Contributions of Electrostatic and Hydrophobic Interactions to the Host–Guest Complexation of Pyrocatecholate Anions with Cationic Cyclodextrins

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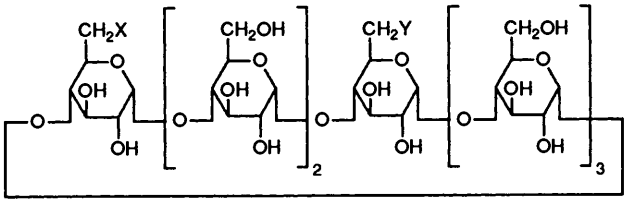
Binding constants of a series of 4-substituted pyrocatechols (1,2-dihydroxybenzenes) to four 6-deoxy, 6-amino derivatives of β-cyclodextrin have been determined from the kinetics of their auto-oxidation at pH 10.0. In several cases, the binding constants at pH 6.0 have been determined by spectrophotometry. Cationic forms of amino derivatives of β-cyclodextrin bind pyrocatecholate anions with equilibrium constants up to 10^5 dm^3 mol^{-1}. The electrostatic and hydrophobic interactions were shown to contribute additively to the free energy of the host–guest complexation.

There is much current interest in the development of host molecules for the specific binding of compounds of definite structure (molecular recognition).[1] Among others, cyclodextrins are widely used for this purpose.[2]

Molecular recognition of ionic guests is a special task, since their binding to cyclodextrins is usually rather weak compared to the related neutral molecules, owing to a decrease in hydrophobicity upon ionization.[3] This effect can be compensated for by introducing oppositely charged groups into the cyclodextrin. Thus, polypyridinio derivatives of cyclodextrins form fairly stable complexes with simple inorganic anions[4] and ionic dyes,[5] and zwitterionic modified cyclodextrins bind amino acid zwitterions.[6] For a better understanding of how both electrostatic and hydrophobic interactions can be combined to give the most efficient binding of an ionic organic guest, we have studied the binding of a series of pyrocatecholato anions to amino derivatives of β-cyclodextrin of the general formula **1**. Compounds **1c, d** are always cationic, and **1a, b** should exist in the protonated cationic form even in moderately basic solutions.



1

a; X = MeNH, Y = OH
b; X = Y = MeNH
c; X = Me_3N^+, Y = OH
d; X = Y = Me_3N^+

Pyrocatechols are widespread in nature and some of them are used clinically.[7] They are sensitive to oxidation, especially in the deprotonated form, and the rate of pyrocatechol auto-oxidation was found to be affected by cyclodextrins **1**.[8] This makes it possible to evaluate the binding constants from the kinetic data according to Scheme 1, where S stands for a pyrocatechol and CD for cyclodextrin.

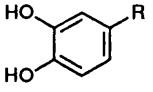
$$S \xrightarrow[O_2]{k_0} \text{products}$$

$$S + CD \rightleftharpoons CDS \quad (K)$$

$$CDS \xrightarrow[O_2]{k_c} \text{products}$$

Scheme 1

This paper reports the binding constants of a series of pyrocatechols **2** at pH 10, where the 1,2-dihydroxybenzene group is mono-deprotonated, and, in a few cases, at pH 6, where this



2

	R
a;	H
b;	CO_2H
c;	CH_2CO_2H
d;	$(CH_2)_2CO_2H$
e;	$CH{=}CHCO_2H$
f;	$CH(OH)CO_2H$
g;	CH_2NH_2
h;	$CH_2CH(NH_2)CO_2H$

group is neutral. An examination of the results shows that the electrostatic and hydrophobic interactions contribute additively to the binding free energy.

Experimental

Materials.—Preparation of cyclodextrins **1a–d** was described elsewhere.[8] Pyrocatechols **2a–h** were purchased from Aldrich and used without further purification. Cinnamic acid and phenol (Reakhim) were purified according to standard procedures.[9]

Kinetic Measurements.—Rate constants were measured spectrophotometrically using a Hitachi 150-20 UV–VIS spectrophotometer equipped with a thermostatted cell holder, as described in ref. 8.

Results and Discussion

Binding of the Neutral Catechols.—In the pH range 2.0–8.5, pyrocatechols are sufficiently stable in aqueous solution to allow spectrophotometric study of their binding to cyclodextrins. Variations in absorbance (A) upon addition of cyclodextrin (CD) were analysed according to Scheme 1, with k_0 = 0, k_c = 0, using eqn. (1) where $[S]_t$ and $[CD]_t$ are the total

$$(A - \varepsilon_{CD}[CD]_t - \varepsilon_S[S]_t)/[S]_t = \frac{(\varepsilon_{CDS} - \varepsilon_S)K[CD]_t}{1 + K[CD]_t} \quad (1)$$

concentrations of a catechol and cyclodextrin, respectively, ε_S and ε_{CD} are their extinction coefficients, and ε_{CDS} is the extinction coefficient of the complex. The positions of absorption maxima chosen to determine the binding constants and differential extinction coefficients, ($\varepsilon_S - \varepsilon_{CDS}$) are given in Table 1.

Table 1 Binding constants (K) of several pyrocatechols with cyclodextrins determined spectrophotometrically at wavelengths λ using differential extinction coefficients ($\varepsilon_S - \varepsilon_{CDS}$) at pH 6.0 and 25 °C

Pyrocatechol	log K/dm^3 mol^{-1} β-CD	**1a**	**1b**	λ/nm	$\varepsilon_S - \varepsilon_{CDS}$/dm^3 mol^{-1} cm^{-1}
2b	<2	<2		285	250
2c	<2	2.27		280	75
2d	<2	2.66		280	100
2e	2.24	2.49	2.64	285	2700

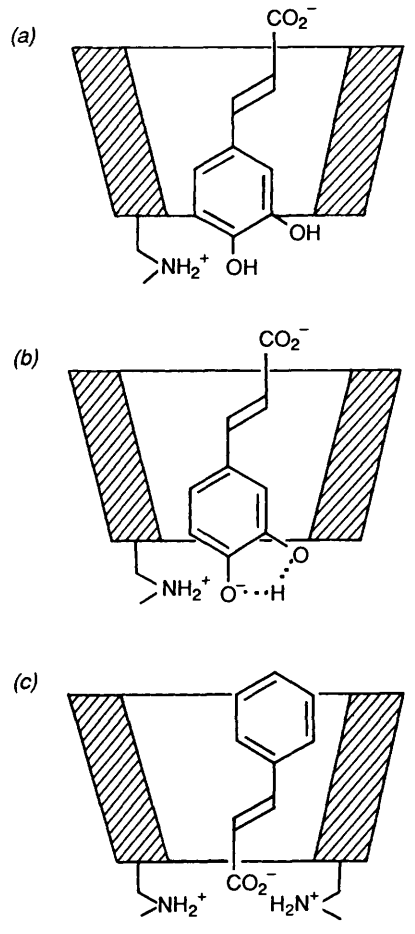


Fig. 1 Proposed structures of complexes of **1** in the protonated form with mono- (*a*) and di- (*b*) anions of caffeic acid as well as with the anion of cinnamic acid (*c*)

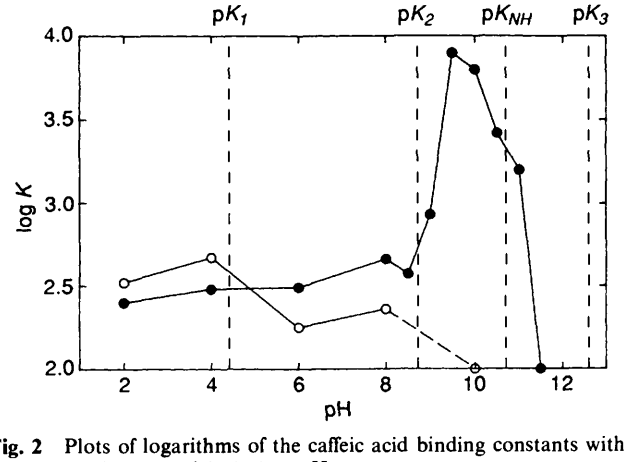


Fig. 2 Plots of logarithms of the caffeic acid binding constants with β-cyclodextrin (○) and **1a** (●) *vs.* pH

The binding constants thus obtained are summarized in Table 1. The cationic cyclodextrins are seen to bind pyrocatechol carboxylic acids, existing at pH 6 as their respective monoanions with a deprotonated carboxyl group, several times better than the unsubstituted cyclodextrin. This effect can be attributed to the electrostatic interactions of the carboxylate anion with ammonium groups of **1a,b**.

Recently Schneider and Theis[10] found the mean free energy of electrostatic interaction $\Delta G_{ip} = -5$ kJ mol^{-1} per every ionic pair in host–guest complexes of various structures. This corresponds to a ten-fold increase in the value of K. We suppose that the much smaller effects observed in this system are a result of electrostatic interactions at a longer distance than in an intimate ionic pair. The separation of the charges results from an unfavourable orientation of a pyrocatechol carboxylate anion, as shown in Fig. 1(*a*).

Binding of the Pyrocatecholate Anions.—At pH >8.5, all pyrocatechols studied undergo auto-oxidation with measurable rates. Cyclodextrins **1a–d** influence auto-oxidation of the pyrocatechols and the binding constants can be evaluated from the rate–cyclodextrin concentration profiles[8] using eqn. (2), which follows from Scheme 1.

$$k_{obs} = (k_0 + k_c K[CD]_t)/(1 + K[CD]_t) \quad (2)$$

Rate and equilibrium constants were calculated from the experimental results by nonlinear regression. Unsubstituted β-cyclodextrin did not affect catechol auto-oxidation due to very weak binding of pyrocatecholate anions, see below. Cyclodextrins **1a–d** exerted inhibitory effects (k_c/k_0 ratio within 0.07–0.4) on the rates of auto-oxidation of catechols **2b**, **e**, and accelerating effects (k_c/k_0 ratio 1.3–8.0) for other pyrocatechols. The nature of these effects will be described elsewhere. Here, we present a discussion of the binding constants.

Fig. 2 shows the pH dependence of the binding constants of caffeic acid to β-cyclodextrin and **1a** found both spectrophotometrically and kinetically. Binding to β-cyclodextrin becomes very weak at pH >4 when caffeic acid is converted to its monoanion (pK_a = 4.5[11]) and is negligible at pH >8 when the 1,2-dihydroxyphenyl group undergoes deprotonation. This is the expected behaviour (see introductory section) since caffeic acid loses its hydrophobicity upon ionization. With cationic cyclodextrin **1a** the binding constant does not decrease at pH 4–5 and reaches a maximum at pH 10.

The electrostatic interaction of carboxylate anion with the cyclodextrin ammonium group evidently compensates for the decrease in the pyrocatechol hydrophobicity at pH 4–5 and an additional interaction with the deprotonated 1,2-dihydroxyphenyl group (pK_a = 8.76[11]) gives an increase in K at pH >8.5. An expected pK_a value for the secondary amino group of **1a** is *ca.* 10.7. [For deoxy-6-(hexadecylamino)-β-cyclodextrin, also bearing a secondary amino group, the pK_a was found to be 7.5–8.0.[12] This compound, however, behaves as a cationic surfactant[12] forming micelles with a positive surface charge, which can lower the observed pK_a value.[13]] This explains the decrease in K at pH >10.5 since deprotonation of the amino group makes **1a** neutral.

The binding constants for the whole series of the pyrocatechols at pH 10.0, when **2** (not less than 94%) and **1** (not less than 80%) are deprotonated and protonated, respectively, are summarized in Table 2.

Several trends are evident from an examination of the data in Tables 1 and 2.

The binding of catecholate anions to doubly charged cyclodextrins is always better than to singly charged except for **2a**, where an opposite effect is observed, and **2g**, where the binding constants with both forms are practically the same. An

Table 2 Binding constants (K) of pyrocatechols **2** with cyclodextrins **1** determined *via* the cyclodextrin effect on rates of catechol auto-oxidation at pH 10.0 and 25 °C

Pyro-catechol	log K/dm^3 mol^{-1} **1a**	**1b**	**1c**	**1d**	log P_{oct} [a]
2a	3.76	3.19			2.15
2b	3.02				1.87
2c	3.22	4.43			1.41
2d	4.15	4.77			1.90
2e	3.80	4.72	2.40[b]	2.78	2.10
2f	2.11	2.82			0.40
2g	3.48	3.54			1.09
2h	< 1	1.30			−1.43

[a] Logarithms of partition constants of the respective benzene derivatives between octanol and water.[14] [b] log K = 2.64 at pH 11.0.

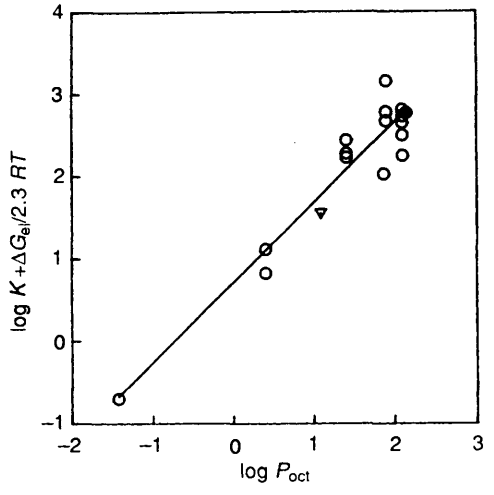


Fig. 3 Data from Tables 1 and 2, plotted according to eqn. (4); (○) **2b–f, h**, (●) **2a** with **1a**, (▽) **2g** with **1b**

obvious structural feature of **2a, g** compared to the other guests is the absence of the carboxylate group. Therefore, at first, only the results for carboxylate pyrocatechols **2b–f, h** will be discussed.

For a quantitative account of the electrostatic contribution ΔG_{el}, the approach of Schneider and Theis[10] predicts that eqn. (3) holds, where n is the number of all possible ionic pair

$$\Delta G_{el} = \Delta G_{ip} n \qquad (3)$$

interactions in the host–guest complex. To evaluate ΔG_{ip}, we averaged all increments in the binding free energy accompanied by the appearance of an additional unit charge, *e.g.* the difference in binding constants of **2e** to **1a** and **1b** corresponds to $\Delta G_{ip} = -5.3$ kJ mol^{-1}. Assuming the structure of inclusion complexes with **2b–f, h**, shown in Fig. 1(*b*) and neglecting the long distance interaction with the carboxylate group, one can calculate the mean value of ΔG_{ip} as -5.9 ± 1.8 kJ mol^{-1} for interactions between the proximate anionic pyrocatecholate group and ammonium groups of **1a, b**.

The nature of the ammonium group is important: cyclodextrins with quaternary ammonium groups, **1c, d**, form much less stable complexes than those bearing secondary groups, **1a, b**, Table 2. This effect is explicable by the loss of the ability to form hydrogen bonds with the anionic guest on passing from the secondary to quaternary ions.

Another feature of the quaternary ammonium group is that it does not dissociate at high pH values. This allows us to check the explanation of the maximum in Fig. 2 given above. The binding constant of **2e** to **1c** does not actually decrease at pH > 10, see footnote in Table 2.

Besides electrostatics, the hydrophobicity of the guests should be taken into account. A widely used measure of the hydrophobicity is the logarithm of the partition constant of the given compound between a non-aqueous solvent, *e.g.* octanol (P_{oct}), and water.[14] Since there are no sufficient data for partitioning of the pyrocatechols, we used for correlation the P_{oct} values for the respective benzene derivatives: benzoic acid for **2b**, phenylacetic acid for **2c** and so on, given in Table 2.

The equation describing both electrostatic and hydrophobic contributions is given as eqn. (4) where $\Delta G_{ip} = -5.9$ kJ mol^{-1},

$$\log K = a - \Delta G_{ip} n/(2.3\,RT) + b \log P_{oct} \qquad (4)$$

$2.3\,RT = 5.7$ kJ mol^{-1}, and a, b are constants. Fig. 3 shows the data of Tables 1 and 2 for **2b–f, h** in the coordinates of eqn. (4). The slope of the line in Fig. 3 is equal to $b = 1.0 \pm 0.1$, and the intercept $a = 0.71 \pm 0.15$. Thus, the final equation takes the form of eqn. (5) which describes the experimental results with a standard deviation $S = 0.30$.

$$\log K = 0.71 + 1.0n + 1.0 \log P_{oct} \qquad (5)$$

The behaviour of non-carboxylic pyrocatechols **2a, g** is explicable as follows. The pK_a of benzylamine is 9.33.[15] If the amino group of **2g** has the same basicity, it should be deprotonated at pH 10.0 and the predominant form of **2g** should be a monoanion. The latter is likewise true for **2a**. Application of eqn. (4) to these compounds shows that the binding constants of **2a** to **1a**, as well as **2g** to **1b** are in keeping with those expected from the correlation with **2b–f, h**. The respective points are indicated in Fig. 3.

The binding of **2a** to **1b** is too weak and the binding of **2g** to **1a** is too strong. A possible reason for these deviations is a change of orientation of the guests lacking the carboxylate group upon inclusion in the cyclodextrin cavity. The anionic deprotonated carboxylate group, with a strong tendency to remain in an aqueous environment, may ensure more or less invariable orientation of **2b–f, h** inside cyclodextrins of the type shown in Fig. 1(*a*), (*b*). Unsubstituted pyrocatechol **2a** may penetrate deeper into the cyclodextrin cavity, being in close contact with the ammonium group of **1a** and in an unfavourable position with respect to the second ammonium group of **1b**. In this case, the second ammonium group would only decrease the hydrophobicity of the cavity. For **2g**, the structure of the inclusion complex may be influenced by hydrogen bonding between the amino group of pyrocatechol and cyclodextrin hydroxy groups. If such a bond is formed more readily in the complex with **1a** then with **1b**, the former receives some extra stabilization.

Binding of some Related Compounds.—To see the extent to which cyclodextrins **1a, b** 'recognize' pyrocatecholate anions, we have studied the binding of cinnamic acid and phenol under the same conditions. The former is structurally related to caffeic acid, **2e**, but lacks aromatic hydroxy groups. The latter is related to pyrocatechol, **2a**, but has only one aromatic hydroxy group. The binding constants determined by concurrent methods are summarized in Table 3.

The standard procedure with phenolphthalein as a competing dye[16] was employed for β-cyclodextrin. The binding constants to **1a, b** were determined kinetically using competitive inhibition of the auto-oxidation of caffeic acid by phenol or cinnamic acid.

β-Cyclodextrin forms a more stable complex with cinnamic acid than with **2e**, as expected in view of the higher hydro-

Table 3 Binding constants (K) of cinnamic acid and phenol with cyclodextrins determined by concurrent methods at pH 10.0 and 25 °C

	log K/dm^3 mol^{-1}		
Guest	β-CD	**1a**	**1b**
Cinnamic acid	2.72	2.34	3.40
Phenol	1.18[a]	3.10	3.66
	2.11[b]		

[a] Phenolate anion.[3b,c] [b] Phenol.[3c]

phobicity of the former. In contrast to **2e**, the binding constant of cinnamic acid decreases on passing from β-cyclodextrin to **1a**, probably due to a lowering of the cavity hydrophobicity by the ammonium group. This effect is absent for **2e** since it contacts with the ammonium group through hydrophilic hydroxy groups [both cinnamic and caffeic acids are thought to form inclusion complexes of the type shown in Fig. 1(*a*)]. So, **1a** discriminates between cinnamic and caffeic acids at pH 10 with a factor of *ca.* 30, *cf.* Tables 2 and 3.

The same discrimination factor was found with **1b** as a host (Tables 2 and 3). Unexpectedly, **1b** binds cinnamic acid much better than **1a**. We suppose that in this case, the reorientation of the bound cinnamate anion occurs as shown in Fig. 1(*c*). This mode of inclusion maximizes the electrostatic contribution but is unfavourable for hydrophobic interaction. As a result, the binding constant to **1b** is only 5 times higher than to β-cyclodextrin, while **2e** binds to **1b** 300 times better than to β-cyclodextrin.

The results for phenol will be discussed only qualitatively since phenol is semi-ionized at pH 10. Its binding constants are comparable to those of **2a** and the electrostatic contribution on passing from β-cyclodextrin to **1a** (log K = 1.7 for binding of **2a** in neutral form to β-cyclodextrin[17]) is evident. Thus, cyclodextrins **1a**, **b** are specific for aryloxo anions and can not discriminate phenolate and catecholate structures.

Conclusion

The above results show that for series of related compounds, *e.g.* **2b–f**, **h**, both electrostatic and hydrophobic interactions contribute additively to the binding free energy, eqn. (5). Furthermore, interactions of each type are utilized here to a maximum extent, as is evident from the unity coefficients of the respective terms of eqn. (5). A combination of these interactions provides a fairly strong and specific binding with the equilibrium constants reaching 10^5 dm^3 mol^{-1} for cyclodextrin **1b**.

The additivity of electrostatic and hydrophobic interactions is not trivial, since the hydrophobicity generally depends on the presence of ionic groups.[1a] The structural feature of cyclodextrins **1a–d**, which allows the independent electrostatic and hydrophobic binding, supposedly lies in the fact that the ionic ammonium groups are separated from the cavity and can interact with an ionic group of the guest jutting out from the cavity, Fig. 2(*b*).

Acknowledgements

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