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trans-Cinnamic Acid–α-Cyclodextrin System as Studied by Solubility, Spectral, and Potentiometric Techniques

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Abstract □ Complex formation in aqueous solutions of *trans*-cinnamic acid or *trans*-cinnamate ion (the substrate, S) and α-cyclodextrin (the ligand, L) can be described quantitatively as the 1:1 and 1:2 complexes, SL and SL_2. The solubility, spectral, and potentiometric data over a wide range of ligand concentrations yielded consistent estimates of the complex association constants. For cinnamic acid at 25°, $K_{11} = 2260\ M^{-1}$, $\Delta H^\circ_{11} = -9.3$ kcal/mole, and $\Delta S^\circ_{11} = -8$ e.u.; and $K_{12} = 60\ M^{-1}$, $\Delta H^\circ_{12} = -12$ kcal/mole, and $\Delta S^\circ_{12} = -26$ e.u. For cinnamate ion at 25°, $K_{11} = 110\ M^{-1}$, $\Delta H^\circ_{11} = -1.9$ kcal/mole, and $\Delta S^\circ_{11} = +11$ e.u.; and $K_{12} = 15\ M^{-1}$, $\Delta H^\circ_{12} = -9$ kcal/mole, and $\Delta S^\circ_{12} = -15$ e.u. (all entropy changes are unitary quantities). Thermodynamic cycles for the complexes, using solubility data, reveal that complex formation in the solid phase is thermodynamically spontaneous but that complex stability is greater in aqueous solution than in the solid phase.

Keyphrases □ Complexation—*trans*-cinnamic acid with α-cyclodextrin, thermodynamic analysis, stoichiometry, stability equilibria, determined by spectral, solubility, and pH measurements □ Thermodynamics—complexation analysis, determined by spectral, solubility, and pH measurements, *trans*-cinnamic acid–α-cyclodextrin complex □ *trans*-Cinnamic acid—analysis of complexation with α-cyclodextrin, thermodynamics □ α-Cyclodextrin—analysis of complexation with *trans*-cinnamic acid, thermodynamics □ Solubility measurements—complexation of *trans*-cinnamic acid with α-cyclodextrin □ UV spectrometry—complexation of *trans*-cinnamic acid–α-cyclodextrin □ pH measurements—complexation of *trans*-cinnamic acid–α-cyclodextrin

The chemical and physical properties of *trans*-cinnamic acid and its derivatives make these compounds highly useful substrates[1] in studies of molecular complex formation (1–7). Preliminary studies in this laboratory on the *trans*-cinnamic acid–α-cyclodextrin (cyclohexaamylose) system in aqueous solution showed that simple 1:1 stoichiometry in the complex does not adequately describe the equilibrium system. This observation seemed to be worth pursuing because cinnamic acid reportedly forms a 1:1 complex with α-cyclodextrin (8) and because this substrate does not seem to be an atypical one in such studies; hence,

[1] The substrate is the compound whose measured properties constitute the dependent variable; the ligand is the substance (α-cyclodextrin in this case) whose concentration is the independent variable.

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knowledge of its stoichiometric relationships may be of wider utility. Attempts to correlate and to explain complex stability or to explicate complex structures and the forces involved cannot be successful unless the stoichiometries and stabilities of the complexes are known.

Although 1:1 stoichiometric ratios commonly are found for cyclodextrin complexes, different ratios have been reported for some substrates. Among organic acids and acid anions, solid phase stoichiometries other than 1:1 have been found for cinnamic acid (acid–β-cyclodextrin, 2:1) (9), salicylic acid (acid–β-cyclodextrin, 3:1) (10), and some normal aliphatic acids (11). More recently, Laufer and coworkers (12–14) studied some acids and acid anions and found 1:2 (SL_2) complexes of 4-biphenylcarboxylate and *p*-methylcinnamate ions with α-cyclodextrin in aqueous solution.

The present approach to sorting out stoichiometric relationships in complex systems is to use more than one experimental technique capable of providing estimates of stability constants (15). Disagreement of apparent 1:1 stability constants evaluated by several methods is definitive evidence for the presence of at least one complex with a stoichiometry other than 1:1; quantitative agreement among several methods strengthens the argument for a model leading to such consistency. In this study, solubility (16), spectral (17), and potentiometric (18) methods were used.

EXPERIMENTAL

Materials—α-Cyclodextrin[2] was used directly. Drying to constant weight at 90° indicated 9.77% water, corresponding closely to the hexahydrate[3] ($[\alpha]_D^{25} = 150.4 \pm 2.1°$). *trans*-Cinnamic acid was recrystallized from water, mp 133.5–133.7° [lit. (19) mp 133–134°]. Tris(hydroxymethyl)aminomethane base and hydrochloride[4] were used directly. Other chemicals were reagent grade. Water was deionized and distilled from alkaline permanganate.

Apparatus—Spectrophotometric measurements were made on spectrophotometers[5] fitted with jacketed cell compartments for temperature control. The pH measurements were made with a combination electrode[6]. Solubility studies were carried out in a constant-temperature water bath equipped to rotate the sample vessels end-over-end at 32 rpm.

Solubility Method—Equal amounts (50 mg) of cinnamic acid were weighed into each of several 10-ml glass ampuls. To each ampul were added 0.50 ml of 0.10 *M* HCl and graded measured volumes of an aqueous stock solution of α-cyclodextrin. Water was added to make the total volume 5.0 ml. The ampuls were sealed and rotated end-over-end in a 25.0° water bath until solubility equilibrium was reached. (Twenty-four hours was adequate in the initial rising portion of the phase diagram, whereas 10 days was necessary in the plateau regions, as determined by numerous trials.)

The ampuls then were placed upright and motionless at 25° overnight to permit the precipitate to settle. A portion of the clear supernate was withdrawn with a 1-ml disposable syringe equipped with a 3.8-cm (1.5-in.) 20-gauge needle. A 2.0-ml sample of the supernate was titrated to the phenolphthalein end-point with standard sodium hydroxide; a correction was applied to account for the hydrochloric acid present in the sample solution.

The solid phases in the ampuls were collected by filtration and were dried; weighed samples were analyzed for cinnamic acid by titration. The cyclodextrin content was obtained by difference.

Spectral Method—Unionized cinnamic acid was studied by weighing increasing amounts of α-cyclodextrin into each of several 10-ml volumetric flasks; to each flask were added 1.0 ml of 0.1 *M* HCl, 5.0 ml of a stock solution of cinnamic acid, and water to bring to volume. Reference solutions were prepared for each sample solution, containing the same concentrations of cyclodextrin and hydrochloric acid but no cinnamic acid. Absorbance was measured at 279 nm in a 1.0-cm cell. For studies at temperatures other than 25°, concentrations at the experimental temperature were corrected from the concentrations at the temperature of preparation using the density of water.

Cinnamate anion was studied similarly, except that the pH was brought to 9 with tris(hydroxymethyl)aminomethane buffer, ensuring that essentially all of the substrate (pKa 4.35) was ionized whereas essentially none of the α-cyclodextrin (pKa 12) (20) was in anionic form. [Separate potentiometric measurements showed that this buffer does not significantly complex with α-cyclodextrin[7].] Measurements were made at 269 nm. The ionic strength in these and all studies was constant at 0.01 *M*. Control of pH with sodium hydroxide led to essentially the same results as with tris(hydroxymethyl)aminomethane buffers.

Potentiometric Method—A stock solution was prepared to contain equal concentrations of cinnamic acid and sodium cinnamate by exactly half-neutralizing a weighed sample of cinnamic acid with standard sodium hydroxide. The ionic strength was brought to 0.01 *M* with sodium chloride. Increasing amounts of α-cyclodextrin were weighed into each of several flasks, to which equal volumes of the cinnamic acid–cinnamate-ion stock solution were added. The pH of each solution, measured at 25°, was interpreted as pKa′, the apparent pKa of cinnamic acid in that solution.

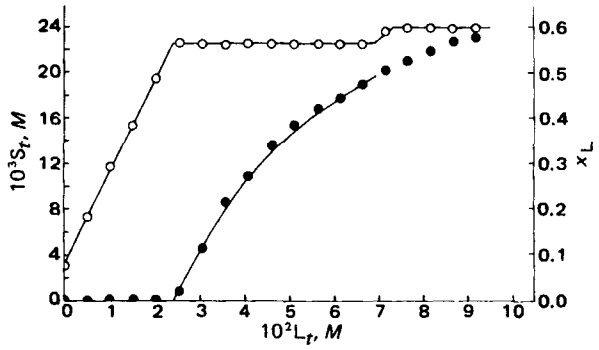

Figure 1—*Solubility of the* trans*-cinnamic acid–α-cyclodextrin system at 25°. Key:* ○ *(left vertical axis), total solution phase concentration of substrate; and* ● *(right vertical axis), mole fraction of cyclodextrin in the solid phase. The total added concentration of ligand (cyclodextrin) is given on the horizontal axis. The smooth lines were calculated as described in the text.*

RESULTS

Solubility Study—Figure 1 shows the experimental results of the solubility study. On the abscissa is specified L_t, the total molar concentration of ligand (α-cyclodextrin) in the system. The left-hand ordinate gives S_t, the total molar concentration of substrate found in the solution phase after equilibration. The right-hand ordinate gives the mole fraction of ligand in the solid phase.

The appearance of the solution phase diagram proves that at least two complexes of different stoichiometry are present, and the solid phase data indicate that a complex is present with an L/S ratio greater than unity. Therefore, these observations suggest that the simplest description of the system will invoke the complexes SL and SL_2. This conclusion may seem inconsistent with the linear nature of the initial rising portion of the solution data, but this will be shown not to be a problem.

To extract numerical estimates from Fig. 1, a quantitative description is required. The complex formation equilibria and stability constants are defined as:

$$S + L \rightleftharpoons SL$$

Scheme I

$$SL + L \rightleftharpoons SL_2$$

Scheme II

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

[2] Lot 106C-0046-1, Sigma.
[3] Aldrich.
[4] Sigma.
[5] Cary model 14 or 16.
[6] Orion model 801 pH meter.
[7] A. B. Wong, unpublished results in these laboratories.

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

where K_{11} and K_{12} are concentration constants at the experimental ionic strength of 0.01 *M*. Assumptions include the constancy of activity coefficients, a negligible general medium effect, and negligible volume changes (this last assumption was the least well founded but was a relatively unimportant factor).

The following variables need to be defined: S_t is the total solution molar concentration of S, L_t is the total formal molar concentration of L, s_0 is the molar solubility of free S, s_{11} is the molar solubility of SL, and s_{12} is the molar solubility of SL_2. Throughout the initial rising portion of the solution phase diagram, solid substrate is present; hence, the concentration of S is held constant at the solubility limit. The mass balance on S gives:

$$S_t = [S] + [SL] + [SL_2] \qquad \text{(Eq. 3)}$$

which, with the given condition and Eqs. 1 and 2, becomes:

$$S_t = s_0 + K_{11}s_0[L] + K_{11}K_{12}s_0[L]^2 \qquad \text{(Eq. 4)}$$

where [L] is the free ligand concentration. The mass balance on L gives:

$$L_t = [L] + [SL] + 2[SL_2] \qquad \text{(Eq. 5)}$$

which becomes:

$$L_t = [L] + K_{11}s_0[L] + 2K_{11}K_{12}s_0[L]^2 \qquad \text{(Eq. 6)}$$

Eliminating [L] between Eqs. 4 and 6 yields:

$$S_t = s_0 + \frac{L_t}{2} + \frac{ab}{c} - \frac{b}{c}(a^2 + 8K_{11}K_{12}s_0L_t)^{1/2} \qquad \text{(Eq. 7)}$$

where:

$$a = 1 + K_{11}s_0$$

$$b = \frac{a}{2K_{11}s_0} - 1$$

$$c = 4K_{12}$$

Equation 7, which is valid only for finite values of K_{11} and K_{12}, is the equation of the rising portion of the solubility curve for the SL + SL_2 system before the solubility limit of either complex has been reached. The slope is given by dS_t/dL_t (Eq. 8):

$$\frac{dS_t}{dL_t} = \frac{1}{2}\left[1 - \frac{8bK_{11}K_{12}s_0}{c(a^2 + 8K_{11}K_{12}s_0L_t)^{1/2}}\right] \qquad \text{(Eq. 8)}$$

Since a and c must be positive, the curvature in the slope depends upon the sign of b. If $b = 0$, the slope is constant with the value 0.5; this implies that $K_{11} = 1/s_0$. If b is negative, the slope decreases as L_t increases; if b is positive, the slope increases as L_t increases. Whether these cases can be distinguished experimentally will be determined by the magnitudes of the parameters and the range of L_t over which the variable S_t can be observed. The value of the slope at $L_t = 0$ obtained from Eq. 9 is:

$$\left(\frac{dS_t}{dL_t}\right)_{L_t=0} = \frac{1}{2} - \frac{1}{2}\left[\frac{1 - K_{11}s_0}{1 + K_{11}s_0}\right] \qquad \text{(Eq. 9)}$$

The discontinuity giving rise to the segment that is invariant in S_t (the first plateau) needs to be considered. As L_t increases, S_t rises because of the formation of soluble SL and SL_2 complexes. Ultimately, the solubility limit of one of these complexes is reached (as long as the solid S phase is not exhausted first and the solubility limit of L is not reached). Suppose that the solubility of SL is reached before that of SL_2. Then [S] = s_0 and [SL] = s_{11}, and from Eq. 3 one gets:

$$S_t = s_0 + s_{11} + \frac{K_{12}(s_{11})^2}{K_{11}s_0} \qquad \text{(Eq. 10)}$$

That is, S_t becomes invariant as soon as one of the complexes reaches its solubility limit, with the complexation equilibria ensuring that the second complex concentration then also is fixed. This conclusion is general for any number of complexes and no matter which complex reaches its solubility first as long as solid S is present. Thus, the first plateau is accounted for. Of course, the interpretation of S_t in this region depends on which complex has reached its solubility limit. Because of the solid phase data, the argument leading to Eq. 10 appears to apply to Fig. 1.

As L_t continues to increase, the solid S eventually is depleted by its conversion into complexes and its precipitation as solid SL. At the point where solid S disappears, the constraint on S_t is lost (although the condition [SL] = s_{11} still applies). An equation for the dependence of S_t on L_t can be obtained (21); but since it plays no role in the quantitative interpretation of the present data, it can be overlooked. The S_t value rises until the solubility limit of the second complex (SL_2) is reached, when S_t again becomes invariant; its value, given by Eq. 11, is reached by an argument similar to that leading to Eq. 10:

$$S_t = \frac{K_{12}(s_{11})^2}{K_{11}s_{12}} + s_{11} + s_{12} \qquad \text{(Eq. 11)}$$

Thus, a "second plateau" is observed. (If L_t could be made sufficiently large, eventually all solid SL would disappear, and S_t would decrease to a final value approximately equal to s_{12}.)

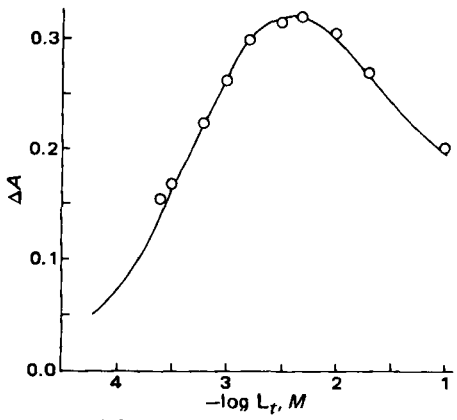

Figure 2—*Spectra of the* trans*-cinnamic acid–α-cyclodextrin system at 25°. The vertical axis gives the change in absorbance in a 1-cm path length of cinnamic acid at 279 nm produced by a total concentration L_t of ligand. The total substrate concentration, S_t, was 8.48 × 10^{-5} M. The ΔA values at the two points corresponding to the highest L_t were corrected for a general medium effect based on an equivalent weight/volume concentration of α-methylglucoside; at L_t = 0.1 M, this correction was 0.009 aufs. The smooth curve was calculated with Eqs. 15 and 16.*

The composition of the solid phase is now considered. From $L_t = 0$ up to the discontinuity marking the beginning of the first plateau, it is postulated that the complex species are soluble so that only solid substrate is present; thus, the mole fraction of cyclodextrin in the solid phase, x_L, should be zero, as is found experimentally (Fig. 1). Throughout the first plateau, the essential feature is that solid S is being replaced by solid SL; therefore, at the beginning of the plateau, $x_L = 0$; at the end of this plateau, $x_L = 0.5$, as is observed. At the beginning of the second plateau, the value of x_L should begin to rise as solid SL is replaced by solid SL_2, the eventual value of x_L being 0.667 (if L_t can be made large enough). Figure 1 shows that x_L does increase in this region, reaching a value of 0.58 at the highest ligand concentration studied.

Equations can be derived relating x_L to L_t in these regions (21), and the smooth line in the first plateau region was calculated with one of these equations, the experimental quantities, and system parameters estimated from the solution phase data. Some ambiguity is introduced by the varying solution phase and total system volumes in these calculations; hence, the qualitative interpretation previously given will suffice for present purposes.

The solubility data were not interpreted independently of the spectral and potentiometric data; rather, for unionized cinnamic acid, the solubility and spectral studies were analyzed to give K_{11} and K_{12} values that best fit both sets of results. These K_{11} and K_{12} values then were imposed as constraints on the potentiometric results. Therefore, uncertainties attached to these values represent limits outside of which the quantitative consistency among the independent sets of data is seriously degraded. These results are obtained at 25°: $K_{11} = 2260 \pm 50\ M^{-1}$, $K_{12} = 60 \pm 5\ M^{-1}$, $s_0 = 3.01 \pm 0.05 \times 10^{-3}\ M$ (the uncertainty is the standard deviation of 11 determinations for s_0), $s_{11} = 1.7 \pm 0.2 \times 10^{-2}\ M$, and $s_{12} = 5.5 \pm 0.5 \times 10^{-3}\ M$ or $1.4 \pm 0.6 \times 10^{-3}\ M$ (because of the ambiguity in a quadratic solution).

Spectral Study—The UV spectrum of cinnamic acid is altered in the presence of α-cyclodextrin. At low cyclodextrin concentrations, isosbestic points are observed at 231 and 296 nm, but these isosbestic points are lost at ligand concentrations greater than ~0.003 *M*. (At such low ligand concentrations, a general medium effect on the spectra cannot account for departure from the isosbestic behavior, as demonstrated by incorporation of α-methylglucoside.) This finding is evidence for at least two

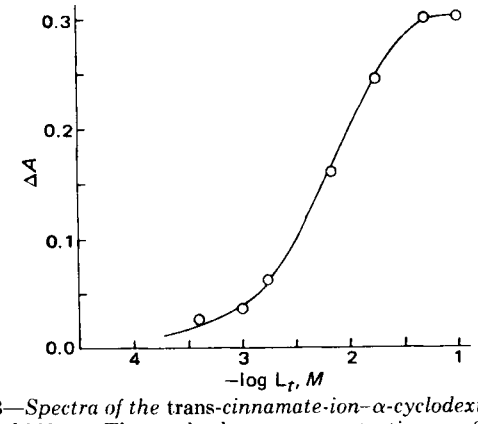

Figure 3—*Spectra of the* trans-*cinnamate-ion–α-cyclodextrin system at 25° and 269 nm. The total substrate concentration was 8.56 × 10^{-5} M. The smooth curve was calculated with Eqs. 15 and 16.*

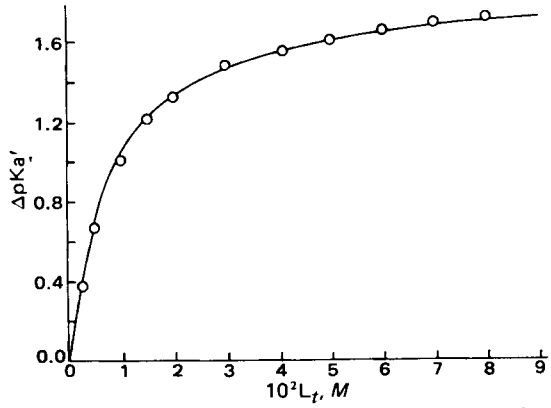

Figure 4—*Potentiometric study of the* trans-*cinnamic acid–α-cyclodextrin system at 25°. The smooth curve was calculated with Eqs. 26 and 34. The total substrate concentration, A_t, was 4.83 × 10^{-3} M.*

complexes. Figure 2 shows the change in absorbance at a fixed wavelength produced by α-cyclodextrin over a wide concentration range.

The analysis of this system assumes SL plus SL_2 complexes for consistency with the solubility results. This system has been treated before (15, 22) but primarily in terms of conventional double-reciprocal graphical analysis, which is not effective for extracting the stability constants. If adherence to Beer's law is assumed for all species, the absorbance for unit path length in a substrate solution containing nonabsorbing ligand is:

$$A = \epsilon_S[S] + \epsilon_{11}[SL] + \epsilon_{12}[SL_2] \qquad \text{(Eq. 12)}$$

In a solution without ligand, it is:

$$A_0 = \epsilon_S S_t \qquad \text{(Eq. 13)}$$

where ϵ_i is the molar absorptivity of species i. The total substrate concentration, S_t, is held constant. Defining $\Delta A = A_0 - A$, $\Delta\epsilon_{11} = \epsilon_S - \epsilon_{11}$, and $\Delta\epsilon_{12} = \epsilon_S - \epsilon_{12}$ and incorporating the relationship between S and free S:

$$S_t = [S](1 + K_{11}[L] + K_{11}K_{12}[L]^2) \qquad \text{(Eq. 14)}$$

relates ΔA to the free ligand concentration:

$$\Delta A = \frac{S_t K_{11}[L](\Delta\epsilon_{11} + \Delta\epsilon_{12}K_{12}[L])}{1 + K_{11}[L] + K_{11}K_{12}[L]^2} \qquad \text{(Eq. 15)}$$

From the mass balance on L:

$$L_t = [L] + \frac{S_t K_{11}[L](1 + 2K_{12}[L])}{1 + K_{11}[L] + K_{11}K_{12}[L]} \qquad \text{(Eq. 16)}$$

It is not practical to express ΔA as an explicit function of L_t, but a numerical curve-fitting procedure is straightforward. Estimates of K_{11}, K_{12}, $\Delta\epsilon_{11}$, and $\Delta\epsilon_{12}$ are obtained from preliminary trials or from supplementary data (*i.e.*, the solubility study). An arbitrary but realistic value of [L] is selected, and the corresponding quantities ΔA and L_t are calculated with Eqs. 15 and 16. These quantities are compared with the experimental values, and the parameters are adjusted until a satisfactory agreement is achieved. As noted earlier, K_{11} and K_{12} were not permitted to vary freely but were constrained by the condition that the final values must account for both the spectral and the solubility data. The smooth curve in Fig. 2 was calculated in this way; at 25°, the results are $K_{11} = 2260\ M^{-1}$, $K_{12} = 60\ M^{-1}$, $\Delta\epsilon_{11} = 4700$, and $\Delta\epsilon_{12} = 1900$. (At 279 nm, $\epsilon_S = 1.98 \times 10^4$.)

Figure 3 is the plot of ΔA *versus* log L_t for the cinnamate-ion–α-cyclodextrin system at 25°. This curve has a sigmoidal shape characteristic of simple 1:1 complexing, and a Benesi–Hildebrand plot of $1/\Delta A$ *versus* $1/L_t$ is linear; evidently, the system can be described in terms of a 1:1 complex. The potentiometric data, however, appear to require the presence of both the SL and SL_2 complexes in the cinnamate-ion system; hence, both complexes are considered to be present in the spectral study. The K_{11} and K_{12} values were required to give consistent fits to both the spectral and the potentiometric data. The smooth curve in Fig. 3 was drawn with Eqs. 15 and 16 and the values $K_{11} = 110 \pm 3\ M^{-1}$, $K_{12} = 15 \pm 2\ M^{-1}$, $\Delta\epsilon_{11} = 4300$, and $\Delta\epsilon_{12} = 3300$. (At 269 nm, $\epsilon_S = 2.04 \times 10^4$.)

Potentiometric Study—The potentiometric method for studying cyclodextrin complexes of weak acids was introduced in 1976 and applied to some 1:1 complexing systems (18). Let pKa represent the negative logarithm of the dissociation constant of the weak acid in the absence of ligand (pKa refers to the experimental conditions and is not the thermodynamic constant), and let pKa′ be the corresponding quantity in the presence of cyclodextrin. Define ΔpKa = pKa′ − pKa. Figure 4 shows the dependence of ΔpKa′ on L_t for the cinnamic acid–α-cyclodextrin system. This curve cannot be accounted for satisfactorily in terms of only 1:1 complexes between cyclodextrin and the unionized and ionized forms of the substrate. Therefore, the theory of this method for the SL + SL_2 case was developed.

Let HA, A^-, and L represent cinnamic acid, cinnamate ion, and cyclodextrin, respectively. Then the three acid–base equilibria are presented as:

$$HA \rightleftharpoons H^+ + A^-$$
Scheme III

$$HAL \rightleftharpoons H^+ + AL^-$$
Scheme IV

$$HAL_2 \rightleftharpoons H^+ + AL_2^-$$
Scheme V

with their acid–base dissociation constants given by:

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad \text{(Eq. 17)}$$

$$K_{a11} = \frac{[H^+][AL^-]}{[HAL]} \qquad \text{(Eq. 18)}$$

$$K_{a12} = \frac{[H^+][AL_2^-]}{[HAL_2]} \qquad \text{(Eq. 19)}$$

The four complexation equilibria are given by:

$$HA + L \rightleftharpoons HAL$$
Scheme VI

$$A^- + L \rightleftharpoons AL^-$$
Scheme VII

$$HAL + L \rightleftharpoons HAL_2$$
Scheme VIII

$$AL^- + L \rightleftharpoons AL_2^-$$
Scheme IX

with their corresponding stability constants:

$$K_{11a} = \frac{[HAL]}{[HA][L]} \qquad \text{(Eq. 20)}$$

$$K_{11b} = \frac{[AL^-]}{[A^-][L]} \qquad \text{(Eq. 21)}$$

$$K_{12a} = \frac{[HAL_2]}{[HAL][L]} \qquad \text{(Eq. 22)}$$

$$K_{12b} = \frac{[AL_2^-]}{[AL^-][L]} \qquad \text{(Eq. 23)}$$

The apparent acid dissociation constant, K_a', is defined by:

$$K_a' = \frac{[H^+][A^-]_{total}}{[HA]_{total}} = \frac{[H^+]([A^-] + [AL^-] + [AL_2^-])}{[HA] + [HAL] + [HAL_2]} \qquad \text{(Eq. 24)}$$

Table I—Thermodynamic Quantities for Cinnamic Acid–α-Cyclodextrin and Cinnamate-Ion–α-Cyclodextrin Complexes

T, °K	Complex[a]	K, M^{-1}	ΔH°, kcal/mole	ΔS°, e.u. Molar Basis	ΔS°, e.u. Mole Fraction Basis
		trans-Cinnamic Acid			
289	SL	3700 ± 100	−9.3 ± 0.6	−16 ± 2	−8
298	SL	2260 ± 50			
308	SL	1350 ± 80			
318	SL	850 ± 50			
289	SL_2	120 + 10	−12 ± 1	−34 ± 4	−26
298	SL_2	60 ± 5			
308	SL_2	30 ± 3			
318	SL_2	17 ± 2			
		trans-Cinnamate Ion			
298	SL	110 ± 3	−1.9 ± 0.6	+3 ± 2	+11
308	SL	100 ± 3			
318	SL	90 ± 3			
298	SL_2	15 ± 2	−9 ± 3	−23 ± 9	−15
308	SL_2	10 ± 1			
318	SL_2	6 ± 1			

[a] S = substrate (cinnamic acid or anion) and L = ligand (cyclodextrin).

Substitution leads to:

$$K_a' = K_a \frac{[1 + K_{11b}[L] + K_{11b}K_{12b}[L]^2]}{[1 + K_{11a}[L] + K_{11a}K_{12a}[L]^2]} \quad \text{(Eq. 25)}$$

or:

$$\Delta pKa' = \log \frac{[1 + K_{11a}[L] + K_{11a}K_{12a}[L]^2]}{[1 + K_{11b}[L] + K_{11b}K_{12b}[L]^2]} \quad \text{(Eq. 26)}$$

As in the spectral study, it is not feasible to find ΔpKa′ as an explicit function of L_t, but L_t can be related to [L] to give a practical method for evaluating the stability constants. The mass balances on ligand and substrate are written as:

$$L_t = [L] + [HAL] + [AL^-] + 2[HAL_2] + 2[AL_2^-] \quad \text{(Eq. 27)}$$

$$A_t = [HA] + [A^-] + [HAL] + [AL^-] + [HAL_2] + [AL_2^-] \quad \text{(Eq. 28)}$$

Substitution with the equilibrium expressions yields functions of L_t and A_t in terms of the variables [L], [HA], and [H^+]. Under the experimental conditions, [H^+] = K_a', so:

$$C = \frac{K_a}{[H^+]} = \frac{K_a}{K_a'} = \frac{1 + K_{11a}[L] + K_{11a}K_{12a}[L]^2}{1 + K_{11b}[L] + K_{11b}K_{12b}[L]^2} \quad \text{(Eq. 29)}$$

giving:

$$L_t = [L] + [HA](K_{11a}[L] + CK_{11b}[L] + 2K_{11a}K_{12a}[L]^2 + 2CK_{11b}K_{12b}[L]^2) \quad \text{(Eq. 30)}$$

and:

$$A_t = [HA](1 + C + K_{11a}[L] + CK_{11b}[L] + K_{11a}K_{12a}[L]^2 + CK_{11b}K_{12b}[L]^2) \quad \text{(Eq. 31)}$$

Define:

$$K_{11}' = K_{11a} + K_{11b}C \quad \text{(Eq. 32)}$$

and:

$$K_{12}' = K_{11a}K_{12a} + K_{11b}K_{12b}C \quad \text{(Eq. 33)}$$

Then Eq. 34 is obtained:

$$L_t = [L]\left[1 + \frac{K_{11}'A_t + 2K_{12}'A_t[L]}{1 + C + K_{11}'[L] + K_{12}'[L]^2}\right] \quad \text{(Eq. 34)}$$

The procedure is to assign a value to [L] and, with Eqs. 26 and 34 and estimates of the stability constants, to calculate ΔpKa′ and L_t for comparison with the experimental results.

Not all of the constants defined for the system are independent. From the definitions, one obtains:

$$K_{11a}K_{a11} = K_aK_{11b} \quad \text{(Eq. 35)}$$

and:

$$K_{12a}K_{a12} = K_{a11}K_{12b} \quad \text{(Eq. 36)}$$

The smooth curve in Fig. 4 was calculated with Eqs. 26 and 34. In this calculation, K_{11a} and K_{12a} were fixed at 2260 and 60 M^{-1}, respectively, as determined from the solubility and spectral studies; thus, only K_{11b} and K_{12b} were treated as adjustable parameters. Even these parameters, however, were not completely free since they had to account for both the potentiometric data and the spectral study on the cinnamate ion. The final values, as given previously, were K_{11b} = 110 M^{-1} and K_{12b} = 15 M^{-1}. It was necessary to incorporate a finite value for K_{12b} to achieve a satisfactory fit at the higher L_t range in Fig. 4.

Thermodynamic Quantities—Since the comparative studies demonstrated that these systems can be described in terms of SL and SL_2 complexes, any of the experimental techniques suffices to make subsequent estimates of the stability constants. The spectral technique was applied to the cinnamic acid–α-cyclodextrin and cinnamate-ion–α-cyclodextrin systems at several temperatures to obtain enthalpy and entropy changes for the complexation equilibria. The data were analyzed in terms of SL and SL_2 complexes (Table I). Entropy changes on a mole fraction basis (unitary entropy changes) (23) were obtained for Schemes I and II from:

$$\Delta S° \text{ (unitary)} = \Delta S° \text{ (molar)} + 8.0 \text{ e.u.} \quad \text{(Eq. 37)}$$

DISCUSSION

Complex Stoichiometry and Stability—Several kinds of evidence show that the cinnamic acid–α-cyclodextrin system cannot be described solely in terms of 1:1 complexes:

1. The isosbestic points seen at low ligand concentrations are lost at higher concentrations.
2. The shape of the plot of Δ*A versus* log L_t (Fig. 2) requires some process in addition to 1:1 complexation.
3. Apparent 1:1 stability constants, evaluated assuming only 1:1 stoichiometry and using data at low ligand concentration, are 1300 M^{-1} for the solubility method and 2900 M^{-1} for the spectral method, the disagreement being inconsistent with the assumption.
4. The increase of total dissolved substrate after the initial plateau portion of the solubility diagram cannot occur if only 1:1 complexation occurs.
5. The solid phase composition in the solubility study requires an L/S ratio greater than 1:1.
6. The potentiometric curve cannot be fitted with the assumption of 1:1 stoichiometry.

Few modern workers use the solubility method to study molecular complexes, and the technique has been criticized because it does not permit the estimation of stability constants unless the stoichiometry of the interaction is known. This criticism is correct, but the limitation applies equally to every other experimental technique. In the present investigation, the solubility method was the single most useful tool because it not only showed that more than one type of complex must be present, but it also indicated the probable stoichiometric ratios. But the strongest evidence for the interpretation of the cinnamic acid–α-cyclodextrin system in terms of SL and SL_2 complexes is the quantitative

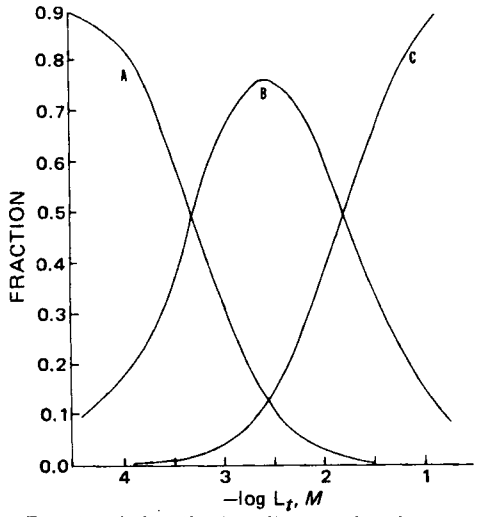

Figure 5—*Fractional distribution diagram for the* trans-*cinnamic acid–α-cyclodextrin system at 25°. The total cinnamic acid concentration was 8.5 × 10⁻⁵* M. *Curves A, B, and C are S, SL, and SL_2, respectively.*

agreement among the three experimental techniques when analyzed according to this model. Such comparative study by independent techniques is one desirable feature of investigations into complex stoichiometries and stabilities; another advantage is extension of the measurements over as wide a range of ligand concentrations as is feasible, preferably over essentially the full binding isotherm.

Should the small values of K_{12} relative to K_{11} (Table I) be thought to render the incursion of SL_2 formation negligible except at a high ligand concentration, it should be noted that the fractional distribution of species S, SL, and SL_2 depends on the product $K_{11}K_{12}$. These distributions (*e.g.*, for SL_2) can be calculated with Eqs. 16 and 38:

$$\text{fraction of } SL_2 = \frac{[SL_2]}{S_t} = \frac{K_{11}K_{12}[L]^2}{1 + K_{11}[L] + K_{11}K_{12}[L]^2} \quad \text{(Eq. 38)}$$

Figure 5 is a fractional distribution diagram for the cinnamic acid–α-cyclodextrin system at 25° with $S_t = 8.5 \times 10^{-5}$ *M*, corresponding to the conditions for the spectral study. At the relatively low total cyclodextrin concentration of 0.01 *M*, about 37% of the cinnamic acid is present as the ternary SL_2 complex. Diagrams of this type should be valuable in selecting experimental conditions for studying complex properties.

The evidence for a ternary SL_2 complex in the cinnamate-ion system is less compelling, consisting of the need to include the term containing K_{12b} in Eq. 26 to achieve a satisfactory fit to the potentiometric data.

Complex Properties and Structures—The symbolism of Schemes VI–IX is adopted here to discuss the several complexes with brevity and without ambiguity. Thus, HA represents cinnamic acid and A^- is cinnamate ion.

Several properties of the complexes (other than the standard thermodynamic quantities to be considered later) were measured in the present study and may be helpful in determining the complex structures. The complex molar absorptivities for HAL, HAL_2, AL^-, and AL_2^- were obtained but only at a single wavelength. The general spectral shifts in the presence of cyclodextrin are not dramatic and do not seem helpful at this point.

With Eqs. 35 and 36, one calculates $pKa_{11} = 5.66$ and $pKa_{12} = 6.27$, compared with pKa = 4.35 for uncomplexed cinnamic acid. Complexing between carboxylic acids and α-cyclodextrin leads to a decrease in the acid strength of the substrate; this is equivalent to saying that the unionized carboxylic acid complexes more strongly than does the carboxylate ion (18). Since the two statements are equivalent, a rationalization of one of them equally accounts for the other. Consider the acidity of HAL (*i.e.*, the acidity of complexed cinnamic acid) relative to that of cinnamic acid in water. Spectroscopic data (24) indicate that the α-cyclodextrin cavity is apolar. If the cinnamic acid group is included within the ligand cavity, it experiences an environment that is comparable (except for "end" effects and restricted molecular motion) to that provided by a solvent such as dioxane or a ketone. In such a medium, having an effective dielectric constant smaller than that of water, the extent of ionization of the carboxylic acid will be less than in water. Therefore, it is anticipated that $K_{a11} < K_a$ for neutral carboxylic acids, from which it follows that $K_{11a} > K_{11b}$, as is observed for these substrates[8]. The same argument applies to the ternary complex HAL_2, which also must be an inclusion complex[9].

The solubility of HAL was determined to be 1.7×10^{-2} *M*. If the substrate HA is included within the cavity, it is not surprising that HAL would have a solubility intermediate to that of the hydrophobic HA ($s_0 = 3.01 \times 10^{-3}$ *M*) and the hydrophilic L ($s_L = 0.125$ *M*). The solubility of HAL_2 is 5.5×10^{-3} *M* (or 1.4×10^{-3} *M*), considerably smaller than that of L or HAL and comparable with that of HA. If HA is included in a channel formed by an end-to-end association of two cyclodextrins, the solubility of HAL_2 will be determined in part by solvent interactions with a dimer-like species L_2; this species is twice as large as L or HAL and provides no additional sites for solvent interaction; hence, its solubility probably will be smaller.

The solubility of the cinnamate-ion–α-cyclodextrin complex was not determined; if it is an inclusion complex with the carboxylate group buried in the cavity, its solubility might be as low as that of HAL. On the other hand, if the carboxylate function is exposed, the complex may be as soluble as the substrate; sodium cinnamate had a solubility of 0.505 *M*. These limits will be used later.

The picture that results from these several kinds of evidence and lines of reasoning is of binary (1:1) inclusion complexes, probably with the side chain in the cavity. The ternary (1:2) complexes may have the substrate in a cavity formed by two ligand molecules associated end-to-end. Although α-cyclodextrin does not form a dimer (28), the SL_2 complex is formed in the presence of cinnamic acid or cinnamate ion. In a sense, cyclodextrin dimerization is promoted by the substrate molecule, the complex structure possibly being much like a dowel joint. Such SL_2 complexes are formed by cinnamic acid, cinnamate ion, *p*-methylcinnamate ion (13), and 4-biphenylcarboxylate ion (12) whereas benzoic acid, benzoate ion (14), and *p*-nitrophenolate ion (18, 27) apparently form only 1:1 complexes. The length of the substrate molecule is possibly a factor determining whether an SL_2 complex forms (11).

Thermodynamic Analysis—The values of free energy and entropy changes depend on the concentration units used in evaluating them, and these quantities can be corrected for the entropy of mixing if the concentrations are expressed on the mole fraction scale (29). The resulting values are called unitary quantities. Table I gives unitary entropy changes (in the last column) for the process studied here[10].

The favorable free energy changes are manifested for the HAL, HAL_2, and AL_2^- complexes as favorable $\Delta H°$ and unfavorable $\Delta S°$ values; for the AL^- complex, the entropy change is favorable and the enthalpy change is very small. Another observation is that, for each substrate, $\Delta H°$ is more favorable and $\Delta S°$ is less favorable for the 1:2 complexes than for the 1:1 complex. (The $\Delta H°$ and $\Delta S°$ for the 1:2 complexes refer to the stepwise process, $SL + L \rightleftharpoons SL_2$, not to the overall process, $S + 2L \rightleftharpoons SL_2$.)

The conventional interpretation of favorable enthalpy changes and of entropy decreases invokes the role of water structure or ordering in the complexation. The negative $\Delta S°$ may signify an increase in solvent ordering in the product state (complex) compared with the individual solvated species. Nonpolar solutes, upon association, show entropy increases, but moderately polar solutes lead to entropy decreases. Jencks (31) pointed out that a favorable $\Delta H°$ may result from solute–solute interactions or from increased solvent–solvent interactions, which are made possible by the greater solvent order. Cyclodextrins are rather special solutes because the cavity contains highly ordered solvent molecules, which must be displaced upon inclusion of a substrate. Van Etten *et al.* (24) suggested that these water molecules cannot form the normal complement of hydrogen bonds because of steric restrictions and that

[8] The same argument applied to phenols may seem to lead to the same prediction. However, phenols behave differently from carboxylic acids in nonaqueous media. For example, in pyridine, the acid strengths of phenols are enhanced relative to those of carboxylic acids, with the result that, for example, *p*-nitrophenol is a stronger acid than is benzoic acid in this solvent (25). Similar behavior is seen in acetone (26). Thus, it is likely that phenol acidity will be increased in the cyclodextrin cavity, with the consequence that $K_{11b} > K_{11a}$, as is seen (18, 27).

[9] The conclusion that HAL and HAL_2 are inclusion complexes follows from the abnormally large equilibrium constants for their formation. (The overall constant for $HA + 2L \rightleftharpoons HAL_2$ is equal to $K_{11}K_{12}$.) When two species interact noncovalently in solution in a simple stacking manner, the stability constant for the process is proportional to the area of contact between the molecules (6). For a molecule the size of cinnamic acid, the maximum value to be expected is on the order of $10^2\ M^{-1}$. Since K_{11} for cinnamic acid–α-cyclodextrin is much higher that this value, some extraordinary stabilizing phenomenon, presumably inclusion, must be occurring. The ternary complex HAL_2 is formed from L and the inclusion complex HAL, so it too is an inclusion complex.

[10] For other discussions of unitary quantities, see Refs. 23 and 30. The authors thank Professor P. Mukerjee, who first called their attention to unitary quantities.

Table II—Thermodynamic Analysis of the Cinnamic Acid–α-Cyclodextrin System

Process[a]	$\Delta G°$ (unitary)[b], cal/mole
1. $S(s) \rightleftharpoons S(aq)$	$+5820 \pm 10$
2. $L(s) \rightleftharpoons L(aq)$	$+3610 \pm 50$
3. $SL(aq) \rightleftharpoons SL(s)$	-4800 ± 70
4. $S(aq) + L(aq) \rightleftharpoons SL(aq)$	-6950 ± 15
5. $S(s) + L(s) \rightleftharpoons SL(s)$	-2320 ± 90
6. $SL(s) \rightleftharpoons SL(aq)$	$+4800 \pm 70$
7. $L(s) \rightleftharpoons L(aq)$	$+3610 \pm 50$
8. $SL_2(aq) \rightleftharpoons SL_2(s)$	-5460 ± 100 (-6280 ± 260)
9. $SL(aq) + L(aq) \rightleftharpoons SL_2(aq)$	-4810 ± 50
10. $SL(s) + L(s) \rightleftharpoons SL_2(s)$	-1860 ± 140 (-2680 ± 280)

[a] The *s* represents solid phase and aq represents solution. [b] Uncertainties represent standard deviation estimates calculated from experimental estimates of the uncertainties in the solubilities and stability constants.

Table III—Thermodynamic Analysis of the 1:1 Cinnamate-Ion–α-Cyclodextrin Complex

Process	$\Delta G°$ (unitary)[a], cal/mole
1. $M^+S^-(s) \rightleftharpoons M^+S^-(aq)$	$+2790 \pm 120$
2. $L(s) \rightleftharpoons L(aq)$	$+3610 \pm 50$
3. $M^+SL^-(aq) \rightleftharpoons M^+SL^-(s)$	[-2790 ± 120 to -4800 ± 70][b]
4. $M^+S^-(aq) + L(aq) \rightleftharpoons M^+SL^-(aq)$	-5160 ± 20
5. $M^+S^-(s) + L(s) \rightleftharpoons M^+SL^-(s)$	[-1550 ± 200 to -2430 ± 150]

[a] Uncertainties represent standard deviation estimates calculated from experimental estimates of the uncertainties in solubilities and stability constants. [b] Estimated limits.

their release to bulk solvent is manifested as a favorable enthalpy change for complex formation. Restriction of rotational motion of an included substrate molecule could lead to an entropy decrease.

Such rationalizations do not provide much predictive power. For the AL^- complex, an entropy increase is seen, and the enthalpy change is very small. If the carboxylate enters the cavity, the net result may be a considerable decrease in solvent order by release of water from the cavity and from the carboxylate group. Presumably, the counterion remains outside the cyclodextrin. Komiyama and Bender (32) interpreted similar $\Delta H°$ and $\Delta S°$ values in complexation of 1-adamantanecarboxylate with α-cyclodextrin as evidence for apolar binding of the nonpolar substrate to the top of the cavity. This interpretation does not seem applicable to the cinnamate-ion system.

The $\Delta H°$ and $\Delta S°$ values for HAL_2 and AL_2^- are similar, considering the uncertainties. In each case, the process may be much the same: a ligand cavity is evacuated, the ligand slides over the portion of substrate protruding in an SL complex, S readjusts its position within the L_2 aggregate, and solvent reordering occurs.

The solubility data provide an opportunity for a different kind of analysis. Table II gives unitary free energy changes for the processes described in this paper. Processes 1–4 are for the formation of complex HAL; these processes, when added, give the net Process 5. Process 5 is solvent independent (if solid L is understood to represent the hydrated ligand); it is expected that the same value would be obtained for its $\Delta G°$ if corresponding experimental values were available for Processes 1–4 in another solvent (such as a mixed aqueous–organic solvent). Thus, $\Delta G°$ for Process 5 can be compared with $\Delta G°$ for Process 4, indicating that the complex formation is more favorable in water than in the solid state. Tables II and III show a similar relationship for the HAL_2 and AL^- complexes. Formation of these complexes in the solid phase is thermodynamically favorable.

These processes can be represented as a cycle as for the complex SL (Scheme X):

$$\begin{array}{ccccc} S(aq) & + & L(aq) & \rightleftharpoons & SL(aq) \\ \Updownarrow & & \Updownarrow & & \Updownarrow \\ S(s) & + & L(s) & \rightleftharpoons & SL(s) \end{array}$$

Scheme X

Just as other solvents should lead to the same free energy change for the solid phase process, the solid phases in this cycle also could be replaced by liquid phases, and rather than using solubilities in the model, partition coefficients would be employed. This cycle suggests why, for a series of closely related compounds, limited correlations between complex stability constants and substrate solubility or partition coefficient may be observed.

This analysis suggests new lines of experimental work. It will be necessary to establish that the free energy change for the solid phase process is, in fact, independent of the solvent used. It will be interesting to partition this free energy change into enthalpy and entropy changes. Since a long-term goal is to predict complex stability constants in aqueous solution (*i.e.*, Process 4 in Table II), these thermodynamic cycles offer a promising route; after study of numerous systems, it may be easier to estimate the free energy change of Process 5 than of Process 4. The cycle then provides a path for the calculation of the free energy change of Process 4.

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