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ACKNOWLEDGMENTS AND ADDRESSES

Received April 4, 1969, from *Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan.*
Accepted for publication April 30, 1969.
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Binding Specificity between Small Organic Solutes in Aqueous Solution: Classification of Some Solutes into Two Groups According to Binding Tendencies

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Abstract □ Experimental data have been obtained which appear to show that the binding between organic species dissolved in water apparently takes place most effectively *between* members of two large, distinct classes of structures, classified arbitrarily as A and B types. Typical examples of Class A are the uncharged alkylxanthines and tetramethylpyrimidopteridinetetrone. Among the compounds in Class B are various benzene derivatives, salicylates, and *trans*-cinnamic acid anions. Many drugs may be included in the present classification system; some examples for which data are available are caffeine, theophylline, and prednisolone in Class A, and phenacetin, promethazine, and menadione in Class B. The complexing tendencies of series of systems involving pairs of interacting organic molecules in aqueous solution were investigated by the phase-solubility technique. Stability constants for some caffeine interactions were evaluated by means of partitioning studies.

Keyphrases □ Organic solute binding specificity—aqueous solution □ Solutes, small organic—binding tendency classification □ Stability constants—solute binding □ Solubility—solute interaction effect □ Spectrophotometric analysis—organic solutes

Water strongly stabilizes a large number of molecular complexes, apart from its participation in hydrophobic bonding (1). Water provides a medium which seems to be unique for the molecular binding tendencies of organic molecules (2–10), many of them of great biological and pharmacological importance. In the last few years a number of papers have reported the properties of pyrimidines, purines, and the important nucleoside and nucleotide polymers in the aqueous environment. It has conclusively been shown, for example, that the bases associate to varying degree in aqueous solution, evidently through plane-to-plane stacking (11–16). It is believed that molecular interactions between adjacent bases in nucleic acid strands to a major extent are responsible for the structural stability of nucleic acids in solution (17–19).

The exact nature of the force or balance of forces operating between the complex components in aqueous solution still is the subject of controversial discussions in the literature. The observed binding between organic molecules in water is, however, firmly believed to be strictly physical in nature. As pointed out earlier (1, 5, 6), the observed intensity of binding cannot be rationalized on the basis of simple charge-transfer-type interactions (the binding constants are extremely low in alcohol, dioxane, and purely nonpolar solvents), dispersion forces (little or no interactive tendency is evident among systems of low polarizability), hydrophobic associations (very small contributions from flexible alkyl side chains), or hydrogen bonding alone. The matter is, of course, complicated by the possible interplay of different interacting forces. The problem is obviously related to the structure of liquid water, which in itself is a very intricate one, and many safe conclusions have not been made so far (20–25).

In this article the authors present their most recent observations carried out on series of systems involving pairs of interacting molecules in aqueous solution. These results have strongly reinforced a growing belief

that the observed binding in water takes place most effectively *between* members of two large classes of structures. Typical examples of these two apparently natural classes, arbitrarily called Classes A and B, are:

Class A	Class B
Caffeine	Benzoates and salicylates
Theophylline	Cinnamates
Prednisolone	Cinnamamides
Tetramethylpyrimido-pteridinetetrone	Naphthoic acids
	Phenols and naphthols
	Aromatic aminoacids
	Phenacetin
	Menadione
	Tryptophan

Although members of Classes A and B bind noticeably with others within their own class, the strongest interactions seem to be between the two groups.

For the purpose of expressing the extent of interaction between the complexing pairs, in this report the authors have assumed that essentially only 1:1 and 1:2 complexes were formed. The experimental data have been analyzed to yield stability constants for formation of these species. Most of the equilibrium constants were obtained through a systematic investigation using tetramethylpyrimidopteridinetetrone (TMPPT), cinnamamide, and/or *N,N*-dimethylcinnamamide as model compounds. TMPPT has a planar molecular structure similar to the alkylxanthines. The binding behavior of this compound for organic molecules appeared to be very strong, and it was felt that it would provide a useful insight into the general phenomenon of molecular association in water.

EXPERIMENTAL

Materials—Caffeine, theophylline, and phenacetin of USP grade and antipyrine NF were used directly. Caffeine and theophylline were dried at 110° prior to use. *N,N*-Dimethylcinnamamide was synthesized from cinnamoyl chloride and dimethylamine in ether and recrystallized from water–methanol, m.p. 102–103°. 1,3,7,9-Tetramethylpyrimido(5,4-*g*)pteridine-2,4,6,8(1*H*,3*H*,7*H*,7*H*,9*H*)-tetrone (TMPPT),[1] m.p. > 320°, was used usually without further purification.[2] All other compounds used were from commercial sources and were purified by recrystallization from appropriate solvents. Their melting points were found to deviate no more than 2° from literature reports. 7-(2-Dimethylaminoethyl)-theophylline,[3] phenylbutazone (lot SN 34514), and 10-(2-dimethylaminopropyl)phenothiazine hydrochloride (promethazine hydrochloride, control no. F-663110) were used.[4]

All reagents used for buffered solutions and the organic solvents used were of analytical grade. The water was purified by redistillation.

[1] Aldrich Chemical Co.

[2] The material as received was found to contain no significant amount of interfering impurities from analysis of its saturated solution. An appropriately diluted sample of an aqueous solution in equilibrium with a large excess of unpurified material revealed the same spectral characteristics as those found in solutions of the recrystallized substance. Accurately prepared solutions from both untreated and recrystallized material showed identical, highly characteristic spectra over the range 220–400 mμ; λ_{max}. 233 mμ (ε 49,000), 264 (ε 11,875), 271 (ε 12,000), 362 (ε 25,500); λ_{min}. 303 mμ (ε 1500). The equilibrium solubility in water of unpurified and recrystallized material was found to be the same. Since this is a characteristic physical property of a pure compound, just as is the melting point, the unpurified substance was considered to be essentially free of interfering impurities.

Anal.—Untreated material: Calcd. for $C_{12}H_{12}N_8O_4$: C, 47.37; H, 3.98; N, 27.62. Found: C, 47.21; H, 3.87; N, 27.82.

[3] Dimethazan.

[4] Supplied by Endo Laboratories Inc., Geigy Pharmaceuticals, and Wyeth Laboratories Inc., respectively.

Procedure—*Solubility Studies*—The experimental method for most of the systems investigated was the phase-solubility technique, which recently was reviewed in detail (26). An equal amount of the slightly soluble material to be tested, in considerable excess of its normal solubility, was added into each of several 15-ml. screw-cap vials. Increments of a stock solution of the complexing agent (the ligand) were pipeted into the vials, and the solution in each vial was brought to a constant final volume of 10 ml. with the necessary amount of the solvent. The vials were closed, and the screws were securely sealed with parafilm and masking tape.

When the hydroxy-substituted benzoic and cinnamic acids were tested, the vials were closed and sealed under slight nitrogen pressure to prevent oxidation of the acids.

Solubility equilibrium was obtained by tumbling the vials in a water bath thermostated at 25.0 ± 0.1° for at least 48 hr. It was determined that this was sufficient time to ensure solubility equilibrium for all of the substances tested.

When the ligand was the ionized form of an acid (and the solid phase material was a neutral molecule over a wide pH range), a stock solution was prepared *in situ* upon dissolving the acid in 0.1 *M* sodium bicarbonate; pH was adjusted to 8.3 with the necessary amount of sodium hydroxide. The vials were then prepared so that the resulting solution in each also was 0.1 *M* with respect to the bicarbonate. When the ligand was the unionized form of the acid, a 0.005 *M* sulfuric acid solution was used as the solvent to depress ionization of the ligand. A phosphate buffer of pH 6.5 was used for the theophylline (pKa 8.8) systems to ensure that essentially all theophylline was in its neutral form. Stock solutions of the aromatic acids were then made up by adding an equivalent amount of base and phosphate buffer to the desired volume. For some of the acids, pH 6.7–6.8 was maintained in the theophylline systems. For 8-chlorotheophylline, apparent pKa 5.3 (27), in its neutral form, a monochloroacetate buffer of pH 2.9 was used. Theophyllinate and 8-chlorotheophyllinate systems were tested at pH 11.3 and in 0.1 *M* bicarbonate, respectively.

The pKa of phenylbutazone, determined spectrophotometrically in water (25.0°), was found to be 4.56 ± 0.02. A stock solution of the sodium salt was prepared *in situ* by dissolving the compound in a solution containing an equivalent amount of sodium hydroxide.

The pKa of TMPPT, determined by the solubility technique outlined by Albert and Serjeant (28), in hydrochloric acid solutions was found to be −0.55 ± 0.10.

Following equilibration, the content of the vials was filtered through Pyrex sintered-glass filters and analyzed for total solid solubilized.

Analysis—An aliquot of the filtered samples diluted with methanol was determined spectrophotometrically in most instances on a Cary 16 spectrophotometer. (Sometimes a Cary 15 or a Cary 14 instrument was used.) The analytical wavelength chosen for the determination of the solubilized material was one where the ligand did not absorb. In some cases, small absorbances due to the presence of the ligand were subtracted from the observed values.

The dilution of the filtered samples with methanol prior to analysis was consequently carried out for all systems, because the complex formation in this solvent was practically negligible. In this way the complexes formed in water were essentially broken down so that the measured absorbances were due to uncomplexed substances. In water, noticeable spectral perturbations occurred with some of the compounds upon complex formation. In all cases, an exactly known concentration of the analyzed compound in methanol was used as the standard. The maximum water content in the diluted samples was 2%.

When theophylline was the solid phase material, it was separated from the complexing agent by extraction with a mixture of 3 parts chloroform and 1 part isopropyl alcohol and measured spectrophotometrically in this solvent.

Partitioning Studies—Since it has been shown (29) that caffeine undergoes self-association rather markedly in water, a phase diagram with caffeine as the solid phase cannot be unambiguously interpreted. For this reason a partitioning technique was employed so that the binding of caffeine in relative low concentration with some of the ionized aromatic acids could be studied. The experimental operation was essentially the one described by Guttman and Higuchi (29), but the total caffeine concentration was kept constant. The distribution of caffeine between 10% chloroform in isooctane and a 0.1 *M* sodium bicarbonate solution containing varying amounts of the ionized form of the acid was studied at 25.0 ± 0.2°.

Calculation of Equilibrium Constants—*Solubility Method*—Phase diagrams were made by plotting the total molar concentration found in solution of the substance to be tested, S_t, *versus* the total concentration of ligand, L_t. The quantitatve description of the different types of phase diagrams was treated by Higuchi and Connors (26). In the cases where the diagrams indicated formation of only soluble complexes and appeared to be first order with respect to the ligand, the apparent $K_{1:1}$ stability constants were evaluated according to Eq. 1:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \qquad \text{(Eq. 1)}$$

where S_0 is the equilibrium solubility in the absence of L and thus equal to the intercept in the plot. The data were treated by the method of least squares to get the best fit values for slope and intercept. For this purpose, a computer program was used on an Olivetti-Underwood Programma 101.

Methods are available to obtain the individual stability constants from phase diagrams exhibiting a positive curvature (26), and some assumptions usually are made depending upon the extent of complex formation. Therefore, the calculations applied when this type of diagram was observed in the present study will be outlined.

The assumption was made that only two complexes were formed, SL and SL_2,[5] with the stability constants given by:

$$K_{1:1} = \frac{[SL]}{[S][L]} \qquad \text{(Eq. 2)}$$

$$K_{1:2} = \frac{[SL_2]}{[SL][L]} \qquad \text{(Eq. 3)}$$

Concentrations in molar units are represented by the brackets. The mass balance equations are:

$$S_t = [S] + [SL] + [SL_2] \qquad \text{(Eq. 4)}$$

$$L_t = [L] + [SL] + 2[SL_2] \qquad \text{(Eq. 5)}$$

By combining Eqs. 2–4 and since $[S] = S_0$,

$$\frac{S_t - S_0}{[L]} = K_{1:1}S_0 + K_{1:1}K_{1:2}S_0[L] \qquad \text{(Eq. 6)}$$

and a plot of the left-hand term of Eq. 6 *versus* $[L]$ gives both $K_{1:1}$ and $K_{1:2}$ from slope and intercept. However, free ligand concentration, $[L]$, is not known. To obtain the estimate of $[L]$, the first step is to assume that all complexed was in the SL form. Then Eq. 5 reduces to:

$$[L] = L_t - (S_t - S_0) \qquad \text{(Eq. 7)}$$

and Eq. 6 takes the form:

$$\frac{S_t - S_0}{L_t - (S_t - S_0)} = K_{1:1}S_0 + K_{1:1}K_{1:2}S_0\,[L_t - (S_t - S_0)] \qquad \text{(Eq. 8)}$$

The first estimate of $K_{1:1}$ and $K_{1:2}$ came from a plot of the left-hand side of Eq. 8 *versus* $L_t - (S_t - S_0)$.

Combining Eqs. 2–5 and solving for $[L]$,

$$[L] = \frac{-(K_{1:1}S_0 + 1) + \sqrt{(K_{1:1}S_0 + 1)^2 + 8K_{1:1}K_{1:2}S_0L_t}}{4K_{1:1}K_{1:2}S_0} \qquad \text{(Eq. 9)}$$

The quadratic form of Eq. 9 was used in computing the free ligand concentration from the known values of S_t and L_t and the preliminary stability constants obtained from Eq. 8. Thus, the stability constants obtained from the exact Eq. 6 should represent better values than those obtained from Eq. 8. The procedure of getting better $K_{1:1}$ and $K_{1:2}$ values involved repeated computations in using Eqs. 9 and 6 successively, after the initial $K_{1:1}$, $K_{1:2}$, and $[L]$ were calculated from Eqs. 8 and 9.

Convergent values for slope and intercept (constant values for the stability constants) were usually obtained after three to four iterations. The data for the linear plots were treated by a computer-programmed least-squares method, and the calculations of $[L]$ were also programmed.

Partitioning—It was assumed that the ligand, for all practical purposes, was present only in the aqueous phase, since the aromatic acids would be ionized at the pH used in these studies. The free caffeine was distributed between the two solvents. For the interaction of the substance S with the ligand L in the aqueous solution,

$$K_{1:1} = \frac{[SL]}{[S]_{aq.}[L]} \qquad \text{(Eq. 10)}$$

where $[SL]$, $[S]_{aq.}$, and $[L]$ are the molar concentrations of the complex, free S, and L species.

The partition coefficient (PC) was defined as: PC = molar concentration of S in aqueous phase/molar concentration of S in organic phase, or

$$PC = \frac{S_{t,aq.}}{[S]_{org.}} = \frac{S_t - [S]_{org.}}{[S]_{org.}} \qquad \text{(Eq. 11)}$$

where S_t is the total molar concentration of the substance S in the system, and $[S]_{org.}$ is the molar concentration of S in the organic phase. The mass balance equations for the aqueous phase are:

$$S_{t,aq.} = [S]_{aq.} + [SL] \qquad \text{(Eq. 12)}$$

$$L_t = [L] + [SL] \qquad \text{(Eq. 13)}$$

Defining for simplicity,

$$(PC)_0 = \frac{[S]_{aq.}}{[S]_{org.}} \qquad \text{(Eq. 14)}$$

Combining Eqs. 10, 11, and 12,

$$PC = (PC)_0 + K_{1:1}(PC)_0[L] \qquad \text{(Eq. 15)}$$

As a first approximation, the authors set $[L] = L_t$ and got a preliminary estimate of $K_{1:1}$ from slope and intercept by plotting PC against $[L]$ in Eq. 15, $K_{1:1}$ = slope/intercept.

Now, combining Eqs. 10 and 13 with 14,

$$[L] = \frac{L_t}{1 + K_{1:1}(PC)_0[S]_{org.}} \qquad \text{(Eq. 16)}$$

Free ligand concentration could then be calculated from Eq. 16 from the initial estimate of $K_{1:1}$ and the known values of $(S)_{org.}$

Equations 15 and 16 were used successively until convergent values for the slope were obtained (note that the intercept is a constant in the system), *i.e.*, constant values of $[L]$ and $K_{1:1}$. The linear plots were fitted by a programmed least-squares method. Provided only a single 1:1 complex is present in the system under investigation, the final $K_{1:1}$ value should represent the true stability constant.

RESULTS

The stability constants calculated from phase-solubility studies for the binding of a variety of compounds to TMPPT, cinnamamide, and *N,N*-dimethylcinnamamide are listed in Table I. Data for some other interacting systems are given in Table II. Typical increases observed in the solubility of the pyrimidopteridine compound in the presence of benzoate and cinnamate anions are shown in Fig. 1. The solubility of the compound is increased more than 25 times with 0.05 *M* ferulic acid anion. The upward curvature in the plot is also easily recognized, indicating the formation of higher order associations with the added material. Another apparent feature of the TMPPT complexes of this type was their yellow color, which increased in intensity with the number of substituents on the ligand. Unsubstituted *trans*-cinnamate and benzoate did not appear to form colored complexes. Spectrophotometric studies showed a shift toward longer wavelengths of the 362-mμ absorption band of TMPPT in the presence of the cinnamate anions.

In general, the solubility diagrams for TMPPT as the solid phase material were either linear over the whole concentration range of ligand used or showed a positive curvature, as in Fig. 1. In a few instances, notably for *p*-anisidine, 2,6-dihydroxybenzoic acid, 4-

[5] It is not possible to prove that the equilibria occurring in solution are restricted to the formation of only two complexes; neither is it possible to show that these equilibria are actually occurring. Nevertheless, with the assumptions made, it is possible to describe accurately the phase-solubility diagrams and thus express the extent of complexation.

Table I—Stability Constants (liters/mole) for the Interaction of Various Compounds with TMPPT and Cinnamamides in Water at 25°

Ligand	TMPPT	Cinnamamide	*N,N*-Dimethylcinnamamide
1 Caffeine	11.9	37.5	37.6
2 Theophylline	12.8	27.3	27.5
3 8-Methoxycaffeine	19.1	48.5	54.0
4 8-Chlorotheophylline	18.5	39.3	
5 Theophylline-7-acetic acid	5.0	22.7	
6 Theophylline-7-acetate	5.9	20.0	17.3
7 Phenylbutazone, sodium salt	6.0	5.0	
8 Antipyrine	1.9	3.6	
9 Imidazole	2.3	0.8	
10 Theophyllinate	56.2 (7.1)[a]	14.3	
11 8-Chlorotheophyllinate	154.1 (11.6)	25.8	
12 Phenacetin	43.3	8.0	6.8
13 Phenol	13.1 (5.7)	1.7	
14 4-Chlorophenol	29.2	2.7	
15 Sodium sorbate	4.2	1.4	
16 *p*-Anisidine	14.9[b]	2.4	
17 10-(2-Dimethylaminopropyl) phenothiazine hydrochloride	65.5 (2.7)	11.5	
18 7-(2-Dimethylaminoethyl) theophylline	10.0		31.0
19 β-Hydroxyethyltheophylline	7.8		27.3
20 Sodium salicylate	44.3 (4.7)		1.9
21 2,6-Dihydroxybenzoate	102.1 (37.1)		5.9
22 2,6-Dihydroxybenzoic acid	96.6[b]		6.1
23 Nicotinamide	5.5		3.9
24 4-Hydroxycoumarin	99.0[b]		10.8

[a] The numbers in parentheses are the 1:2 stability constants for TMPPT–ligand, also expressed in l./mole. [b] Calculated from initial increase in solubility of TMPPT. An insoluble complex is also formed.

hydroxycoumarin, and 3-methoxy-4-hydroxymandelic acid anion, there was an initial linear increase in the solubility of TMPPT with ligand concentration, followed by a plateau region where precipitation of an insoluble complex occurred. The slopes were always considerably less than one for the diagrams or portions of them exhibiting increasing linear behavior or at any point in the diagrams showing curvature. The calculation of stability constants from the diagrams where a linear increase in the solubility of TMPPT was observed was based on the assumption that a single 1:1 complex was formed. Both 1:1 and 1:2 stability constants were evaluated from the phase diagrams showing positive curvature. Studies of the partitioning of TMPPT between water and an organic phase composed of 25% chloroform in isooctane (v/v) indicated that this compound did not self-associate to a detectable extent in water. This observation was given the interpretation that higher order associations of the compound should not interfere with the evaluation of reliable stability constants from the phase-solubility diagrams.

The interactions of the unionized form of *trans*-cinnamic, caffeic, and ferulic acid with TMPPT gave insoluble complexes. These interactions were not investigated further since the authors were primarily interested in the associations taking place in solution. The formation of insoluble complexes has also been found for theophylline interactions with a series of unionized benzoic acids (5).

The 1:1 stability constants in Table II for the interaction of caffeine with some of the ionized aromatic acids require some additional explanation. The calculated constants from the partitioning data were dependent upon the total caffeine concentration. To get the most reliable 1:1 constants for these systems, several experiments were carried out with different caffeine concentrations in

Table II—Stability Constants for the Interaction of Some Organic Molecules in Water at 25°

Interacting System	$K_{1:1}$ l./mole	$K_{1:2}$ l./mole
TMPPT–sodium benzoate	9.2	
TMPPT–*trans*-cinnamic acid anion	40.7	10.4
TMPPT–*d,l*-mandelic acid anion	4.2	
TMPPT–mandelamide	2.8	
TMPPT–5-phenyl-2,4-pentadienoic acid anion	128	25.9
TMPPT–*p*-aminohippuric acid anion	29.2	
TMPPT–*p*-coumaric acid anion	106	26.1
TMPPT–3-methoxy-4-hydroxybenzoic acid anion	50.5	14.6
TMPPT–3-methoxy-4-hydroxymandelic acid anion	139[a]	
TMPPT–caffeic acid anion	202	75
TMPPT–ferulic acid anion	228	85
TMPPT–cinnamamide	70.0	
TMPPT–*N,N*-dimethylcinnamamide	57.4	
TMPPT–β-naphthaleneacetic acid anion	141	16.0
TMPPT–4-methoxyphenylacetic acid anion	10.9	1.7
TMPPT–3-(*p*-methoxyphenyl)propionic acid anion	14.1	3.6
TMPPT–*N,N*-dimethyl-*p*-anisidine	8.2	
Caffeine–3-methoxy-4-hydroxybenzoic acid anion	17.8	
Caffeine–ferulic acid anion	46.0	
Phenacetin–caffeine	17.0	
Theophylline–caffeic acid anion	39.5	
Theophylline–2,6-dihydroxybenzoic acid anion	300	
Cinnamamide–ferulic acid anion	8.0	
N,N-Dimethylcinnamamide–caffeic acid anion	7.2	

[a] See *Footnote* [b] in Table I.

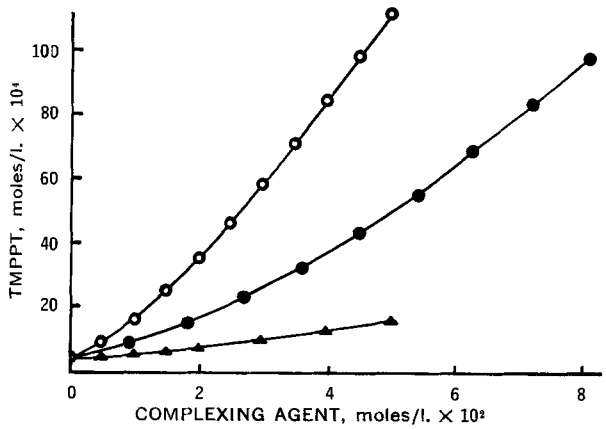


Figure 1—*Increase in solubility of TMPPT produced by addition of ferulic acid anion* (○), *2,6-dihydroxybenzoate* (●), *and trans-cinnamate* (▲) *in 0.1 M sodium bicarbonate at 25°.*

each. Figure 2 shows the data for the interaction of caffeine with ferulic acid anion with the apparent $K_{1:1}$ constants, calculated as outlined in the *Experimental* section, plotted against the caffeine concentration. The increase in stability constants with increasing caffeine concentration strongly suggests that higher order caffeine complexes are formed. This is not surprising, because caffeine has been found to exist in aqueous solution as monomer, dimer, and tetramer (29), and all the caffeine species might possibly associate with the aromatic anion. This was supported with data from the solubility technique, which showed that one molecule of the anion brought close to two molecules of caffeine into solution. When the linear plot in Fig. 2 was extrapolated to zero caffeine concentration, the value of 46.0 for the $K_{1:1}$ constant was believed to represent the best "true" constant.

When the two cinnamamides were used as solid phase material in the solubility technique, only a linear increase in the solubility was observed. The slopes of the solubility diagrams were in every instance less than one. Partitioning studies between water and 10% chloroform in isooctane (v/v) indicated that self-association of cinnamamide and *N,N*-dimethylcinnamamide in the aqueous phase did not occur. The calculation of stability constants from the phase diagrams was made assuming formation of a single 1:1 complex in the systems.

Studies were attempted with vitamin A acid anion and phenothiazine as complexing agents, but they did not lead to definitive results. The salt form of vitamin A acid apparently formed micelles at very low concentrations, and phenothiazine was unstable in solution.

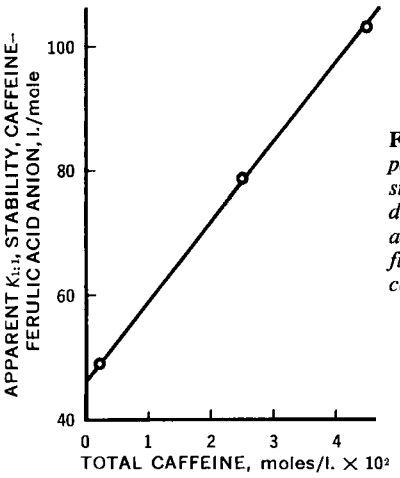


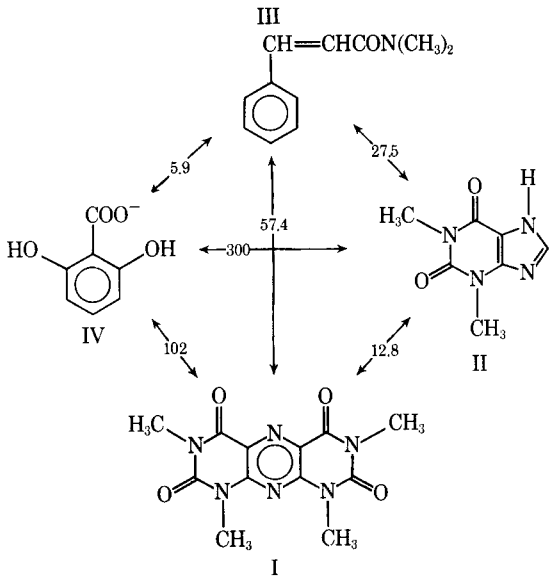
Figure 2—*Plot of apparent* $K_{1:1}$ *stability constants from partitioning data of the caffeine–ferulic acid anion system as a function of total caffeine concentration at 25°.*

DISCUSSION

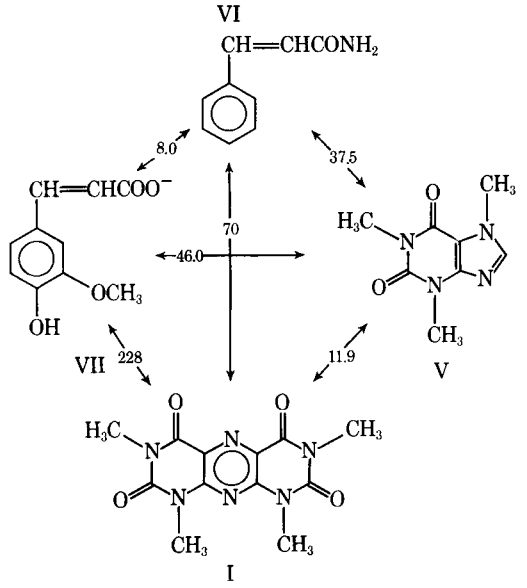
Earlier studies indicated that there is relatively little specificity in the type of interactions under present consideration. For example, if the stability constants of complexes formed by Compound 1 with a series of other species were plotted against the stability constants derived using Compound 2 for the same series of interactants, it has been shown that the points fall essentially on a straight line passing through the origin (6, 7). The present investigation strongly suggests, however, that there are at least two broad classes, labeled here as Class A and Class B. The data collected indicate that although members of Class A and Class B will interact with others within their own class, the strongest binding seems to be between the two groups.

Thus, for example, TMPPT and theophylline have been classified as being in Class A. *N,N*-Dimethylcinnamamide and γ-resorcylic acid anion are considered to be in Class B. As listed in Table I, the former two interact weakly, K equal to 12.8 l./mole, with each other; the latter pair interact even more weakly with K less than 6 l./mole. The four constants for the crossinteractions are substantially larger, being 27.5, 57.4, 102, and 300 l./mole. This is shown diagrammatically in Scheme I. The same trend is revealed by examining Scheme II, where the TMPPT–caffeine system represents the A–A interaction with K equal to 11.9 l./mole. The cinnamamide–ferulic acid anion system, with a K of 8.0 l./mole, is the B–B interaction. The four possible A–B interactions have 1:1 stability constants of 37.5, 46.0, 70, and 228 l./mole.

The possible existence of such a generic difference in the binding behaviors of these compounds is strongly supported by the plots shown in Figs. 3 and 4. Here the stability constants listed in Table I for the interactions of the various complexing agents with cinnamamide (Class B) and *N,N*-dimethylcinnamamide (Class B) have been compared with those for the interactions of the same compounds with TMPPT (Class A). Both Figs. 3 and 4 show that all the compounds fall readily in two groups, those binding the cinnamamides strongly and TMPPT weakly, and those characterized by the opposite binding tendencies. Thus, these plots demonstrate that a large number of organic compounds may be classified as belonging to either of the two categories, A or B. Such a classification is apparently valid among the compounds tested that showed relatively strong binding tendencies. However, the classification may, of course, be somewhat obscure for compounds that tended to bind rather weakly with any interactant. Examples of the latter type



Scheme I—A–B system interactions in water at 25°. The numbers on the arrows represent the 1:1 stability constants for the interacting species. I, TMPPT; II, theophylline; III, N,N-*dimethylcinnamamide; and IV,* 2,6-*dihydroxybenzoate*



Scheme II—A–B system interactions in water at 25°. The numbers on the arrows represent the 1:1 stability constants for the interacting species. I, TMPPT; V, caffeine; VI, cinnamamide; and VII, ferulic acid anion

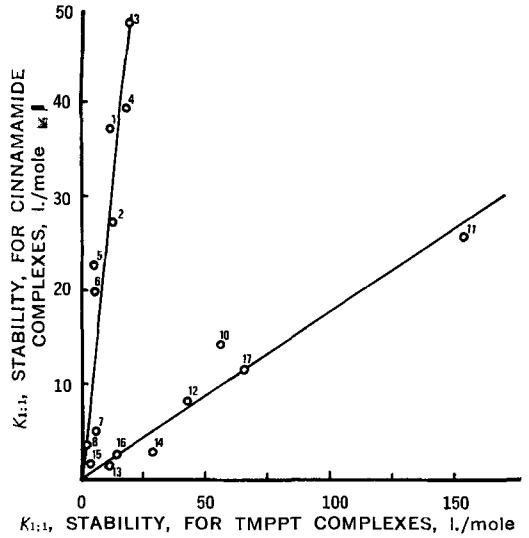


Figure 3—*A plot of the 1:1 stability constants for cinnamamide and TMPPT with each of the indicated compounds. The numbers refer to the compounds listed in Table I.*

from the present data are phenylbutazone, antipyrine, imidazole, sodium sorbate, and nicotinamide.

From Figs. 3 and 4, it is seen that the apparent two classes of compounds fall essentially on their own straight line passing through the origin in the plots, thus indicating a reasonable linear free energy relationship for the A–B interactions. The rationale for this type of plot is that the difference in the free energy ($\delta\Delta G°$) for the binding of one class of compounds to TMPPT compared to the cinnamamides is expressed by the slope. That a linear free energy relationship exists does not, of course, imply that a single binding mechanism is operating among the two categories of interactants. It has been found that simple linear free energy relationships are not necessarily limited to closely related reactions but might apply to entire groups of reactions (30). The free energy changes involved in these systems, in general, are small. From the slopes of the lines drawn in Figs. 3 and 4, it was calculated that the binding of Class A compounds to the cinnamamides (Class B) was favored by about 600 cal./mole compared to the binding of the same class to TMPPT (Class A). For the binding of Class B compounds, TMPPT was favored over the cinnamamides by 1000–1500 cal./mole.

The present data suggest that all the investigated neutral xanthines and the pyrimidopteridine compounds constitute the same class, Class A. Examination of previously reported stability constants for prednisolone and hydrocortisone interactions (5, 6) indicates that these compounds also apparently may be classified as being in the same A category. Class B consists of a large number of aromatic compounds. Apparently all benzoate derivatives, phenols, naphthols, cinnamates, and naphthoates, for which data are available, form much stronger complexing pairs with the Class A compounds than with members of their own group. In Class B the cinnamates appear to be among the strongest binders. Since molecular complexation is believed to be a possible mechanism for drug–receptor interactions *in vivo*, it is interesting to note that drugs like phenacetin and salicylate, according to the present classification system, are typical B compounds. The observed binding of the phenothiazine derivative (promethazine hydrochloride) that was tested indicated that it apparently fit into the same Class B. The strong binding to TMPPT of the biologically important metabolite 3-methoxy-4-hydroxymandelic acid anion (K equal to 139 l./mole) showed that it most likely belongs to Class B. The stability constants reported for the interaction of menadione and tryptophan with some alkylxanthines (7, 31) further suggest that both these compounds may be members of Class B.

Some insight into factors that confer characteristics of A and B compounds can be gained by correlating the data for the theophylline systems. The ionized form of theophylline and 8-chlorotheophylline bind TMPPT stronger than cinnamamide, whereas the reverse is true for the uncharged form of these xanthines. While the neutral xanthines were labeled Class A compounds, Fig. 3 shows that their ionized forms fit nicely into Class B. Recently, it was also shown that theophyllinate binds stronger to 8-methoxycaffeine than its neutral form (32), and neutral theophylline had a stronger affinity for some cinnamate esters than theophyllinate (10). For a long time it has been assumed, and in some cases confirmed by experiment (5, 8, 32), that the stronger solvation around the ions compared to the neutral molecules should decrease the complexing capability in water. The theophyllinate behavior cannot be explained in this way. Although the binding with cinnamamide is reduced, theophyllinate and 8-chlorotheophyllinate bind TMPPT markedly stronger than do the corresponding neutral forms. The 1:1 stability constants were found to be 12.8 and 18.5 l./mole for the interaction with theophylline and 8-chlorotheophylline, respectively, compared to 56 l./mole for the binding of TMPPT to theophyllinate and 154 l./mole for the interaction with 8-chlorotheophyllinate. On the other hand, data for the neutral and ionized forms of theophylline-7-acetic acid show that when the negative charge is adjacent but isolated from the imidazole ring in theophylline, there is no significant change in the binding strength with either cinnamamide or TMPPT. The neutral and ionized molecules interacted with cinnamamide to give K equal to 22.7 and 20.0 l./mole, respectively. The corresponding constants for the interaction with TMPPT were 5.0 and 5.9 l./mole.

An important factor regarding the observed behavior of the theophyllinates appears to be the great change in the resonance structures of both theophylline and 8-chlorotheophylline upon formation of their anions. In theophylline-7-acetic acid, the resonance character of the heterocyclic ring system of the molecule cannot be affected by ionization of the carboxylic group. The binding of the theophyllinates, in general, resembled the binding of the

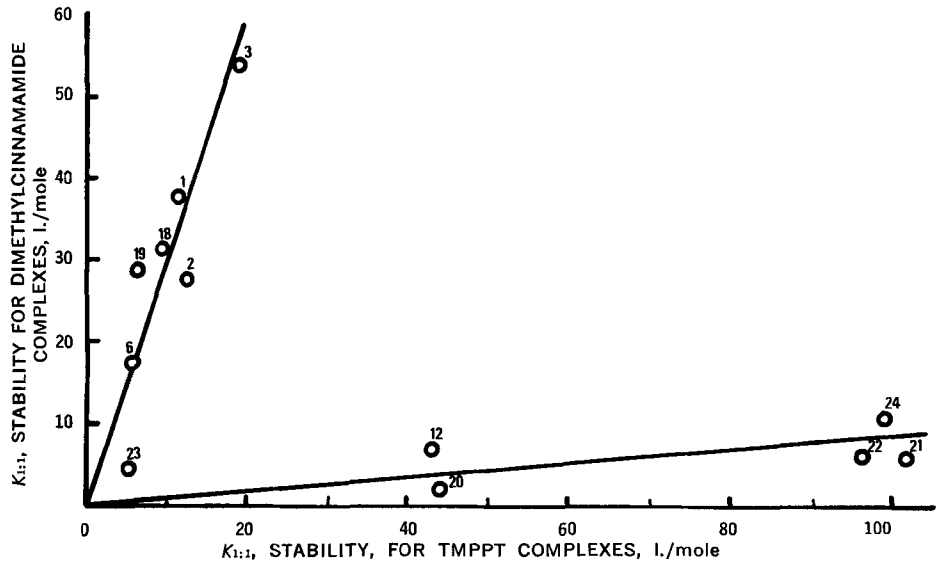


Figure 4—*A plot of the 1:1 stability constants for* N,N-*dimethylcinnamamide and TMPPT with each of the indicated compounds. The numbers refer to the compounds listed in Table I.*

benzoates and the cinnamates with TMPPT. All these ligands gave yellow, soluble complexes with TMPPT, and the solubility diagrams were all characterized by an upward curvature. The neutral alkylxanthines, on the other hand, interacted in a first-order manner with TMPPT, and no colored complexes were formed.

Since the difference in electronic character of the two forms of theophylline crudely appears to be responsible for their differences in binding with the A–B system compounds, one is tempted to speculate if this reflects any important property which would clearly distinguish an A from a B compound. Still there are contradicting opinions about the electronic properties of, for example, the purine derivatives. Although Pullman and Pullman (33) found from MO calculations that a correlation existed between the electron donating properties of the purines and their solubilizing effect on hydrocarbons (33), the influence of the medium was not taken into account. Hanna and Sandoval (34) carried out NMR studies of caffeine in complexes with benzene and mesitylene in carbon tetrachloride, and they concluded that caffeine acts as a typical acceptor in these complexes. This is, of course, contrary to the predictions made from MO theory. It is evident that the effect of the solvent environment on the extent of complex formation has to be taken into account (1). In attempts to rationalize the observed data, many problems arise because knowledge about this refinement is far from complete.

The interacting species listed in Tables I and II represent structurally a spectrum of different planar aromatic and heterocyclic molecules. Both neutral and ionized species have been tested. The substituents on the compounds are differently oriented, and some would be expected to exert an electron-withdrawing effect while others should be donating electrons and, in any case, affect the π-orbitals of the aromatic and heterocyclic ring systems. The variations in structure appear to be pronounced within each of the proposed two classes of interacting species. In general, therefore, it seems impossible to ascribe the interactions to any localized binding site. The binding of one class of compounds to a member of the other class must be mainly nonspecific in nature. The same reasoning is applicable to the observed weak binding between members of the same class. However, since the collected data clearly demonstrated that there are at least two broad classes of compounds, some selectivity must be operating among the interactants. In other words, a particular molecule must be able to recognize an A from a B compound. This property of the interactants seems to eliminate the important contribution to the stability of the complexes due to dispersion forces. The dispersion energy of attraction can be expressed in terms of a single parameter, the product of the polarizabilities of the interacting molecules (35, 36). Thus, if dispersion forces were important for the binding in water, one would not expect to see the observed selectivity since all the molecules would be more or less "blind."

At the present time, the authors believe that charge–transfer complexes of the formal type (37) are not a major factor in stabilizing these complexes in water. Experiments have shown that the binding in these systems is considerably reduced in media less polar than water (1), and this is the opposite effect of what should be expected if charge–transfer was a major factor (38). But the possibility cannot be excluded that a mechanism of nonclassical "donor–acceptor" type, peculiar to water, may be operating in the interactions being considered. It is clear that factual information about the role of water in these interactions is lacking. This may be the main reason why no satisfactory explanation has emerged as to the exact nature of the forces involved, although numerous complex interactions in aqueous media have been reported.

The complex geometry of the systems investigated cannot be described with great assurance since they exist only in solution in equilibrium with the uncomplexed components. With all the possible solute–solute, solvent–solvent, and solute–solvent interactions taking place simultaneously in aqueous solution, any detailed structural or mechanistic interpretation can only be speculative and may involve assumptions that are far from safe. However, some progress in understanding the spatial arrangement of complexes of organic molecules in aqueous solution is evident from previous studies. In general, it has been shown that planarity of the interacting molecules is important (5–7), and it has been observed that expansion of the ring system from a benzenoid to the naphthalene structure led to substantial increased binding with, for example, theophylline (5, 6). Recently, it was reported that a reasonable linear correlation existed between the standard unitary free energy change and the estimated planar area of the smaller neutral interactants for theophylline complexes with a series of cinnamate esters and related compounds, and it was postulated that the 1:1 complexes were most likely of a plane-to-plane orientation (10). NMR measurements of the tryptophan–caffeine complex have suggested that the geometry apparently corresponded to plane-to-plane stacking (7). The effect of increased planar area upon the complex stability is substantially confirmed by the present study. For example, by increasing the planar surface from that of a typical alkylxanthine like caffeine or theophylline to the three-membered heterocyclic ring system in the pyrimidopteridine compound, the more favorable is the observed binding in water. The coplanarity of the benzene ring with the ethylenic bond and the carboxylate in the series of cinnamic acid anions increases the planar surface area of these molecules compared to the benzoates and other benzene derivatives. It is evident from the present data that the cinnamic acid anions, in in general, are much stronger binders than the latter type of compounds, notably toward the Class A compounds. However, it cannot be ascertained at the present time if the strong binding tendency of the cinnamates is due to the increase in surface area or to the extended conjugation with increased π-electron delocalization in the molecular system. It may be suggested that a combination of both the effects contributes favorably to the observed binding.

Since the strongest binding, in general, is seen with compounds that are polycyclic in nature (of which there are examples of both Class A and Class B compounds in the present study) or contain extended conjugation, the view that planar area overlap of the partners in the complex takes place has considerable appeal. But it is not possible to make any conclusions, which at this time may be more than speculative, regarding the preferred mutual orientation of the components in these complexes in solution. Again it is felt that the role of water may be essential for the modes of orientation.

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 13, 1970, from the *Department of Analytical Pharmaceutical Chemistry and Pharmaceutics, School of Pharmacy, University of Kansas, Lawrence, KS 66044*

Accepted for publication June 2, 1970.

This investigation was supported in part by Grant GM-05830 from the National Institutes of Health.

H. K. gratefully acknowledges partial financial support from the Norwegian Research Council for Science and the Humanities.

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Alkaloids of Tylophora II: Structural Studies

KOPPAKA V. RAO

Abstract □ Structural studies on the six alkaloids isolated from *Tylophora crebriflora* (N. O. Asclepiadaceae) are described here. Spectral data indicate that five of these alkaloids (A–E) possess the dibenzo[*f,h*]-pyrrolo[1,2*b*]isoquinoline skeleton known to be present in tylocrebrine. They differ in the number, nature, and distribution of the oxygen-bearing substituents and in the presence or absence of a benzylic-type hydroxyl. An oxygen substitution pattern of 3, 4, 6, and 7 is suggested for Alkaloids A, B, and C and that of 2, 3, 4, 6, and 7 for Alkaloids D and E. Alkaloid F is shown to be a seco analog of tylocrebrine with a 1,2-diphenyl *cis* stilbene skeleton.

Keyphrases □ Alkaloids—*Tylophora crebriflora* □ Structural studies—*T. crebriflora* alkaloids □ NMR spectroscopy—structure □ IR spectrophotometry—structure □ UV spectrophotometry—structure

The isolation of six new alkaloids designated as A, B, C, D, E, and F, together with the known compounds tylocrebrine and tylophorine from *Tylophora crebriflora*, S. T. Blake (N. O. Asclepiadaceae), was described in Part I (1). An examination of the analytical and spectral data indicated that Compounds A–E resemble tylocrebrine (I) in that they possess the dibenzo[*f,h*]-pyrrolo[1,2*b*]isoquinoline skeleton with four or five oxygen-bearing substituents. The studies that provided evidence for the structures of these new members are described in this paper.

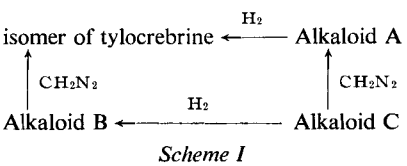
DISCUSSION

Alkaloid A, $C_{24}H_{27}NO_5$, resembles tylocrebrine in its UV and IR spectra and in having four methoxyl groups. The extra oxygen atom is in the form of a hydroxyl group, as shown by the formation of a monoacetate (bands at 1725 and 1225 cm.$^{-1}$ in IR and a sharp 3-proton peak at τ 7.85 in the NMR spectrum). Clemmensen reduction or catalytic hydrogenation converts Alkaloid A to a nonhydroxylic compound $C_{24}H_{27}NO_4$, thus indicating that the hydroxyl is benzylic. In its spectral and chromatographic behavior, the reduction product is almost indistinguishable from tylocrebrine, but the mixed melting (decomposition) point seems to indicate that the two may not be identical.

The presence of the benzylic hydroxyl in Alkaloid A is analogous to the case of tylophorinine (II, R = OH), another known member of this group (2). In this compound, the hydroxyl was placed at 14 instead of 9 (for numbering, see Structure I), because the latter structure would have represented a highly labile carbinolamine system and the stability of the compound was inconsistent with such a structure (2). This was later confirmed by synthesis (3). In an analogous manner, the stability of Alkaloid A strongly suggests the location of the hydroxyl to be 14. This is also supported by the following NMR spectral evidence: the chemical shifts of the benzylic CH(OH) protons in tylophorinine and Alkaloid A are very close: τ 3.96 and 3.87, respectively. The corresponding chemical shift of the same proton in the acetates of both compounds is τ 3.48; in both cases, it is split as a doublet, as would be expected. It is, therefore, concluded that Alkaloid A has the hydroxyl at 14.

Alkaloid B, $C_{23}H_{25}NO_4$, has three methoxyl groups. The fourth oxygen is part of a phenolic group (UV spectral shift in base and band at 3540 cm.$^{-1}$). This is supported further by the formation of a monoacetate (1750 cm.$^{-1}$ in IR and a 3-proton peak at τ 7.59). Methylation with diazomethane leads to a tetramethoxy compound, $C_{24}H_{27}NO_4$, which is almost indistinguishable from tylocrebrine on the basis of spectral and chromatographic data, but the mixed melting (decomposition) point suggests that the two may not be identical.

Alkaloid C, $C_{23}H_{25}NO_5$, shows features similar to both A and B. It has three methoxyls and forms a diacetate (1725 and 1760 cm.$^{-1}$ in IR and 3-proton peaks at τ 7.87 and 7.59 for the alcoholic acetate and phenolic acetate groups, respectively). It can be converted by methylation to Alkaloid A and by Clemmensen reduction to B. Hence, this compound is the desmethyl derivative of Alkaloid A, and the position of the phenolic hydroxyl in B and C is the same. The transformations are shown in Scheme I.



Scheme I

Alkaloids D and E have the compositions $C_{25}H_{29}NO_6$ and $C_{25}H_{29}NO_5$, respectively. They each have five methoxyl groups. Clem-