Effect of Structure of Pyridinecarboxylic Acids and Hydroxypyridines on Molecular Interaction in Water

KIICHIRO KAKEMI, HITOSHI SEZAKI, MASAHIRO NAKANO*, and KIICHIRO OHSUGA

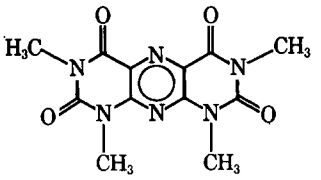
Abstract □ Complexing tendencies of pyridinecarboxylic acids and hydroxypyridines toward 8-methoxycaffeine or 1,3,7,9-tetramethylpyrimido(5,4 - *g*)pteridine - 2,4,6,8(1*H*,3*H*,7*H*,9*H*) - tetrone (TMPPT) were found to be much smaller than those of corresponding benzene derivatives. The results were interpreted to be attributable to the fact that pyridinecarboxylic acids and 3-hydroxypyridine are present predominantly in zwitterionic form in water. *Para*-aminobenzoic acid was found to complex with 8-methoxycaffeine and TMPPT to a much greater extent than *m*-aminobenzoic acid. The complexing tendency of these aminobenzoic acids was also found to qualitatively agree with the extent of molecular form present in water. The smaller complexing tendency of 4-hydroxypyridine toward 8-methoxycaffeine and TMPPT was discussed on the basis of possible structural similarity between two interacting species.

Keyphrases □ Pyridinecarboxylic acids, hydroxypyridines interaction—structure effect □ Complex formation, aqueous solution—pyridinecarboxylic acids, hydroxypyridines □ UV spectrophotometry—analysis □ Solubility analysis—spectrophotometer

As a part of investigations into the effect of molecular structure on molecular interaction in solution (1–4), the present study was undertaken to quantitatively examine complexing tendencies of pyridinecarboxylic acids and hydroxypyridines and to relate them to the structures of these species in aqueous solution.

It has been shown earlier that the ionization of molecules usually results in reduction of complexing tendency (3). It was shown that pyridine and aniline complexed with 8-methoxycaffeine, whereas pyridinium ion and anilinium ion were devoid of this property. Similarly, hydrocinnamate ion and phenethylammonium ion were found to be less effective complexing agents toward 8-methoxycaffeine than hydrocinnamic acid and phenethylamine, respectively. Reduction in complexing ability by ionization of benzoic acids has also been noted earlier by Higuchi and Drubulis (5).

If pyridinecarboxylic acids and hydroxypyridines are present predominantly in zwitterionic form in water, a certain reduction in complexing tendencies will be expected in comparison with corresponding benzene derivatives which do not undergo such tautomerization. The present investigation has been designed to further test this hypothesis in some pyridine derivatives. Specifically, the complexing behaviors of pyridinecarboxylic acids, benzoic acid, aminobenzoic acids, hydroxypyridines, and phenol with 8-methoxycaffeine and another structurally related compound, 1,3,7,9-tetramethylpyrimido(5,4-*g*) pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetrone (abbreviated as TMPPT), have been determined in order to assess the degree of interaction in water on the assumption that the increase in solubility is entirely attributed to complex formation for the system under study. 8-Methoxycaffeine has been



TMPPT

shown to be a suitable solute[1] for solubility studies (3). The selection of TMPPT was based on its simplicity in spectrophotometric assay and on its resemblance to alkylxanthines in its complexing behavior (3).

EXPERIMENTAL

Materials—The following compounds (all of reagent grade) were employed as complexing agents[1]: benzoic acid, m.p. 122°; nicotinic acid, m.p. 237°; isonicotinic acid, m.p. 319°; *p*-aminobenzoic acid, m.p. 187°; *m*-aminobenzoic acid, m.p. 174°; phenol, m.p. 40°; 3-hydroxypyridine, m.p. 129°; and 4-hydroxypyridine, m.p. 148°. Compounds used as solutes were 8-methoxycaffeine (Eastman, m.p. 179°) and TMPPT (Aldrich, m.p. >340°). Sodium hydroxide, carbon tetrachloride, and methanol were also of reagent grade. Distilled water was used as solvent throughout this study.

Solubility Studies—With the exception of the interactions of *p*- and *m*-aminobenzoic acids with 8-methoxycaffeine, the experimental procedure employed in this study was similar to that described in an earlier publication (1). The solubility of 8-methoxycaffeine in the presence of *p*- and *m*-aminobenzoic acids was determined in the following manner. 8-Methoxycaffeine was equilibrated with aminobenzoic acid solutions, an aliquot of the supernatant liquid (1 ml.) was withdrawn, and it was placed in a test tube with a ground-glass stopper. The solution was then made alkaline with a 0.01 *N* NaOH solution (9 ml.) and it was shaken with a 3-ml. portion of carbon tetrachloride using an aliquot shaker.[2] An aliquot portion (1 ml.) of the organic layer was withdrawn by means of a micro pump,[3] diluted with methanol, and assayed spectrophotometrically for 8-methoxycaffeine content. No aminobenzoic acids were detected in the organic layer under the present experimental condition. 8-Methoxycaffeine solutions of known concentrations were simultaneously extracted and they served as standard solutions. Wavelengths chosen for spectrophotometric determination were 281 mμ for 8-methoxycaffeine and 360 mμ for TMPPT.

RESULTS AND DISCUSSION

Pyridinecarboxylic Acids and Benzoic Acid—The solubility diagrams of 8-methoxycaffeine in the presence of benzoic acid, nicotinic acid, and isonicotinic acid are shown in Fig. 1. The figure evidently indicates a much smaller complexing tendency of the pyridinecarboxylic acids as compared with that of benzoic acid. Since such a difference in complexing tendencies was not observed between nicotinamide and benzamide, a mere difference between the pyridine ring and benzene ring does not seem to account for the observed results. Stability constants for the nicotinamide-8-methoxycaffeine

[1] In the present solubility studies a compound added in excess of its solubility is designated as a solute, while a compound added in order to increase the solubility of a solute is designated as a complexing agent.

[2] Lab-Tek, Ames Lab-Tek, Inc. Westmont, Ill.

[3] Ingenjörsfirma Pumpett, Åby, Sweden; Distributor in U. S.: Lapine Scientific Co., Chicago, Ill.

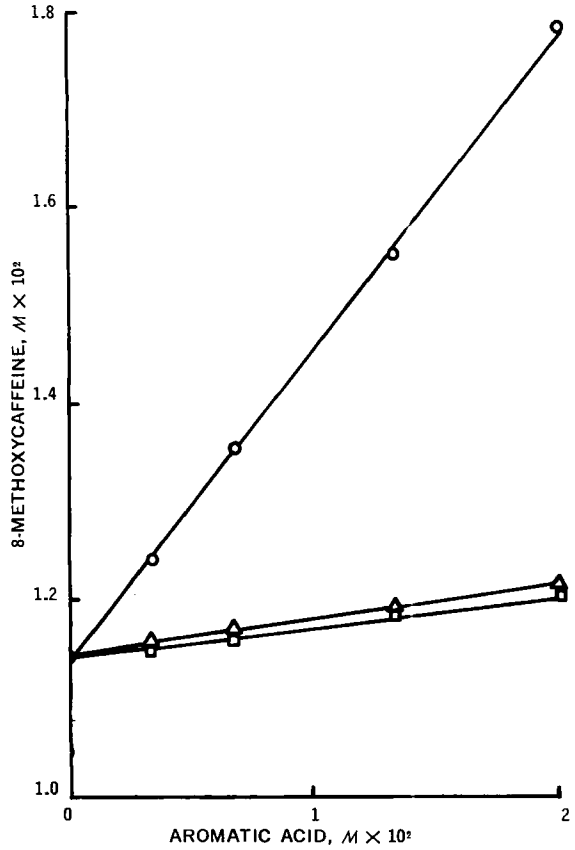


Figure 1—*The apparent solubility of 8-methoxycaffeine in the presence of benzoic acid* (○), *nicotinic acid* (△), *and isonicotinic acid* (□) *in water at 25°.*

and benzamide-8-methoxycaffeine complexes were 28 and 21 M^{-1} respectively, in water at 25° (6). Furthermore methyl nicotinate (K = 14 M^{-1} at 25°) was found to be a much better complexing agent than nicotinic acid (K = 3.7 M^{-1}) toward 8-methoxycaffeine (2). Since the acids, and not the amides and also most likely esters, of pyridine series appear to be substantially less effective complexing agents than those of the corresponding benzene series, the difference

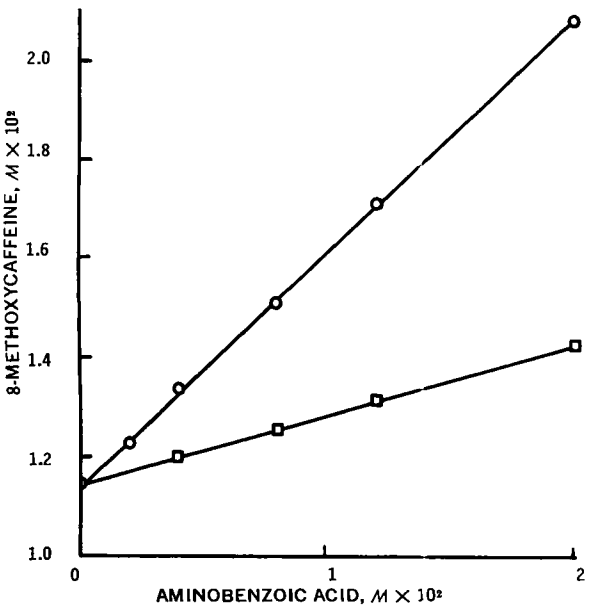
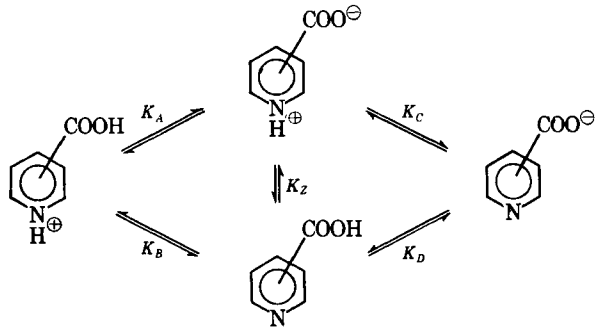


Figure 2—*The apparent solubility of 8-methoxycaffeine in the presence of* p-*aminobenzoic acid* (○) *and* m-*aminobenzoic acid* (□) *in water at 25°.*

may be considered to arise from characteristics related to only the structure of acids in aqueous solution. Reduced complexing ability of the pyridinecarboxylic acids should rather be attributed to their tautomeric structures in aqueous solution. Both nicotinic acid and isonicotinic acid were reported to be present in water predominantly in the zwitterionic forms (7), whereas benzoic acid exists primarily in molecular form.

In an aqueous solution of pyridinecarboxylic acids, the following equilibria (Scheme I) must be considered:



Scheme I

The equilibrium constants involved are interrelated by the following equations: $Ka_1 = K_A + K_B$, $1/Ka_2 = 1/K_C + 1/K_D$, and $K_Z = K_A/K_B = K_D/K_C$, where Ka_1 = an apparent proton gained Ka and Ka_2 = an apparent proton lost Ka. With the value of pKa_1 = 2.07, pKa_2 = 4.81, and K_Z = 10 for nicotinic acid (7), calculations (8) show that only 8% of nicotinic acid is in unionized form, while 84, 4, and 4% of the substance are present as zwitterion, cation, and anion, respectively, at pH 3.4 (resultant pH of the solution). Isonicotinic acid with apparent pKa's of 1.84 (proton gained) and 4.86 (proton lost) and K_Z = 25 (7) gives about 4% unionized molecule, 90% zwitterion, 3% cation, and 3% anion at pH 3.4. Benzoic acid with pKa = 4.17 (9), on the other hand, is 94 to 96% in unionized form when it is dissolved in water, resultant pH of the solution being 2.8–3.0.

Since ionic forms of acids and bases were shown to have smaller complexing tendencies toward 8-methoxycaffeine than the acids or bases themselves (3), it may be expected that the zwitterionic forms of these pyridine compounds also have only a little complexing power. The present results may be supported by Poole and Higuchi's studies concerning sarcosine anhydride complexes (10). While isonicotinic acid showed little complexing tendency with sarcosine anhydride, the solubility of benzoic acid was increased by 19% in a 1% sarcosine anhydride solution over that in water.

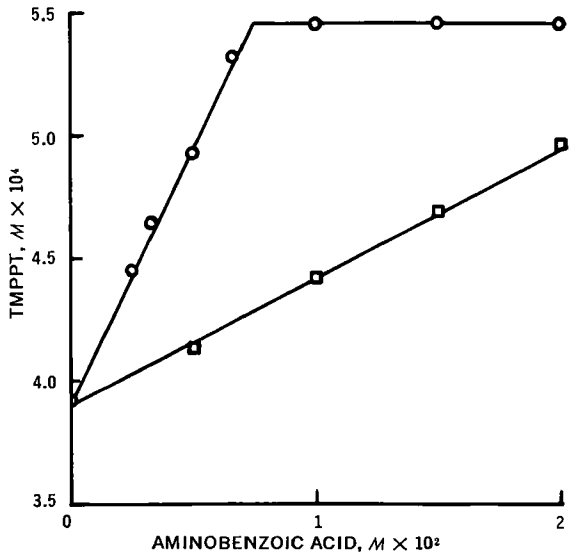


Figure 3—*The apparent solubility of TMPPT in the presence of* p-*aminobenzoic acid* (○) *and* m-*aminobenzoic acid* (□) *in water at 25°.*

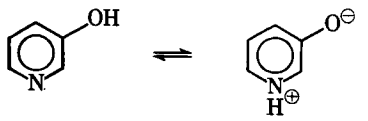
Aminobenzoic Acids—Amino carboxylic acids which exist predominantly in molecular form are, however, expected to display good complexing tendencies according to the foregoing interpretation that molecular form is primarily responsible for complexation. The solubility diagrams for the interactions of *p*-aminobenzoic acid and *m*-aminobenzoic acid with 8-methoxycaffeine are presented in Fig. 2. *Para*-aminobenzoic acid displayed more than five times as great solubilizing power as its *meta*-isomer. The same trend is apparent in their interactions with TMPPT as shown in Fig. 3. Although the *p*-aminobenzoic acid-TMPPT complex was found to have a limited solubility in water, it is evident in the figure that *p*-aminobenzoic acid has approximately four times as great solubilizing power as its *meta*-isomer at its lower concentration range. K_Z values have been estimated to be 0.09 for *p*-aminobenzoic acid and 1.4 for its *meta*-isomer (11). Simple calculations (8) show that *m*-aminobenzoic acid with $pKa_1 = 3.07$ and $pKa_2 = 4.79$ (11) is about 44% zwitterionic, 32% molecular, 12% cationic, and 12% anionic at pH 3.9 (resultant pH of the solution), whereas *p*-aminobenzoic acid with $pKa_1 = 2.43$ and $pKa_2 = 4.85$ (11) is about 7% zwitterionic, 81% molecular, 6% cationic, and 6% anionic at pH 3.6 (resultant pH of the solution). The difference in the percentages of molecular form between *m*- and *p*-aminobenzoic acids is consistent with the proposed interpretation to account for the observed difference in their complexing powers. *Para*-aminobenzoic acid which exists predominantly in molecular form is a much better complexing agent than its *meta*-isomer which exists only one-third in molecular form, although a part of the difference may be attributed to an intrinsic difference in complexing tendencies between *meta* and *para* isomers.

Poole and Higuchi (10) have earlier shown that the solubility of *p*-aminobenzoic acid increased by 26% in a 1% sarcosine anhydride solution over that in water whereas the increase in the solubility of the *meta*-isomer was only 7% under the same condition. Their results may also be rationalized on the same basis.

Aminophenols are, on the other hand, known to be ordinary amphoteric substances (8) and all of them are entirely in molecular form in water at neutral pH. Thus no significant difference in complexing tendency is expected between *m*-aminophenol and its *para*-isomer. The observed increase in solubility of *p*-aminophenol in the presence of 1% sarcosine anhydride and that of the *meta*-isomer were reported to be 15 and 14%, respectively (10), which is in accord with expectation.

Although aromatic α-amino acids (tryptophan, tyrosine, and phenylalanine) are 100% zwitterionic in water, they have been found to interact appreciably with alkylxanthines in water (4). In these compounds, the zwitterionic parts are well separated from the aromatic groups which are considered to be the major site of interaction (4, 12). In pyridinecarboxylic acids and *m*-aminobenzoic acid, ionic charges are either on or adjacent to the aromatic systems. Therefore in the former class of compounds the retarding effect of zwitterion on the complexing capacity of organic compounds is considered to be much less than the latter class of compounds.

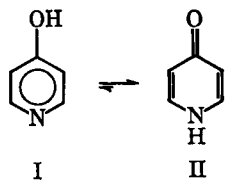
Hydroxypyridines and Phenol—Investigations were extended to some hydroxypyridines and their complexing tendencies were compared with phenol. Solubility diagrams of 8-methoxycaffeine in the presence of phenol, 3-hydroxypyridine, and 4-hydroxypyridine (4-pyridone) are presented in Fig. 4. A trend similar to that for pyridinecarboxylic acid complexes was obtained with these hydroxypyridines, phenol being a better complexing agent than the hydroxypyridines. While phenol with pKa = 9.98 (13) is 99.99% in unionized form in water (resultant pH of the solution being about 6.0), 3-hydroxypyridine was shown to be an equilibrium mixture of the following tautomers (Scheme II) (14). 3-Hydroxypyridine with



Scheme II

$pKa_1 = 5.10$, $pKa_2 = 8.60$, and $K_Z = 1.17$ (14) can be calculated to be 52.4% zwitterionic, 44.6% molecular, 1.5% cationic, and 1.5% anionic at pH 6.9 (resultant pH of the solution). Smaller complexing tendency of 3-hydroxypyridine as compared to that of phenol may thus be explained to be ascribable to the existence of more than a half of the molecules in zwitterionic form in water in the same way as interpreted the smaller interactive tendency of pyridinecarboxylic acids in comparison with that of benzoic acid.

4-Hydroxypyridine, on the other hand, is known to exist predominantly in vinylogous amide form (15, 16) rather than in zwitterionic form. Thus a different explanation must be sought for the observed small complexing ability of 4-hydroxypyridine. Experiments were carried out using TMPPT as a solute instead of 8-methoxycaffeine to confirm the results obtained with 8-methoxycaffeine and the results are as shown in Fig. 5. These results seem to essentially reproduce the trend obtained with 8-methoxycaffeine.



I II

A structural feature of interacting molecules may be considered to have some bearing on the extent of these interactions. Although molecular interactions in water appear to be largely nonspecific as compared with hydrogen-bonding interactions in nonpolar solvents, a certain class of compounds (aromatic compounds such

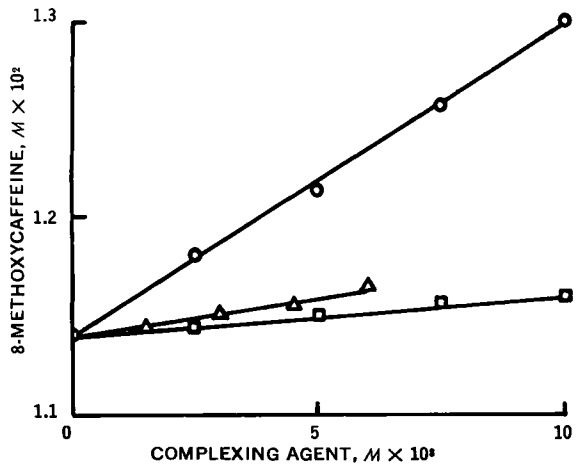


Figure 4—*The apparent solubility of 8-methoxycaffeine in the presence of phenol* (○), *3-hydroxypyridine* (△), *and 4-hydroxypyridine* (□) *in water at 25°.*

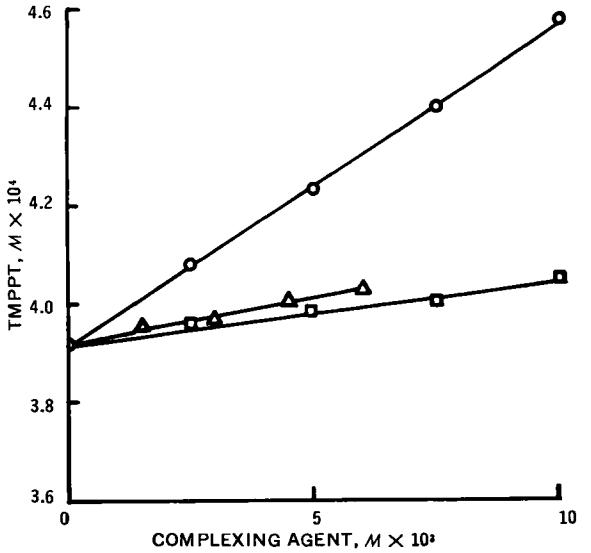


Figure 5—*The apparent solubility of TMPPT in the presence of phenol* (○), *3-hydroxypyridine* (△), *and 4-hydroxypyridine* (□) *in water at 25°.*

Table I—Apparent 1:1 Stability Constants (M^{-1}) in Water at 25° as Determined by the Solubility Method

Complexing Agent	Solute	
	8-Methoxycaffeine	TMPPT
Benzoic acid	40	—
Nicotinic acid	3.7	—
Isonicotinic acid	3.2	—
p-Aminobenzoic acid	73	55
m-Aminobenzoic acid	13	14
Phenol	17	18
3-Hydroxypyridine	4.6	5.4
4-Hydroxypyridine	2.3	3.3

as benzoic acids, phenols, benzamides) interact more strongly with another class of compounds (cyclic amides such as alkylxanthines, uracil, TMPPT) than do two compounds of the same class (12). 4-Hydroxypyridine in its vinylogous amide form(II) resembles the structure of uracil which is a cyclic amide. Uracil and 1,3-dimethyluracil were found to complex with TMPPT, a cyclic amide, to a much less extent than with phenazine, an aromatic compound (17). Structural similarity of the uracils to TMPPT (both belong to the same class) was proposed to be a possible reason for the smaller interactive tendency observed between these compounds than between the uracils and phenazine. The extent of interaction of 4-hydroxypyridine with TMPPT or 8-methoxycaffeine may likewise be considered to be smaller than that of phenol with these solutes on the basis of structural similarity between two interacting species. This is, however, only speculative at this stage. The preliminary study of the interaction of 4-hydroxypyridine with an aromatic compound, phenazine, has provided a rather encouraging result. The stability constant of the 4-hydroxypyridine-phenazine complex was calculated to be 5.8 M^{-1} at 25°, which is significantly greater than that of the 4-hydroxypyridine-TMPPT complex (3.3 M^{-1}).

Apparent stability constants calculated by means of the phase-solubility technique (18) are summarized in Table I in order to facilitate quantitative comparison of the complexing tendency of the compound used. Failure in the exact correlation between the percentage of molecular form present and the extent of molecular interaction in the systems discussed above may arise from the following factors among others: (*a*) the determination of K_Z values is usually made only indirectly and under the assumption that the p*Ka* (proton gained) of unionized molecule (pK_B) is equal to the pKa of its ester (7); and (*b*) the observed solubility of a solute can be somewhat less than the expected solubility calculated from the percentage of molecular form since zwitterions may salt out the solute (19).

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* Present address: Faculty of Pharmacy and Pharmaceutical Sciences, The University of Alberta, Edmonton, Alberta, Canada. To whom all correspondence should be addressed.