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Interaction of Pharmaceuticals with Schardinger Dextrins V

Interaction with a Series of Phenyl-Substituted Carboxylic Acids

By WAYNE A. PAULI* and JOHN L. LACH

Interactions were observed between beta-cyclodextrin and a series of phenyl-substituted carboxylic acids in aqueous solution. By use of the solubility method of analysis, definite interactions were found to occur with each of the acids. When phase diagrams allowed, stoichiometric ratios were calculated for the complexes and the corresponding K_f and ΔF^0 values determined. Stoichiometries from the analysis of various isolated complexes agreed quite closely with those obtained from the phase diagrams. Several of the inclusion complexes exhibit extremely high formation constants indicative of thermodynamically favorable interactions. Even though a complex mechanism consisting of pure inclusion and other attractive forces is expected for these interactions, the experimental data indicate the relative importance of the separation between the carboxyl and phenyl groups in the net interactions observed. Saturated acids were found to be far more reactive with beta-cyclodextrin than were the corresponding unsaturated acids. This finding could have important pharmacological and biochemical implications, as beta-cyclodextrin has been used as an enzyme model by numerous investigators.

COMPLEX FORMATION, by means of molecular inclusion formation, has only recently been recognized as a promising area in the field of pharmacy. Schlenk (1) defines inclusion compounds as addition products in which one of the components fits into and is surrounded by the crystal lattice of a second. They are probably best described in a negative way, as they do not form by means of ionic, covalent, or coordinate covalent bonds, and are thus often referred to as "no-bond" interactions. Actually inclusion is believed to be the result of the ability of one compound, because of its peculiar stereochemical properties and possibly its polarity, to enclose a second compound spatially. The terms "guest" and "host" have been applied to the enclosed molecule and the enclosing molecular network, respectively. For inclusion formation to occur, the host must be capable of forming a solid structure containing hollow spaces large enough to accommodate a prospective guest species.

An inclusion compound will have a stability largely dependent on the spatial arrangement and fit between the guest and the host. Powell (2) notes that the important factor in inclusion formation is geometry rather than chemistry, and therefore the geometrical features of the interacting species are more critical than are their chemical characteristics. Pauling (3) has pointed out that often the host network is formed through the intermolecular hydrogen bonding of the individual host units.

The cyclodextrins, frequently called the Schardinger dextrins because they were originally prepared by him in 1903 (4), are macrocyclic nonreducing glucosyl polymers produced by the

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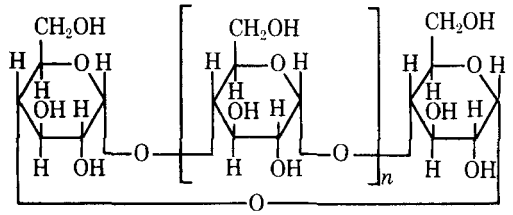
enzymatic partial hydrolysis of starch. They contain six or more residues bonded by alpha 1–4 linkages resulting in structure I.



I

The first three cyclodextrins, commonly known as alpha, beta, and gamma dextrin, differ structurally only in the values of n which are 4, 5, and 6, respectively. At the present time, the more popular system of nomenclature designates the various homologs by the names cyclohexa-, cycloheptа-, and cycloocta-amylose, etc., the Greek prefix corresponding to the degree of polymerization. The physical and chemical properties of the cyclodextrins have been rereported by Schardinger (5), Freudenberg (6), McClenahan (7), Cramer (8), and French (9).

As a result of their cyclic structure and internal cavities (6 Å. for alpha, 8 Å. for beta, and 10–11 Å. for gamma), the cyclodextrins readily interact with a wide variety of compounds forming monomolecular inclusion compounds. Because of their different ring sizes, they differ in degree of interaction with different sized molecules. Advantage is taken of this preferential behavior to separate them from one another by use of the proper organic reagents, as many of the resulting inclusion compounds are insoluble.

Many pharmaceutical agents have been studied regarding their interactions with the cyclodextrins (10–13). Labile compounds such as vitamin A and unsaturated fatty acids have been stabilized by combination with the cyclodextrins (14), indicating a promising application for these macrocyclic polymers in the pharmaceutical area.

An interesting aspect of cycloamylose chemistry is the ability of these materials to induce stereoselective precipitation (15).

Since 1950, interest has grown in the use of cyclodextrin clathrate compounds as model systems for more complicated and unexplained phenomena (16–19). Recent attention has been directed toward *Bacillus macerans* amylase, the enzyme used in the biosynthesis of the Schardinger dextrins (20).

In view of the work done in the pharmaceutical area utilizing the cyclodextrins, it appears that more attention need be given to the nature of these interactions in an effort to gain additional insight into the mechanisms involved. Since it has been definitely shown that interactions can occur through pure clathration alone or in combination with other attractive forces, it becomes increasingly important to clarify this phase of cyclodextrin technology.

EXPERIMENTAL

Reagents

Beta-cyclodextrin, $[\alpha]_D^{25°}$ in water = +162.5 ± 0.5; phenylacetic acid, recrystallized, m.p. 76–77°; cinnamic acid, recrystallized, m.p. 132–133°; hydrocinnamic acid, recrystallized, m.p. 47–48°; 4-phenylbutyric acid, recrystallized, m.p. 51–52°; 5-phenylvaleric acid, recrystallized, m.p. 58–59°; 5-phenyl-2,4-pentadienoic acid, recrystallized, m.p. 166–167°; phenylundecanoic acid, b.p. 183–186°/1.5 mm.

Apparatus

A constant-temperature bath maintained at 30 ± 0.5° with rotating spindle; 5-dram tinted prescription vials with plastic screw caps, parafilm sheeting used as cap liners; Beckman DU spectrophotometer; Beckman DK-2 spectrophotometer; 1-cm. quartz cells.

Procedure

Preparation of the Beta-Cyclodextrin.—The beta-cyclodextrin was prepared by the action of *B. macerans* amylase on potato starch. The method of preparation used in this laboratory is similar to the procedure originally presented by French and his group (9, 10).

Solubility Studies.—The solubility method of Higuchi and Lach (21) was used to study complex formation. Excess quantities of the substances to be complexed were accurately weighed into 5-dram prescription vials together with varying concentrations of beta-cyclodextrin. To each reaction vial, 10 ml. of distilled water then was added, after which they were sealed with parafilm and capped. The vials were then rotated on a mechanical spindle in a constant-temperature bath set at 30° for 24 hr. until the system reached equilibrium. Aliquot portions of the supernatant liquid then were removed, properly diluted, and analyzed spectrophotometrically. The beta-cyclodextrin showed no absorption at the wavelengths and concentrations employed. If the vials contained solid material at the end of equilibration, sintered-glass filters were used on the pipet tips. Absorptivities had previously been determined for each of the compounds at the following wavelengths: phenylacetic acid, 257 mμ; cinnamic acid, 272 mμ; hydrocinnamic acid, 258 mμ; 4-phenylbutyric acid, 258 mμ; 5-phenylvaleric acid, 259 mμ; 5-phenyl-2,4-pentadienoic acid, 300 mμ; phenylundecanoic acid, 258 mμ.

RESULTS AND DISCUSSION

Definite interactions were observed between all of the acids and beta-cyclodextrin. The relationships between the equilibrium solubilities of the various acids and beta-cyclodextrin concentration are clearly seen by the linear interaction isotherms. The presence of plateau regions in several of the

solubility plots, indicative of the formation of insoluble complexes, made possible the determination of stoichiometries between interacting species. Apparent formation constants and free energies of formation could then be calculated for these particular systems. Further verification of the stoichiometries obtained from the solubility diagrams was accomplished by the isolation and analysis of each of the insoluble complexes. Figures 1–7 represent the interactions of the phenyl-substituted carboxylic acids with beta-cyclodextrin. The slopes of these isotherms are shown in Table I.

TABLE I.—SLOPES OF ISOTHERMS OF INTERACTIONS OF THE PHENYL-SUBSTITUTED CARBOXYLIC ACIDS WITH BETA-CYCLODEXTRIN

Phenylacetic acid	0.77
Hydrocinnamic acid	0.93
4-Phenylbutyric acid	1.10
5-Phenylvaleric acid	1.25
Phenylundecanoic acid	1.90
Cinnamic acid	0.67
5-Phenyl-2,4-pentadienoic acid	0.14

Phenylacetic acid, the smallest saturated member of the series, would be expected to show the greatest interaction with beta-cyclodextrin, and therefore the largest slope if pure inclusion formation were the only mechanism involved. But since interactions between the larger saturated acids and beta-cyclodextrin are characterized by greater slopes, other interactive forces are probably involved.

Because of the large number of alcoholic hydroxyl groups within the cyclodextrin structure, it is quite possible that hydrogen bonding between these groups and the carboxyl groups of the various acids could be responsible in part for the observed interactions. If this were so, one might further conclude that removal of the bulky phenyl ring from the vicinity of the carboxyl group would greatly enhance

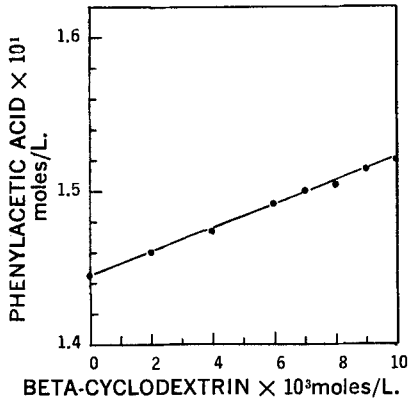


Fig. 1.—Interaction of phenylacetic acid with beta-cyclodextrin at 30°.

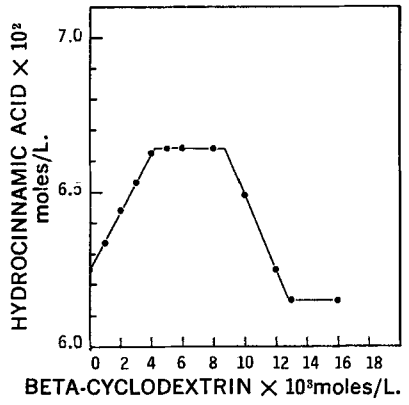


Fig. 2.—Interaction of hydrocinnamic acid with beta-cyclodextrin at 30°.

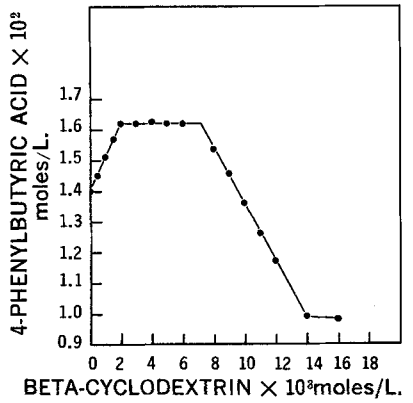


Fig. 3.—Interaction of 4-phenylbutyric acid with beta-cyclodextrin at 30°.

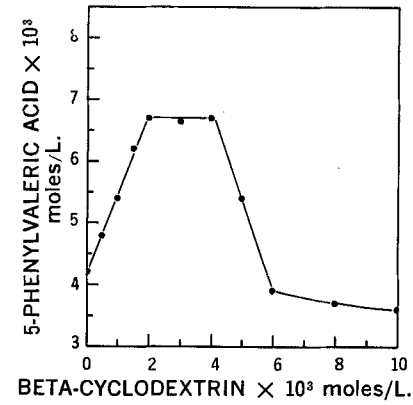


Fig. 4.—Interaction of 5-phenylvaleric acid with beta-cyclodextrin at 30°.

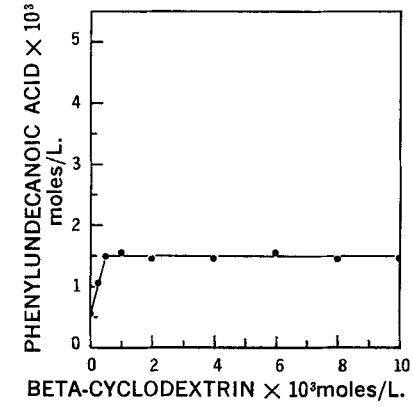


Fig. 5.—Interaction of phenylundecanoic acid with beta-cyclodextrin at 30°.

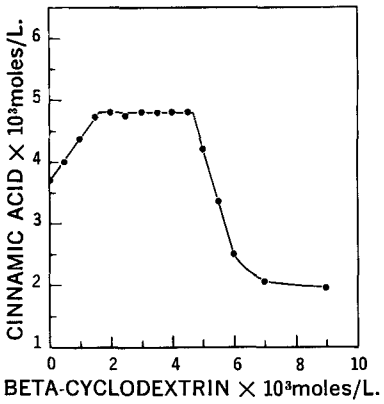


Fig. 6.—Interaction of cinnamic acid with beta-cyclodextrin at 30°.

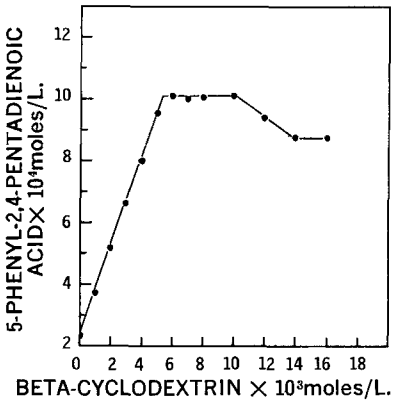


Fig. 7.—Interaction of 5-phenyl-2,4-pentadienoic acid with beta-cyclodextrin at 30°.

the relative reactivity of the acid by reducing possible steric hindrance. Interactions of the saturated acids, differing only in the number of methylene units separating the phenyl ring and the carboxyl group with beta-cyclodextrin, show a definite trend—the greater the separation, the stronger the corresponding interaction. This phenomenon can be clearly seen in Fig. 8, which illustrates the effect of acid chain length on the interaction slope. A nearly linear relationship is observed up to four methylene groups, but on further lengthening of chain, a leveling off results. As the chain length increases, the probability of steric hindrance exists resulting from an increased flexibility in the chain which could allow the phenyl group to approach the reactive end of the acid molecule. The shorter chain lengths would be more rigid and would therefore prevent or retard possible interference of this nature. If inclusion of the phenyl group were the predominant mechanism for these complexes, a similar degree of interaction would be expected for all of the acids. But since the least soluble acids are shown to interact to a greater degree, the importance of phenyl ring enclosure as the predominant mechanism in these systems is therefore minimized. Figure 9 illustrates the effect of initial solubility of the complexed acids on the slopes of their corresponding interaction isotherms.

To investigate the effects of unsaturation in the

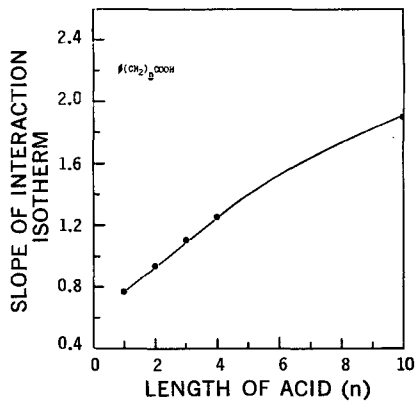


Fig. 8.—Effect of chain length on the relative reactivities of a series of phenyl-substituted carboxylic acids with beta-cyclodextrin at 30°.

chain separating the phenyl and carboxyl groups, cinnamic acid and 5-phenyl-2,4-pentadienoic acid were studied concerning their interactions with beta-cyclodextrin. When the interaction slopes for these acids were compared with those for the corresponding saturated acids, they were found to be significantly lower indicating reduced interaction tendencies for the unsaturated compounds. The reduced reactivity could be attributed to the increased bulk and rigidity of the chain resulting from the conjugated ethylenic linkages. Rigidity introduced by the chain adds to the bulkiness of the acid molecule increasing the possibility of steric interference as the acid approaches the cyclodextrin cavity. The more flexible chain found in the saturated acids would permit a greater freedom of movement by the components of the molecule and could therefore allow the acid to asume an optimum configuration for combination with the cyclodextrin. With respect to these unsaturated acids, an additional steric problem could arise due to the pi electron clouds associated with each of the double bonds, as the cyclodextrin void has been reported to be electronegative in nature (22). Figure 10 shows the effect that ethylenic groups, in conjugation with the ring, have on the reactivities of the unsaturated acids with beta-cyclodextrin. The deviation of the unsaturated acids from the linear pattern observed for the saturated series can also be seen in Fig. 9. Cinnamic acid and 5-phenyl-2,4-pentadienoic acid show significantly weaker interactions with beta-cyclodextrin than the corresponding parent saturated acids. Also, as the initial solubility of the complexed unsaturated acid decreases, the interaction slope decreases. This is opposite to the pattern observed with the group of saturated acids. This reduced reactivity observed for the unsaturated acids could have important biochemical and pharmacological implications. Beta-cyclodextrin has been reported to exhibit behavior similar to that shown by particular enzyme systems regarding interaction with various molecules. Because of this similarity in activity, cyclodextrins have been used as model systems in the study of enzyme mechanisms (16–19). It is quite possible that certain correlations could be established with regard to the *in vivo* metabolic fate of saturated and unsaturated fatty acids, and therefore, work is in progress in these labora-

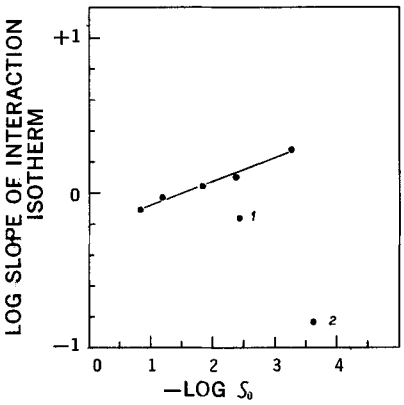


Fig. 9.—Relationship of slope of interaction isotherms of various phenyl-substituted carboxylic acids with beta-cyclodextrin to initial solubility at 30°. Key: *1*, cinnamic acid; *2*, 5-phenyl-2,4-pentadecanoic acid.

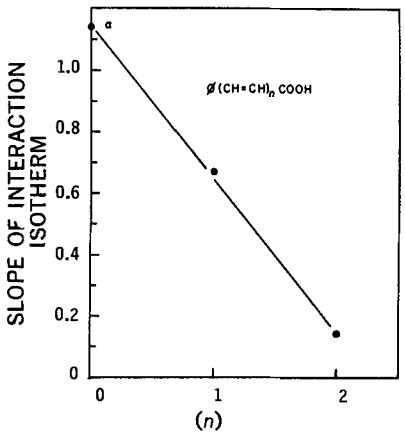


Fig. 10.—Effect of unsaturation on the relative reactivities of several phenyl-substituted carboxylic acids with beta-cyclodextrin at 30°. Key: a, data reported in *Reference 10.*

tories to further elucidate this unique interaction specificity shown by beta-cyclodextrin.

When no plateau region is present in the interaction isotherm, the stoichiometry of the resulting complex cannot be calculated because the concentration of free drug in the system is an invariant. However, five of the acids did exhibit plateau regions in their solubility plots for interactions with beta-cyclodextrin. The stoichiometries for these complexes are listed in Table II. Verification of the

TABLE II.—STOICHIOMETRIES OF THE PHENYL-SUBSTITUTED CARBOXYLIC ACID–BETA-CYCLODEXTRIN (β-CD) COMPLEXES

Compd.	From the Phase Diagram (Acid β-CD)	Analysis of the Isolated Complex (Acid β-CD)
Hydrocinnamic acid	1.00:1.00	1.02:1.00
4-Phenylbutyric acid	1.00:1.00	1.01:1.00
5-Phenylvaleric acid	2.04:1.00	1.99:1.00
Cinnamic acid	2.06:1.00	2.01:1.00
5-Phenyl-2,4-pentadienoic acid	1.02:1.00	1.03:1.00

stoichiometric ratios was accomplished by analysis of the isolated complexes. These values are also included in Table II. The presence of 1:1 and 2:1 stoichiometries for different systems containing similar guest compounds points toward the expected complexity of these systems and to the multitude of factors that could contribute to the observed interactions.

Once the stoichiometry is known for a complex, its formation constant and free energy of formation can be determined. These values were obtained for each of the insoluble complexes and are listed in Table III. The formation constants were calculated in a manner analogous to those employed in previous studies (23). Formation constants were also determined by a method presented by Thoma and Stewart for 1:1 complexes (24). Due to the probable complexity of these interactions, the absolute values of the formation constants should be accepted with reservation, although the magnitude of the constants does indicate the existence of extremely stable complexes. These values are in agreement with the large constants previously reported for other interactions involving the cyclodextrins (10, 12). It is interesting to note that formation constants representing interactions with other complexing agents have been reported in the order of 1–100, considerably lower than the results obtained in this study (23, 25). The large negative free energies of formation also point toward extremely stable complex formation, and indicate that the fit between the cyclodextrin and the various acids must be quite favorable.

Although it has been speculated that the degree of ionization of the acids involved might parallel their interactive tendencies with beta-cyclodextrin, the authors at this time cannot rationalize the observed interactions on this basis alone due to the similar magnitude of the pKa's of the various acids.

CONCLUSION

Inclusion compounds are characterized by the lack of bonding between guest and host components, while organic molecular complexes form as a result of weak forces between the interacting species. Although the cyclodextrins are characterized by their ability to form monomolecular inclusion com-

TABLE III.—FORMATION CONSTANTS AND FREE ENERGIES OF FORMATION OF THE PHENYL-SUBSTITUTED CARBOXYLIC ACID–BETA-CYCLODEXTRIN COMPLEXES AT 30°

Compd.	Kf	Kf^a	ΔF^0 (cal./mole)
Hydrocinnamic acid	2.0×10^2	2.1×10^2	−3190
4-Phenylbutyric acid	"infinite"	"infinite"	...
5-Phenylvaleric acid	1.1×10^5	...	−6991
Cinnamic acid	3.5×10^4	...	−6301
5-Phenyl-2,4-pentadienoic acid	7.2×10^2	7.1×10^2	−3963

[a] Values determined by the method of Thoma and Stewart for 1:1 complexes only.

pounds, it has been shown that the net interactions observed with many guest compounds are a combination of pure spatial inclusion formation and other attractive forces. Molecules too large to be included within the cyclodextrin cavities were shown to interact with the cyclodextrins, indicating a mechanism other than pure inclusion formation for these systems (11).

Even though the mechanism involved in the interactions of the phenyl-substituted carboxylic acids with beta-cyclodextrin is believed to consist of both pure inclusion and other attractive forces, the importance of the separation between the carboxyl and phenyl groups in the net interactions observed is suggested by the experimental data. It also was shown that due to the similar magnitude of the pKa's for the various acids, differences in relative acidity could not totally account for the observed differences in these interactions, assuming the interaction is due primarily to the undissociated acid molecule. Unsaturated acids were found to be far less reactive with beta-cyclodextrin than were the corresponding saturated acids. This unique specificity shown by beta-cyclodextrin is being investigated further in these laboratories.

Even though a possible mechanism is suggested, the only manner by which the true structures of the resulting complexes can be established is by an X-ray examination of the isolated interaction products.

The large formation constants and free energies of formation for some of these systems suggest a high degree of stability for the complexes and favorable combining conditions between the acids and beta-cyclodextrin.

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Pharmacological Studies on Some New Acrylic Acid Amide Derivatives

By HUGH J. BURFORD and JAMES B. GILL

A group of newly synthesized acrylic acid amides have been evaluated pharmacologically and chemically for their similarity to the tranquilizer reserpine. The amides differ from reserpine in their behavorial depression effects in that they cause no ptosis at doses which yield decreased locomotor ability and electroshock-threshold lowering. The amides also differ markedly from reserpine in potency. Their therapeutic index (LD_{50}/TD_{50}) is about 2 compared with a value of 19 for reserpine. Finally, the amides differ from reserpine in that their TD_{50}/ED_{50} is around 1 as compared to a value of 3.5 for reserpine. The conclusion is that central nervous system depression and electroshock-threshold lowering by the amides may possibly be mediated by a similar neural mechanism. Also the role of methoxy group substitution on electroshock-threshold lowering potency is discussed.

AZLACTONES, 5,4-oxazolones, and their amide derivatives are interesting, easily synthesized compounds. They have been well studied chemically, especially in relation to the chemistry of penicillin (1–4). However, few pharmacological studies of azlactones or their derivatives have appeared. Cardiac activity was studied by Schueler and Hanna (5) and sedative properties of an amide derivative have been reported by Cronheim *et al.* (6). More recently Robison and Schueler (7) have studied substituted acrylic acid amide derivatives of unsaturated azlactones and reported them to be primarily convulsants, although one member of their series was found to be a long-acting depressant.

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