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Complexes of Disubstituted Benzene Positional Isomers with α-Cyclodextrin

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Stability constants for complex formation between α-cyclodextrin and some *ortho*-, *meta*-, and *para*-disubstituted benzenes were measured in aqueous solution at 25°C by potentiometry, spectrophotometry, competitive spectrophotometry, and solubility. All systems form 1 : 1 complexes, some *para* substrates form 1 : 2 complexes (one substrate to two cyclodextrins), but no *meta* substrates form 1 : 2 complexes. *Ortho* substrates form weak complexes. These observations are accounted for in terms of a binding site molecular model. On the average over many systems, K_{11} (*para*) and K_{11} (*meta*) are approximately equal. Major discrepancies (greater than a factor of two) are diagnostic of significantly different electronic or steric effects in the complexing abilities of the isomeric substrates.

INTRODUCTION

Cyclodextrins are cyclic oligomers of D-glucose produced by the action of certain enzymes on starch. They have been studied by many laboratories as enzyme models, because they (and chemically modified cyclodextrins) possess a binding site (the cavity of the cyclodextrin) and functional groups capable of carrying out chemical reactions. The chemistry and applications of cyclodextrins have been widely reviewed (*1–10*).

Satisfactory interpretations of such enzyme modelling studies, as well as of applications in pharmaceuticals, require knowledge, and even predictive capability, of fundamental matters including complex stoichiometries and structures, binding forces, solution thermodynamics, and kinetics. Our laboratory has carried out systematic studies on the stabilities of complexes formed between α-cyclodextrin and 1,4-disubstituted benzenes in aqueous solution (*11–14*). These studies have provided us with the ability to account for stoichiometric relationships and complex stabilities in these systems. Interpretation of the results is aided by a binding site model in which α-cyclodextrin is described as a one-site ligand and the 1,4-disubstituted benzene as a two-site substrate. Then the system may contain the complexes X′Y, XY′, and X′Y′, where XY is the uncomplexed substrate, and a superscript prime represents a site complexed by the ligand. The experimen-

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tally measured stability constants K_{11} and K_{12} are related to microscopic binding constants by

$$K_{11} = K_{X'Y} + K_{XY'} \quad [1]$$

and

$$K_{12} = \frac{a_{XY}K_{X'Y}K_{XY'}}{K_{11}}, \quad [2]$$

where a_{XY} is a parameter describing interaction between the two sites in 1:2 complex formation; a_{XY} is, in fact, the equilibrium constant for this reaction:

$$X'Y + XY' \overset{a_{XY}}{\rightleftharpoons} X'Y' + XY.$$

If, therefore, the two substrate binding sites are independent, $a_{XY} = 1$. It is possible to make reasonable estimates of the isomeric binding site constants, $K_{X'Y}$ and $K_{XY'}$, and of the interaction parameter, a_{XY}, and thus to describe the detailed solution equilibria of this type of system for 1,4-disubstituted benzenes.

There is a strong interest in extending such studies to the corresponding 1,2- and 1,3-disubstituted benzene substrates, because of the positional selectivity of the cyclodextrins functioning as enzyme model catalysts; for example, catalysis of ester groups *meta* to a binding site is more effective than for the *para* isomer (*15*). The present paper describes a study of the complex stabilities of α-cyclodextrin complexes of some 1,2-, 1,3-, and 1,4-disubstituted benzenes in aqueous solution.

EXPERIMENTAL PROCEDURES

Materials. α-Cyclodextrin (Sigma) was dried for 3 h at 105°C to give the anhydrous form. Nitrazine yellow (Aldrich) was recrystallized from water. All substrates were from commercial sources and were recrystallized before use (except for salicyclic acid, which was purified by sublimation). Melting points agreed with literature values (*16*).

Apparatus. Spectrophotometric measurements were made with a Perkin–Elmer Model 559 or a Cary–Varian Model 2200 spectrophotometer. Potentiometric measurements were made with an Orion Model 701A pH meter and a Corning semimicro combination electrode.

Procedures. Several techniques were used for the measurement of stability constants; all of these have been described elsewhere. Most of the substituted benzoic acids were studied by potentiometry (*11, 13*), and the nonionizable substrates by the solubility method (*14, 17*). Other methods used were ultraviolet spectrophotometry and competitive spectrophotometry (*16, 18*). The spectrophotometric and potentiometric methods were modified in their data treatment by making use of a Taylor's series calculation of free ligand concentration (*19*). The cited references include discussions of the precision in the stability constants.

All measurements were at 25.0 ± 0.1°C in aqueous solutions having ionic strength 0.10 M (made up with NaCl). Solubility studies on benzoic acids were in 0.10 M HCl.

TABLE 1

STABILITY CONSTANTS FOR 2-, 3-, AND 4-SUBSTITUTED BENZOIC ACIDS WITH α-CYCLODEXTRIN AT 25°C[a]

Substituent	Position	K_{11a} (M^{-1})	K_{12a} (M^{-1})	K_{11b} (M^{-1})
OH	2	32.3 (1.6)	18.7 (1.20)	7.8 (0.3)[c]
OH	3	455 (6)	0	6.3 (0.2)
OH	4	1100 (8)	0	16.1 (0.2)
OCH_3	2	[b]	—	7.2 (0.2)[c]
OCH_3	3	855 (3)	0	14.9 (0.30)
OCH_3	4	897 (4)	0	2.7 (0.1)
CN	2	[b]	—	7.6 (0.1)[c]
CN	3	365 (5)	0	60.5 (1.1)
CN	4	435 (10)	25.2 (1.0)	63.7 (3)[c]
NO_2	2	[b]	—	4.1 (0.3)[c]
NO_2	3	109 (5)	0	29.7 (0.6)
NO_2	4	305 (8)	26.9 (2.9)	68 (7)
CH_3	3	496 (4)	0	14.9 (0.1)
CH_3	4	1201 (1)	1.9 (0.10)	—
Cl	3	1165 (27)	0	68.1 (2.0)
Cl	4	703 (7)	21.0 (1.4)	—
COOH	3	233 (3)	0	—
COOH[d]	4	1344 (17)	23.8 (0.6)	

[a] Standard deviations in parentheses.
[b] $\Delta pH \approx 0$ in potentiometric method.
[c] Competitive indicator method.
[d] From Ref. (*14*).

RESULTS

2-, 3-, and 4-Substituted benzoic acids. Eighteen of these compounds were studied, these being the *ortho, meta,* and *para* isomers of hydroxy-, methoxy-, cyano-, and nitrobenzoic acids, and the *meta* and *para* isomers of chloro-, methyl-, and carboxybenzoic acids. Of these, the *m*- and *p*-carboxybenzoic acids and the *p*-methyl- and *p*-chlorobenzoic acids were studied only in their conjugate acid forms; the other substrates as their conjugate base forms constituted 14 additional substrates. The stepwise stability constants are designated K_{11a}, K_{12a}, and K_{11b}, the subscript denoting the complex stoichiometry and the form (acid or base) of the substrate. The results are given in Table 1.

Most of these systems were studied potentiometrically, but some were investigated by the solubility method because of their low solubilities. The *ortho* methoxy-, cyano-, and nitrobenzoic acids all yielded no significant pH change in the potentiometric method, implying that $K_{11a} \approx K_{11b}$. For all systems K_{12b} is not significantly different from zero. The results for *p*-cyano-, *p*-nitro-, and *m*-nitrobenzoic acids are means of two or more independent measurements.

TABLE 2

STABILITY CONSTANTS OF NONIONIZABLE SUBSTRATES WITH α-CYCLODEXTRIN[a]

Substrate	1,3		1,4[b]	
	K_{11} (M^{-1})	K_{12} (M^{-1})	K_{11} (M^{-1})	K_{12} (M^{-1})
Dimethylphthalate	63.4 (0.6)	0	443 (13)	102 (3)
Dicyanobenzene	19.5 (2.0)	0	33.1 (1)	7.2 (0.7)
Dinitrobenzene	22.1 (1.4)	0	35.8 (1)	4.6 (0.4)

[a] At 25°C; standard deviations in parentheses.
[b] From Ref. (*14*).

Nonionizable substrates. In Table 2 are listed stability constants obtained by the solubility method for some nonionizable substrates of the 1,3-X-C_6H_4-X and 1,4-X-C_6H_4-X type. Terephthalic acid and isophthalic acid (last two entries in Table 1) are also substrates of this type, and these also were studied by the solubility method. The values of K_{12a} given as zero in Tables 1 and 2 were not significant at the 95% level, except for *m*-dinitrobenzene and dimethylisophthalate, where they were not significant at the 90% level.

DISCUSSION

Several comparisons will now be made between the stabilities of cyclodextrin complexes of *meta* and *para* isomers. For some of these there exists ambiguity concerning the nature of the stability constants, according to the binding site model, but the comparisons are not precise, and no serious problems are introduced. Thus, for example, a 1,3-disubstituted benzene is really a three-site substrate (the 5 position being the third potential binding site), but binding at the unsubstituted site is expected to make a minor contribution to K_{11}.

A recent study of complexes of symmetrical 1,4-disubstituted benzenes with α-cyclodextrin showed that their K_{11} values, at 25°C, could be well described by the empirical correlation

$$\log K_{11} = -0.636 \log s_0 - 0.231\mu + 0.524, \quad [3]$$

where s_0 is the substrate equilibrium molar solubility and μ is the binding site dipole moment (*14*). This same equation is now found to give a satisfactory account of the 1,3-X-C_6H_4-X systems, as shown in Table 3. This good agreement suggests that the 1,3-substrates behave similarly to the 1,4 substrates when their different properties (solubility and polarity in this case) are taken into account. It appears that the positional effects that lead to increased solubility of the 1,3 isomer lead also to decreased complex stability.

TABLE 3

COMPARISON OF CALCULATED AND EXPERIMENTAL COMPLEX STABILITIES FOR 1,3-X-C_6H_4-X SUBSTRATES

X	K_{11} (M^{-1})	
	Calculated[a]	Observed
CN	15.0	19.5
NO_2	16.7	22.1
COOH	243	233
$COOCH_3$	71.6	63.4

[a] Calculated with Eq. [3].

Figure 1 is a Hammett plot of K_{11a} and K_{11b} for 3- and 4-substituted benzoic acids. (For isophthalic and terephthalic acids $K_{11a}/2$ is plotted.) This plot, for just the *para* substrates, had earlier (*11*) been interpreted to mean that K_{11a} mainly describes binding at the COOH site in substrate X-C_6H_4-COOH, with a small contribution from X-site binding; K_{11b}, however, describes essentially pure X-site binding in X-C_6H_4-COO^-. Figure 1 shows that 3-substituted benzoic acids behave in essentially the same way as 4-substituted benzoic acids. Of course for many *meta*/*para* pairs there is a substantial difference in complexing ability; the meaning of the statement is that, given a stability constant, it is not possible to conclude definitely whether it describes the *meta* or the *para* isomer.

This observation is pursued in Fig. 2, which shows K_{11} for the *para* isomer plotted against K_{11} for the corresponding *meta* isomer for the α-cyclodextrin complexes of 28 *meta*/*para* pairs. Figure 2 includes results from the present paper as well as data from Refs. (*11–15, 20–24*). The line is drawn with a slope of 1. Of the 28 points, 14 fall within 0.3 log unit of the line, or within a factor of 2 of the

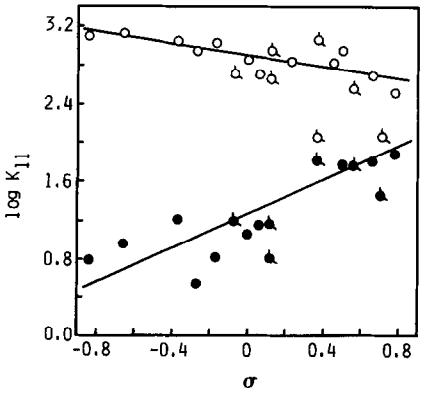

FIG. 1. Hammet plots of K_{11a} and K_{11b} for 3- and 4-substituted benzoic acids. The open circles show K_{11a}, the filled circles K_{11b}. 3-Substituted substrates are represented by circles having dashes oriented at 120°.

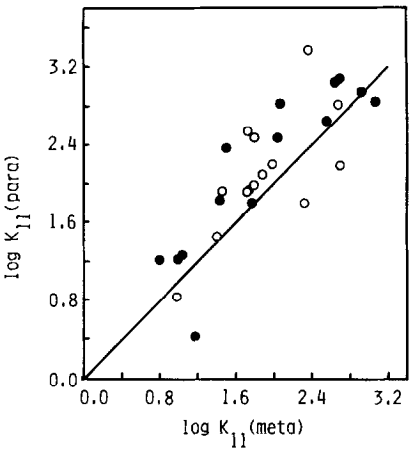

FIG. 2. Plot of K_{11} (*para*) against K_{11} (*meta*) for 28 pairs. Data from present study (filled circles) and Refs. (*11–15, 20–24*). The line has a slope of one.

relationship K_{11} (*para*) = K_{11} (*meta*). Modest deviations from this simple equality may be expected because of the different extents to which electronic effects are transmitted from the substituent to the binding site in the *meta* and *para* isomers.

Major deviations from the line in Fig. 2 are diagnostic of significant differences in complexing between the two isomers. Thus, we anticipate that binding to a *meta* substrate may be subject to steric interference from the 3 substituent, whereas this effect is absent in the *para* substrate; this will decrease K_{11} (*meta*) relative to K_{11} (*para*). Resonance charge delocalization is more effective from the *para* position, and this may lead to a significant effect. For example, the topmost point in Fig. 2 is for the *p*-nitrophenolate/*m*-nitrophenolate pair; the binding site is the nitro group (*13, 25*), and the phenolate is the substituent. Evidently the combination of the powerfully electron-withdrawing nitro binding site with an electron-rich substituent in the *para* position yields an unusually large K_{11} (*para*). The terephthalic acid/isophthalic acid case, however, which also shows a large positive deviation, reflects the steric effect. It had earlier (*14*) been concluded, on other grounds, that the carboxylic acid group is very deeply inserted into the cyclodextrin cavity in the 1 : 1 terephthalic acid complex. It is therefore anticipated that in the *meta* isomer, isophthalic acid, this deep penetration will be prevented by steric interaction with the *meta* substituent, thus sharply decreasing K_{11} (*meta*), and raising the ratio K_{11} (*para*)/K_{11} (*meta*). It is noteworthy, in this connection, that K_{12} (*meta*) is essentially zero even for systems having a finite K_{12} (*para*); this is consistent with the preceding argument.

ortho-Disubstituted benzenes behave quite differently from their *meta* and *para* isomers, giving quite small stability constants. Figure 3 is a molecular interpretation of all the effects that have been discussed. The 1,4-, 1,3-, and 1,2-disubstituted substrates are shown, with outlines of the cyclodextrin cavity as it might be located at potential binding sites. Thus in a 1,4-XY substrate K_{11} is composed, according to Eq. [1], of contributions from each site, and K_{12} may be observable

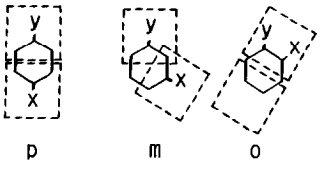

FIG. 3. Model of α-cyclodextrin complex formation with 1,4-, 1,3-, and 1,2-disubstituted benzenes. The approximate position of the cyclodextrin cavity is shown by rectangles over potential binding sites.

because the two sites are sufficiently separated to allow two cyclodextrin molecules to bind simultaneously. [This does not mean that there is no interaction between the sites; this question has been treated in detail elsewhere (*14*).] As Fig. 3 suggests, however, the 1,3-XY substrate presents a different possibility. If the substituents are not very bulky and if the binding site penetration is not deep, K_{11} (*meta*) may be very similar to K_{11} (*para*), modified by transmission of electronic effects. If, however, interaction of the 3 substituent with the rim of the cyclodextrin occurs, K_{11} (*meta*) may be much smaller than K_{11} (*para*), or possibly larger if attractive interactions are possible. Moreover, as seen in the figure, 1 : 2 complexation is not likely because of the probability that two cyclodextrins will sterically interfere.

In 1,2-disubstituted substrates the entrance of either X or Y into the cyclodextrin cavity will be severely restricted, and complexation may be limited to the remaining binding site, namely the unsubstituted end of the molecule. A typical value for a stability constant at such a site may be about 10 M^{-1} [for example, benzoate ion or aniline (*11, 12*).] If X and Y are not bulky, or if they interact intramolecularly to form a compact group, it is conceivable that this may function as a combined binding site, giving the possible complexing pattern shown in Fig. 3. This may happen with salicyclic acid; indeed, this is the only *ortho*-substituted substrate that shows both 1 : 1 and 1 : 2 complexing (Table 2), and this can be accounted for by the model of Fig. 3.

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