Stoichiometric Model of α-Cyclodextrin Complex Formation

THOMAS W. ROSANSKE and KENNETH A. CONNORS ˣ

Received October 9, 1979, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.* Accepted for publication January 7, 1980.

Abstract □ The solubility, spectral, and kinetic methods were used to study complexing between α-cyclodextrin (ligand, L) and 3,5-dimethoxycinnamic acid, benzalacetone, and methyl cinnamate (substrates, S). In aqueous solution at 25° and with an ionic strength of 0.01 *M*, the following stability constants were found (K_{11} for SL and K_{12} for SL_2): 3,5-dimethoxycinnamic acid, $K_{11} = 1965\ M^{-1}$ and $K_{12} = 0$; benzalacetone, $K_{11} = 105\ M^{-1}$ and $K_{12} = 15\ M^{-1}$; and methyl cinnamate, $K_{11} = 1200\ M^{-1}$ and $K_{12} = 50\ M^{-1}$. A model of complex formation is proposed that can account for the observed stoichiometry and that yields stability estimates for two isomeric 1:1 complexes in the systems in which a 1:2 complex forms. For cinnamic acid, benzalacetone, and methyl cinnamate, stability constants are inversely correlated with the substrate dipole moment.

Keyphrases □ α-Cyclodextrin—complexation with 3,5-dimethoxycinnamic acid, benzalacetone, and methyl cinnamate, stoichiometric model □ Stability constants—complexes of α-cyclodextrin with cinnamic acid, benzalacetone, and methyl cinnamate □ Complexation—α-cyclodextrin, stoichiometric model

A previous study (1) showed that *trans*-cinnamic acid forms 1:1 (SL) and 1:2 (SL_2) complexes[1] with α-cyclodextrin (cyclohexaamylose) in aqueous solution. Therefore, knowledge of the factors that influence the stoichiometric relationships in such a system and that control the magnitudes of the complex stability constants was desired. For this purpose, the complexing of several substrates structurally related to cinnamic acid was studied in aqueous α-cyclodextrin solutions. These substrates are 3,5-dimethoxy-*trans*-cinnamic acid (I), benzalacetone (*trans*-4-phenyl-3-buten-2-one, II), and methyl *trans*-cinnamate (III). The complexing behavior was studied by the solubility, spectral, and kinetic techniques.

EXPERIMENTAL

Materials—3,5-Dimethoxy-*trans*-cinnamic acid[2] was recrystallized twice from water, mp 174.6–175°. Benzalacetone[2] was recrystallized twice from *n*-hexane, mp 39.7–40.7° [lit. (2) mp 41–42°]. Methyl *trans*-cinnamate[3] was distilled under reduced pressure, bp 95–97° (10–12 mm Hg), mp 32.9–33.9° [lit. (3) mp 33.5–34.5°]. α-Cyclodextrin[4] was used directly; drying to constant weight at 90° indicated 9.77% water content, corresponding closely to the hexahydrate (9.99% water); $[\alpha]_D^{25}$ +150.4 ± 2.2° (10 measurements) [lit. (4) $[\alpha]_D^{25}$ +150.5°].

Procedures—The solubility and spectral studies were carried out as described for cinnamic acid (1), except that in the solubility technique the supernatant solution was analyzed spectrophotometrically after appropriate dilution. For the 3,5-dimethoxycinnamic acid, this dilution was made a basic pH to minimize spectral perturbations caused by complexing since it is known (5) that acid anions complex less strongly than the parent carboxylic acid.

A kinetic method was used to study methyl cinnamate. Increasing amounts of α-cyclodextrin were weighed accurately into a series of 10-ml volumetric flasks and were dissolved in pH 10.36 carbonate buffer. To each flask, 0.2 ml of a saturated aqueous solution of methyl cinnamate was added. The solutions were brought to volume with the carbonate buffer. At recorded times, samples were analyzed spectrophotometrically at 295 nm. The kinetics were apparent first order.

All studies were carried out at 25.0° and an ionic strength of 0.01 *M*. The stability constants are defined by:

$$K_{11} = \frac{[SL]}{[S][L]} \qquad \text{(Eq. 1)}$$

$$K_{12} = \frac{[SL_2]}{[SL][L]} \qquad \text{(Eq. 2)}$$

RESULTS

3,5-Dimethoxycinnamic Acid—UV spectra of 3,5-dimethoxycinnamic acid with varying concentrations of α-cyclodextrin revealed isosbestic points at 231, 240, and 300 nm; these isosbestic points were preserved even at high ligand concentrations. This behavior is consistent with 1:1 stoichiometry, and the Benesi–Hildebrand double-reciprocal plot is shown in Fig. 1. Least-squares analysis gave $K_{11} = 1970\ M^{-1}$.

Figure 2 is the solubility diagram for this system. The linear rise in total solubility is consistent with 1:1 complexing, and the stability constant evaluated from the line (excluding the three highest points) was $K_{11} = 1960\ M^{-1}$, in excellent agreement with the result from the spectral study. (The deviation of the three terminal points is attributed to a small spectral perturbation caused by complexation in the analytical solution.) The well-behaved isosbestic points, the linear spectral and solubility plots, and the agreement of K_{11} values from the independent spectral and solubility studies all indicate that this system is described fully by 1:1 stoichiometry.

Benzalacetone—Figure 3 shows the solubility diagram for the benzalacetone system. The initial rising portion is followed by a plateau region, and then a decrease in S_t occurs with a shoulder unusual in such diagrams. The shape of this curve will be discussed. Analysis of the solid phases gave the following results for $10^2 L_t$ and x_L, where x_L is the mole fraction of cyclodextrin in the solid: 2.90, 0.26; 4.90, 0.60; 6.01, 0.61; 6.90, 0.65; 8.02, 0.66; and 10.1, 0.67. These data show that at least one complex with a stoichiometric L to S ratio of greater than unity must be present.

Interpretation of the initial rising portion in terms of 1:1 plus 1:2 complexes, as described previously (1, 6), shows that the slope of the line is 0.50, which implies that $K_{11} = 1/s_0$, where s_0 is the solubility at $L_t = 0$. This relationship gives $K_{11} = 105\ M^{-1}$; with $K_{11} = 1/s_0$, the data can be fitted regardless of the value of K_{12}. Thus, the solubility study showed that a complex of higher L to S stoichiometry must be present and yielded an estimate of K_{11}.

The spectral data showed isosbestic points at 241 and 304 nm; these points were lost at cyclodextrin concentrations of greater than ~0.007

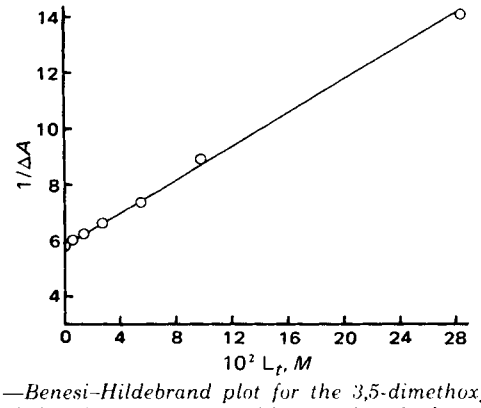

Figure 1—*Benesi–Hildebrand plot for the 3,5-dimethoxycinnamic acid–α-cyclodextrin system at 25° with a wavelength of 291 nm, a path length of 1 cm, a total substrate concentration of 4.54 × 10⁻⁵ M, and a pH of 2.3; L_t represents the total ligand concentration (moles per liter), and ΔA is the change in absorbance.*

[1] The substrate (cinnamic acid in this example) is represented by S, and the ligand (cyclodextrin) is represented by L.

[2] Aldrich.

[3] Eastman.

[4] Sigma lot 106C-0046-1.

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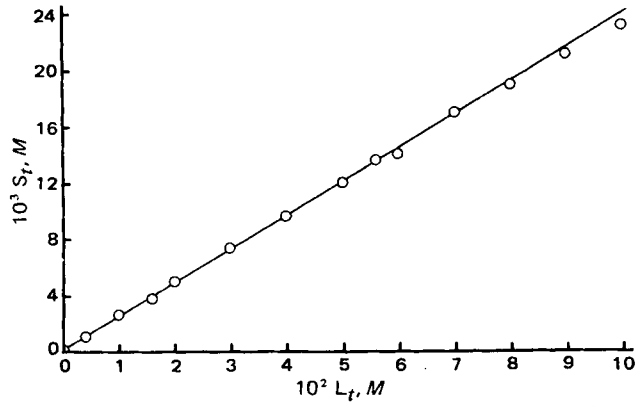


Figure 2—*Solubility study of the 3,5-dimethoxycinnamic acid–α-cyclodextrin system at 25°. The substrate solubility at $L_t = 0$ is 1.51×10^{-4} M; S_t represents the total substrate concentration.*

M, indicating the presence of at least two complexes. The spectral results at 291 nm are given in Fig. 4. The smooth curve was calculated by assuming 1:1 and 1:2 complexes, fixing K_{11} at 105 M^{-1} (from the solubility data), and allowing K_{12} and the complex molar absorptivities to function as adjustable parameters, as described previously (1). The resulting values were $K_{11} = 105\ M^{-1}$, $K_{12} = 15\ M^{-1}$, $\Delta a_{11} = 4500$, and $\Delta a_{12} = 6600$, where $\Delta a_{11} = a_S - a_{11}$, *etc.*, with *a* representing the designated molar absorptivity.

Methyl Cinnamate—The solubility plot for methyl cinnamate (Fig. 5) is qualitatively similar to that of benzalacetone. The solid phase analysis again gave evidence of a 1:2 complex ($10^2 L_t$ and x_L): 2.00, 0.17; 4.01, 0.56; 4.90, 0.60; 6.01, 0.62; 6.80, 0.66; and 10.1, 0.67. Treatment of the initial rising portion in terms of SL and SL_2 complexes gives $K_{11} = 1200\ M^{-1}$ and $K_{12} = 50\ M^{-1}$.

The kinetic method used to study this system is based on the inhibitory effect of cyclodextrin on the rate of alkaline hydrolysis of methyl cinnamate. Scheme I shows the assumed kinetics.

$$S + OH^- \xrightarrow{k_S} \text{products}$$

$$SL + OH^- \xrightarrow{k_{11}} \text{products}$$

$$SL_2 + OH^- \xrightarrow{k_{12}} \text{products}$$

Scheme I

The following equations then are readily derived, as was shown previously (6, 7):

$$L_t = [L] + \frac{S_t(K_{11}[L] + 2K_{11}K_{12}[L]^2)}{1 + K_{11}[L] + K_{11}K_{12}[L]^2} \qquad \text{(Eq. 3)}$$

$$\frac{k_S - k'_S}{k_S} = \frac{q_{11}K_{11}[L] + q_{12}K_{11}K_{12}[L]^2}{1 + K_{11}[L] + K_{11}K_{12}[L]^2} \qquad \text{(Eq. 4)}$$

where L_t is the total ligand concentration, [L] is the free (uncomplexed) ligand concentration, k'_S is the apparent second-order rate constant in the presence of ligand, $q_{11} = 1 - k_{11}/k_S$, and $q_{12} = 1 - k_{12}/k_S$. It is not practical to obtain an expression for k'_S as an explicit function of L_t, so a curve-fitting procedure is used. Figure 6 shows the kinetic data; the smooth line was calculated with Eqs. 3 and 4, taking $K_{11} = 1200\ M^{-1}$ and $K_{12} = 50\ M^{-1}$ (from the solubility study) and treating q_{11} and q_{12} as adjustable parameters. The method is used to assign reasonable values to [L] and to calculate corresponding values for L_t and k'_S for comparison with the experimental results. The line in Fig. 6 was obtained using $q_{11} = 0.75$ and $q_{12} = 0.86$.

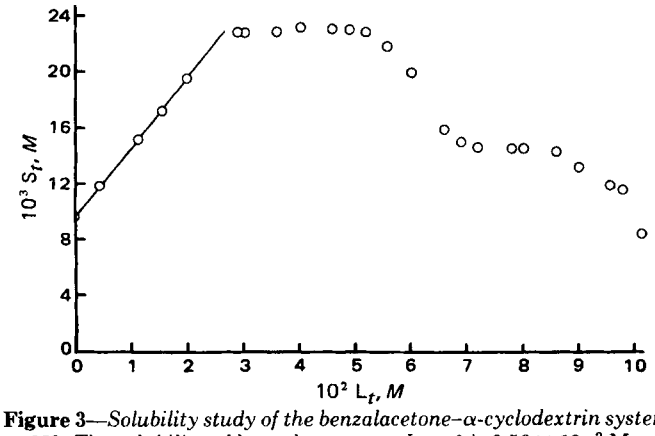


Figure 3—*Solubility study of the benzalacetone–α-cyclodextrin system at 25°. The solubility of benzalacetone at $L_t = 0$ is 9.56×10^{-3} M.*

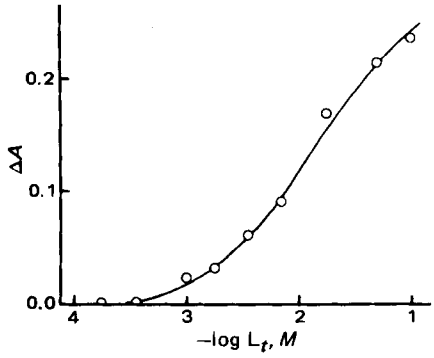


Figure 4—*Change in absorbance at 291 nm in the benzalacetone–α-cyclodextrin system with a path length of 1 cm, a total substrate concentration of 4.39×10^{-5} M, and a pH of 2.3.*

DISCUSSION

Complex Stoichiometry and Stability—Each substrate was investigated by two experimental techniques because such comparative studies have been effective in elucidating stoichiometric relationships (1, 7). The important pieces of information are the isosbestic point behavior, the solid phase compositions, and the quantitative consistency between the two methods. The essential conclusion is that solutions of α-cyclodextrin with 3,5-dimethoxycinnamic acid can be described fully in terms of 1:1 complex formation whereas the benzalacetone and methyl cinnamate systems require 1:1 and 1:2 stoichiometries for their description.

The shapes of the solubility curves for benzalacetone and methyl cinnamate have not been explained fully. In systems containing 1:1 and 1:2 complexes, there are several possibilities for solubility behavior: (*a*) the solubility of SL will be reached first, (*b*) the solubility of SL_2 will be reached first, (*c*) the solubilities of SL and SL_2 will be reached essentially simultaneously, and (*d*) SL and SL_2 will form solid solutions. Case *a* is observed with cinnamic acid and apparently does not apply here (1). Cases *b* and *c* both require, as argued previously (6), that $x_L = 0.67$ at the point terminating the plateau; hence, these cases do not account for the solid phase analyses. The formation of a solid solution from solid SL and SL_2 does not seem unlikely if these are inclusion complexes; X-ray evidence for solid cyclodextrin complexes with channel-like structures was reported (8, 9). This reaction decreases the number of solid phases by one; then upon depletion of solid S, only one solid phase remains (the solid solution), and the constraint on S_t is removed.

The q_{11} and q_{12} values obtained from the kinetic analysis may be interpreted as the fractional decrease in reactivity of the complexed substrate relative to the uncomplexed substrate. If the system actually contains two isomeric 1:1 complexes, then q_{11} is a weighted average of the corresponding quantities for the individual complexes (7). It is quite possible for one of these quantities to be positive ($k_{11} < k_S$) while the other is negative ($k_{11} > k_S$). The value of q_{12} seems somewhat low

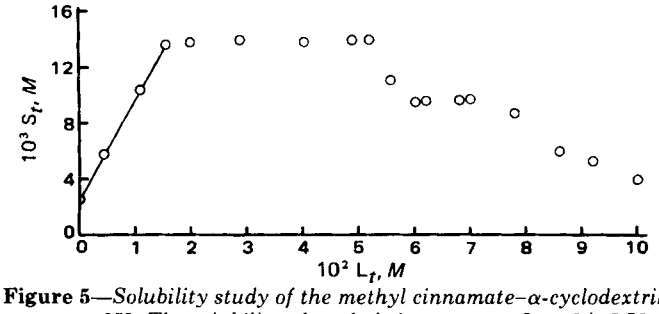


Figure 5—*Solubility study of the methyl cinnamate–α-cyclodextrin system at 25°. The solubility of methyl cinnamate at $L_t = 0$ is 2.50×10^{-3} M.*

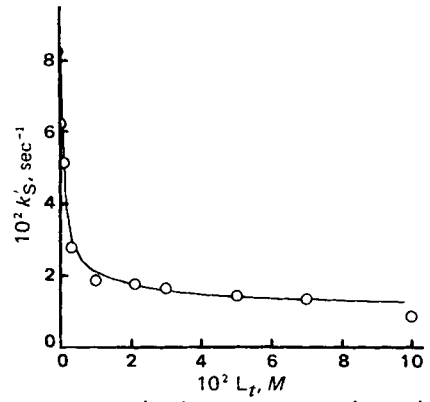


Figure 6—*Apparent second-order rate constants for methyl cinnamate hydrolysis as a function of the α-cyclodextrin concentration; the rate constant at* $L_t = 0$ *was* 8.33×10^{-2} M^{-1} sec^{-1}.

(complete inhibition, $q_{12} = 1$, is anticipated for the fully included substrate); however, as Fig. 6 shows, considerable uncertainty (at least 10%) may be associated with this value because of imprecision in the rate data. Table I summarizes the stability constant findings for these substrates.

Stoichiometric Model of Complexing—As a framework for describing and explaining complex stoichiometry and stability in these systems, the following argument may be useful. Let h-H represent the cyclodextrin (host) molecule, the lower and upper case letters indicating that the two ends of the cavity are structurally different, and let g-G represent a linear nonsymmetrical substrate (guest) molecule. Then all possible 1:1 inclusion complexes may be represented as follows, where it is assumed that, within each orientation, the energy distribution of positional isomers is narrow so that only a single complex need be considered:

1:1 complexes	symbol
g-G h-H	gH
g-G H-h	gh
G-g h-H	GH
G-g H-h	Gh

It also is possible, in principle, to form 2:1 and 1:2 inclusion complexes:

2:1 complexes	symbol
g-G G-g H-h	HGGh
g-G g-G H-h	HGgh
G-g g-G H-h	Hggh
G-g G-g H-h	HgGh

1:2 complexes	symbol
g-G h-H h-H	gHhG
g-G H-h h-H	ghhG
G-g h-H H-h	GHHg
G-g H-h H-h	GhHg

Now suppose that, for the systems being considered, no 2:1 complexes exist. In the present study, there was no necessity to invoke S_2L complexes, and there appears to be no literature report of S_2L complexes with α-cyclodextrin. Then it is reasonable to infer that the guest molecule can enter only one end of the host. As a consequence of this inference, there can be only two 1:1 complexes and only one 1:2 complex. For example, suppose only the H end of the host can be entered. In this case, the only possible complexes are gH, GH, and GHHg. The model thus has led, for this experimental case, to a great simplification of the stoichiometric possibilities. Other special cases of the general model can be described but are not required in this treatment.

Table I—Stability Constants for α-Cyclodextrin Complexes at 25°

Substrate	K_{11}, M^{-1}	K_{12}, M^{-1}
Cinnamic acid[a]	2260	60
Cinnamate ion[a]	110	15
3,5-Dimethoxycinnamic acid	1965	0
Benzalacetone	105	15
Methyl cinnamate	1200	50

[a] From Ref. 1.

It is known (7) that the observed K_{11} value obtained from any experimental technique is the sum of the stability constants for all isomeric 1:1 complexes; hence, for the case being considered:

$$K_{11} = K_{gH} + K_{GH} \qquad \text{(Eq. 5)}$$

To carry the analysis further, the assumption is made that the 1:2 complex is formed by adding a host molecule to a 1:1 complex without perturbing the structure (or energy) of the 1:1 complex. The 1:2 complex can be formed *via* the gH route (Scheme II) or the GH route (Scheme III).

$$gH + h\text{-}H \overset{K'}{\rightleftharpoons} GHHg$$

Scheme II

$$GH + h\text{-}H \overset{K''}{\rightleftharpoons} GHHg$$

Scheme III

The observed stability constant for 1:2 complex formation therefore is given by:

$$K_{12} = \frac{[GHHg]}{([gH] + [GH])[h\text{-}H]} \qquad \text{(Eq. 6)}$$

which becomes:

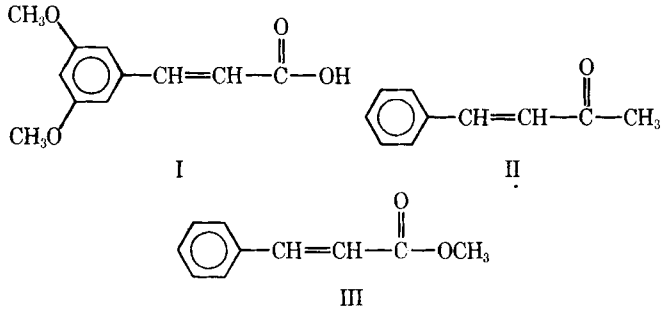
$$K_{12} = \frac{K'K''}{K' + K''} \qquad \text{(Eq. 7)}$$

The assumption that addition of the second host molecule does not perturb the preformed 1:1 complex structure is equivalent to assuming $K' = K_{GH}$ and $K'' = K_{gH}$; thus:

$$K_{12} = \frac{K_{gH}K_{GH}}{K_{gH} + K_{GH}} \qquad \text{(Eq. 8)}$$

With Eqs. 5 and 8, K_{gH} and K_{GH} can be evaluated[5].

Table II lists K_{gH} and K_{GH} values for the substrates of Table I. The assignment of symbols is arbitrary. Because of the structural likenesses among the members of this series of substrates, it seems reasonable to attribute all larger values to one mode (K_{GH}) and the smaller constants to the other (K_{gH}).



Interpretation of the data for these substrates (I–III) in terms of the model suggests that the two isomeric 1:1 complexes are formed by inclusion of either the side chain or the phenyl ring in the cyclodextrin cavity. Molecular models indicate that the disubstituted phenyl ring of I cannot penetrate the ligand cavity; hence, the stoichiometric model predicts that one of the isomeric 1:1 constants will be zero and, therefore, that $K_{12} = 0$. This behavior is observed; therefore, the assignments in Table II signify that K_{GH} describes binding of the side chain whereas K_{gH} describes binding of the phenyl ring. A further prediction is that K_{GH} for I should be very similar to that for cinnamic acid, as is seen.

[5] Equation 8 can be written as $1/K_{12} = 1/K_{gH} + 1/K_{GH}$; *i.e.*, the 1:2 dissociation constant is equal to the sum of the 1:1 dissociation constants.

Table II—Calculated Isomeric 1:1 Stability Constants [a]

Substrate	K_{GH}, M^{-1}	K_{gH}, M^{-1}
Cinnamic acid	2199	61.5
Cinnamate ion	92.1	17.9
3,5-Dimethoxycinnamic acid	1965	0
Benzalacetone	86.9	18.1
Methyl cinnamate	1148	52.3

[a] Calculated with Eqs. 5 and 8 from the data in Table I.

All substrates in Table II other than I evidently offer the possibility of binding at both sites, so significant values of both K_{11} and K_{12} are anticipated and observed. Since the model has no provision for substituent effects, it does not provide quantitative predictions for these substrates. The advantages of the model are in transforming a potentially very complicated system into a fairly simple one on the basis of experimental observation and reasonable approximations and in providing qualitative stoichiometric predictive ability and a means for deriving quantitative estimates of isomeric stability constants when certain assumptions are met.

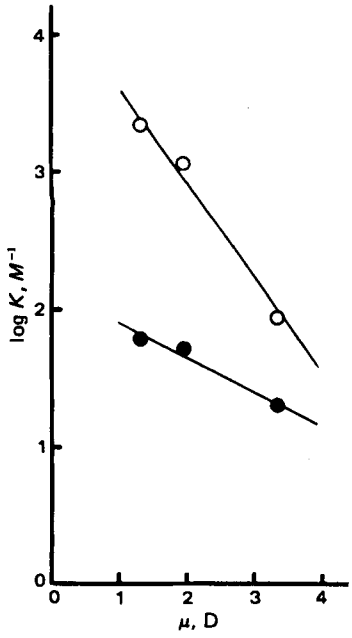


Figure 7—*Plot of log* K_{GH} (○) *and log* K_{gH} (●) *against the substrate dipole moment for II (μ = 3.34 D), III (μ = 1.95 D), and cinnamic acid (μ = 1.31 D). Dipole moments are from Ref. 10. The lines have no theoretical significance.*

Dipole Moment Correlation—The stability constants for the substrates in Table II, particularly K_{GH}, vary more than might be expected based on the apparent structural similarity of these compounds. The behavior of benzalacetone, in particular, seems anomalous. However, the constants in Table II for II, III, and cinnamic acid correlate well with the substrate dipole moment, as shown in Fig. 7; the larger the dipole moment, the smaller is the stability constant. This behavior is consistent with the view that the interior of the cyclodextrin cavity is less polar than the surrounding aqueous medium; hence, the more polar the substrate, the less is its tendency to partition into the ligand cavity.

The relative slopes of the plots for K_{GH} and K_{gH} in Fig. 7 substantiate the structural assignments of these quantities, the more sensitive quantity (K_{GH}) representing the side chain upon which the structural change is made and, therefore, responding more to the change than does K_{gH}, which describes binding far removed from the structural alteration. That the constant assigned to the side chain, which is the more polar end of the substrate molecule, is larger than that for the phenyl ring may seem inconsistent, but the comparison overlooks other factors, particularly the sizes of these two binding sites.

The cinnamate-ion system is qualitatively consistent with this interpretation. The essentially identical stability constants for cinnamate and benzalacetone suggest that a methyl ketone may be a good model for the polarity of a carboxylate ion.

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ACKNOWLEDGMENTS

Supported in part by National Science Foundation Grant GP-36567 and in part by a grant from The Upjohn Co.