REFERENCES

(1) C. Fuehrer, *Chem.-Ing.-Tech.*, **43**, 849(1971).

(2) J. A. Hersey and J. E. Rees, Particle Size Analysis Conference, Bradford, England, 1970.

(3) D. Train, *Trans. Inst. Chem. Eng.*, **35**, 258(1957).

(4) D. Train and J. A. Hersey, *Powder Metall.*, **6**, 20(1960).

(5) D. Train, *J. Pharm. Pharmacol.*, **8**, 745(1956).

(6) E. Kristoffersson and V. E. Krogerus, *Farm. Aikak.*, **79**, 1(1970).

(7) K. Kawakita and Y. Tsutsumi, *Bull. Chem. Soc.*, **39**, 1364(1966).

(8) R. W. Heckel, *Trans. Metall. Soc.*, **221**, 671(1961).

(9) J. N. C. Healey, M. H. Rubinstein, and V. Walters, *J. Pharm. Pharmacol.*, **26**, 41p(1974).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 5, 1974, from the *School of Pharmacy, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, England.*

Accepted for publication June 3, 1975.

The author thanks Dr. R. W. Galloway for preparing the radiographs.

Effect of Cycloamyloses on Apparent Dissociation Constants of Carboxylic Acids and Phenols: Equilibrium Analytical Selectivity Induced by Complex Formation

KENNETH A. CONNORS ˣ **and JOHN M. LIPARI**

Abstract □ Apparent dissociation constants of organic acids were determined by potentiometric titration in the presence of cyclohexaamylose or cycloheptaamylose. The quantity $\Delta pKa' = pKa'$ (cycloamylose) − pKa (water) was positive or zero for all carboxylic acids studied and negative or zero for all phenols. The term $\Delta pKa'$ can be related to the cycloamylose concentration, K_{11a}, and K_{11b}, where K_{11a} and K_{11b} are 1:1 stability constants for complexes of the acid and the anion, respectively. From the dependence of $\Delta pKa'$ on cycloamylose concentration, estimates of K_{11a} and K_{11b} can be obtained. If $\Delta pKa' \neq 0$, then $K_{11a} \neq K_{11b}$; for carboxylic acids, $K_{11a} \geq K_{11b}$; for phenols, $K_{11b} \geq K_{11a}$. Because of variable pKa′ shifts, it is possible to carry out differentiating titrations of some acid mixtures in cycloamylose solutions, whereas the same acids cannot be differentiated in water. If an acid is weakened by cycloamylose, its conjugate base is strengthened, and some carboxylate salts can be readily titrated in the presence of a cycloamylose.

Keyphrases □ Cycloamyloses—effect on apparent dissociation constants of carboxylic acids and phenols, stability constants for complex formation □ Dissociation constants, apparent—carboxylic acids and phenols, effect of cycloamyloses, complex formation □ Carboxylic acids—apparent dissociation constants, effect of cycloamyloses □ Phenols—apparent dissociation constants, effect of cycloamyloses

Cycloamyloses (also called cyclodextrins or Schardinger dextrins) are cyclic oligomers containing six or more D-glucose units linked 1 → 4; they are produced by the action of *Bacillus macerans* amylase on starch. The six-unit and seven-unit substances are called cyclohexaamylose (α-cyclodextrin) and cycloheptaamylose (β-cyclodextrin), respectively. These molecules are doughnut shaped, and their possession of a cavity of fixed size and shape has led to considerable interest in their chemical properties. The production, purification, and chemistry of the cycloamyloses have been reviewed (1, 2). The catalytic properties of cycloamyloses, which have been widely studied as enzyme models, also have been discussed (3).

Any molecule smaller than the cavity of a cycloamylose molecule can enter the cavity and there undergo noncovalent interaction with the atoms lining and rimming the cavity. The resulting association product is called an inclusion compound or complex. The cycloamylose is thus a "host" for the smaller "guest" molecule. A 1:1 stoichiometry is typical, although other ratios have been observed and interpreted as the result of another guest molecule lying across the cavity opening. The dimensions of the cavity are such that many mono- and disubstituted benzene derivatives can fit into the cyclohexaamylose cavity, but polysubstituted benzenes may be excluded.

By forming an inclusion complex with a guest molecule, a cycloamylose is capable of altering some physical and chemical properties of the guest substance; it is by such changes that the complex formation is recognized and studied. Among the solution properties that can be modified are solubility, chemical reactivity, vapor pressure, and the electronic absorption spectrum. This capability for changing the chemical and physical behavior of solutes through complex formation may have analytical potential, and the present study examines the effects of cyclodextrins on the apparent dissociation constants of organic acids in aqueous solution.

It has been well established that many organic acids interact with the cycloamyloses (4–10), although little is known about the effects of complexing on acid dissociation equilibria. The potentiometric titration curve of monophenylphosphoric acid in the presence of cycloamylose reveals changes in pKa values, and even in the stoichiometric consumption of base, that require specific intermolecular interactions for their interpretation (2, 11). The pKa of

Table I—Apparent Dissociation Constants of Carboxylic Acids and Phenols in the Presence of 0.02 *M* Cyclohexaamylose[a]

Compound	pKa'	pKa	ΔpKa'
Acetic acid	4.83	4.76	+0.07
Propionic acid	5.16	4.97	+0.19
Monochloroacetic acid	3.30	3.20	+0.10
Maleic acid	3.40, 6.38	3.32, 6.23	+0.08, +0.15
Malonic acid	3.69, 5.92	3.60, 5.70	+0.09, +0.22
Benzoic acid	5.20	4.11	+1.09
Salicylic acid	3.63	3.40	+0.23
m-Hydroxybenzoic acid	4.95	4.10	+0.85
p-Hydroxybenzoic acid	5.83	4.58	+1.25
o-Methoxybenzoic acid	4.20	4.15	+0.05
m-Methoxybenzoic acid	5.13	4.18	+0.95
o-Nitrobenzoic acid	3.20	3.19	+0.01
m-Nitrobenzoic acid	3.83	3.60	+0.23
p-Nitrobenzoic acid	4.53	3.74	+0.79
p-Fluorobenzoic acid	4.98	4.18	+0.80
o-Phthalic acid	3.50, 5.34	3.40, 5.20	+0.10, +0.14
Nicotinic acid	4.85	4.85	0.00
Picolinic acid	5.33	5.31	+0.02
Gallic acid	4.53	4.20	+0.33
Cinnamic acid	5.80	4.43	+1.37
o-Hydroxycinnamic acid	5.82	4.69	+1.13
m-Hydroxycinnamic acid	5.64	4.49	+1.15
p-Hydroxycinnamic acid	5.88	4.40	+1.48
o-Methoxycinnamic acid	5.20	4.70	+0.50
m-Methoxycinnamic acid	5.76	4.47	+1.29
p-Methoxycinnamic acid	6.24	4.90	+1.34
Phenol	9.81	9.81	0.00
o-Nitrophenol	7.21	7.21	0.00
m-Nitrophenol	8.00	8.29	−0.29
p-Nitrophenol	6.15	7.09	−0.94
p-Bromophenol	9.20	9.20	0.00
1-Naphthol	9.20	9.20	0.00
2-Naphthol	9.30	9.40	−0.10

[a] At 25.0° in aqueous solution.

p-nitrophenol decreases by one unit upon its complexation with cyclohexaamylose (12).

In this study, the apparent dissociation constants of carboxylic acids and phenols in the presence of cycloamyloses were determined by potentiometric titration. This experimental approach has been elaborated into a systematic technique for studying molecular complexes of acids and bases.

EXPERIMENTAL

Materials—Organic acids were from commercial sources and were used directly or were recrystallized before use until a satisfactory melting point was obtained. Purities were established by potentiometric titration. α-Cyclodextrin[1] and β-cyclodextrin[2] were used directly. All water was freshly boiled, deionized, and distilled. Cyclodextrin concentrations are expressed on the anhydrous basis.

Potentiometric Titration—An aqueous solution was prepared to contain appropriate concentrations of the sample acid and cyclohexaamylose or cycloheptaamylose. A 25.0- or 50.0-ml portion of this solution was transferred to the titration vessel, a glass-jacketed beaker thermostated at 25°. Through a stopper on the beaker were passed an inlet tube for flushing the system with nitrogen, a combination glass–saturated calomel electrode[3], and a 5-ml buret graduated to 0.01 ml. Stirring was accomplished by magnetic stirrer.

Titration was with 0.07 or 0.1 *N* sodium hydroxide. The initial concentration of acid in the sample solution was in the 0.001–0.01 *N* range.

[1] Eastman Lot No. A4X (mp 262–265°) or Aldrich Lot No. 011747 (mp 260–264°).
[2] Lot No. 5022, Nutritional Biochemical Corp.
[3] Corning 476051; the pH meter was a Radiometer model 25SE.

Table II—Apparent Dissociation Constants of Acids in the Presence of 0.014 *M* Cycloheptaamylose[a]

Compound	pKa'	ΔpKa'
Benzoic acid	4.95	+0.84
o-Nitrobenzoic acid	3.33	+0.13
p-Nitrobenzoic acid	4.02	+0.28
o-Methoxybenzoic acid	4.48	+0.28
Salicylic acid	3.68	+0.28
Cinnamic acid	5.08	+0.65
o-Hydroxycinnamic acid	5.33	+0.58
o-Methoxycinnamic acid	5.48	+0.78
m-Methoxycinnamic acid	4.88	+0.41
p-Methoxycinnamic acid	5.29	+0.39
Potassium hydrogen phthalate	5.55	+0.17
p-Nitrophenol	6.70	−0.39
2-Naphthol	9.05	−0.35

[a] At 25.0° in aqueous solution.

pKa' Determination—The apparent acid dissociation constant, K_a', was determined using:

$$pKa' = pH - \log \frac{V}{V_{ep} - V} \quad \text{(Eq. 1)}$$

where V is the volume of titrant added, pH is the corresponding value of pH, and V_{ep} is the volume of titrant required to reach the end-point. Alternatively, pKa' was determined as the pH corresponding to the midpoint of the titration. The ionic strength changed during the titration but was never greater than 0.005 *M* at the titration midpoint.

RESULTS

Dissociation Constants—Equation 2 defines ΔpKa', the change in pKa value caused by incorporating a cycloamylose in the aqueous solution of the acid:

$$\Delta pKa' = pKa' - pKa \quad \text{(Eq. 2)}$$

where pKa' is the value observed in the presence of cycloamylose, and pKa is the corresponding value in the absence of cycloamylose. (Although pKa is itself an apparent constant rather than the thermodynamic constant in water, pKa' and pKa were determined under identical conditions except for the presence or absence of the cycloamylose.)

According to Eq. 2, positive and negative values of ΔpKa' correspond to a weakening or strengthening, respectively, of the acid by the cycloamylose. The term ΔpKa' is a function of cyclodextrin concentration; but for purposes of screening a large number of acids, ΔpKa' was measured at a fixed cycloamylose concentration. Table I shows these results for cyclohexaamylose. Several polyprotic acids with dissociation constants too similar to be readily resolved were not included in Table I; for these acids (citric, oxalic, 3-nitrophthalic, succinic, and terephthalic), the pH at half-neutralization indicated very small acid-weakening effects by cyclohexaamylose. This same quantity was about +0.5 for fumaric acid; 0.12 *M* dextrose had no effect on the pKa of cinnamic acid.

Table II shows some data for cycloheptaamylose.

The dependence of ΔpKa' on cycloamylose concentration was studied, and Fig. 1 shows the results for benzoic acid and *p*-nitrophenol. A quantitative interpretation of these curves is given in the *Discussion* section.

Potentiometric Titrations—Essentially no alkali was consumed by the cycloamyloses, and the accuracy and precision of potentiometric titration in the presence of a cycloamylose were comparable with those observed in its absence. For example, nine titrations of 0.115 mEq of benzoic acid in the presence of cyclohexaamylose gave a mean recovery of 99.9% with a standard deviation of 0.23%.

One interesting feature of the data in Tables I and II is the wide variation in ΔpKa' values, which do not seem to be simply related to pKa. This behavior suggested the possibility of carrying out certain differentiating titrations in the presence of cycloamylose, titrations that would be impossible or difficult in its absence. Although it is generally recognized that successful differentiating titrations of two acids can be made if the ratio of acid dissociation constants is at least 10^4 (13, 14), useful titrations are possible with

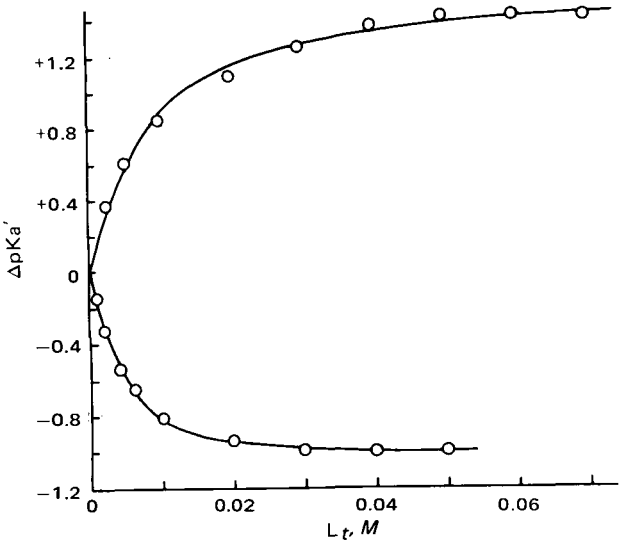


Figure 1—*Dependence of ΔpKa' on total cyclohexaamylose concentration (L_t) for benzoic acid (upper curve) and* p*-nitrophenol (lower curve). The lines were drawn with Eq. 10 as described under* Discussion. *The experimental points are at 25° and ionic strength 0.002* M.

much smaller ratios. The accuracy and precision depend upon the quality of the data and their treatment.

Many pairs of the acids included in Table I cannot be differentiated in aqueous solution; but in the presence of cyclohexaamylose, the resulting selective shifts of acid strength generate ratios of dissociation constants adequate for differentiating potentiometric titration. Table III shows some of these pairs of acids, none of which can be differentiated in water. In 0.02 *M* cyclohexaamylose, however, these pairs of acids can be quantitatively analyzed for both components by potentiometric titration. Figures 2 and 3 show some of these titrations.

These data suggest that effective differentiating titrations can be performed if the ratio of dissociation constants is about 10^2 (accuracy is also dependent upon the ratio of concentrations and individual strengths—the titration in Fig. 3 exemplifies a borderline case), and they demonstrate that this condition sometimes can be met simply by incorporating a cycloamylose in the titration mixture. From the data in Table I it is easy to predict which acids can be successfully differentiated by this means. Percent recoveries of the acids in Table III were within 1% of theory, except for mixtures of benzoic and salicylic acids in a 1:5 ratio. The precision is also satisfactory; five titrations of a mixture of 0.114 mEq of salicylic acid and 0.119 mEq of *p*-hydroxybenzoic acid gave standard deviations of 0.27% (salicylic) and 0.45% (*p*-hydroxybenzoic).

If the strength of an acid is decreased by treatment with cycloamylose, then the strength of its conjugate base should be increased since pKb = pKw − pKa. Therefore, the salts of some carboxylic acids were titrated potentiometrically with standard acid in the presence of cyclohexaamylose (Fig. 4). Evidently, the cyclohexaamylose treatment gives a simple and accurate analytical method for some carboxylate salts in aqueous solution. The pKa' values obtained by titration of the salt with hydrochloric acid were in agreement with those found from titration of the acid with sodium hydroxide.

DISCUSSION

ΔpKa and Complex Stability—The magnitudes and variability of pKa' shifts given in Tables I and II rule out a nonspecific medium effect, and it is instead assumed that molecular complex formation takes place between the substrate (acid) and ligand (cycloamylose). Let HA represent the unionized substrate and L the ligand. Suppose only 1:1 stoichiometry occurs; then the solution may contain the unionized and ionized forms of the substrate, and both of these species may be complexed with the ligand. Schemes I and II show the acid dissociation equilibria for the uncomplexed and complexed forms of HA, respectively, and Eqs. 3 and 4 give the corresponding dissociation constants:

Table III—Pairs of Acids Differentiated by Potentiometric Titration in 0.02 *M* Cyclohexaamylose

Acids	pKa Difference: In Water	pKa Difference: In 0.02 *M* Cyclohexaamylose
Cinnamic–chloroacetic	1.23	2.20
Benzoic–*o*-nitrobenzoic	0.92	2.00
Benzoic–salicylic	0.71	1.57
Salicylic–*p*-hydroxybenzoic	1.18	2.20
Cinnamic–*o*-nitrobenzoic	1.24	2.60
p-Nitrophenol–*m*-nitrophenol	1.20	1.85

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

Scheme I

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

Scheme II

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad \text{(Eq. 3)}$$

$$K_a{}^c = \frac{[H^+][AL^-]}{[HAL]} \qquad \text{(Eq. 4)}$$

The complexation equilibria and complex stability constants (K_{11a} and K_{11b} are 1:1 stability constants for the acid and anion, respectively) are as follows:

$$HA + L \rightleftharpoons HAL$$

Scheme III

$$K_{11a} = \frac{[HAL]}{[HA][L]} \qquad \text{(Eq. 5)}$$

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

Scheme IV

$$K_{11b} = \frac{[AL^-]}{[A^-][L]} \qquad \text{(Eq. 6)}$$

These equations are easily combined to give:

$$\frac{K_a}{K_a{}^c} = \frac{K_{11a}}{K_{11b}} \qquad \text{(Eq. 7)}$$

which shows that only three of the four constants can be independent. Cramer *et al.* (12) verified Eq. 7 for the *p*-nitrophenol–cyclohexaamylose system.

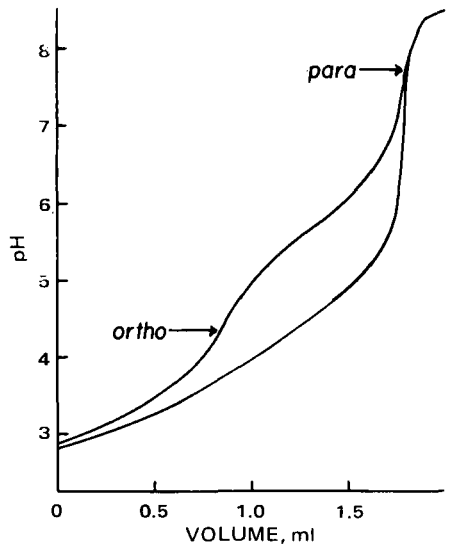


Figure 2—*Potentiometric titration of a mixture of 14.0 mg of salicylic acid and 13.8 mg of* p*-hydroxybenzoic acid with 0.1131* N *NaOH. Key: lower line, in water; and upper line, in 0.02* M *cyclohexaamylose. Arrows indicate end-points in the differentiating titration.*

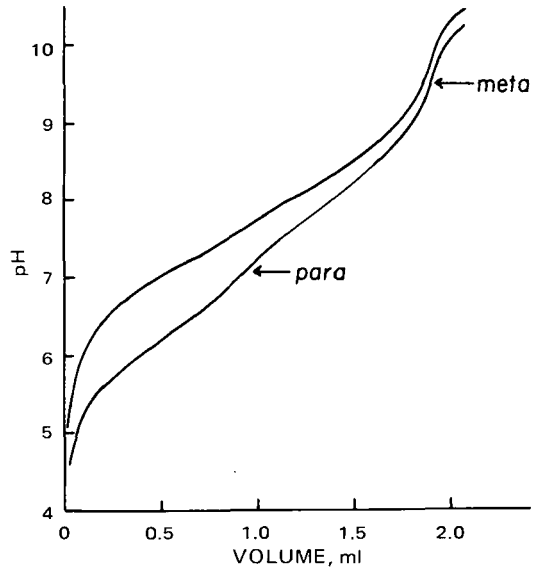


Figure 3—*Potentiometric titration of 15.2 mg of* p-*nitrophenol and 14.5 mg of* m-*nitrophenol with 0.1131* N *NaOH. Key: upper line, in water; and lower line, in 0.02* M *cyclohexaamylose. Arrows indicate end-points in the differentiating titration.*

In the present study, an apparent constant K_a' was measured. This constant is not identical with K_a^c, since in the potentiometric measurements the substrate is present in both the free and complexed forms. It is necessary to find the relationship between K_a' and the ligand concentration. The apparent acid dissociation constant can be defined by:

$$K_a' = \frac{[H^+]([A^-] + [AL^-])}{[HA] + [HAL]} \quad \text{(Eq. 8)}$$

Then, substitution from Eqs. 3, 5, and 6 gives:

$$K_a' = K_a \frac{(1 + K_{11b}[L])}{(1 + K_{11a}[L])} \quad \text{(Eq. 9)}$$

which may also be written:

$$\Delta pKa' = \log \frac{(1 + K_{11a}[L])}{(1 + K_{11b}[L])} \quad \text{(Eq. 10)}$$

In the limit as the ligand concentration becomes very large, Eq. 9 approaches Eq. 7. The observation of a ΔpKa' value significantly different from zero implies that $K_{11a} \neq K_{11b}$.

To estimate stability constants with the aid of Eq. 10, it is necessary to know [L], the free ligand concentration. Let L_t and A_t be the total (formal) molar concentrations of ligand and acid, respectively; then these mass balance equations can be written:

$$L_t = [L] + [HAL] + [AL^-] \quad \text{(Eq. 11)}$$

$$A_t = [HA] + [A^-] + [HAL] + [AL^-] \quad \text{(Eq. 12)}$$

If $L_t >>> A_t$, as in many complexing studies, it may be acceptable to set $[L] \approx L_t$. This was not possible in the present study, however, since A_t was in the 0.001–0.01 *M* range and L_t was 0.001–0.07 *M*. Expanding Eqs. 11 and 12 with the aid of the equilibrium constants defined earlier gives:

$$L_t = [L] + K_{11a}[HA][L] + K_aK_{11b}[HA][L]/[H^+] \quad \text{(Eq. 13)}$$

$$A_t = [HA] + K_a[HA]/[H^+] + K_{11a}[HA][L] + K_aK_{11b}[HA][L]/[H^+] \quad \text{(Eq. 14)}$$

Since $K_{11a} \neq K_{11b}$ and an increasing fraction of the substrate is in the anion form as titration proceeds, the effective stability constant[4] varies throughout the titration and [L] varies according to these equations. Therefore, from Eq. 9, pKa' must vary.

Experimentally, it is found that pKa', evaluated with Eq. 1 in the buffer region of the titration, does vary but not markedly (0.05 unit or less while the pH changes more than 0.5 unit; part of this variation is an activity coefficient effect). Therefore, it is necessary

[4] The effective stability constant can be defined as $K_{11a}f_a + K_{11b}f_b$, where f_a and f_b are the fractions of uncomplexed substrate in the acid and anion forms, respectively.

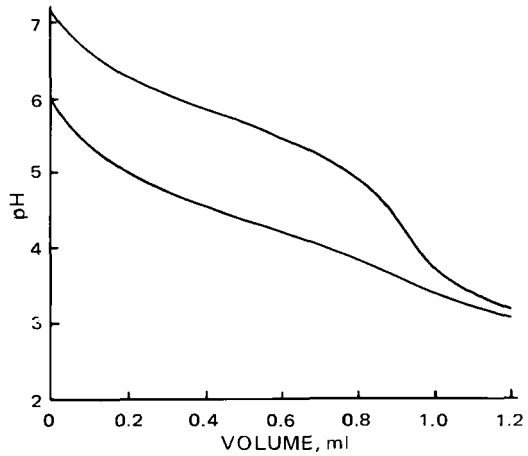


Figure 4—*Potentiometric titration of 0.0675 mEq of sodium cinnamate with 0.0722* N *HCl. Key: lower line, in water; and upper line, in 0.02* M *cyclohexaamylose.*

to choose a value of $[H^+]$ for the solution of Eqs. 13 and 14; the reasonable value of $[H^+] = K_a'$ was selected. The experimental observations were made at this condition, *i.e.*, the titration midpoint. With this condition, Eqs. 9, 13, and 14 are combined to yield:

$$L_t = [L]\left\{1 + \frac{K_{11}A_t}{1 + C + K_{11}[L]}\right\} \quad \text{(Eq. 15)}$$

where:

$$K_{11} = K_{11a} + K_{11b}C \quad \text{(Eq. 16)}$$

and:

$$C = \frac{1 + K_{11a}[L]}{1 + K_{11b}[L]} \quad \text{(Eq. 17)}$$

The procedure is to assign a reasonable value to [L] and, with preliminary estimates of K_{11a} and K_{11b}, to calculate L_t using Eq. 15. The corresponding value of ΔpKa' is then calculated with the relation $\Delta pKa' = \log C$. This procedure is repeated with additional values of [L] until the entire experimental range of L_t is covered. The experimental data points then can be compared with the calculated curve. The constants K_{11a} and K_{11b} are treated as adjustable parameters, and the calculation is continued until a reasonable agreement is achieved.

Preliminary estimates of K_{11a} and K_{11b} can be obtained from the data. From Eq. 10, the limiting value of ΔpKa' at large L_t is log (K_{11a}/K_{11b}); the ratio K_{11a}/K_{11b} estimated in this way is a minimum value (if $K_{11a} > K_{11b}$) or a maximum value (if $K_{11b} > K_{11a}$). Estimates of the individual constants can be made as follows. Equation 9 can be written:

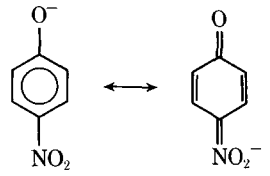
$$\frac{K_a'}{K_a} = 1 + \frac{(K_{11b} - K_{11a})[L]}{1 + K_{11a}[L]} \quad \text{(Eq. 18)}$$

which can be rearranged into several forms suitable for linear plotting. Equation 19 shows the common "double reciprocal" (Benesi–Hildebrand or Lineweaver–Burk) form:

$$\frac{K_a}{K_a' - K_a} = \frac{1}{[L](K_{11b} - K_{11a})} + \frac{K_{11a}}{(K_{11b} - K_{11a})} \quad \text{(Eq. 19)}$$

The free concentration [L] is not known but may be approximated by $(L_t - A_t)$. From a plot of $K_a/(K_a' - K_a)$ against $1/(L_t - A_t)$, the constants are evaluated for use in the calculation as described. (If $K_{11a} > K_{11b}$, a similar form of Eq. 19 is used.)

Figure 1 shows the dependence of ΔpKa' on cyclohexaamylose concentration for benzoic acid and *p*-nitrophenol. The solid curves were calculated as already described. Evidently, Eq. 10 gives a quantitative description of the *p*-nitrophenol system. Agreement between the calculated curve and the experimental points is fair for the benzoic acid system. For the *p*-nitrophenol–cyclohexaamylose system, these constants are found: $K_{11a} = 200\ M^{-1}$ and $K_{11b} = 2200\ M^{-1}$ at 25°. These values can be compared with the values $K_{11a} = 290\ M^{-1}$ and $K_{11b} = 2230\ M^{-1}$ found spectrophotometrically by Cramer *et al.* (12) (calculated for 25° from the data of Cramer *et al.*, these are at ionic strength 0.5 *M*). The benzoic acid curve yields the constants $K_{11a} = 1400\ M^{-1}$ and $K_{11b} = 38\ M^{-1}$.



Scheme V

Casu and Rava (9) found $K_{11a} = 1050\ M^{-1}$ for benzoic acid in 0.08 *N* HCl using a spectrophotometric technique.

The potentiometric method described here appears to be a valuable technique that has not heretofore been applied to the study of organic molecular complexes. It is, of course, equivalent to the classical potentiometric method for studying complexes of metal ions with weak bases (15, 16). The data treatment suggested here, in terms of apparent dissociation constants, is different from, and rather simpler than, a treatment using the conventional Bjerrum formation function. The extension of this method to systems containing complexes of higher orders is easy in principle, but much more experimental work will be necessary to establish the practical value of the technique to these complicated systems.

Complex Stability and Interactant Structure—One striking feature of the data in Tables I and II is the relationship of ΔpKa′ to molecular structure. For several aromatic acids, ΔpKa′ seems to be determined mainly by the location of a substituent group rather than by its identity. Such effects are often observed with cycloamyloses, and they have been widely interpreted in terms of inclusion complex formation, with the stability of the complex being determined in part by the spatial compatibility of the guest molecule and the cycloamylose cavity. Of course, ΔpKa′ is not a simple measure of complex stability (see Eq. 10), and its dependence upon both K_{11a} and K_{11b} introduces another possibility for dispersion within a series.

Another notable characteristic of these data is that, for substances showing a nonzero value of ΔpKa′, carboxylic acids have positive ΔpKa′ values and phenols have negative values. That is, for carboxylic acids, $K_{11a} > K_{11b}$ (the acid complexes more strongly than does the anion); for phenols, $K_{11b} > K_{11a}$. Even if this generalization should be found to have exceptions, the observation is sufficiently arresting in individual examples to invite discussion.

For example, *p*-nitrobenzoic acid (ΔpKa′ = +0.79 at 0.02 *M* cyclohexaamylose) can be compared with *p*-nitrophenol (ΔpKa′ = −0.94). The result for the phenol is the unexpected one; it is unusual for an anion to complex (with a common neutral ligand in aqueous solution) more strongly than the uncharged species. Viewed another way, the *p*-nitrophenolate ion "partitions" into the cycloamylose cavity to a greater extent than does *p*-nitrophenol, in contrast with the reverse situation for the benzoic acid. The extensive charge delocalization in the *p*-nitrophenol anion possibly is responsible for this behavior (Scheme V).

The carboxylic acid anion is incapable of this type of charge distribution and may be more strongly solvated than is the phenolate. *p*-Nitrophenol itself is a good hydrogen-bond donor, and its affinity for the solvent may destabilize its complex with the cycloamylose.

If ΔpKa′ at a fixed ligand concentration is taken as a rough measure of complex stability, the comparative data of Tables I and II for cycloheptaamylose and cyclohexaamylose seem qualitatively reasonable. The cavity of cycloheptaamylose has a larger diameter than that of cyclohexaamylose, which will lead to stronger complexes with substrates (such as some 1,2-disubstituted aromatics and naphthols) too large to fit the cyclohexaamylose cavity and to weaker complexes with substrates (monosubstituted, 1,3- and 1,4-disubstituted aromatics) that fit snugly into the cyclohexaamylose cavity.

Analytical Applications—These applications fall into two classes:

1. Potentiometric titration of aqueous solutions of substances whose apparent acid or base strength is increased by the addition of a cycloamylose. Some phenols show an increase in acidity, and some carboxylate salts become stronger bases. These applications may be quite practical, because the analysis of dilute aqueous solutions of these substances can be difficult. A sample concentration range of 0.001–0.01 *M* is suitable, and the substances for which useful effects can be expected are shown or suggested by the data in Tables I and II.

2. Differentiating potentiometric titration of mixtures of acids, with the addition of a cycloamylose being the means of "tuning" the pKa′ values of the mixture to optimize the selectivity. The equilibrium selectivity of a process can be defined as log (K_2/K_1) or $pK_1 - pK_2$ (17). This value should be 2 or greater for titrations of acids in aqueous solution, and addition of a cycloamylose has been shown capable of producing this selectivity.

Other uses can be expected for these effects. For example, the effective buffer ranges of acids are shifted in the presence of cycloamyloses. The pKa′ shifts could be useful for separating acids in aqueous chromatographic systems, as in ion exchange.

REFERENCES

(1) D. French, *Advan. Carbohyd. Chem.*, **12**, 189(1957).

(2) J. A. Thoma and L. Stewart, in "Starch: Chemistry and Technology," vol. I, R. L. Whistler and E. F. Paschall, Eds., Academic, New York, N.Y., 1965, p. 209.

(3) D. W. Griffiths and M. L. Bender, *Advan. Catal. Relat. Subj.*, **23**, 209(1973).

(4) H. Schlenk and D. M. Sand, *J. Amer. Chem. Soc.*, **83**, 2312(1961).

(5) J. Cohen and J. L. Lach, *J. Pharm. Sci.*, **52**, 132(1963).

(6) J. L. Lach and J. Cohen, *ibid.*, **52**, 137(1963).

(7) J. L. Lach and T.-F. Chin, *ibid.*, **53**, 69(1964).

(8) W. A. Pauli and J. L. Lach, *ibid.*, **54**, 1745(1965).

(9) B. Casu and L. Rava, *Ric. Sci.*, **36**, 733(1966).

(10) S. Takenaka, N. Matsuura, and N. Tokura, *Tetrahedron Lett.*, **1974**, 2325.

(11) F. Cramer, *Angew. Chem.*, **73**, 49(1961).

(12) F. Cramer, W. Saenger, and H.-C. Spatz, *J. Amer. Chem. Soc.*, **89**, 14(1967).

(13) S. Bruckenstein and I. M. Kolthoff, in "Treatise on Analytical Chemistry," part I, vol. I, I. M. Kolthoff and P. J. Elving, Eds., Interscience, New York, N.Y., 1959, p. 470.

(14) J. S. Fritz and G. H. Schenk, Jr., "Quantitative Analytical Chemistry," 2nd ed., Allyn and Bacon, Boston, Mass., 1969, p. 174.

(15) M. M. Jones, "Elementary Coordination Chemistry," Prentice-Hall, Englewood Cliffs, N.J., 1964, chap. 8.

(16) M. T. Beck, "Chemistry of Complex Equilibria," Van Nostrand Reinhold, London, England, 1970.

(17) K. A. Connors, "Reaction Mechanisms in Organic Analytical Chemistry," Wiley–Interscience, New York, N.Y., 1973, p. 178.

ACKNOWLEDGMENTS AND ADDRESSES

Received February 24, 1975, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*

Accepted for publication June 3, 1975.

Supported by National Science Foundation Grant GP-36567.

[x] To whom inquiries should be directed.