions on the Solubility of 8-Methoxycaffeine

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The effect of pyridine, aniline, hydrocinnamic acid, phenethylamine, propionic acid, or theophylline on the solubility of 8-methoxycaffeine was compared with that of the respective salt at 25°. In most cases the free acids or bases showed greater binding tendencies toward 8-methoxycaffeine than the corresponding ionic forms. Reduction in solubilizing capacity of the ionic forms may be attributed mainly to prevention by the solvent shell from complex formation rather than just the salt-out effect due to the change in the activity coefficient of 8-methoxycaffeine in the presence of organic salts. Theophylline was ,on the other hand, unusual in a sense that its ionic form (theophylline ion) bounds 8-methoxycaffeine with a greater stability constant than its free form. Theophyllinate ion was demonstrated to interact more strongly than theophylline with another structurally similar compound. The structural similarity between the two interactants in aqueous media may be responsible for this reverse phenomenon.

Although effects of inorganic salts on the solubility of organic species in aqueous solutions have been known,[2] few detailed studies can be found in the literature dealing with effects of organic salts on the solubilities of other organic compounds.[3] As a part of investigations to elucidate the mode of molecular interaction in aqueous solutions the present study was initiated to obtain information on the solubility of a model compound, 8-methoxycaffeine, in the presence of an ionic solubilizer[4] with respect to the following cases; (a) the aromatic ring of a solubilizer is in its ionic form, (b) the ionic group of a solubilizer is adjacent to the ring, (c) the ionic group of a solubilizer is somewhat removed from the aromatic group, (d) an aliphatic compound is a solubilizer, and (e) a solubilizer is structurally similar to a solubilizate.[4]

8-Methoxycaffeine was employed as a model solubilizate because of (a) its relatively low solubility in water, which eliminates complications associated with the self-association at saturation like in caffeine,[5] (b) its strong absorption at around 280 mμ convenient for the spectrophotometric determinations of its concentrations, (c) its existence in the free form over the pH range studied, *i.e.* the solubility and molar absorptivity being independent of pH, and (d) its great complexing ability[6] which may serve to differentiate the solubilizing abilities of closely related solubilizers.

Experimental

Materials——All compounds were from commercial sources in their highest quality available and were purified whenever necessary. 1,3,7,9-Tetramethylpyrimido (5,4-*g*) pteridine-2,4,6,8 (1H,3H, 7H,9H)-tetrone

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[1] Location: *Yoshida, Sakyo-ku, Kyoto.*

[2] J.T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. 1, Academic Press, New York, N.Y., 1958, pp. 263—275; F.A. Long and W.F. McDevit, *Chem. Rev.*, **51**, 119 (1952).

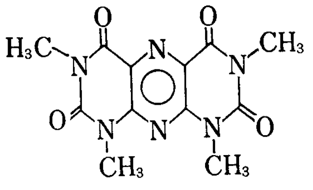
[3] *a*) T. Higuchi and A. Drubulis, *J. Pharm. Sci.*, **50**, 905 (1961); *b*) S. Ueda, *Chem. Pharm. Bull.* (Tokyo), **14**, 29 (1966).

[4] Throughout this paper the term "solubilizer" is to be understood as a substance added to a solution to increase the solubility of a poorly soluble substance, "solubilizate".

[5] D. Guttman and T. Higuchi, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 4 (1957).

[6] Private communication, M. Nakano and Ph.D. Thesis, University of Wisconsin, Madison, Wisconsin, 1967.

(I, TMPPT) was obtained from Aldrich Chemicals, Milwaukee, Wisconsin. 8-Methoxycaffeine was purchased from Eastman Organic Chemicals, Rochester, N.Y.



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Solubility Studies——Either a 0.1 N NaOH or 0.1N H_2SO_4 solution was added to adjust the pH of the solution containing a known amount of solubilizer so that the solubilizer is essentially in its either ionic or unionized form. No buffer was added to eliminate the effect of extra ionic species. An excess of 8-methoxycaffeine was placed with a volume of the above stock solution in each of a series of screw-capped tubes, and the volume was made up to 10 ml with an aqueous solution, the pH of which had been adjusted to the same value as that of the stock solution. The tubes were sealed with "Parafilm" and dipped in a water bath. Mixing was accomplished by a horizontal mechanical shaking of the tubes at 25° for 48 hr. Attainment of equilibrium was checked by the method of Robinson and Jencks.[7] After equilibration, an aliquot of the supernatant liquid was withdrawn and analyzed spectrophotometrically at 280 mμ on dilution with water. Small absorption due to the known amount of solubilizer was subtracted from the total observed absorbance. Since the amount of the complex was essentially negligible in the concentration range employed in the spectrophotometric determintions (*i.e.*, concentration of 8-methoxycaffeine was less than 2×10^{-5} M), there was no difference in molar absorptivity between 8-methoxycaffeine alone and that with the solubilizer when account was taken of the small absorption due to the solubilizer. When theophylline or its salt was the solubilizer, 8-methoxycaffeine was extracted from alkaline aqueous solution into chloroform layer while theophyllinate ion remaining in the aqueous layer. Determination of 8-methoxycaffeine in chloroform was made at 280 mμ. Apparent 1:1 stability constants were computed according to the phase-solubility technique.[8]

Studies with TMPPT——Interactions of TMPPT with theophylline and theophyllinate ion were investigated by both solubility and spectrophotometric methods. The solubility of TMPPT and stability constants of the TMPPT complexes were determined in a manner similar to that described for the 8-methoxycaffeine complexes. Determinations of stability constants by spectrophotometry were carried out at 377 mμ for theophylline—TMPPT system and at 362 mμ for theophyllinate—TMPPT system. The method of Foster, *et al.*[9] was used with the data obtained from the difference spectra. The temperature of sample solutions was 24°.

Results and Discussion

a) When the Charge is on the Aromatic Ring

The influences of pyridine (at pH 8.8) and pyridinium ion (at pH 2.2) on the solubility of 8-methoxycaffeine are presented in Fig. 1. Pyridinium ion did not display any significant

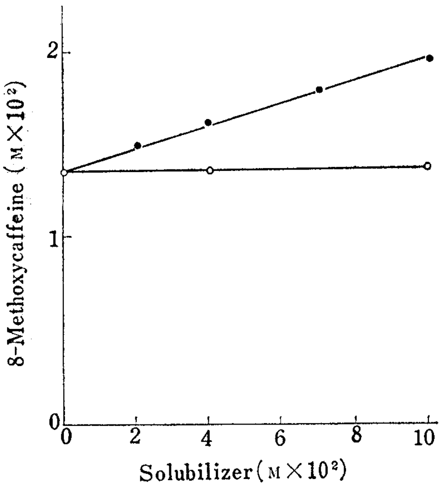

Fig. 1. The Apparent Solubility of 8-Methoxycaffeine in the Presence of Pyridine (•) and Pyridinium Ion (○) at 25° in Water

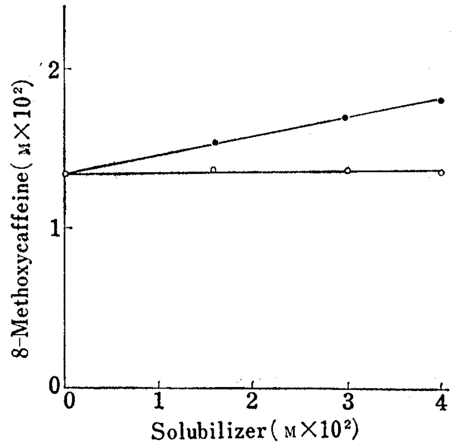

Fig. 2. The Apparent Solubility of 8-Methoxycaffeine in the Presence of Aniline (•) and Anilinium Ion (○) at 25° in Water

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[7] D.R. Robinson and W.P. Jencks, *J. Am. Chem. Soc.*, **87**, 2462 (1965).

[8] T. Higuchi and K.A. Connors, *Advan. Anal. Chem. Instr.*, **4**, 117 (1965).

[9] R. Foster, D. Ll. Hammick, and A.A. Wardley, *J. Chem. Soc.*, **1953**, 3817.

solubilizing ability, while pyridine did. Reduction in complexing tendency when a charge is on aromatic ring is in accord with the observation made by Mollica and Connors$^{10)}$ that the anionic form of theophylline was a poor interactant in comparison with unionized theophylline toward methylcinnamate. The charge on aromatic ring, which is considered to be the main site of interaction, seems to reduce the solubilizing capacity of solubilizers.

b) When the Charge is Adjacent to the Aromatic Ring

The effects of aniline (at pH 8.0) and anilinium ion (at pH 2.0) on the solubility of 8-methoxycaffeine is recorded in Fig. 2. The conversion of neutral aniline to its cation nullified its effectiveness. This may be attributed to both (a) the reduced π-electron conjugation (anilinium ion has essentially the same electronic structure as toluene whose complexing tendency is expected to be very small) and (b) strong solvation around the ion (the solvation shell is assumed to decrease the complexing capability of interactants). Similar results were also obtained by Higuchi and Drubulis$^{3a)}$ on solubilizing tendencies of benzoic acids and their salts on steroids and phenacetin. They observed the acid forms to be stronger binders than the salts.

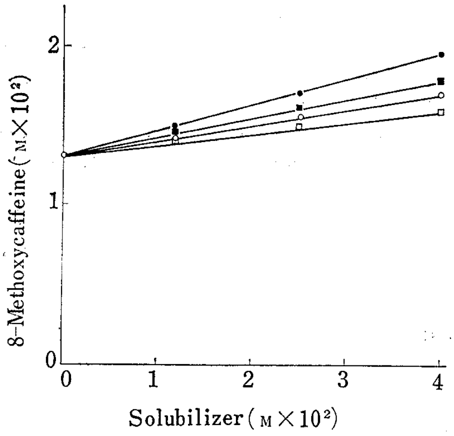

Fig. 3. The Apparent Solubility of 8-Methoxycaffeine in the Presence of Hydrocinnamic Acid (•), Hydrocinnamate Ion (○), Phenethylamine (▪), and Phenethylammonium Ion (□) at 25° in Water

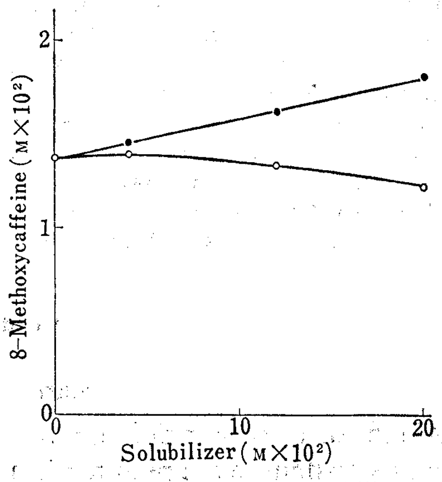

Fig. 4. The Apparent Solubility of 8-Methoxycaffeine in the Presence of Propionic Acid (•) and Propionate Ion (○) at 25° in Water

c) When the Charge is on the Aliphatic Substituent of Aromatic Compounds

Solubility diagrams of 8-methoxycaffeine in the presence of hydrocinnamic acid (at pH 2.0) and its salt (at pH 7.1): phenethylamine (at pH 12.2) and its ion (at pH 7.1) are shown together in Fig. 3. It is apparent that the ionized forms of the acid and base are much weaker binders than the corresponding unionized forms. The salts are considered still to possess solubilizing ability since their charges are removed from the main site of interaction.

d) When the Solubilizers are Aliphatic Ions

Minor effects on the solubility of 8-methoxycaffeine in the presence of aliphatic species are observed as shown in Fig. 4. The acid showed a slight solubilizing tendency, while the salt displayed a minor salting out effect.$^{2)}$

The apparent stability constants calculated from the phase-solubility technique for the systems discussed above are summarized in Table I. Since the reductions in solubilizing capacities of the aromatic compounds on ionization are much more than those which can be expected from the changes in the solvent properties, it may be assumed that the major effect

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10) J.A. Mollica and K.A. Connors, *J. Am. Chem. Soc.*, **89**, 308 (1967).

Table I. Apparent 1:1 Stability Constants of 8-Methoxycaffeine Complexes at 25° in Water

Solubilizer	Stability constant (M^{-1})	Solubilizer	Stability constant (M^{-1})
Pyridine	4.8	Hydrocinnamate ion	8.4
Pyridinium ion	[a]	Phenethylamine	9.8
Aniline	9.8	Phenethylammonium ion	5.8
Anilinium ion	[a]	Propionic acid	1.6
Hydrocinnamic acid	14.7	Propionate ion	[a]

[a] little or no solubilizing ability

is attributed to the reductions in complexing tendencies due to the interference by solvent shells around the ions. The pronounced reductions in solubilizing capacity of pyridinium ions compared with those of phenethylamine can be rationalized by the hypothesis that the site of interaction is primarily the aromatic ring, the charged group on or next to the aromatic ring being most effective in inhibition from complexing.

e) When Solubilizers are Structurally Similar to Solubilizates

The effects of theophylline (at pH 5.5) and theophyllinate ion (at pH 10.3) on the solubility of 8-methoxycaffeine are presented in Fig. 5. Contrary to the preceeding cases, theophyllinate ion formed unmistakably a more stable complex with 8-methoxycaffeine than theophylline. The authors believe that this is the first definite demonstration of a greater binding tendency of an ionized substance toward a neutral molecule than its unionics form. Although Wadke and Guttman[11] have observed that ionized 8-chlorotheophylline displayed a greater perturbation than its unionic form in the spectra of some analogs of riboflavin, dependence of the magnitude of spectral perturbation of pH, however, does not necessarily correlate with dependence of the magnitude of stability constant of pH. Thus their work does not establish any definite proof of the phenomenon.

In order to explore this reverse phenomenon, TMPPT has been employed as a solubilizate instead of 8-methoxycaffeine because of its structural similarity to theophylline and of its convenient

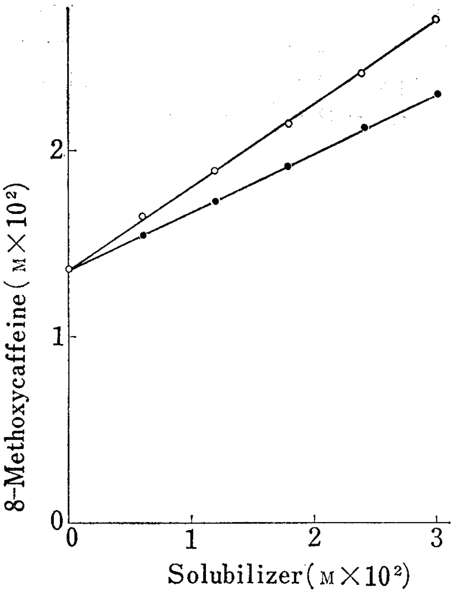

Fig. 5. The Apparent Solubility of 8-Methoxycaffeine in the Presence of Theophylline (•) and Theophyllinate Ion (○) at 25° in Water

Table II. Apparent 1:1 Stability Constants of Theophylline Complexes Determined by the Solubility Technique at 25° in Water

Complex	Stability constant (M^{-1})
Theophylline-8-methoxycaffeine	34
Theophyllinate ion-8-methoxycaffeine	58
Theophylline-TMPPT	13 (13[a])
Theophyllinate ion-TMPPT	69 (58[a])

[a] Determined by spectrophotometry at 24°.

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[11] D.A. Wadke and D.E. Guttman, *J. Pharm. Sci.*, **54**, 1293 (1965).

properties for the present purposes. The stability constants summarized in Table II show that theophyllinate ion again exhibited a much stronger binding tendency than theophylline in the interactions with TMPPT (the former appears to interact approximately four times as much with TMPPT as does the latter).

The relative complexing tendencies of theophylline and theophyllinate ion toward TMPPT were independent of the method of study as manifested by the favorable comparisons between the values of stability constants obtained by the solubility and spectrophotometric methods.

It is important to note that the above phenomenon is not of general nature in the interactions of theophylline and its anion with other substances. Theophylline has been shown to interact more faborably with methylcinnamate10) or phenazine12) than does theophyllinate ion. Thus this effect can not be ascribed to the inherent characteristics of theophylline, but rather appears to depend upon the choice of interactants.

As pointed out by Higuchi and Nakano6) earlier, bonding tendencies between structurally similar compounds are seemingly much smaller than those between compounds belonging to different classes. For example, the dimerization constant of caffeine5) is much smaller than that expected from its great complexing ability with a number of compounds.8) In some unknown manner, similar compounds do not associate greatly. Conversion of the neutral theophylline molecule to its anion may reduce the similarity between theophylline and 8-methoxycaffeine or TMPPT. This effect, although speculative in view of a limited number of systems studied, may be considered to be responsible for the phenomenon at this time. This aspect of molecular interactions is being vigorously pursued and will constitute the next report from this laboratory.

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12) M. Nakano, unpublished results ($K_{1:1}=190$, $K_{1:2}=25$ for phenazine–theophylline complexes and $K_{1:1}=88$, $K_{1:2}=9$ for phenazine–theophyllinate complexes at 25° in water).