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Interaction of Pharmaceuticals with Schardinger Dextrins III

Interactions with Mono-Halogenated Benzoic Acids and Aminobenzoic Acids

By JOHN L. LACH and TING-FONG CHIN

A series of mono-halogenated benzoic acids and aminobenzoic acids were shown to undergo definite interactions with the cyclodextrins. The complexes formed are considered to be due, in part, to inclusion formation and to other attractive forces existing between the guest and host molecules. Data are presented to illustrate the effect of steric hindrance, polarity, resonance structure and inductive effect with respect to this interaction. Some stoichiometric data are also presented and several formation constants calculated.

THE CYCLIC STRUCTURE of the dextrins confers upon these compounds the ability to form mono-molecular inclusion compounds, where the guest is enclosed within the cyclodextrin void. In the case of these cyclodextrins there is just one host molecule formed by several constituents (glucose units) united through ordinary chemical bonds, which enclose one single molecule of the guest. This makes the cage of a permanent nature as opposed to clathrates (these may also have a cage-like hollow space in the center as in the dextrins) which are derived from crystalline lattices.

Generally speaking, formation of solid crystalline inclusion compounds can be explained on the basis that the molecules are held together by virtue of their spatial configuration, and these compounds are defined as chemically inert toward each other. True inclusion formation therefore implies that no attractive force or forces are of paramount importance between guest and host. However, in aqueous solution inclusion compounds of the cyclodextrins and various organic substances do exist (as reported from these laboratories) (1, 2), indicating that some attractive force or forces are operative which stabilize the guest molecule in the cage-like hollow space or the guest molecules are bonded to the cyclodextrin in some manner other than inclusion formation. The purpose of this investigation was to study this interaction further in terms of polarity, inductive effect, resonance structure, and steric hindrance with respect to the degree of interaction of these dextrins with various agents. A series of mono-halogenated and aminobenzoic acid derivatives were selected for this study.

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EXPERIMENTAL

Reagents:

α-Cyclodextrin $[\alpha]_D^{25}$ in water = +150.5 ± 0.5; β-cyclodextrin $[\alpha]_D^{25}$ in water = +162.5 ± 0.5;

p-chlorobenzoic acid, recrystallized, m.p. 235–236°; *m*-chlorobenzoic acid, recrystallized, m.p. 158–159°; *o*-chlorobenzoic acid, recrystallized, m.p. 140–141°; *p*-bromobenzoic acid, recrystallized, m.p. 250–251°; *m*-bromobenzoic acid, recrystallized, m.p. 154–155°; *o*-bromobenzoic acid, recrystallized, m.p. 148–150; *p*-iodobenzoic acid, recrystallized, m.p. 263–265°; *m*-iodobenzoic acid, recrystallized, m.p. 185–187°; *o*-iodobenzoic acid, recrystallized, m.p. 161–162°; *p*-aminobenzoic acid, recrystallized, m.p. 186° dec.; *m*-aminobenzoic acid, recrystallized, m.p. 175° dec.; *o*-aminobenzoic acid, recrystallized, m.p. 145–146°; cinnamic acid, recrystallized, m.p. 133°, were utilized.

Apparatus:

A constant temperature water bath, set at 30 ± 0.5° with rotating spindle; 20-ml. capacity vials with gum rubber stoppers and aluminum caps; Beckman DU spectrophotometer with 1-cm. silica cells.

Procedures:

Preparation and Separation of Cyclodextrins.—The method used for the preparation and separation of cyclodextrins is the modified method of French (3).

Determination of Molecular Complex Formation.—The solubility method of Higuchi and Lach (4) was used to study complex formation. The total concentration of the drug in the solution was measured spectrophotometrically since the cyclodextrins show no absorption at the wavelengths and concentration employed. Absorptivities had previously been determined for each of the drugs studied at the following wavelengths: *p*-chlorobenzoic acid, 238 mμ; *m*-chlorobenzoic acid, 280 mμ; *o*-chlorobenzoic acid, 238 mμ; *p*-bromobenzoic acid, 240 mμ; *m*-bromobenzoic acid, 280 mμ; *o*-bromobenzoic acid, 240 mμ; *p*-iodobenzoic acid, 244 mμ; *m*-iodobenzoic acid, 282 mμ; *o*-iodobenzoic acid, 244 mμ; *p*-aminobenzoic acid, 278 mμ; *m*-aminobenzoic acid, 300 mμ; *o*-aminobenzoic acid, 318 mμ· and cinnamic acid, 270 mμ.

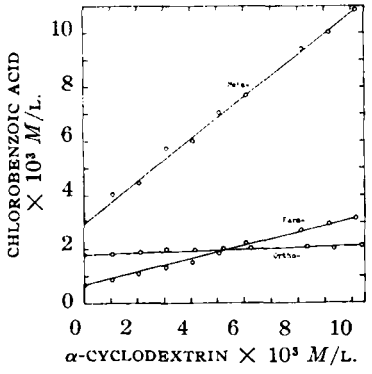

Fig. 1.—Interaction of chlorobenzoic acids with α-cyclodextrin at 30°.

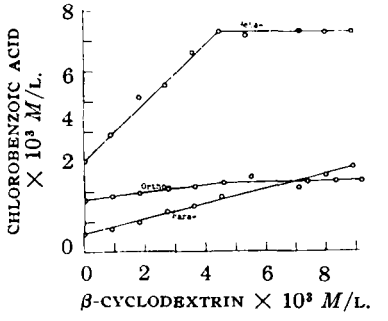

Fig. 2.—Interaction of chlorobenzoic acids with β-cyclodextrin at 30°.

RESULTS AND DISCUSSION

A number of methods were tested for this molecular complexation study. These methods included partition distribution, equilibrium dialysis, and solubility method in nonaqueous solutions.

The partition distribution method proved unsuccessful. The addition of various concentrations of cyclodextrin to a chloroform-water system containing benzoic acid resulted in no change in the distribution of this organic acid between the two phases, indicating that no interaction of the dextrin

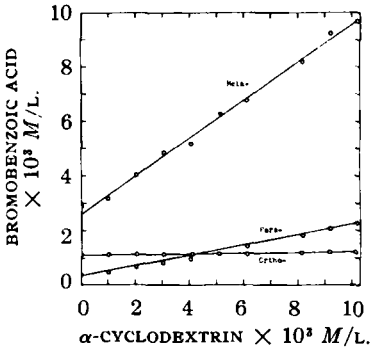

Fig. 3.—Interaction of bromobenzoic acids with α-cyclodextrin at 30°.

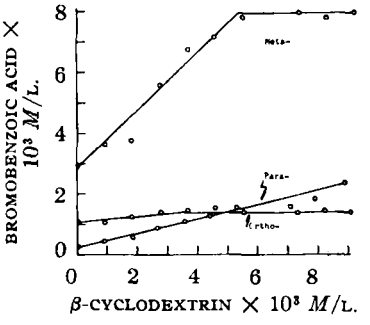

Fig. 4.—Interaction of bromobenzoic acids with β-cyclodextrin at 30°.

and benzoic acid had taken place. The use of ether, carbon tetrachloride, skellysolves, and benzene in this distribution study was also unsuccessful. This was somewhat surprising since benzoic acid and cyclodextrin readily interact in aqueous solution. This lack of interaction in the two-phase system is probably because the affinitive force of cyclodextrin with water and the affinitive force of benzoic acid with the organic solvent are greater than the attractive force of the cyclodextrin and benzoic acid.

A solubility method employing organic solvents to minimize any interaction due to hydrogen bonding or polar effects was also investigated. The solubility effect of benzoic acid as a function of cyclo-

dextrin in solvents such as chloroform, carbon tetrachloride, ether, 1,4 dioxane, skellysolves, and benzene was studied. However, as with the partition study, no interaction was observed. This lack of interaction of benzoic acid with the dextrins in such solvents does indicate that attractive forces must play a major role in interactions of this type in solution. However, it should be pointed out that this lack of reactivity may also be partly because benzoic acid forms dimers in many organic solvents (5). This lack of reactivity was also noticed by Schlenk (6), who pointed out that water seems to be a necessary component for complex formation of the cyclodextrins. Schlenk reported no interaction when the anhydrous cyclodextrins were heated and shaken with myristic acid in methanol. Also, these cyclodextrins failed to react with dry vapor of tri-

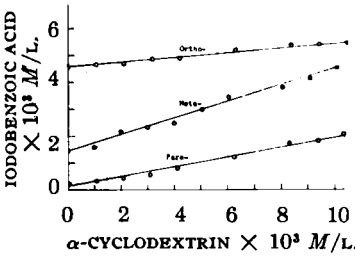

Fig. 5.—Interaction of iodobenzoic acids with α-cyclodextrin at 30°.

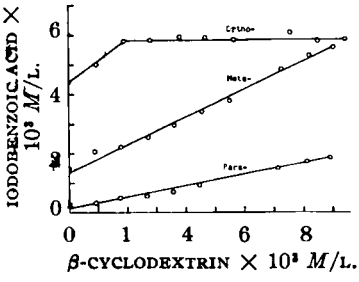

Fig. 6.—Interaction of iodobenzoic acids with β-cyclodextrin at 30°.

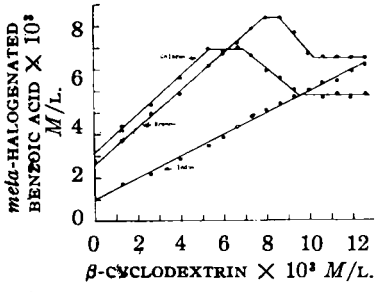

Fig. 7.—Interaction of *meta*-halogenated benzoic acids with β-cyclodextrin at 30°.

chloroethylene or bromobenzene, both known to be typical adduct formers.

The solubility method employing an aqueous solution was, therefore, used to study these interactions. Two series of agents were employed—mono-halogenated benzoic acid derivatives to study inductive and polar effects, and aminobenzoic acid derivatives to study resonance structure effects with respect to this cyclodextrin interaction. The results of these interactions are shown in

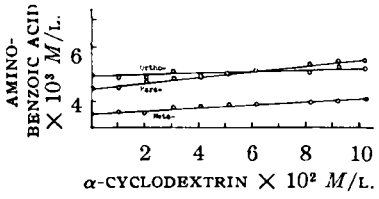

Fig. 8.—Interaction of aminobenzoic acids with α-cyclodextrin at 30°.

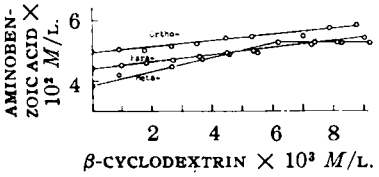

Fig. 9.—Interaction of aminobenzoic acids with β-cyclodextrin at 30°.

Figs. 1–9. A definite increase in the equilibrium solubility of the mono-halogenated benzoic acids as a function of cyclodextrin added is observed in Figs. 1–6. Since the concentration of the free benzoic acids in solution is an invariant, exact stoichiometric relationships cannot be calculated from these phase diagrams; however, the linear dependency of these interactions is indicative of a first-order relationship with respect to the cyclodextrin concentration. Since the solubility limit of β-cyclodextrin was not exceeded in this system it was felt that some information regarding the stoichiometric relationship would be obtained using this higher dextrin concentration. Figure 7 shows a phase diagram obtained for the interaction of *m*-chlorobenzoic acid, *m*-bromobenzoic acid, and *m*-iodobenzoic acid with β-cyclodextrin. From the plateau region of these diagrams the stoichiometry of *m*-chlorobenzoic acid and *m*-bromobenzoic acid can be calculated according to the method of Higuchi and Lach (4). A 1 to 1 relationship is obtained for these two acids. The formation constant for both acids was calculated to be 2.24×10^3. A formation constant of this magnitude is considerably greater than those previously reported for interactions of this type (7–9). This is certainly indicative of the formation of a strong cyclodextrin complex.

A comparison of the interactions of the halogenated derivatives and the cyclodextrins can be approximated from the slopes of these isotherms and are presented in Tables I and II.

An examination of data presented in these tables reveals the following observations:

1. The degree of interaction for chlorobenzoic acid is greater than bromobenzoic acid which in turn is greater than iodobenzoic acid.

2. The interaction of *meta* halogenated benzoic acids is greater than for the *para* halogenated benzoic acids which is in turn greater than the *ortho* halogenated benzoic acids.

3. The interaction of β-cyclodextrin is greater than that of the α-cyclodextrin.

It is difficult at this time to interpret these data mathematically. However, certain physical and chemical properties of these halogenated acids are evident. As already pointed out, the degrees of interaction of chlorobenzoic acids are greater than

TABLE I.—SLOPES OF ISOTHERMS OF INTERACTION OF THE MONO-HALOGENATED BENZOIC ACIDS WITH α-CYCLODEXTRIN

Position of Halogen Substituted	Benzoic Acid Derivatives		
	Chloro	Bromo	Iodo
Meta	0.76	0.68	0.31
Para	0.24	0.19	0.18
Ortho	0.03	0.01	0.08

those of bromobenzoic acids which in turn are greater than those of iodobenzoic acids. This order of reactivity may be due, in part, to inclusion formation. Since the void space in the cyclodextrin molecule is fixed, it is certainly reasonable that the smaller molecule would interact to a greater degree by virtue of its ease of entrance into the void. Attempts to correlate this activity of the mono-halogenated *para* and *meta* benzoic acids with the

TABLE II.—SLOPES OF ISOTHERMS OF INTERACTION OF THE MONO-HALOGENATED BENZOIC ACIDS WITH β-CYCLODEXTRIN

Position of Halogen Substituted	Benzoic Acid Derivatives		
	Chloro	Bromo	Iodo
Meta	0.96	0.93	0.47
Para	0.25	0.24	0.19
Ortho	0.13	0.09	0.74

Hammett sigma constants was only partially successful suggesting that, in addition to hydrogen bonding, steric factors and other attractive forces are also operative in this interaction. Interestingly, the order of reactivity of these halogenated aromatic acids parallels the electronegativity of the halogen substituent on the aromatic ring and not solely on the polarity of the carboxyl group. This may be rationalized on the basis of an inductive effect brought about by the halogen substituent. Since the inductive effect of the *meta* isomer is greater than that of the *para* isomer, the result is a lower electron density at the carboxyl group, facilitating a greater degree of intermolecular hydrogen bonding. This difference in degree is evident in the greater interaction of the *meta* isomer. Although the electron density of the carboxyl group is lowest in the *ortho* acid, the low degree of interaction can be rationalized on the basis of a steric effect. The bulky halogen atom in this *ortho* position decreases the hydrogen bonding tendency of the carboxylic acid and increases the overall size of the terminal position of the molecule to hinder the ease of entrance into the void space of the cyclodextrin. However, the reason for the *o*-iodobenzoic acid interaction with β-cyclodextrin being greater than the *para* and *meta* isomers cannot be explained at this time.

Data obtained in a study of a series of aminobenzoic acids are presented in Figs. 8 and 9. Table III lists the slopes of these isotherms.

The results of this aminobenzoic acid series are, at this time, somewhat difficult to understand, particularly in the interactons of the α-cyclodextrin. The order of reactivity of the *para* and *meta* isomers does not parallel the order observed in the halogenated and hydroxylated series (1). It does, as expected, parallel this order in the β-cyclodextrin interaction. This may be due, in part, to the greater freedom of entrance of the interacting molecule afforded by the larger void in the β-cyclodextrin; consequently, steric considerations would be less than in the restricted α-cyclodextrin molecule. The greater observed activity of the *para* isomer in the α-cyclodextrin over the *meta* isomer is also not readily obvious. A consideration of the resonance effect with respect to the polarity of the carboxyl group in the *para* isomer should lead to a lesser degree of interaction than the *meta* isomer. Since this is not

TABLE III.—SLOPE OF THE ISOTHERMS OF INTERACTION OF THE AMINOBENZOIC ACIDS WITH CYCLODEXTRINS

Aminobenzoic Acids	α-Cyclodextrin	β-Cyclodextrin
m-Aminobenzoic acid	0.58	2.25
p-Aminobenzoic acid	0.98	1.09
o-Aminobenzoic acid	0.29	0.91

the case the data suggest that the steric effect is more dominant in the α-cyclodextrin containing the smaller void.

It is interesting to compare the cyclodextrin interaction with cinnamic acid (Fig. 10) and that of benzoic acid which has been reported by Lach and Cohen (1). The difference in these two molecules lies in a carbon double bond. Lach and Cohen reported a slope of approximately 1.0 for the ben-

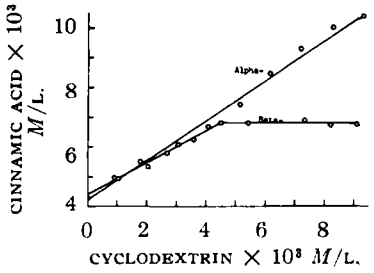

Fig. 10 — Interaction of cinnamic acid with cyclodextrins at 30°.

zoic acid interaction. A slope of approximately 0.6 is obtained here for the cinnamic acid study. The greater degree of interaction of benzoic acid may be attributed to the higher polarity of the carboxylic group due to resonance consideration. Comparison of this interaction based on size only should not produce such a great change in slope which is a measure of the degree of reactivity.

Investigations of this type do indicate the complexity of such interactions. Studies with other molecules and the cyclodextrins are being conducted in these laboratories to elucidate further the nature of these interactions.

CONCLUSION

Data presented show that the cyclodextrins form molecular complexes with the mono-halogenated benzoic acids and the aminobenzoic acids in aqueous solution. It is difficult, at this time, to explain fully the mechanism involved in this interaction since no crystallographic data of the complexes formed are available. However, it can be pointed out that, in addition to inclusion formation, other attractive forces must also be operative in the net interaction. Several formation constants and stoi-

chiometric ratios have been calculated. The higher order of magnitude of these formation constants indicates a high degree of stability of the complex.

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Potential Hypoglycemic Sulfonylureas

By KARL A. NIEFORTH†, GLENN L. JENKINS, and ADELBERT M. KNEVEL

A series of *N'*-alkyl-*N*-*p*-phenylbenzenesulfonylureas and *N,N'*-bis(*N*-alkylcarbamyl) 4,4'-biphenyldisulfonamides has been prepared for pharmacological studies of their hypoglycemic activities.

ALTHOUGH SULFONYLUREAS have been reported in the literature for several years, it was not until 1955 that the hypoglycemic activity of these compounds was recognized and reported. Since that time, the interest in synthesizing compounds of this type has increased considerably and has resulted in a few marketable compounds. The purpose of this paper is to extend research in this area to include the biphenyl nucleus.

Early work in the area of hypoglycemics was centered around derivatives of guanidine (1, 2). Several years later, various derivatives of isopropylthiadiazole were investigated and found to be active hypoglycemics (3). This work (3) may also be considered to be the start of the use of sulfonamides in controlling the symptoms of diabetes. In 1955, a sulfonylurea compound was found to be very active in lowering the blood sugar concentration in diabetics (4). After this work was reported, new active compounds began appearing at a rapid rate (5–8). It is now generally accepted that the sulfonylurea function is a source of hypoglycemic compounds. The work in this paper is based on that assumption and is conducted in two parts. The first part is the synthesis of *N'*-alkyl-*N*-*p*-phenylbenzenesulfonylureas (VII); the second part is the synthesis of *N,N'*-bis(*N*-alkylcarbamyl)4,4'-biphenyldisulfonamides (XIV).

Biphenyl (I) was sulfonated according to a published procedure (9) and gave good yields of 4-biphenylsulfonic acid (II). This in turn was reacted with phosphorus pentachloride using carefully controlled conditions to form 4-biphenylsulfonyl chloride (III). If the conditions were not controlled, varying amounts of 4,4'-biphenyldisulfonyl chloride (X) were found in the reaction mixture. The sulfonyl chloride was heated in ammonia water forming 4-biphenylsulfonamide (IV). The sulfonylurea (V) was prepared (10) upon reaction of the sulfonamide with *n*-butylisocyanate in the presence of triethylamine. Because of the difficulty in preparing the various isocyanates needed in this project, an alternate route was used to prepare the remainder of the compounds in Part I (11).

In Part II, biphenyl(VIII) was sulfonated with an excess of sulfuric acid to form 4,4'-biphenyldisulfonic acid (IX) (12). The reaction between 4,4'-biphenyldisulfonic acid and phosphorus pentachloride was controlled to prevent the formation of 4,4'-dichlorobiphenyl. 4,4'-Biphenyldisulfonyl chloride (X) was heated with ammonia water to form 4,4'-biphenyldisulfonamide (XI). From this point, the reactions in Part II are the same as those utilized in Part I. One compound (XII) was synthesized by the reaction of 4,4'-biphenyldisulfonamide and *n*-butylisocyanate. The remainder of the compounds was prepared by the alternate route (XI-XIV).

EXPERIMENTAL[1]

4-Biphenylsulfonic Acid and 4,4'-Biphenyldisulfonic Acid.—These compounds were prepared by reacting biphenyl with sulfuric acid. The quantities of sulfuric acid depended on the desired acid (9, 12). The salts of the sulfonic acids were formed by dissolving the acids in alcohol and adding concentrated solutions of potassium or sodium hydroxide to the alcoholic solutions.

4-Biphenylsulfonyl Chloride.—Twenty grams

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[1] Sulfur determinations were carried out by Dr. G. Weiler and Dr. F. B. Strauss, Microanalytical Laboratory, 164 Banbury Road, Oxford, England, and Alfred Bernhardt, Mikroanalytisches Laboratorium im Max-Planck-Institut fur Kohlenforschung, Hohenweg 17, Mulheim (Ruhr), Germany.