

Interaction of Pharmaceuticals with Schardinger Dextrins I

Interaction with Hydroxybenzoic Acids and *p*-Hydroxybenzoates

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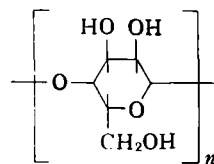
By means of the solubility method of analysis, the Schardinger dextrins were shown to interact in aqueous solution with benzoic acid derivatives. A definite interaction occurred with all compounds tested. Some stoichiometric data are presented and several formation constants calculated. It is postulated that both hydrogen bonding and inclusion formation are responsible for the main interaction observed.

SCHLENK (1) defines inclusion compounds as addition compounds in which one entity fits into and is surrounded by the crystal lattice of the other. These inclusion compounds may be called "no bond" complexes and are characterized by the lack of adhesive forces between the components of the complex. It is not a chemical interaction which causes an inclusion compound to form, although this may be a factor in the net complexation observed.

The steric configurations of the molecules are such that the enclosing, or "host," molecule can spatially enclose the included, or "guest," molecule, leaving unaffected the bonding systems of the components. Geometrical rather than chemical characteristics of the molecules are limiting factors in the interaction.

The chief forces involved in an inclusion compound are those holding host molecules to each other forming the space which encloses the guest molecule. Such is not the case with monomolecular inclusion compounds formed by one molecule of host enclosing one or more molecules of guest. This type of inclusion formation is typified by the Schardinger dextrins.

The Schardinger dextrins are a series of homologous oligosaccharides obtained from the breakdown of starch by the action of *Bacillus macerans* amylase. They are also known (2) as the cycloamyloses, cyclodextrins, and cycloglucans. They are homogeneous cyclic molecules composed of six or more alpha-D-glucopyranose units linked 1-4 as in amylose (3). Their structure is



where n is 6, 7, or 8, known as the alpha, beta, and gamma cyclodextrins, respectively.

The cyclic structure of the Schardinger dextrins confers upon the compound the ability to form monomolecular inclusion compounds where the guest is enclosed within the cyclodextrin void. These inclusion compounds may be grouped within the class of clathrate compounds where the host assumes a cage-like shape. Because of their cyclic structures, and the relatively large open space within each molecule (6 Å. for alpha, 8 Å. for beta, and 10–11 Å. for gamma), they have been reported to form complexes with crystal violet (4), carbon dioxide (5), 1-isopropyl azulene, cinnamaldehyde, hexachlorocyclohexane, pentamethylene tetrazole, and diethylaminoethyl-*p*-amino benzoate (6), ethylene (7), methyl orange (8), tetrachlorosalicylate, butter yellow, and adrenaline (9), and various gases (10).

Because of their different internal ring sizes, the cyclodextrins show different degrees of inclusion formation with different sized molecules. For example (11), alpha has been shown to form adducts with chlorine, bromine, iodine, hydrocarbons, and benzene derivatives with small substituents. Beta interacts with bromine, iodine, and almost any organic molecule. Gamma interacts with iodine and only large organic molecules. Alpha forms an inclusion compound with ethylene (7), while beta does not.

The clathrate forming power of the cyclodextrins has been utilized in various applications. They have been shown (12) to retard the cleavage of glycosides by inclusion. Cramer

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(13) has carried out basic catalyses with the cyclodextrins. They have been used as indicators (14) in paper chromatography. By means of the cyclodextrins (15), optically active compounds have been separated and solutions of labile compounds have been stabilized. They have been used for the resolution of racemates (16). The cyclodextrins form clathrates with many labile drugs such as unsaturated fatty acids and vitamin A (17). Since the drug included is, in essence, shielded by the host from its surroundings, stability of the drug can be greatly increased.

In view of the properties of these dextrans, this study was undertaken as a preliminary investigation into the possible utilization of the Schardinger dextrans in pharmaceutical systems.

EXPERIMENTAL

Reagents.—Benzoic acid, recrystallized, m.p. 119–121°; *o*-hydroxybenzoic acid, recrystallized, m.p. 155–157°; *m*-hydroxybenzoic acid, recrystallized, m.p. 198–200°; *p*-hydroxybenzoic acid, recrystallized, m.p. 212–214°; methylparaben, m.p. 124–126°; ethylparaben, recrystallized, m.p. 112–115°; propylparaben, recrystallized, m.p. 95–97°; butylparaben, recrystallized, m.p. 67–69°; alpha cyclodextrin; beta cyclodextrin; *Bacillus macerans*, strain 7069, American Type Culture Collection; agar; dextrose; nutrient broth; Quaker oats; calcium carbonate, reagent grade; potato starch; trichloroethylene; bromobenzene; and *n*-propanol were used as reagents.

Apparatus.—A constant temperature water bath, set at 30°, $\pm 1/2^\circ$, with rotating spindle; 20-ml. capacity vials with gum rubber stoppers and aluminum caps; Beckman DU spectrophotometer; 1-cm. and 5-cm. silica cells comprised the necessary apparatus.

PROCEDURE

Preparation of the Schardinger Dextrans.—The Schardinger dextrans are prepared by the action of *Bacillus macerans* amylase on one or another of the common starches.

Our method of preparation was based on the experiences of others, notably French and his group (18) and Tilden and Hudson (19). The complete process is quite involved and time consuming and need not be gone into in detail, although the basic procedure will be summarized.

Bacillus macerans was grown on a rolled oats medium, buffered with calcium carbonate, at 40° for 2 to 3 weeks to permit production of the amylase enzyme. This enzyme-containing solution was then decanted into 4% starch (potato) paste and stored, with intermittent agitation, at 40° for 24 hours. At the end of this period, marked by a noticeable decrease in the viscosity of the starch paste, the production of the Schardinger dextrans was completed.

The Schardinger dextrans were precipitated out of solution as trichloroethylene complexes. The alpha,

beta, and gamma cyclodextrins were then separated by taking advantage of their differing solubilities in water and in the presence of bromobenzene. The individual cyclodextrins were then purified by repeated crystallizations from water and 60% *n*-propanol.

The greatest yields obtained were of beta, with lesser but satisfactory yields of alpha. Gamma was produced in such small quantities that no experimentation with this cyclodextrin was possible.

The cyclodextrins were checked for purity by the microscopic method of French (18) and by carbon-hydrogen analysis (Table I). These were then dried

TABLE I.—CARBON-HYDROGEN ANALYSIS OF THE CYCLODEXTRINS

Cyclo-dextrin	% C Calcd.	% C Det.	% H Calcd.	% H Det.
Alpha	44.44	43.79	6.22	6.16
Beta	44.44	43.84	6.22	6.46

in an oven at 70° and kept in a desiccator until used.

Solutions of the cyclodextrins were freshly prepared, with the aid of heat, before each run because of the tendency toward mold growth in these solutions.

Solubility Studies.—The procedure used in this study was the solubility method of Higuchi and Lach (20).

Quantities in excess of the normal solubilities of the drugs studied were weighed into 20-ml. vials. Quantities of 3% alpha or 1% beta cyclodextrin were accurately measured in, and water added to make a volume of 5 ml. of solution in each vial. A more concentrated solution of beta was not used because of its limited solubility in water. The vials were sealed with gum-rubber stoppers and aluminum caps and rotated on a mechanical shaker in a constant temperature water bath at 30° for 24–48 hours until the system reached equilibrium.

At the end of the equilibration time, 1- or 2-ml. aliquots of the supernatant solutions were removed, diluted with water, and analyzed for total drug concentration by ultraviolet spectrophotometry. Since several of the vials showed solid material present, a cotton plug was used as a filter on the pipet tips.

Absorptivities had previously been determined for each of the drugs studied at the following wavelengths: benzoic acid, 272.5 $m\mu$; *o*-hydroxybenzoic acid, 298 $m\mu$; *m*-hydroxybenzoic acid, 290 $m\mu$; *p*-hydroxybenzoic acid, 251 $m\mu$; methyl *p*-hydroxybenzoate, 256 $m\mu$; ethyl *p*-hydroxybenzoate, 256 $m\mu$; propyl *p*-hydroxybenzoate, 255 $m\mu$; and butyl *p*-hydroxybenzoate, 256 $m\mu$. It was determined that the cyclodextrins did not interfere with the spectrophotometric measurements at the concentrations employed.

RESULTS AND DISCUSSION

In order that some insight be gained into the mechanism of interaction of organic compounds with the Schardinger dextrans, two homologous series of acid derivatives, the hydroxybenzoic acids and the *p*-hydroxybenzoates, were tested for complexing activity with the cyclodextrins.

A definite interaction occurred with each of these eight compounds with both alpha and beta cyclodextrins. The straight-line plots obtained show that the increase in solubility of the drug is a function of the concentration of the cyclodextrin. All of the isotherms show a positive slope and several show a plateau. Plots showing this plateau are indicative of a relatively insoluble complex being formed. Phase diagrams of this type, with calculation of stoichiometries and formation constants, have been explained by Higuchi and Lach (20).

The Hydroxybenzoic Acids.—Figures 1 and 2 represent interactions of the hydroxybenzoic acids with the cyclodextrins. Table II represents the

TABLE II.—SLOPES OF ISOTHERMS OF INTERACTIONS OF THE HYDROXYBENZOIC ACIDS WITH THE SCHARDINGER DEXTRINS

	Alpha	Beta
<i>o</i> -Hydroxybenzoic acid	0.305	0.885
<i>p</i> -Hydroxybenzoic acid	0.813	1.044
Benzoic acid	1.004	1.135
<i>m</i> -Hydroxybenzoic acid	1.085	1.192

slopes of these plots. Several conclusions can be drawn from these data.

Data for these interactions indicate no clear cut mechanism of complexation. If these interactions were pure, that is classic, inclusion formation, the smallest molecule, unsubstituted benzoic acid, would show the greatest degree of inclusion and therefore the greatest slope. Since it does not, other forces are probably involved. This is reasonable in view of the multiplicity of hydroxyl groups on the cyclodextrin molecule and the likelihood of hydrogen

bonding between the cyclodextrin and the hydroxybenzoic acids in the aqueous system employed. This is further substantiated by the lowest complexing activity, as evidenced by the lowest slope, being a property of the *ortho*-hydroxy acid in the case of both alpha and beta interaction. *ortho*-Hydroxybenzoic acid possesses intramolecular hydrogen bonds, thus decreasing the number of hydroxyl and carboxyl groups available for hydrogen bonding with the cyclodextrin. Hydrogen bonding therefore would seem to be a factor of importance in the cyclodextrin-hydroxybenzoic acid interaction, especially since we note that the *meta*- and *para*-hydroxy acids interact to approximately the same degree as benzoic acid itself. Since these substituted acids are larger than the parent acid, we would expect them to show less tendency towards inclusion formation. However, they possess an extra hydroxyl group, causing greater intermolecular hydrogen bonding with the cyclodextrins, and a greater degree of interaction than expected is observed.

It would seem, therefore, that the interactions observed are not true inclusion formation. Inclusion formation as such involves molecules associated by spatial relationships only, while in the interactions observed, hydrogen bonding seems to play a significant role. Thus the interaction data must be considered from a semiquantitative standpoint since the interaction is probably a result of more than one mechanism; attempts to interpret the data assuming inclusion formation only would not be wholly successful.

Results of Schlenk and Sand (21) support this conclusion. Their work on the association of the Schardinger dextrins with organic acids showed that the mole ratio of the cyclodextrins to certain benzoic acid derivatives was a function of the physical state of the complex. The stoichiometry of

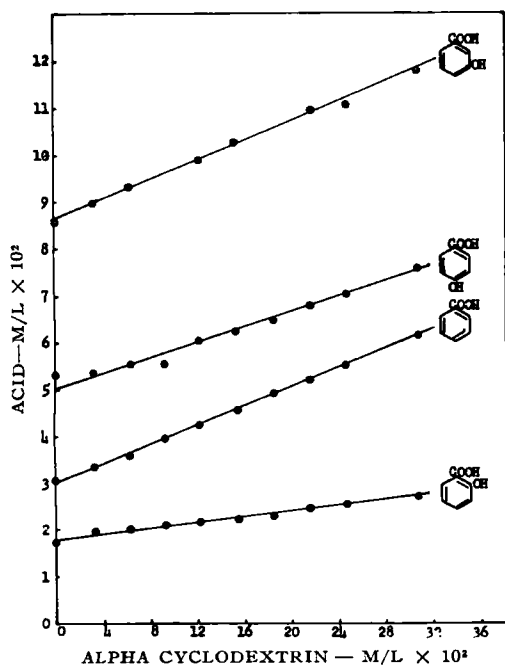


Fig. 1.—Interaction of the hydroxybenzoic acids with alpha cyclodextrin at 30°.

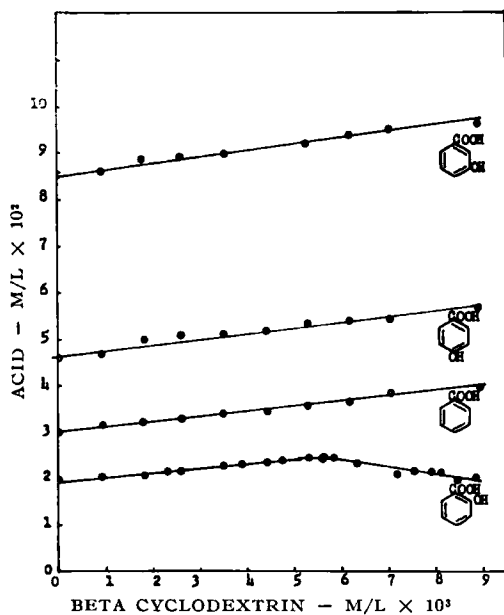


Fig. 2.—Interaction of the hydroxybenzoic acids with beta cyclodextrin at 30°.

the crystallized solid complex was not the same as that of the associated compound in solution. This indicates a complex mechanism of interaction in the aqueous system studied. It should be emphasized here that this study was conducted in aqueous solution where conditions for hydrogen bonding are favorable. In nonpolar systems, hydrogen bonding would not be as significant a factor in the net interaction.

Certain plots obtained through solubility data provide information concerning both stoichiometry and formation constant of the complex formed.

Where no plateau region is obtained, stoichiometry cannot be determined because the concentration of free drug present in solution is an invariant. However, linear dependence of the complexation upon cyclodextrin concentration is indicative of a first order dependency of the interaction upon cyclodextrin concentration. In cases such as this, stoichiometry with respect to the drug molecule is difficult to calculate since the data were obtained at constant drug activity because of the drug excess present.

Of the hydroxybenzoic acids, only the salicylic acid-beta cyclodextrin plots shows a plateau region which is indicative of formation of a relatively insoluble complex. From the plateau region of a phase diagram of this type, the stoichiometry of the complex can be determined and its formation constant calculated (20). Analysis of this region indicates three salicylic acid to one beta cyclodextrin complex, with a formation constant on the order of 5×10^4 . This value is accepted with reservation because of the probable complexity of the interaction. The magnitude of this formation constant does indicate, however, the existence of a complex of great stability. In other studies of interactions of

the hydroxybenzoic acids (22-25), calculated formation constants were all on the order of 1-100, calculated on the basis of a 1:1 interaction. Since the cyclodextrin interaction constant is much higher, an interaction of greater stability than the previously reported intermolecular attractive forces is indicated. This is reasonable in view of the complexity of these interactions and the probability of more than one interaction mechanism being involved.

The *p*-Hydroxybenzoates.—In the study of the interactions of the cyclodextrins with esters of *p*-hydroxybenzoic acid, strong interactions are also observed (Figs. 3 and 4).

Alpha forms soluble complexes with the parabens while the beta isomer forms complexes of limited solubility. The complexes formed with alpha are all soluble due to the greater solubility of alpha over beta. For the beta interactions, the data indicate that the less the solubility of the paraben, the less the solubility of the complex. Thus, methyl forms a soluble complex while butyl forms a relatively insoluble complex. The ethylparaben and propylparaben complexes show intermediate stages of solubility.

For both alpha and beta interactions with the parabens, a decrease in slope with increasing molecular weight is observed, except for the propyl and butyl esters (Table III). This latter phenomenon

TABLE III.—SLOPES OF ISOTHERMS OF INTERACTIONS OF THE *p*-HYDROXYBENZOATES WITH THE SCHARDINGER DEXTRINS

	Alpha	Beta
Methylparaben	0.911	1.044
Ethylparaben	0.510	0.896
Propylparaben	0.295	0.687
Butylparaben	0.357	0.747

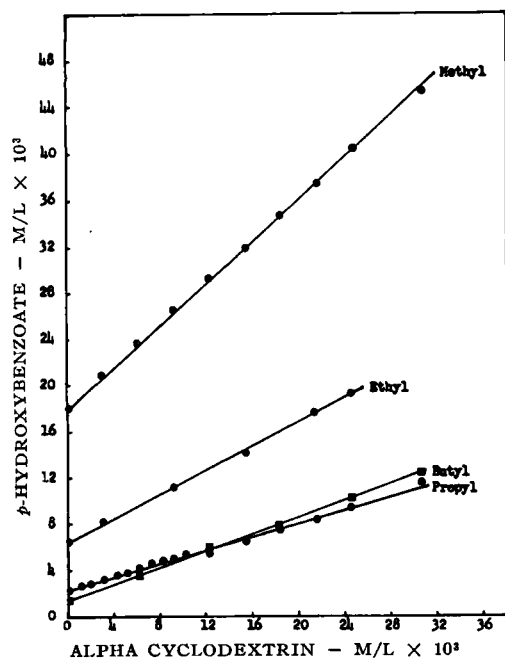


Fig. 3.—Interaction of the *p*-hydroxybenzoates with alpha cyclodextrin at 30°.

requires further elucidation of the mechanism of interaction before any explanations can be attempted. The overall tendency towards generally decreasing slope can be attributed to spatial filling; as the ester chain becomes longer and the molecule therefore larger, less inclusion of the ester takes place.

Calculation of stoichiometry along the propyl-beta and butyl-beta plateaus shows a molecular ratio in each case of 1:1. On this basis, stoichiometries of the methyl-beta and ethyl-beta complexes are tacitly assumed to be 1:1, and formation constants calculated (Table IV).

TABLE IV.—FORMATION CONSTANTS OF THE PARABEN-BETA COMPLEXES

Compound	K_f "infinite"
Methylparaben	1.38 $\times 10^3$
Ethylparaben	9.23 $\times 10^2$
Propylparaben	2.04 $\times 10^3$
Butylparaben	

These formation constants are quite high compared to those previously reported in pharmaceutical interaction studies. Stability constants reported for paraben complexes at 30° are on the order of 50 for complexes with caffeine (21), and are generally below 50 for complexes of drugs with other complex-

ing agents when studied by the solubility method (26). The work of Poole and Higuchi (27) with sarcosine anhydride shows very low formation constants, with some values approaching the magnitude of our results but usually falling below 100.

Formation constants of the magnitude reported here indicate formation of a complex of increased stability. Thus an interaction involving more than hydrogen bonding or dipole-dipole attraction is likely involved and a combination of these plus inclusion formation is a distinct probability.

Unpublished results of Pauli (28) indicate that alpha cyclodextrin has a greater stabilizing effect on the stability of benzocaine to alkaline hydrolysis than any other complexing agent reported. This too is indicative of a stable complex and lends additional support to the above conclusions.

CONCLUSION

Molecular complexes exist where there are forces of an electrostatic nature drawing molecules together. Inclusion formation involves molecules held together by virtue of their spatial configurations; these compounds have been defined as being chemically inert toward each other since true inclusion formation involves no attractive forces between guest and host.

This criterion for inclusion formation may be valid for crystalline and solid complexes, but when the complex exists in aqueous solution, as in this study, attractive forces such as hydrogen bonds come into play. Evidence for this is

provided by the hydroxybenzoic acid interactions where hydrogen bonding appears to play a significant role in the net interaction observed. Therefore, in these interactions of the cyclodextrins with organic molecules, we assume that hydrogen or other attractive bonds act to bring the molecules together in solution and play a part in holding them together.

The net interaction observed in this study is believed to be a combination of attractive forces and inclusion formation. No distinctions as to the relative contributions of the two forces can be obtained from the data presented. Since X-ray examination of solid compositions is the only method known by which unequivocal identification of inclusion compounds can be made (29), this question is deferred for further study.

High formation constants observed in this study indicate a more stable complex than is usually observed in pharmaceutical studies of this type. Since, undoubtedly, some attractive forces are to be considered in this study, we feel that the interaction of the Schardinger dextrins with organic molecules is probably a combination of hydrogen bonding, inclusion formation, or other forces acting as factors in the net observed interaction.

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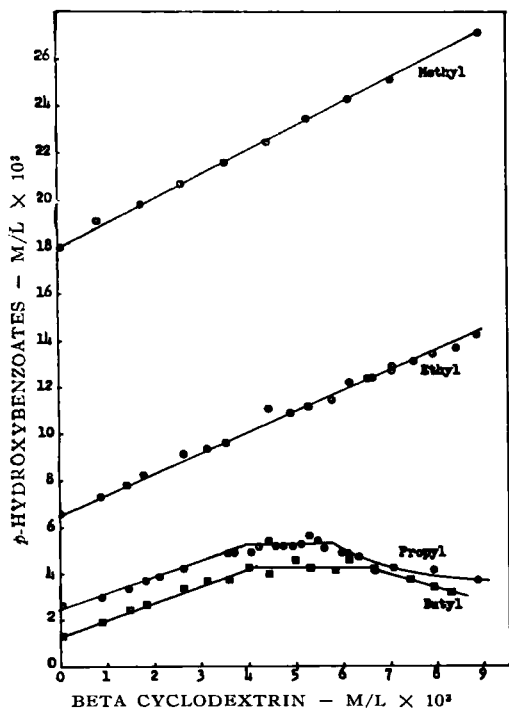


Fig. 4.—Interaction of the *p*-hydroxybenzoates with beta cyclodextrin at 30°.