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Selective Aromatic Substitution by Hydrophobic Binding of a Substrate to a Simple Cyclodextrin Catalyst[1,2]

RONALD BRESLOW AND PETER CAMPBELL

Department of Chemistry, Columbia University, New York, New York 10027

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The reaction of anisole with HOCl is changed in the presence of α-cyclodextrin (cyclohexaamylose). The anisole binds into the cyclodextrin cavity; within the complex, *ortho*-chlorination is completely suppressed while *para*-chlorination is catalyzed. Thus, with appropriate cyclodextrin concentrations the 60/40 *p*/*o* product ratio for reaction of free anisole with HOCl can be changed so that only *p*-chloroanisole is produced. In the complex of anisole with β-cyclodextrin (cycloheptaamylose) *o*-chlorination is also blocked, but *p*-chlorination proceeds at the same rate as in free solution. The actual relative values of the pseudo-first-order rate constants, k_{free} and k_{bound}, depend on the HOCl concentration, since chlorination in the complex is first-order in HOCl, while chlorination of anisole in free solution is second-order in HOCl. These findings strongly support a mechanism in which the cyclodextrin reacts with HOCl to form a hypochlorite, and delivers this chlorine selectively to anisole bound in its cavity. Studies of other aromatic chlorinations, and of a diazo coupling reaction in the presence of α-cyclodextrin, further support this enzyme-like mechanism.

INTRODUCTION

Enzymatic reactions are more selective than simple organic reactions in at least two ways. There is, first of all, the well-recognized preference of a particular enzyme for some substrates and not others, based on the required geometry of the enzyme–substrate complex. The other selectivity is a preference within the enzyme–substrate complex for attack or reaction at only certain atoms of the substrate. Again the preference is dictated by geometrical, and not intrinsic reactivity, considerations. In a sense, enzymatic reactions are dominated in their selectivity by the geometric requirements of the catalyst, while this is seldom true in simple organic chemical reactions. In almost all cases, these latter are dominated by the intrinsic reactivity of the substrate; when geometric factors come in, they are generally simply in the form of "steric hindrance."

We have initiated a program of trying to copy the style of biochemical reactions by designing processes in which the geometry of the reagent–substrate complex would dominate any questions of intrinsic reactivity within the substrate; one example of this approach is the "remote oxidation reactions" we have described elsewhere (*1–3*). Enzymes, in general, bind substrates chiefly by the use of hydrophobic forces. It occurred to us that it might be possible to use the known hydrophobic binding of aromatic compounds into the cavity of the cyclodextrins (*4*) in order to achieve geometric control of aromatic substitution reactions.

[1] Taken in part from the Ph.D. thesis of Peter Campbell, Columbia University, 1970.

[2] For a preliminary communication of some of these results, see R. BRESLOW AND P. CAMPBELL, *J. Amer. Chem. Soc.* **91**, 3085 (1969). Financial support of this work by the National Institutes of Health is gratefully acknowledged.

Cyclodextrins, or cycloamyloses, have played an increasingly important role in the study of model systems for enzymatic reactions, and the complexes of these materials with aromatic compounds are well studied and utilized in a number of hydrolytic model reactions (*5–7*). However, up to this point no attempt had been made to modify the selectivity of aromatic substitution reactions by taking advantage of the geometry of these complexes. As Fig. 1 illustrates, a substrate bound into the cavity of a cyclodextrin (in one likely conformation (*8*) of the complex) has its *ortho*- and *meta*-positions very

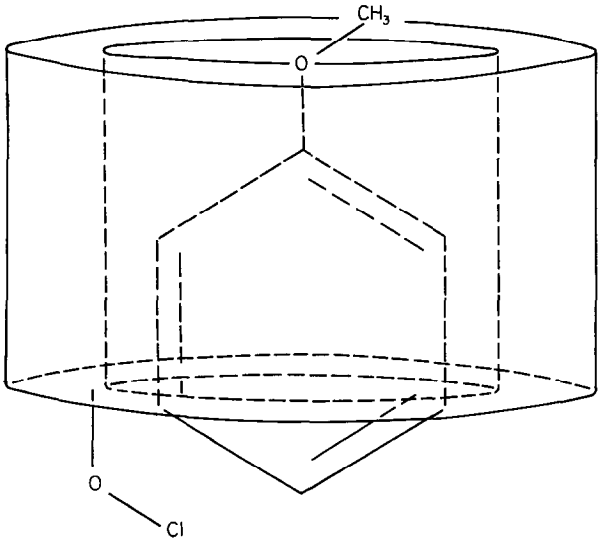


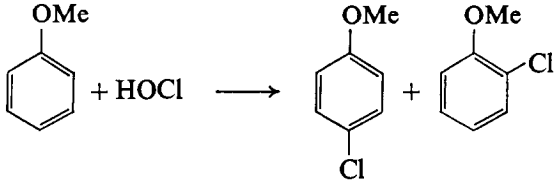
FIG. 1. Schematic drawing of the anisole–cycloamylose inclusion complex. One of the hydroxyls which ring the cavity is shown as a hypochlorite, to illustrate the likely mechanism of the chlorination reactions.

well shielded, while the *para*-position is potentially accessible and might be substituted in an appropriate reaction. This expectation is related to the known geometric requirements for binding of aromatic compounds into these cavities, in which *para*-disubstituted aromatic compounds are well bound compared to *ortho*-disubstituted compounds. Of course, such complexes are in equilibrium with the unbound reagents. If the reactivity of a substrate bound into a cyclodextrin cavity were lowered as a result of the binding, aromatic substitution might occur only with the amount of this substrate which is free at equilibrium in solution, and no change in the aromatic substitution pattern would then be observed. As it turns out, this was not a general problem. In fact, in the particular aromatic substitution reaction which was studied in detail, not only was the selectivity of the substitution markedly changed, but the substrate was even more reactive in the complex than it was in free solution, so the cyclodextrin was playing a catalytic role and not simply a geometric blocking role.

RESULTS AND DISCUSSION

Since the hydrophobic binding of aromatic substrates into the cavity of a cyclodextrin occurs in water solution, it was necessary to select an aromatic substitution which operates in this medium. Further, we preferred to select a reaction in which the

electrophile does not also bind hydrophobically, and in which the product would be less reactive than the starting material so as to simplify the product and kinetic analysis. We also sought a reaction which proceeds at a convenient rate so that kinetic studies could be carried out. For all these reasons, the reaction selected was the chlorination of anisole [*1*] by hypochlorous acid. Data in the literature (*9*) indicated that under ordinary conditions this reaction affords both *para*- [*2*] and *ortho*-chloroanisole [*3*] so it represents the kind of random reaction which we hoped to make selective by the geometric constraints we have discussed.



[*1*] [*2*] [*3*]

Binding constant. Although many compounds have been observed to give insoluble complexes with the cyclodextrins, a smaller number have been examined in solution to determine the equilibrium binding constant for the formation of a soluble inclusion complex. It was clear from the data available (*4*) that the dissociation constant K_d for anisole would be in the region of 10^{-3} M, but it was necessary for us to determine this constant directly. Several methods are available for doing this. For one thing, the ultraviolet spectrum of anisole is modified by binding into the cyclohexaamylose cavity, so a simple uv spectroscopic method can be used to determine the binding constant. It can be shown that

$$\frac{C_0 S_0}{\Delta \text{Abs}} = \frac{C_0 + S_0}{\Delta \epsilon} + \frac{K_d}{\Delta \epsilon}$$

where C_0 is the initial concentration of the cycloamylose, S_0 is the initial concentration of the substrate, ΔAbs is the change in absorbance at a particular wavelength from the absorbance of the substrate alone at this concentration, and $\Delta\epsilon$ is the difference in molar extinction coefficient for the complex and the pure substrate. Thus, a plot of C_0S_0/ΔAbs vs. $C_0 + S_0$ yields a straight line with intercept/slope $= K_d$. This study demonstrates that the complex of cyclohexaamylose with anisole has 1 : 1 stoichiometry, since two isosbestic points are observed; the 1 : 1 stoichiometry is also indicated by the linearity of our plot to determine K_d.

As a general problem, it may be found that this direct spectrophotometric method cannot be applied to determine binding constants. The spectrophotometric change may be too small, or side reactions may interfere with a clean interpretation of the data. Thus, two other kinetic methods for determining the binding constants have been devised (*6*), which we refer to as a direct kinetic method and an indirect kinetic method. The direct method involves observing the effect of the concentration of added cycloamylose on a reaction in which one and only one of the reactants is bound to the cycloamylose. A kinetic treatment of the situation in which an uncomplexed reagent can react either with the free substrate or with the bound substrate, with respective rate constants of k_{free} or k_{bound}, can be analyzed by plotting $(k_{\text{free}} - k_{\text{observed}})$ vs. $(k_{\text{free}} - k_{\text{observed}})/C$ in which C is the concentration of cycloamylose. Such a plot is linear with

an intercept of ($k_{free} - k_{bound}$) and a slope of K_d (in the expected situation that the rate of dissociation of the cyclodextrin complex is much faster in the forward rate of reaction of that complex with a reagent). This technique was used to determine the binding constant of Br_2 with cyclohexaamylose, which is listed in Table 1. The other general kinetic method for determining binding is to observe competitive inhibition caused by one substrate on the cyclodextrin-catalyzed substrate whose binding constant is known. This is analogous to the well-known situation in enzyme chemistry in which inhibition constants are determined in precisely this way.

Consider the scheme

$$\begin{aligned} C\cdot I &\overset{K_i}{\rightleftharpoons} C + I \\ S &\xrightarrow{k_{free}} P \\ C\cdot S &\overset{K_d}{\rightleftharpoons} C + S \\ C\cdot S &\xrightarrow{k_{bound}} P \end{aligned}$$

where C is cycloamylose, S is a reacting substrate, I is a nonreacting substrate whose binding constant is to be determined, and P represents the products of the reaction. The values k_{free}, k_{bound}, and K_d must be independently determined in a separate experiment by the direct method. We may write

$$\begin{aligned} K_d &= \frac{(C)(S)}{C\cdot S} \\ &= \frac{S}{C\cdot S}(C) \\ &= \frac{S}{C\cdot S}(C_0 - C\cdot I - C\cdot S) \\ &\equiv \frac{S}{C\cdot S}(C_0 - C\cdot I) \end{aligned}$$

Substituting from the equilibrium expression for K_i,

$$K_d = \frac{S}{C\cdot S}\left(C_0 - \frac{(C)(I)}{K_i}\right).$$

And similarly for K_d,

$$\begin{aligned} K_d &= \frac{S}{C\cdot S}\left(C_0 - \frac{K_d}{K_i}\cdot\frac{C\cdot S}{S}(I)\right) \\ &= \frac{S}{C\cdot S}C_0 - \frac{K_d}{K_i}(I) \\ &= \frac{k_{bound} - k_{obs}}{k_{obs} - k_{free}}(C_0) - \frac{K_d}{K_i}(I). \end{aligned}$$

Rearranging,

$$(I) = \frac{K_i C_0}{K_d}\cdot\frac{k_{bound} - k_{obs}}{k_{obs} - k_{free}} - K_i.$$

TABLE 1

DISSOCIATION CONSTANTS OF CYCLOHEXAAMYLOSE COMPLEXES

Substrate	Spectrophotometric method (wave lengths used (nm))	K_d ($M \times 10^3$)	Kinetic methods: Direct — Other reactant	Direct — K_d ($M \times 10^3$)	Indirect — Standard reaction	Indirect — K_d ($M \times 10^3$)
Anisole	278.2, 271.2, 270	3.72 ± 0.5	—	—	*m*-Nitrophenyl acetate hydrolysis	4.0 ± 1.0
p-Methylanisole	285, 279, 277, 276	15.1 ± 3.0	—	—	*m*-Nitrophenyl acetate hydrolysis	13.2 ± 4.0
Anisole[a]	281, 280, 279, 278, 277, 273.5, 272.5, 271.5, 270.5	7.18 ± 0.5	—	—	—	—
Phenol	277, 276, 272	50.1 ± 10 (53[b,c])	—	—	—	—
Phenoxide	352, 350, 344	6.83 ± 1.0	—	—	—	—
p-Cresol	282, 280, 277	83 ± 20	—	—	—	—
p-Cresoxide	354, 351	6.2 ± 1.5	—	—	—	—
Bromine	—	—	Fumarate	2.13 ± 0.3	—	—
Fumarate	—	—	—	—	*m*-Nitrophenyl acetate hydrolysis	>1[d]
m-Nitrophenylacetate	—	—	Hydroxide (hydrolysis)	17.4 ± 4 (19 ± 4[b])	—	—

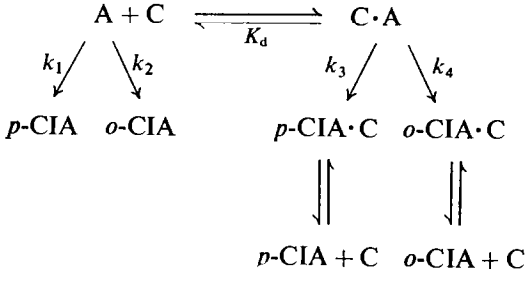
[a] Cycloheptaamylose complex.
[b] Ref. 7.
[c] No error estimate.
[d] Using fumarate bromination as a standard reaction, *p*-diazoniumbenzenesulfonate and hypochlorous acid were also found to have $K_d > 1$.

Thus, a plot of (I) vs. $(k_{bound} - k_{obs})/(k_{obs} - k_{free})$ is linear, and the desired equilibrium constant K_i is the negative intercept.

In the course of this work a variety of binding constants were determined by these methods, and they are listed in Table 1. In cases where two independent methods were applied to the same compound good agreement was obtained, as the table shows.

Chlorination of Anisole by HOCl

When a solution of 5×10^{-4} *M* anisole was treated with 10^{-2} *M* HOCl in unbuffered solution at pH 4.6, conversion was complete in approximately 10 min to a mixture of *o*-chloroanisole and *p*-chloroanisole in a ratio of 1:1.48. A slightly different ratio, $1:1.87 \pm 0.17$, had been reported (*9*) under different conditions involving a two-phase system. When the chlorination was performed in the presence of cyclohexaamylose, a marked change in the product ratio resulted, while a more modest change occurred in the presence of cycloheptaamylose. These results are listed in Table 2 along with the fraction of anisole which is bound in these cyclodextrins, as was determined independently and from the binding constants listed in Table 1. Since a large excess of the cycloamylose was used, the ratio of bound anisole to free anisole, a ratio which we will call γ, is a function only of the cycloamylose concentration and does not vary during the course of the reaction. Thus, with the following kinetic scheme, where A represents anisole, and C cycloamylose



the ratio of *para*/*ortho* product formation, which we denote as ρ, is

$$\rho = \frac{k_1 + \gamma k_3}{k_2 + \gamma k_4} = \frac{\rho k_2 - k_1}{\gamma} = k_3 - \rho k_4.$$

This equation was used to calculate the relative partial rate factors by a plot whose intercept is k_3 and whose slope is k_4. This is the equation which was actually analyzed by computer (*10*) to determine the relative partial rate factors which are listed in Table 3. However, since k_4 is essentially zero, a graphic plot of this equation gives a scatter of points, and a more satisfying graphic expression can be obtained by division by ρ, in which case the plot shown in Fig. 2 is obtained. Application of this treatment to the data of Table 2 results in the relative partial rate constants shown in Table 3, in which arbitrarily k_1(*para*) is set as 1.

It can be seen from these data, or from the raw data in Table 2, that indeed when anisole binds to cyclohexaamylose there is complete suppression of chlorination in the *ortho* position and the product formed from chlorination of this complex is exclusively *para* chloroanisole. However, the striking additional observation is that this mechanism dominates the chemistry almost completely even when only 72% of the anisole is bound, suggesting that the anisole in the complex is more reactive than is the anisole in free solution, which, of course, should still give the normal 40% *ortho* chlorination. The

TABLE 2

ANISOLE CHLORINATION PRODUCTS

	Fraction of anisole bound	Chloroanisole product ratio, *p*/*o*
Cyclohexaamylose ($M \times 10^3$)		
0	0	1.48
0.933	0.20	3.43
1.686	0.33	5.49
2.80	0.43	7.42
4.68	0.56	11.3
6.56	0.64	15.4
9.39	0.72	21.6
Cycloheptaamylose ($M \times 10^3$)		
0.944	0.12	1.69
1.752	0.20	1.88
2.98	0.29	2.16
5.07	0.41	2.63
7.05	0.50	3.08
10.12	0.59	3.77

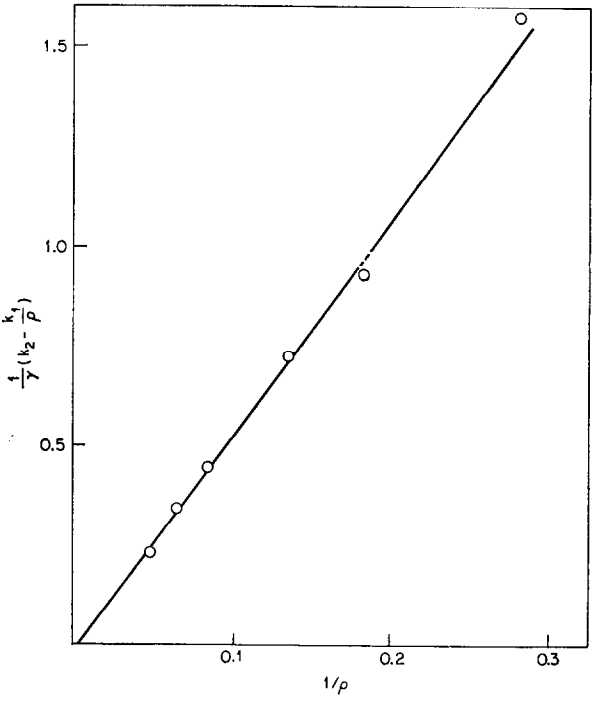


FIG. 2. A plot of the equation by which the partial rate constants of Table 3 can be determined. The symbols are explained in the text.

TABLE 3

PARTIAL RATE CONSTANTS FOR ANISOLE CHLORINATION

	k_{free}	k_{bound} Cyclohexaamylose	k_{bound} Cycloheptaamylose
para	1	5.31 ± 0.05	1.1 ± 0.05
ortho	$0.67/2 \pm 0.02$	0.01 ± 0.05	-0.02 ± 0.05

data in Table 3 analyze this situation quantitatively and indicate that the *ortho* position in the complex is, within experimental error, inert to chlorination, but that the *para* position of anisole is 5.3 times as reactive within the cyclohexaamylose complex as it is in free solution. With the larger cavity of cycloheptaamylose, in which (as the data in Table 1 indicate) the anisole is less well bound, it is also true that there is no detectable chlorination of the *ortho* position of anisole within the complex. However, in this case the *para* position within the complex has within experimental error the same reactivity as that in anisole in free solution, rather than the fivefold increase observed in the cyclohexaamylose complex. Thus, cyclohexaamylose not only blocks *ortho* chlorination, it also catalyzes *para* chlorination.

Kinetic measurements. These conclusions were checked by direct measurements of rates, rather than simple product ratios. Two kinetic techniques were used, the more accurate one involving a monitoring of the absorption change at 276 nm as a function of time, and the less accurate involving quenching of the reaction mixtures at various times and determination of the relative concentrations of anisole and of the product chloroanisoles by vapor phase chromatography. In both cases HOCl was sufficiently in excess that the reaction was pseudo-first-order. The observed pseudo-first-order rate constants are presented in Tables 4 and 5. Using both kinetic methods, controls run with maltose instead of a cycloamylose in concentrations up to 10^{-1} *M* showed no effect of the maltose on the reaction rates.

Two points are immediately apparent from examination of these data. One is that the two methods of determining the rate constants are in general agreement, and the other is that the magnitude of the change in rate on adding the cyclohexaamylose depends on the concentration of HOCl. That is, it is apparent on inspection of the data in Table 4 that the chlorination of free anisole and the chlorination of bound anisole have a different kinetic dependence on the concentration of HOCl, so any comparison of their rates must be done at the same HOCl concentrations. This change in the kinetic dependence on HOCl will be discussed below. The data of Tables 4 and 5 can be analyzed in terms of the pseudo-first-order rate constants for chlorination of free and of bound anisole and these data are in Table 6, along with the ratio of these rate constants expected from our previous determination of product ratios which was summarized in Table 3. (The ratio k_{bound}/k_{free} of 3.0 is the result of combining our more than fivefold increase in the rate of *para* chlorination with the complete disappearance of *ortho* chlorination in the mixed complex.) Thus, the directly observed rates of chlorination under these conditions are completely consistent with those expected from the partial rate factors we deduced by simple product ratio determinations. A similar treatment of the data for the complex with cycloheptaamylose was performed. It will not be detailed here (*11*), but in this case the determination by a uv spectrophotometric kinetic method that k_{bound}/k_{free} equals 0.72 ± 0.2 agrees well with the prediction from the product ratios that the k_{bound}/k_{free} should be 0.66 ± 0.1.

TABLE 4

SPECTROPHOTOMETRIC KINETICS—ANISOLE/CYCLOHEXAAMYLOSE (25.0°C)

Anisole ($M \times 10^4$)	HOCl ($M \times 10^3$)	Cyclohexaamylose ($M \times 10^3$)	Fraction complexed	k_{obs} ($sec^{-1} \times 10^2$)
5.00	10.20	0	0.00	2.91
5.00	10.20	2.63	0.42	5.33
5.00	10.20	10.08	0.72	7.05
5.00	8.16	0	0.00	1.90
5.00	8.16	2.74	0.42	4.07
5.00	8.16	10.28	0.72	5.50
5.00	6.12	0	0.00	1.00
5.00	6.12	2.68	0.42	2.64
5.00	6.12	9.76	0.72	4.10

TABLE 5

VPC KINETICS—ANISOLE/CYCLOHEXAAMYLOSE (25.0°C)

Anisole ($M \times 10^4$)	HOCl ($M \times 10^3$)	Cyclohexaamylose ($M \times 10^3$)	Fraction complexed	k_{obs} ($sec^{-1} \times 10^2$)
5.00	10.20	0	0.00	3.09
5.00	10.20	2.33	0.39	5.66
5.00	10.20	4.85	0.57	7.05
5.00	10.20	7.10	0.66	7.47
5.00	10.20	10.32	0.72	8.26

TABLE 6

RELATIVE CHLORINATION RATES—ANISOLE/CYCLOHEXAAMYLOSE (25.0°C)

Method	HOCl ($M \times 10^2$)	$k_{free} \times 10^2$ (sec^{-1})	$k_{bound} \times 10^2$ (sec^{-1})	k_{bound}/k_{free}
Product ratios	0.95–1.05	—	—	3.0 ± 0.4
uv	1.02	2.91	8.72	3.0 ± 0.4
uv	0.816	1.86	6.97	3.8 ± 0.4
uv	0.612	1.05	5.23	5.0 ± 0.6
VPC	1.02	3.00	9.79	3.5 ± 1.0

In this case, the lower rate within the complex reflects the blocking of any substitution at the *ortho* position. As was described before, the *para* position is of essentially the same reactivity both free and in the complex.

Kinetic order in HOCl. As the data in Table 4 reveal, in the absence of cyclohexaamylose the chlorination of anisole is second-order in HOCl. On the other hand, at a concentration of cyclohexaamylose of 10.0×10^{-3} *M*, in which the reaction is essentially completely dominated by the reaction of the complexed anisole, the overall rate is approximately first-order in HOCl. For this reason, it is, of course, possible to specify the relative rates of bound and free anisole chlorination only with a defined concentration of HOCl, since they have a different kinetic order. Perhaps more importantly,

this change in the kinetic order is completely consistent with the most obvious mechanistic explanation of the other observations we have made on this system. The most likely chlorinating agent for anisole in free solution under our conditions is Cl_2O, formed in equilibrium with 2 moles of HOCl. This is consistent with previous observations (*12*) on the intermediacy of Cl_2O as a chlorinating agent in HOCl reactions. By contrast, the first-order kinetic dependence on HOCl in the reaction of bound anisole indicates that a different chlorinating agent is involved, one which contains only one chlorine atom. Thus, the effect of the cyclodextrin is not simply to block particular positions but also to catalyze attack on other positions. Furthermore, this catalysis is associated with the change in the nature of the chlorinating species.

Mechanism of the reaction. There is no ambiguity about the failure of the *ortho* positions of anisole to be chlorinated in the complex, since models clearly indicate that these positions would be buried inside the cavity and not available to attack. On the other hand, the modest but real increase in rate of chlorination of the *para* position of anisole within the complex requires a mechanistic explanation. Two general possibilities can be considered. In one, the interaction of the anisole with the cyclodextrin changes on reaction, so that the transition state is even more stabilized. Although in an elementary sense one would not expect the positively charged aromatic ring of the transition state to be more stabilized inside the relatively nonpolar cavity, it is possible that particular hydroxyl groups of the cyclodextrin could coordinate to the positively charged ring so as to stabilize the charge. However, this mechanism would not explain why there is a change in the kinetic order in HOCl when the reaction occurs in the complex rather than with free anisole.

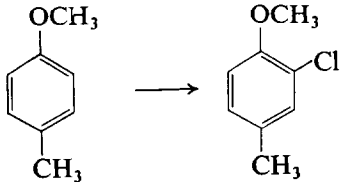
The second possibility is that the cyclodextrin interacts with the chlorinating agent and stabilizes the transition state by being bound at that instant to the species which is delivering chlorine, or to the chlorine itself. This is the mechanism which we believe is strongly indicated by these data. It involves the reversible formation of an alkyl hypochlorite of the cyclodextrin by reaction of one of the hydroxyl groups with HOCl; such rapid equilibrium formation of alkyl hypochlorites is well known (*13*). We propose that such a hypochlorite group, probably on one of the primary hydroxyls of cyclodextrin, is the species which delivers chlorine to the *para* position of the bound anisole. Chlorination in the *para* position occurs not simply because this position is more or less exposed to the solution, but specifically because it is exposed to the hydroxyl groups which rim the cyclodextrin cavity, and thus in particular to a hydroxyl group which has been transformed into a hypochlorite group. Models (as schematically shown in Fig. 1) indicate that such a hypochlorite chlorine atom is in a perfect position to attack the *para* position of a bound anisole (and other hydroxyl groups are in a good position to remove the proton from the aromatic substitution intermediate so as to complete the substitution mechanism).

Whereas chlorination in free solution is slow with HOCl and requires the more reactive Cl_2O, the well-known great increase in effectiveness of neighboring groups (*14*) means that in the intracomplex reaction a simple alkyl hypochlorite, which would be more or less equivalent in reactivity to HOCl, is in this case quite reactive enough to attack the neighboring substrate. Thus, the change in kinetic order in HOCl is expected, since species involving higher orders in HOCl will, in general, not be available in high concentration at equilibrium, and would be involved in the chlorination only if the simple HOCl species or something equivalent to it were not reactive enough. In a sense, we are describing nucleophilic catalysis (*15*) of the chlorination. There is first a substitution on the HOCl molecule by a hydroxyl group of the cyclodextrin, and this nucleophile is then ejected again in the aromatic substitution reaction. In common

with most such cases of nucleophilic catalysis, the rate increase is derived from the entropy advantages of intramolecular or intracomplex reactions.

In the complex with cycloheptaamylose, the *ortho* position was again blocked but in this case the *para* position was essentially of the same reactivity whether anisole was complexed or free. This must, in any case, be an accidental coincidence, since it is unlikely that the *para* position of an included anisole could really be of precisely the same reactivity as the *para* position of anisole in free solution unless several compensating factors were operating. We believe that here, too, the mechanism involves hypochlorite ester formation followed by delivery of chlorine within the complex. In this case the rate advantage is lower since the complex of anisole with cycloheptaamylose is much looser[3] and the neighboring group chlorination reaction within the complex can occur only by freezing out some of the freedom of motion of the anisole bound in the cavity. It is also well known that the rates of such neighboring group reactions generally decrease sharply (*16*) as new degrees of freedom are introduced which must be frozen out in the transition state.

Related reactions. We have examined the reaction of *p*-methylanisole [*4*] with hypochlorous acid, and the product is simply 2-chloro-4-methylanisole [*5*] whether cyclohexaamylose is present or not. However, addition of the cyclohexaamylose slows the rate of reaction. An analysis (*11*) of this indicates that only the free substrate is being chlorinated, and within the complex the *ortho* position is again completely blocked as a result of the complexing. On the other hand, in the chlorination of *p*-cresol [*6*] to 2-chloro-4-methylphenyl [*7*], chlorination is again *ortho* to a substituent on the benzene ring, but now our rate studies (*11*) show that k_{bound}/k_{free} equals 2.4 ± 0.6 (assuming that the reactive species is the *p*-cresoxide ion, and 12.0 ± 2.0 if it is the neutral cresol). This is not an inconceivable result in terms of our previous mechanism, since one would expect the phenoxide oxygen to extrude from the cavity so as to hydro-



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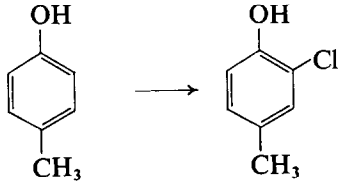
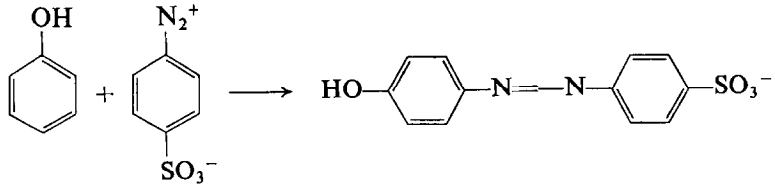


[*6*] [*7*]

gen bond to one of the hydroxyls or to water, and this would actually bring the *ortho* position out of the interior of the cavity and into reach of a cyclodextrin hypochlorite group.

[3] This is clear from models, since the diameter of the cavity is 15% larger in cyclohepta- compared with cyclohexaamylose.

We have also examined (*11*) the effect of cyclohexaamylose on the rate of coupling of phenol [*8*] with *p*-diazoniumbenzenesulfonate [*9*]. In this case, it would be expected that the electrophile could not be delivered from a hydroxyl group of the cyclodextrin,[4] so no increase in rate for this aromatic substitution would be expected if we are correct that such delivery explains the increased rates of chlorinations. The diazonium salt does not bind to the cyclohexaamylose (Table 1). Thus, a direct determination of the rate of the reaction in the presence and absence of cyclohexaamylose, together with the binding constant for phenol and cyclohexaamylose, could be combined to deduce k_{bound}/k_{free}. We found this ratio to be 0.067, which is within experimental error an indication that there is no reaction within the complex. This is consistent with our previous mechanistic idea that fast reactions in the complex involve delivery of the electrophile, which is not possible with this one. They also suggest that there is, in fact, enough hindrance of even the *para* position within a complex that random attack from free solution cannot occur readily.



[*8*] [*9*]

Thus, there is no evidence for the simple idea that complexing with cyclohexaamylose would block the *ortho* position while leaving the *para* position free for attack from solution. However, the desired highly selective substitution reaction does occur for more interesting reasons.

It is amusing that this enzyme-like mechanism—binding of the substrate to the reagent, delivery of a second species from a catalytic group oriented in such a way as to make the delivery quite specific within the complex, followed by dissociation of the catalyst–product complex—produces chloroanisole exclusively substituted in the *para* position. On the other hand, in the only actual enzymatic reaction known (*17*) to chlorinate anisole, involving the enzyme chlorinase, a more or less random 60/40 distribution of *para*- and *ortho*-chloroanisole is produced. It seems very likely that in this latter case the anisole is not bound by the enzyme, and thus our process is much more typical of most enzymatic reactions than is this real enzymatic chlorination.

EXPERIMENTAL (*11*)

Materials. Cycloamyloses were originally obtained as a gift from Prof. Dexter French. Subsequently they were purchased from Pierce Chemical Company, Rockford, Illinois. Some of the lots of cyclohexaamylose were obtained as the crystalline cyclohexane complex. Solutions were prepared from this material by heating a suspension to the boiling point of water, whereupon the material was dissolved. Upon cooling to room temperature, a drop of material, identified by infrared spectrum as cyclohexane, could be detected and removed. Solutions prepared in this way had chemical properties

[4] The electrophile in diazo coupling is unique in this respect. Although it is bulky, the terminal nitrogen is unhindered.

identical to those prepared from pure cyclohexaamylose. Hypochlorous acid was prepared using a modification of the method of Soper and Smith (*18*). Sulfuric acid was slowly added to 500 ml of a solution of 5% sodium hypochlorite, in which 2 g of mercuric oxide was suspended, until the solution was acidic. It was then distilled under vacuum at approximately 35°C into a receiving flask which also contained mercuric oxide. Solutions produced in this way were usually about 5×10^{-2} *M*. They were stored over mercuric oxide near 0°C and proved to be stable indefinitely. Solutions of HOCl in water were analyzed by reaction with potassium iodide and determination of the product I_3^- by spectrophotometry. Anisole, bp 153–154°C, and *p*-methylanisole, bp 173–174°C, were purified by distillation. Further materials were prepared and purified according to literature procedures.

Physical measurements. All pH measurements were made with a Radiometer type TTT 1 titrator equipped with type PHA630 Ta scale expander. A combination electrode, type GK2302C, with glass and calomel electrodes enclosed in a single housing, was standardized by the use of Fisher standard buffers. All physical measurements were made at 25.0 ± 0.1°C, and absorption spectra were measured in a Cary Model 15 spectrophotometer.

Spectrophotometric determination of binding constants. Anisole. A solution of anisole, 5.07×10^{-3} *M*, was prepared by delivering 55.0 μl of pure anisole from a calibrated syringe into a 100-ml volumetric flask and diluting to the mark with water. Five milliliters of this solution was added to each of several 50-ml volumetric flasks into which an accurately weighed quantity of cycloamylose had been placed. The absorption spectrum of each of these solutions was then measured on a Cary Model 15 spectrophotometer, using a cell compartment thermostated to 25.0 ± 0.1°C, and a 1-cm quartz cell. The reference compartment contained a solution of cycloamylose of identical concentration. When maltose, in amounts up to 5×10^{-2} *M*, was added to the anisole solution, no effect on the ultraviolet spectrum could be observed.

Spectra were recorded at several cyclohexaamylose concentrations. The values of C_0S_0/ΔAbs were plotted against $(C_0 + S_0)$. A computer program (*10*) which performs this Hildebrand–Benesi plot (*19*) was used to calculate the intercept $1/\Delta\epsilon$ of the linear least-squares line through the experimental points. Calculations were done at four wavelengths, 280.0, 278.7, 271,3, and 270.0 nm. An average value of K_d was used. Agreement was within 15% of the average.

For determination of the binding constant of anisole and cycloheptaamylose, the wavelengths used were 281.0, 280.0, 279.0, 278.0, 277.0, 273.5, 272.5, 271.5, and 270.5 nm.

p-*Methylanisole.* A procedure essentially identical to that used for anisole was used. The original amount of material was 63.0 ml to produce final solutions of concentration 5.00×10^{-4} *M*. Wavelengths used for measurement were 285.0, 279.0, 277.0, and 276.0 nm.

Kinetic Determination of Binding Constants

Direct method—bromine–cyclohexaamylose. In a typical experiment, a solution of bromine in water, 5.03×10^{-3} *M*, was prepared by direct weighing of bromine in a 50-ml volumetric flask. A second solution was prepared by adding 0.2403 g fumaric acid and 0.3502 g phthalic acid to ca. 70 ml of water. Sodium hydroxide solution was added until solution was complete and the pH had reached 5.50. The solution was quantitatively transferred to a 100-ml volumetric flask and diluted, producing (fumarate species) $= 2.07 \times 10^{-2}$ *M*, (phthalate species) $= 2.11 \times 10^{-2}$ *M*.

The second dissociation constant for fumaric acid is $(3.55 \pm 0.5) \times 10^{-5}$ *M*; thus,

the doubly ionized fumarate accounts for $91.8 \pm 1.2\%$ of the total fumaric acid species.

Two milliliters of the buffered fumarate solution were pipetted into a dry 1-cm quartz cell into which an accurately weighed quantity (0–30 mg) of cyclohexaamylose had been placed. When solution of the cyclohexaamylose was complete, the cell was placed in a thermostated (25.0 ± 0.1°C) cell compartment of a Gilford Model 2400 recording spectrophotometer, and the temperature was allowed to equilibrate for at least 20 min. Meanwhile, the flask containing the bromine solution was immersed in a constant-temperature bath also at 25.0 ± 0.1°C. The reaction was begun by adding 500 μl of the bromine solution to the cell. Mixing was accomplished without removing the cell from its compartment by passing a flat-tipped glass stirring rod through the cell several times. In this way, mixing time could be reduced to about 5 sec. The absorbance of the solution at 385 nm was monitored, using a full-scale range of 0–0.15 absorbance units. Calibrated chart divisions had previously been checked by stopwatch, and were accurate to within 0.3%. The reaction was followed through at least eight half-lives, and the kinetic data were treated with a computer program (*10*) which does a standard first-order plot but allows the infinity reading to be a variable, and which also applies the Guggenheim analysis (*20*) to the data with up to three values of the Guggenheim Δ. All kinetic data produced values for the rate constant which agreed to within 5% when calculated by different methods, which had standard deviations of 1.5% or less, and which had correlation coefficients of 0.998 or greater.

In dummy experiments (all conditions the same except no optical measurements), pH was measured before addition of bromine, immediately (<30 sec) after addition of bromine, and intermittently through eight half-lives. The pH was found to be constant to within 0.02 pH unit.

Each reaction was carried out in triplicate under identical conditions, with the resulting calculated pseudo-first-order rate constants agreeing to within 5%. The concentration of fumarate was varied to confirm that the kinetics followed first-order behavior in that component.

The necessity of using a buffered system presented some difficulties. The chosen system, phthalate/biphthalate, is, *a priori*, a possible complexant with cyclohexaamylose, since various substituted benzoates are known (*4*) to bind to cyclohexaamylose. Although a separate experiment, examination of the cyclohexaamylose-catalyzed hydrolysis of *m*-nitrophenyl acetate for inhibition by phthalate, showed no observable phthalate complex formation, it was still desirable to vary the buffer concentration as a means of verifying that neither phthalate nor biphthalate was affecting the reaction, except as a buffer. Therefore, the concentration of phthalate buffer was changed, reducing it to one-half and one-fifth of its original value, and maintaining a constant ionic strength by addition of appropriate amounts of sodium perchlorate. The resulting rate constants were constant to within 5% (experimental error).

When maltose, in amounts up to 5×10^{-2} *M*, was used as a control, replacing cyclohexaamylose, the observed rate constant for bromine disappearance was identical to k_{free}, the rate constant observed with no cyclohexaamylose present.

The calculated values of $(k_{obs} - k_{free})$ were plotted against $(k_{obs} - k_{free})/C$. A computer program (*10*) to perform this Eadie plot (*21*) was used to calculate the intercept and slope of the linear least-squares line through the experimental points.

Inhibition method. Two reactions were used as bases for the study of binding constants by inhibition methods. The bromination of fumarate, described in the previous section, was used in the study of possible binding of hypochlorous acid and *p*-diazoniumbenzenesulfonate to cyclohexaamylose; and the hydrolysis of *m*-nitrophenyl acetate,

carefully studied by Van Etten et al. (*6*), was used in the study of binding of anisole, *p*-methylanisole, fumarate, and phthalate to cyclohexaamylose.

In the case of inhibition of ester hydrolysis, the procedure of Van Etten (*6*) was followed exactly, except that a Gilford Model 2400 recording spectrophotometer was used to measure the appearance of *m*-nitrophenoxide ion, and sodium perchlorate, rather than potassium chloride, was used to adjust the ionic strength to 0.2. Our data agreed within experimental error with that reported previously (*6*). Anisole and *p*-methylanisole, in amounts up to 7×10^{-3} *M*, had no effect on the hydrolysis of *m*-nitrophenyl acetate in the absence of cyclohexaamylose. The observed rate as a function of the concentration of various inhibitors was treated with a computer program (*10*) according to the procedures described in the Results and Discussion Section.

Chlorination of Anisole by Hypochlorous Acid

Product analysis. The usual anisole concentration was 5×10^{-4} *M*, the largest concentration which could be used while maintaining a large excess of cycloamylose. HOCl concentration was $(0.95\text{–}1.05) \times 10^{-2}$ *M*. As a control experiment, the concentration of anisole was reduced to 2×10^{-4} *M* and 1×10^{-4} *M*. As expected, no change in product ratios was observed. No buffer was used. The initial pH was measured and found to be 4.7.

In a typical experiment, an appropriate amount of cycloamylose was weighed directly in a 10-ml volumetric flask, anisole solution was added, followed by hypochlorous acid solution, and enough water to make 10 ml. At first, mixtures were allowed to react at room temperature overnight; later it was found that the reaction was complete within about 10 min. Even at times up to 12 hr, no change in product distribution could be observed, and no dichloroanisoles could be detected when they were looked for. Thus, at some time after 10 min of reaction, the reaction mixture was poured into a separatory funnel and extracted with 2 ml of ether.

A separate control experiment showed that for all compounds in question, one extraction of an aqueous solution by 1/10 of its volume of ether is at least 99.5% efficient in transferring the anisole or derivative to the ether phase. This efficiency is unimpaired by cycloamylose in concentrations up to 1.2×10^{-2} *M*. The ether phase showed all of the uv chromophore expected, while the aqueous phase contained no detectable chromophore.

The ether phase was dried with anhydrous magnesium sulfate and reduced in volume to ca. 0.5 ml. It was analyzed by VPC, using an Aerograph Hi-Fi chromatograph equipped with flame ionization detector. Nitrogen was used as a carrier gas. The column used was 1/8 in. × 10 ft, packed with 20% w/w tri(*p*-tolyl)phosphate on Chromosorb P. At 140°C the retention times were: anisole (11.2 min), *p*-chloroanisole (39.7 min), *o*-chloroanisole (51.0 min). No *m*-chloroanisole, or dichloroanisoles, could be detected in any reaction.

Peak areas were determined both by triangulation (height times width at half-height) and by cutting and weighing. Agreement between the methods was generally to within 5%. Calibration of the detector was accomplished by injecting solutions containing anisole and *o*- and *p*-chloroanisoles in known relative amounts, determined by direct weight.

Kinetics

Spectrophotometric method. In a typical experiment, 2.0 ml of a solution of hypochlorous acid, 1.13×10^{-2} *M*, was pipetted into a 1-cm quartz uv cell containing an appropriately weighed amount of cycloamylose. The reaction was begun by adding

250 μl of anisole solution, 4×10^{-3} *M*. Absorbance was monitored at 276 nm. In control experiments, maltose, in amounts up to 10^{-1} *M*, had no effect on the observed rate constants.

A separate control experiment showed that solutions of hypochlorous acid and either cyclohexaamylose or cycloheptaamylose are stable indefinitely at room temperature, and that the order of mixing the reactants has no effect on the kinetic behavior of the reaction.

VPC method. In a typical experiment, reactants of identical concentrations to those described for the spectrophotometric method were mixed in identical proportions, scaling all amounts up by a factor of 5 (except 1.0 ml of 5×10^{-3} *M* anisole was used). At 30-sec intervals, 1.0-ml aliquots were withdrawn and quenched by adding them to ca. 10 ml of ca. 0.02 *M* sodium bisulfite solution. The quenched aliquots were then each extracted with 2 ml of ether, the ether phase dried with magnesium sulfate and evaporated to a volume of ca. 0.5 ml. VPC analysis was carried out. The efficiency of quenching was checked by adding sodium bisulfite to a solution of anisole and cyclohexaamylose, then adding hypochlorous acid and proceeding with workup and analysis in the usual way. Under these conditions, no chloroanisoles could be detected.

Chlorination

p-*Methylanisole.* The procedure for product identification used was identical to that used for identification of the products of anisole chlorination, except for VPC conditions. For analysis, a Hewlitt-Packard Model 5750 Research Chromatograph, equipped with dual columns and flame ionization detectors, was used. The column temperature was programmed to rise from 58 to 230°C at a rate of 10°/min, beginning 4 min after injection. The columns were 5% SE-30 on Chromosorb P. Nitrogen was used as carrier gas at a rate of 13 machine units. Retention times, as determined by injection of solutions of authentic samples, were: *p*-methylanisole (11.5 min), 2-chloro-4-methylanisole (15.0 min).

Quenching the reaction at various times with excess sodium bisulfite showed the clean conversion of *p*-methylanisole to 2-chloro-4-methylanisole. No dichloro product or other products were observed in any chlorination reactions. Spectrophotometric and VPC kinetics were done as described above for anisole.

p-*Cresol.* The products were identified by a procedure identical to those used above, except that the reaction was carried out in 0.02 *M* acetate buffer at pH 4.60. The pH stayed constant to within 0.02 pH units. This buffer was also used in the spectrophotometric kinetic measurements on this substrate, monitored at 290 nm.

Diazo Coupling of Phenol and p-*Diazoniumbenzenesulfonate*

A sample of the expected product, 4-hydroxyazobenzene-4′-sulfonate, was prepared in the standard way (*22*). Its spectrum was identical with that developed in the kinetic experiments. In a typical kinetic experiment, 1.0 ml of a solution containing sulfanilic acid, 3.34×10^{-4} *M*, and HCl, 3×10^{-2} *M*, was mixed in a dry uv cell with 1.0 ml of a solution containing sodium nitrite, 3.5×10^{-4} *M*. An appropriate amount of cyclohexaamylose was added, and when the solution was complete, the cell was placed in the thermostated (25.5 ± 0.1°C) compartment of a Gilford Model 2400 recording spectrophotometer. Meanwhile, a solution containing phenol, 1.31×10^{-5} *M*, and disodium hydrogen phosphate, 6×10^{-2} *M*, was equilibrated to the same temperature. After at least 20 min, the reaction was begun by adding 1.0 ml of the phenol solution to the cell. Stirring was accomplished by means of a flat-tipped stirring rod, without removing the cell from its compartment. The increase in absorbance was monitored

at 350 nm, and the kinetic data were analyzed in the usual way. The pH produced by addition of phosphate was 6.90 ± 0.01. When the reaction was monitored by pH meter, the pH did not vary detectably during the course of the reaction.

When maltose, in concentrations up to 0.1 *M*, was used as a control, there was no detectable effect on the rate of the reaction.

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