Effect of Urea Concentrations on the Solubility of the Isomeric Monohydroxybenzoic Acids

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The interactions of urea in varying concentrations with the three isomeric monohydroxybenzoic acids are described. Results indicate that the acids interact in varying amounts with urea; however, the over-all reaction for each isomer is relatively complex. The *meta* compound appears to have the greatest and the *ortho* isomer the least tendency to complex; however, the amount of *m*-hydroxybenzoic acid in solution decreases, within certain limits, with agitating time. In general, the solubility of the complexes is a function of temperature. The solubility characteristics of the interaction products also may be associated with the lower water concentrations at high urea levels.

CERTAIN DIFFERENCES in the physicochemical and/or biologic properties among the isomeric monohydroxybenzoic acids can be rationalized by considering the relative tendencies of these acids to hydrogen bond either intramolecularly or intermolecularly. The structure of the *ortho* compound is such that the hydroxyl and the carboxyl functions can interact through intramolecular hydrogen bonding. The *meta* and *para* isomers of monohydroxybenzoic acid favor intermolecular hydrogen bonds, giving rise to a high degree of association.

Studies of the effect of urea on the denaturation of proteins *via* the breaking of hydrogen bonds have been of special interest to some investigators (1). Other than the evidence given for the action of urea on proteins and certain polymers of amino acids, there appears to be little direct proof that urea in aqueous solution can break hydrogen bonds. Levy and Magoulas studied the effect of urea on the hydrogen bonds in some dicarboxylic acids and concluded that urea in aqueous solution has little or no effect on such hydrogen bonds beyond that associated with the lower water concentrations at high urea concentrations (2).

Since urea is a relatively nontoxic compound and there is still further need of scientific data concerning the mechanistic action of urea as a complexing agent, it was of interest to study the consequences of the interactions between urea and the isomeric monohydroxybenzoic acids as manifested by the effects of certain urea concentrations and temperatures on the solubility of these compounds.

The work of Bolton on the interaction of urea and thiourea with salicylic and benzoic acids at 30°, which was published while the experimental phases of this study were in progress, indicates that urea can serve as a complexing agent for certain aromatic acids (3). Bolton also has reviewed the literature on the use of urea as a complexing agent for oxytetracycline, benzocaine, sulfonamides, wool fat alcohols, quinoxaline, detergents, and barbituric acid derivatives.

This comparative study of the interactions of the three isomeric acids with urea also should afford valuable data regarding the structural arrangements which favor complex formation.

EXPERIMENTAL

Solubility Studies

In accordance with the preceding introductory considerations, it was decided to study experimentally the interaction of the monohydroxybenzoic acid isomers with urea at different concentrations and temperatures using a modification of the Higuchi and Zuck (4–6) solubility method.

Materials.—*o*-Hydroxybenzoic acid (salicylic acid), Baker analyzed reagent, m.p. 159°; *p*-hydroxybenzoic acid (Eastman Organic Chemicals), m.p. 214°; *m*-hydroxybenzoic acid (Eastman Organic Chemicals), m.p. 201°; and urea (crystals), Baker analyzed reagent, m.p. 131.9°–132.8°, were employed.

o-Hydroxybenzoic Acid

Method.—The experimental procedure used to determine the effect of urea concentration and temperature on the solubility of salicylic acid is as follows. Definite quantities of acid (see Table I) and 50-ml. portions of each solution (1–6 *M* urea solutions adjusted to 0.1 *N* [H^+] with hydrochloric acid) were placed in 100-ml. volumetric flasks, the flasks stoppered, and the mixtures shaken for 12 hr. at temperatures of 25.5°, 37°, or 45° utilizing a mechanical shaker and a constant-temperature water bath regulated at ±0.2° with a Brownwill thermostat. This shaking period was selected on the basis of experiments which indicated that all of the solutes in urea solutions up to 5 *M* studied were equilibrated well within the 12-hr. period.

TABLE I.—AMOUNT OF ACID EQUILIBRATED WITH 50 ML. OF THE RESPECTIVE FLUIDS

Acid	Amt. of Acid Used, Gm. 25.5°C.	37°C.	45°C.
o-Hydroxybenzoic acid	2	2	2 and 2.5
p-Hydroxybenzoic acid	2	2	2
m-Hydroxybenzoic acid	3	3	3 and 4.5

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TABLE II.—INTERACTION OF *o*-HYDROXYBENZOIC ACID AND UREA AT VARYING TEMPERATURES (12 HR.)[a]

Original Urea Concn. × 10^1M	Satd. Total Acid Concn. × 10^2M	Acid Concn. as Complex × 10^2M
	At 25.5°C.	
0.0	1.491	...
10.0	1.882	0.391
20.0	2.222	0.731
30.0	3.041	1.550
32.5	3.065	1.574
35.0	3.135	1.644
37.5	2.693	1.202
40.0	2.606	1.115
42.5	2.932	1.441
45.0	2.447	0.956
50.0	2.534	1.043
60.0	2.461	0.970
	At 37.0°C.	
0.0	1.810	...
10.0	2.389	0.579
20.0	3.041	1.231
22.5	3.387	1.577
25.0	3.651	1.841
27.5	4.235	2.425
30.0	4.402	2.592
32.5	4.651	2.841
37.5	4.792	2.982
40.0	4.832	3.022
50.0	4.651	2.841
60.0	4.199	2.389
	At 45.0°C.	
0.0	2.751	...
10.0	3.837	1.086
20.0	4.561	1.810
30.0	6.009	3.258
40.0	7.511	4.760
50.0	7.456	4.705
60.0	7.420	4.669

[a] Data reported are average values for triplicate experiments.

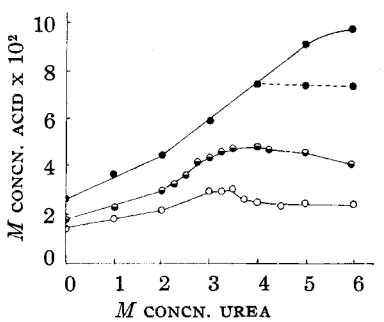

Fig. 1—Effect of urea on the solubility of *o*-hydroxybenzoic acid. Key: ○, 25.5°; ◒, 37.0°; ●, 45.0°; – – – ●– – –, 2 Gm. of acid equilibrated; —●—, 2.5 Gm. of acid equilibrated.

One-milliliter volumetric pipets, the tips of which were wrapped with Whatman No. 1 filter paper and bound with twine, were employed to collect samples free from excess solid acid. Aliquot quantities were removed from the dissolution fluids, diluted to the proper concentration, and assayed spectrophotometrically for the amount of acid in solution using the Beckman DB spectrophotometer at 236 mμ. All samples and controls were run in triplicate. The results obtained for samples equilibrated for 12 hr. are given in Table II and Fig. 1.

p-Hydroxybenzoic Acid

Method.—The experimental procedure employed for determining the extent of interaction between *p*-hydroxybenzoic acid and urea is essentially the same as that used in the salicylic acid and urea complexing study. The amount of solid acid added to the reaction flasks is shown in Table I. As with the *ortho* compound, all samples and controls were run in triplicate. The amount of *p*-hydroxybenzoic acid in solution was determined spectrophoto-

TABLE III.—INTERACTION OF *p*-HYDROXYBENZOIC ACID AND UREA AT VARYING TEMPERATURES (12 HR.)[a]

Original Urea Concn. × 10^1M	Satd. Total Acid Concn. × 10^2M	Acid Concn. as Complex × 10^2M
	At 25.5°C.	
0.0	3.873	...
10.0	6.407	2.534
20.0	8.144	4.271
30.0	9.448	5.575
40.0	10.570	6.697
45.0	11.221	7.348
50.0	11.294	7.421
55.0	11.942	8.069
60.0	12.923	9.050
	At 37.0°C.	
0.0	7.457	...
10.0	11.583	4.126
15.0	13.465	6.008
20.0	14.624	7.167
25.0	15.203	7.746
27.5	18.316	10.859
30.0	17.954	10.497
32.5	18.764	11.307
40.0	18.731	11.274
45.0	18.823	11.366
50.0	19.692	12.235
60.0	21.357	13.900
	At 45.0°C.	
0.0	12.814	...
10.0	18.135	5.321
20.0	22.081	9.267
30.0	27.148	14.334
40.0	27.438	14.624
50.0	27.655	14.841
60.0	29.854	17.040

[a] Data reported are average values for triplicate experiments.

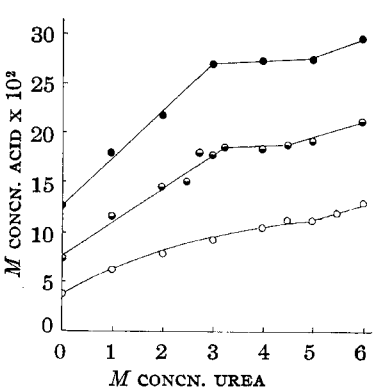

Fig. 2.—Effect of urea on the solubility of *p*-hydroxybenzoic acid. Key: ○, 25.5°; ◒, 37.0°; ●, 45.0°.

TABLE IV.—INTERACTION OF *m*-HYDROXYBENZOIC ACID AND UREA AT VARYING TEMPERATURES (12 HR.)[a]

Original Urea Concn. × $10^1 M$	Satd. Total Acid Concn. × $10^2 M$	Acid Concn. as Complex × $10^3 M$
	At 25.5°C.	
0.0	5.249	...
10.0	6.877	1.628
15.0	8.064	2.815
20.0	8.687	3.438
25.0	9.954	4.705
30.0	10.316	5.067
35.0	12.850	7.601
40.0	14.479	9.230
45.0	16.108	10.859
50.0	18.714	13.465
60.0	21.718	16.469
	At 37.0°C.	
0.0	10.135	...
10.0	13.610	3.475
20.0	18.099	7.964
30.0	22.805	12.670
40.0	26.280	16.145
50.0	30.913	20.778
60.0	32.912	22.777
	At 45.0°C.	
0.0	11.801	...
10.0	16.434	4.633
20.0	22.370	10.569
30.0	27.510	15.709
40.0	32.578	20.777
50.0	38.514	26.713
60.0	44.885	33.084

[a] Data reported are average values for triplicate experiments.

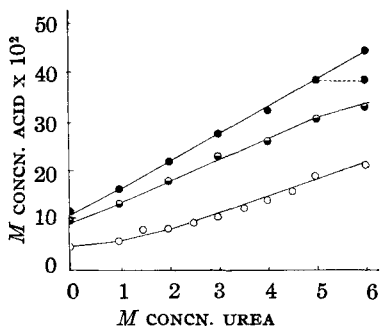

Fig. 3.—Effect of urea on the solubility of *m*-hydroxybenzoic acid. Key: ○, 25.5°; ◒, 37.0°; ●, 45.0°; - - - ● - - -, 3 Gm. of acid equilibrated; —●—, 4.5 Gm. of acid equilibrated.

metrically using the Beckman DB spectrophotometer at 254 mμ. The results are tabulated in Table III and are presented in graphic form in Fig. 2.

m-Hydroxybenzoic Acid

Method.—The experimental procedure employed for determining the extent of interaction between *m*-hydroxybenzoic acid and urea is essentially the same as that used in the previously described complexing studies. Due to the greater solubility of the *meta* isomer, more acid (see Table I) was added to the flasks to assure sufficient excess of acid in the reaction mixtures. The amount of *m*-hydroxybenzoic acid in solution was determined spectrophotometrically at 236 mμ using the Beckman DB spectrophotometer. All samples and controls were run in triplicate. Results are given in Table IV and presented in graphic form in Fig. 3.

Effect of Excess Equilibration Time on the Solubility of the Acids

To determine the effect of agitating time on the solubility of the three isomeric acids, aliquot quantities of certain reaction mixtures were removed at regular intervals of time, diluted, and assayed spectrophotometrically, as previously described. The *ortho* and *para* isomers were equilibrated quickly; therefore, only the results obtained for the *meta* compound at 45° are presented in Fig. 4.

DISCUSSION

***o*-Hydroxybenzoic Acid.**—A study of Fig. 1 and Table II indicates that the solubility of salicylic acid increases with an increase in urea concentrations up to a certain point. This increase in the solubility of the acid can be attributed to the formation of a soluble complex which continues to form as the urea concentration increases up to the saturation point.

The solubility data also demonstrate the precipitation of an insoluble complex at 25.5° and 37° in the presence of high concentrations of urea. This observation appears to be in accordance with Bolton's report (3) of a similar complex formation at 30°; however, the studies conducted at 45° with an additional amount of acid (acid increased from 2 to 2.5 Gm.) do not show precipitation of the complex. At present, the insoluble complex obtained at 25.5° as well as other complexes formed by the interaction of urea with the isomers is under investigation. It is interesting that Higuchi and Zuck reported the formation of an analogous complex of salicylic acid and caffeine (6).

It can be concluded from the preceding data that urea concentrations of approximately 3 *M* at 25.5° and 4 *M* at 37° represent the saturation points with respect to the salicylic acid–urea complex. Any increase in urea concentration beyond the saturation level with respect to the acid–urea complex will effect the precipitation of the complex and thus deplete the acid in solution in accordance with the following equilibrium expression: K_{sp} = [salicylic acid] [urea].

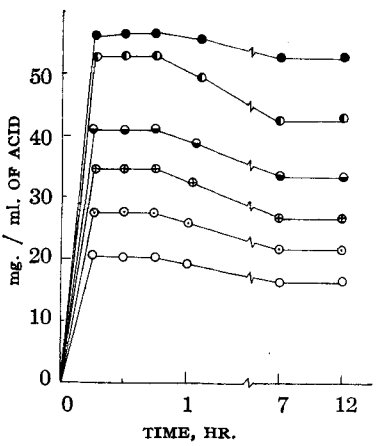

Fig. 4.—Effect of shaking time on the solubility of *m*-hydroxybenzoic acid at 45°. Key: ○, 0 *M* urea; ⊙, 1 *M* urea; ⊕, 2 *M* urea; ◒, 3 *M* urea; ◐, 4 *M* urea; ●, 5 *M* urea.

At these high levels of urea, the solubility characteristics of the complex(es) may be associated also with the lower water concentrations.

p-Hydroxybenzoic Acid.—The results obtained for this acid at lower urea concentrations are similar to those found for the *ortho* isomer. These studies indicate that at all three temperatures and at the lower urea concentrations equimolar amounts of acid and urea complex. However, at approximately 3 and 5 *M* urea concentrations, inflections in the curves are obtained which indicate the possible formation of additional complexes. In can be concluded that the complex(es) is stable and soluble in the urea solutions studied for at least 12 hr. at temperatures of 25.5°, 37°, and 45°.

m-Hydroxybenzoic Acid.—The relatively high water solubility characteristics of the *meta* compound are due to its lesser tendency to hydrogen bond intramolecularly and intermolecularly. Of the three isomers, the *m*-hydroxybenzoic acid possesses the least acidic hydroxyl hydrogen because the electron attracting effect of the carboxyl group is exerted primarily on the *ortho* and *para* positions. On the basis of this rationale, it is not difficult to account for the more pronounced increase in solubility of the *meta* compound by various urea concentrations.

The leveling off of the solubility curve at 45° (with 3 Gm. of acid) demonstrates that all the acid used was consumed in the complexation. This conclusion is confirmed by the observation of the homogeneity of these samples at the end of the agitation period.

GENERAL DISCUSSION

Although the shapes of the solubility curves and an analysis of the acid–urea ratio from some of the phase diagrams, according to the method of Higuchi and Lach (7), might suggest the formation of a 1-to-1 complex, the results of this investigation and those obtained by Bolton (3) point to the uncertainty of the nature of the interaction products.

Attempts were made to calculate the equilibrium constants for the interactions between urea and the three isomers at different temperatures, according to the method of Higuchi and Zuck (4); however, considerable variation in the value for each acid was observed as the concentration of urea was increased. Since the structures of the interaction products over a range of urea concentrations are not known definitely and since urea in aqueous solution may complex or associate with itself (8, 9), especially at high concentrations, it is not too difficult to understand why the equilibrium constants for the systems studied vary within certain limits. This difficulty has been observed by other workers in the field. For example, the effect of the nature of a complexing agent on the equilibrium constant of certain interactions has been discussed by Guttman and Higuchi (10). These workers pointed out that the complexing agent, caffeine, can exist in aqueous solutions as a monomer, dimer, and tetramer, and this in part would account for the variations of equilibrium constant obtained by Higuchi and Zuck (5) with caffeine and benzoic acid.

Since it was difficult to determine a satisfactory over-all equilibrium constant for each isomer at the temperatures studied, no thermodynamic calculations are offered. Attempts to isolate and characterize complexes of the three isomers at different levels of urea concentrations have not been fruitful yet; however, such studies currently are in progress as an extension of the present work. The problem of characterizing such complexes is a difficult one, since many complexes are not stable to recrystallizations from aqueous solutions and often complexes isolated and characterized may not necessarily be the ones present at any of the complexing concentrations studied.

An analysis of the data of the solubility of the isomeric acids in various dissolution fluids as a function of shaking time at three temperature levels reveals that equilibria for the *ortho* and for the *para* compounds are established well within the first hour of agitation, and the solubility of these acids remains constant during the 12-hr. run. However, with the *meta* isomer, different amounts of the acid in solution were found, depending upon the length of time the samples were agitated. These findings are exemplified in Fig. 4. The results of the experiments show that generally more of the *meta* compound is in solution within the first hour than is found upon analysis of the 7- and 12-hr. samples. These differences are more significant for the study carried out at 45° than for those run at 25.5° or 37°. Since it is not known whether these changes in dissolution are due to variations in the solubility and stability of the complex initially formed or to a change in the complex ratio after a certain period of agitation, further work on this phase of the problem is indicated.

SUMMARY AND CONCLUSIONS

Some new information concerning the role of urea as a complexing agent is reported. From the collected data the following conclusions may be made.

1. The over-all reaction of each isomer with urea is relatively complex.

2. Although urea possesses low complexing ability, it is a satisfactory solubilizing agent for the three monohydroxybenzoic acids when less than 3 *M* concentrations are used.

3. Urea increases the solubility of the *para* isomer by forming a complex(es) which is soluble and stable for at least 12 hr. at temperatures of 25.5°, 37°, and 45°.

4. The *meta* compound apparently complexes readily with urea in aqueous solution; however, the concentration of the acid in solution decreases within certain limits with the time the samples are agitated.

5. Of the three isomers, the *meta* compound has the greatest complexing ability with urea; the *ortho* isomer has the least complexing ability. This can be rationalized on the basis of its structure which allows for greater self-stabilization.

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