to the variety of substrate structures examined, hydrogen bonding between cycloheptaamylose and the substrate is eliminated as a major contribution to complex stability.

Implications for Enzyme Specificity and Catalysis. It is generally assumed that the solvation changes resulting from enzyme–substrate binding have some effect upon substrate reactivity. As an example of the possible magnitude of this effect, enzymatic solvent effects have been proposed to be a major source of catalytic action in thiamine pyrophosphate dependent enzymatic reactions.[39] Thus, the enzyme solvent effect may be responsible for a rate acceleration of 10^5–10^6 in pyruvate decarboxylase-catalyzed decarboxylation.[39]

A homogeneous catalyst and substrate system has been presented where the catalytic action is determined solely by the ability of the catalyst to bind the substrate, but catalytic action is independent of the binding constant. One would expect that the conclusions derived from this system should apply to other homogeneous catalysts which have well-defined binding sites and where binding is due to hydrophobic interactions. This implies that, for these systems, (1) the ability to act as a catalyst is totally independent of the binding constant, (2) therefore, catalytic specificity must be a function of the juxtaposition of the catalytic active group(s) and reactive functional group(s), and (3) the solvation change due to binding may be large but it is a constant for bound compounds of similar structures.

Due to the correlation between solvent effects and cycloheptaamylose acceleration, the great variety of possible substrate structures, and the lack of extraneous catalytic effects, decarboxylation rates should prove extremely valuable in examining the polarity and rigidity of protein binding sites. Additional phenomena such as the pH effect upon binding site polarity, specific apolar interactions, and other interactions, including protein conformational changes upon substrate binding which vary binding site polarity, also can be investigated.

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Cycloamyloses as Enzyme Models. The Decarboxylation of Benzoylacetic Acids

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Abstract: Rate constants for the aqueous decarboxylations of eight benzoylacetic acids are determined as a function of pH and, from these data, pK_a values are calculated. Data at the acidic and basic extremes of the pH–rate profile are used to construct Hammett plots, with ρ values of $+0.031$ and $+1.42$, respectively, for benzoylacetic acid and benzoylacetate decarboxylation. Benzoylacetate decarboxylations are accelerated in the presence of cyclohexa- and cycloheptaamylose. These results are interpreted in terms of the cycloamylose microsolvent effect. Decarboxylations of benzoylacetic acids are accelerated by cycloheptaamylose but inhibited by cyclohexaamylose, effects too great to be attributed to microsolvent effects. pH–rate profiles for the cycloheptaamylose-catalyzed and activation parameters for the spontaneous and cycloheptaamylose-catalyzed 4′-methyl-, 3′-chloro-, and benzoylacetic acid decarboxylations are determined. Data for spontaneous decarboxylations are compared with the literature data determined for partially aqueous and nonpolar solvents and are discussed in terms of transition state polarity. Cycloamylose effects on the decarboxylations of benzoylacetic acids are consistent with conformational restraints on the included acid, *e.g.*, conformational catalysis.

The major characteristic of an enzymatic reaction is the enormous rate enhancement observed relative to its nonenzymatic counterpart. This rate enhancement has been credited to many factors.[2-13] An often discussed contribution which has been generally inaccessible to experimental study is the enzyme-imposed conformational restrictions on the bound sub-

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strate. A previous report from this laboratory described the inhibition of intramolecular-catalyzed hydrolysis of mono-*p*-carboxyphenyl esters of 3-substituted glutaric acids on binding to cycloamyloses in nonreactive conformations.[14] We now wish to report the cycloamylose catalysis of benzoylacetic acid decarboxylation where catalysis is attributed to a conformation restriction in the bound acid.

Experimental Section

Reagents. Cyclohexaamylose and cycloheptaamylose were obtained from Corn Products Co. and purified as described previously.[15] Aqueous reaction solutions employed double-distilled water and reagent grade buffers. 2-Propanol (J. T. Baker spectrophometric grade) was dried over calcium hydride, distilled, and stored over molecular sieves. Acetonitrile was Mallinckrodt Nanograde. pH measurements were made on a Radiometer 26 pH meter. Stock solutions of β-keto acids were prepared by dissolving a weighed amount of acid in water and neutralizing with reagent grade sodium bicarbonate.

Benzoylacetic Acid. Benzoylacetic acid was prepared by hydrolysis of ethyl benzoylacetate (Eastman) in 5% potassium hydroxide, ether extraction, and acidification of the aqueous solution. The resultant precipitate was collected in a sintered glass funnel, dried and recrystallized from benzene, mp 101–102° dec (lit. 99.5–100° dec,[16] 103–104° dec[17]).

2-Phenylacetoacetic Acid. Ethyl 2-phenylacetoacetate was prepared by the sodium hydride initiated condensation of ethyl acetate and ethyl phenylacetate in dimethylformamide according to Gittos, *et al.*[18] The product was hydrolyzed by standing it overnight in concentrated sulfuric acid, 5 ml of acid per g of ester, and pouring it over ice. The resultant precipitate was collected, dried over KOH pellets, and recrystallized from benzene–pentane. 2-Phenylacetoacetic acid was recovered as colorless crystals, mp 98–99° (lit.[19] 98°).

2-Benzoylpropionic Acid. Ethyl 2-benzoylpropionate was prepared by the condensation of diethyl carbonate with propiophenone (Matheson, Coleman and Bell) in the presence of sodium ethoxide.[20] The ester was hydrolyzed with a 10% potassium hydroxide solution (1 g of ester per 10 ml of base) by being stirred for 14 hr. The basic solution was extracted with ether and acidified with hydrochloric acid to the Congo red endpoint, and the product was extracted into ether. After drying the ether layer, ether evaporation and recrystallization from carbon tetrachloride gave pure 2-benzoylpropionic acid, mp 78–79° (lit.[21] 77–78°).

4′-Nitrobenzoylacetic Acid. Ethyl 4′-nitrobenzoylacetate was prepared by the method of Bulow and Hailer.[22] The resultant pale yellow plates had mp 71–73° (lit.[22] 75°).

The ester was hydrolyzed according to Hay and Tate.[23] After recrystallization from benzene, the acid had mp 132–133° dec (lit. 132° dec,[23] 135° dec[22]).

4′-Methylbenzoylacetic Acid. 4′-Methylbenzoylacetic acid was prepared from 4′-methylacetophenone (Aldrich) and magnesium methyl carbonate in dimethylformamide according to Stiles.[24] Magnesium methyl carbonate preparation and product isolation were performed according to Finkbeiner and Wagner.[25] After recrystallization from carbon tetrachloride, the acid had mp 84–86° dec (lit.[16] 85–87° dec).

3′-Methylbenzoylacetic Acid. 3′-Methylbenzoylacetic acid was prepared from 3′-methylacetophenone (Aldrich) and magnesium methyl carbonate following the procedure for the 4′-methyl compound. The compound had mp 81–82° dec.

Anal. Calcd for $C_{10}H_{10}O_3$: C, 67.41; H, 5.66. Found: C, 67.52; H, 5.58.

2′-Methylbenzoylacetic Acid. Except for using 2′-methylacetophenone (Aldrich), the procedure for the preparation of 2′-methylbenzoylacetic acid was identical with that used to prepare the 4′-methyl compound. The product had mp 77–79° dec.

4′-Chlorobenzoylacetic Acid. The preparative procedure, with 4′-chloroacetophenone (Aldrich) as the starting material, was identical with that for 4′-methylbenzoylacetic acid. The colorless product had mp 121–122° dec.

Anal. Calcd for $C_9H_7ClO_3$: C, 54.43; H, 3.55; Cl, 17.85. Found: C, 54.35; H, 3.53; Cl, 17.88.

3′-Chlorobenzoylacetic Acid. Using 3′-chloroacetophenone (Aldrich) as the starting material, the preparative procedure was that used for 4′-methylbenzoylacetic acid. The white crystalline product had mp 114° dec.

Anal. Calcd for $C_9H_7ClO_3$: C, 54.43; H, 3.55; Cl, 17.85. Found: C, 54.27; H, 3.36; Cl, 17.76.

Reaction Kinetics. The decarboxylations of all acids and their anions were followed spectrophotometrically by monitoring the decrease in absorption as the reaction proceeded. Plots of log $(A_t - A_\infty)$ against time were linear; rate constants were obtained from the slope, $-k_{obsd}/2.303$. A Gilford 220 recording spectrophotometer with Beckman DU optics was used for kinetic determinations and a Cary Model 14 recording spectrophotometer was used to record spectra. In all cases the product spectrum was identical to that for the appropriate acetophenone. The monitoring wavelength for kinetics was at or near the maximum of the benzoylacetic acid–corresponding acetophenone difference spectrum in 0.1 *N* hydrochloric acid. The wavelengths employed with $\Delta\epsilon$ in parentheses are as follows: benzoylacetic acid, 258 nm (3250); 2-phenylacetoacetic acid, 230 nm (1020); 2-benzoylpropionic acid, 260 nm (4150); 4′-methylbenzoylacetic acid, 271 nm (2970); 3′-methylbenzoylacetic acid, 262 nm (2370); 2′-methylbenzoylacetic acid, 260 nm (1982); 4′-chlorobenzoylacetic acid, 271 nm (2060); 3′-chlorobenzoylacetic acid, 258 nm (2770); 4′-nitrobenzoylacetic acid, 325 nm (521).[26]

Temperature control and general procedure were as previously described.[27] All reactions containing cycloamyloses were performed with cycloamylose concentrations at least tenfold greater than substrate concentrations to maintain pseudo-first-order conditions.

Maximum Rate Constants for Cycloamylose-Catalyzed Reactions and Activation Parameters. The maximum rate constants for cycloamylose-catalyzed reactions, k_c, complex dissociation constants, K_s, activation parameters, and thermodynamic parameters were calculated as described in the preceding paper.[27]

Results

Spontaneous Decarboxylation. The decarboxylations of monobasic β-keto acids are known to occur by simultaneous decomposition of the free acid and the anion[28] (Scheme I). This scheme leads to the rate expression in eq 1. Using the dissociation constant,

$$-d[HA]_T/dt = k_1[HA] + k_2[A^-] \quad (1)$$

$K_A = [H^+][A^-]/[HA]$, and the conservation equation, $[HA]_T \equiv [HA] + [A^-]$, the rate expression is more conveniently expressed as

$$-d[HA]_T/dt = \frac{k_1[H^+] + k_2K_A}{[H^+] + K_A}[HA]_T = k_{obsd}[HA]_T \quad (2)$$

Equation 2 predicts a sigmoidal dependence of k_{obsd} on pH. This dependence is shown in Figure 1 for 4′-methylbenzoylacetic acid decarboxylation at 50.3°. The pH dependencies of decarboxylation rate

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(26) These data refer to the difference spectra in pH 6.85 phosphate buffer.
(27) T. S. Straub and M. L. Bender, *J. Amer. Chem. Soc.*, **94**, 8875 (1972).

Table I. Decarboxylation Rate Constants of Acids and Anions, Ionization Constants in Aqueous Solution and for the Cycloheptaamylose Complex

Acid	T, °C	10^3k_1,[a] sec^{-1}	10^3k_2,[b] sec^{-1}	pK_a[c]	10^3k_{cl}[d]	k_{cl}/k_1	pK_{ca}[d]	10^3K_s,[e] M
$C_6H_5COCH_2CO_2H$	40.8	0.333	0.0203	3.38 ± 0.01	2.41	7.2	3.72	8.3 ± 0.4
	50.3	0.950	0.0740	3.40 ± 0.05	5.90	6.2	3.79	9.8 ± 0.6
	61.0	3.04	0.281	3.43 ± 0.02	17.8	5.9	3.70	14 ± 1.0
$2\text{-}CH_3C_6H_4COCH_2CO_2H$	50.3	0.779	0.113	3.53 ± 0.12				
$3\text{-}CH_3C_6H_4COCH_2CO_2H$	50.3	1.002	0.0555	3.44 ± 0.05				
$4\text{-}CH_3C_6H_4COCH_2CO_2H$	40.8	0.320	0.0111	3.45 ± 0.02	2.17	6.8	3.84	3.3 ± 0.2
	50.3	0.967	0.0359	3.43 ± 0.05	6.04	6.2	3.70	4.6 ± 0.3
	61.0	2.72	0.151	3.57 ± 0.01	15.0	5.5	3.82	6.3 ± 0.4
$3\text{-}ClC_6H_4COCH_2CO_2H$	40.8	0.336	0.0653	3.24 ± 0.03	2.09	6.2	3.64	5.2 ± 0.3
	50.3	0.939	0.225	3.30 ± 0.03	5.20	5.5	3.68	6.0 ± 0.1
	61.0	2.78	0.841	3.34 ± 0.02	13.5	4.9	3.71	8.5 ± 1.9
$4\text{-}ClC_6H_4COCH_2CO_2H$	50.3	0.910	0.112	3.38 ± 0.06				
$4\text{-}NO_2C_6H_4COCH_2CO_2H$	50.3	1.066	0.879	3.24 ± 0.22				
$C_6H_5COCH(CH_3)CO_2H$	50.3	0.587	0.0894	3.58 ± 0.15				
$CH_3COCH(C_6H_5)CO_2H$	40.5	3.04	2.43					

[a] Spontaneous decarboxylation rate constant in 0.1 N hydrochloric acid. [b] Spontaneous decarboxylation rate constant in pH 6.85 phosphate buffer, ionic strength = 0.10. [c] Evaluated *via* eq 3 using rate constants measured at a minimum of eight different pH values. [d] Cycloheptaamylose-un-ionized acid decarboxylation rate constant evaluated as described in text. Cycloheptaamylose accelerations at a minimum of 4 pH values were used for the evaluation. [e] At pH 3.0.

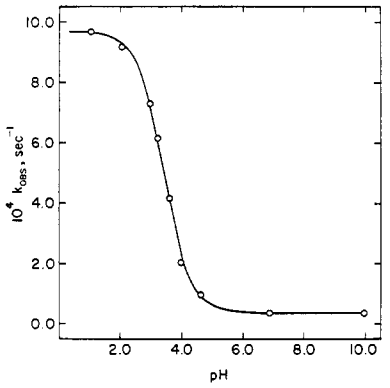


Figure 1. The pH–rate profile for aqueous 4′-methylbenzoylacetic acid decarboxylation at 50.3°. The solid line was drawn using constants from Table I and the expression for k_{obsd} derived from eq 2.

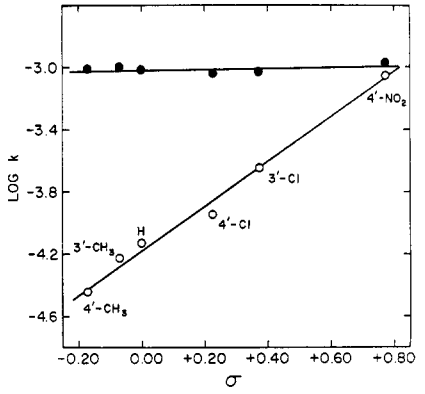
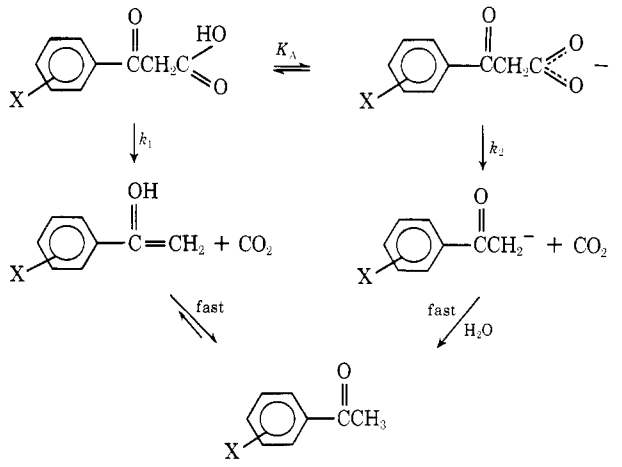


Figure 2. Hammett plot for aqueous benzoylacetic acid (●) and anion (○) decarboxylation at 50.3°. The solid lines have slopes of +0.031 and +1.42 for acid and anion decarboxylation, respectively.

Scheme I



constants for eight benzoylacetic acids and 2-phenylacetoacetic acid were examined. Assuming the rate constants at pH 1.0 are the rate constants for decarboxylation of the un-ionized acids, k_1, and the rate constants at pH 7.0 are rate constants for decarboxylation of the anions, k_2, dissociation constants for these acids can be calculated from eq 3.

$$pK_a = pH - \log [(k_1 - k_{obsd})/(k_{obsd} - k_2)] \quad (3)$$

k_1 and k_2 values and pK_a values determined by eq 3 are collected in Table I. The standard deviations of the pK_a values reflect the dependency of the determination on the magnitude of the difference between decarboxylation rate constants for the un-ionized and ionized acids. The internal consistency of the data is shown in Figure 1, where the solid line was calculated from the values in Table I and eq 2. 2-Phenylacetoacetic acid decarboxylation was not treated in this fashion due to the small k_1/k_2 ratio, 1.25.

Examination of the decarboxylation rates reported in Table I reveals weak substituent effects for decarboxylation of the free acid and strong substituent effects for anionic decarboxylation. The substituent effects are shown more dramatically by the Hammett plots[28] of Figure 2. A least-squares treatment of rates for acid decarboxylation gave the upper line of Figure 2 with a slope or ρ of +0.031 ± 0.031 and a correlation coefficient of only 0.450, indicating that, within experimental error, the decarboxylation rates are independent of substituent. A similar treatment of anionic

(28) H. H. Jaffe, *Chem. Rev.*, 53, 191 (1953).

Table II. Cycloheptaamylose Accelerations of β-Keto Acid Decarboxylation[a]

Acid	pH[b]	10^3k_{un}, sec^{-1} [c]	10^3k_c, sec^{-1} [d,e]	10^3K_s, M [d,e]	k_c/k_{un}	R [f]
$C_6H_5COCH_2CO_2H$	3.01	0.668	5.08 ± 0.19	9.77 ± 0.64	7.6	0.994
	3.61	0.416	3.34 ± 0.19	11.1 ± 1.1	8.0	0.987
	4.03	0.255	2.47 ± 0.20	18.2 ± 2.0	9.7	0.988
	4.64	0.125	0.686 ± 0.060	12.5 ± 1.9	5.5	0.967
$2\text{-}CH_3C_6H_4COCH_2CO_2H$	2.97	0.598	2.45 ± 0.06	15.3 ± 0.7	4.1	0.997
$3\text{-}CH_3C_6H_4COCH_2CO_2H$	2.98	0.762	5.68 ± 0.12	7.64 ± 0.32	7.5	0.997
$4\text{-}CH_3C_6H_4COCH_2CO_2H$	2.98	0.730	4.93 ± 0.14	4.65 ± 0.29	6.8	0.994
	3.22	0.616	4.71 ± 0.14	5.41 ± 0.33	7.6	0.994
	3.60	0.416	3.46 ± 0.07	5.50 ± 0.22	8.3	0.998
	3.99	0.203	1.93 ± 0.02	4.73 ± 0.13	9.5	0.999
$3\text{-}ClC_6H_4COCH_2CO_2H$	2.97	0.704	4.27 ± 0.05	6.02 ± 0.14	6.1	0.999
	3.22	0.602	3.96 ± 0.14	7.22 ± 0.52	6.6	0.992
	3.60	0.473	2.82 ± 0.02	7.82 ± 0.12	6.0	0.999
	3.99	0.352	1.71 ± 0.03	8.60 ± 0.35	4.9	0.998
$4\text{-}ClC_6H_4COCH_2CO_2H$	2.98	0.655	3.42 ± 0.10	6.82 ± 0.43	5.2	0.994
$4\text{-}NO_2C_6H_4COCH_2CO_2H$	2.97	0.965	2.17 ± 0.25	8.4 ± 2.6	2.2	0.848
$C_6H_5COCH(CH_3)CO_2H$	3.01	0.436	1.00 ± 0.02	3.83 ± 0.34	2.3	0.992
$CH_3COCH(C_6H_5)CO_2H$[g]	4.02	2.35	12.1 ± 1.3	14.8 ± 2.6	5.1	0.956

[a] T = 50.3°; ionic strength = 0.10 M; initial β-keto acid concentration = 10^{-4} M. [b] At 25°. [c] Average of two–five determinations, mean error is 2%. [d] Determined from a least-squares treatment of eq 4. Five–six cycloheptaamylose concentrations from 10^{-3} M to 1.5×10^{-2} M were used. [e] Errors are computer calculated standard deviation. [f] Correlation coefficient for the fit to eq 4. [g] T = 40.5°.

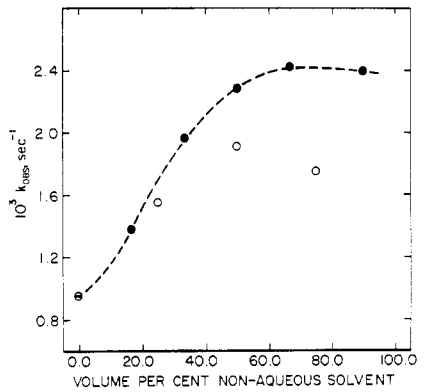


Figure 3. The effect of 2-propanol (●) and acetonitrile (○) on benzoylacetic acid decarboxylation at 50.3°. All solutions were 0.1 M hydrochloric acid.

decarboxylation yielded $\rho = +1.42 \pm 0.07$ for a line with a correlation coefficient of 0.995, indicating that decarboxylation rates are strongly accelerated by electron-withdrawing substituents.

The effects of acetonitrile and 2-propanol addition on benzoylacetic acid decarboxylation rates were determined and are presented in Figure 3. All solutions contained 0.1 N hydrochloric acid to suppress ionization. These results are in good agreement with previous observations that solvent effects for β-keto acid decarboxylations are generally small and are at a maximum at 40–60% by volume organic solvent.[23,29]

β-Keto Acid Decarboxylation with Added Cycloamylose. The decarboxylations of all benzoylacetic acids and anions were accelerated by addition of cycloheptaamylose. Rate enhancements were not a linear function of cycloheptaamylose concentration but were consistent with the Michaelis–Menten scheme often observed in enzymatic systems and with previous studies of cycloamylose accelerated reactions.[14,15,27] Therefore, the effect of cycloheptaamylose, C, at a particular pH, was analyzed according to Scheme II (HA is the total acid, un-ionized and ionized, present) using a treatment derived from that of Colter, *et al.*[30] Rate

(29) G. A. Hall and E. S. Hanrahan, *J. Phys. Chem.*, **69**, 2402 (1965).

Scheme II

$$\begin{array}{ccc} HA + C & \underset{K_s}{\rightleftharpoons} & C{-}HA \\ \downarrow k_{un} & & \downarrow k_c \\ P + CO_2 + C & \rightleftharpoons & C{\cdot}P + CO_2 \end{array}$$

constants for the decarboxylation of the cycloamylose complex, k_c, and the dissociation constants of the complex, K_s, were evaluated by a computer fit of the rate data to Eadie plots[31] based on eq 4. The results at

$$k_{obsd} - k_{un} = (k_c - k_{un}) - K_s(k_{obsd} - k_{un})/[C] \quad (4)$$

50.3° are collected in Table II.

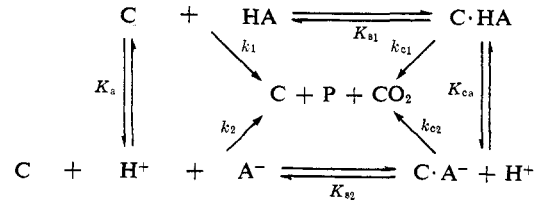
In pH 4.0 acetate buffer solutions, the treatment of the cycloheptaamylose acceleration of 2-phenylacetoacetic acid was analogous to that for benzoylacetic acid decarboxylation. However, when a study was attempted in pH 3.0 formate buffer solutions, precipitation resulted. To determine the reason for precipitation, uv spectra of the acid were determined in 0.1 N hydrochloric acid and in acetonitrile. In acid a shoulder at 252 nm (ε 100) was observed while in acetonitrile a band with λ_{max} at 265 nm (ε 16,500) was observed. By analogy with similar systems,[32] the 265-nm band is attributable to the enol tautomer of 2-phenylacetoacetic acid. The 252-nm shoulder in aqueous solution is typical of the n → π* transition of ketones[33] and is retained with somewhat diminished intensity upon decarboxylation. Therefore, precipitation can be attributed to the preferential binding of the enol tautomer with cycloheptaamylose. An enol shift has previously been used to explain the cycloamylose catalysis of α-hydroxy ketone oxidation.[34] Complete ionization at pH 4.0 is indicated by the invariance of decarboxylation rates between pH 4 and 10, and ultraviolet spectra indicate that the keto form of the anion is preferred. The absence of any effects attributable to tautomerism for benzoylacetic acid decarboxylation

(30) A. K. Colter, S. S. Wang, G. H Megerle, and P. S. Ossip, *J. Amer. Chem. Soc.*, **86**, 3106 (1964).
(31) G. S. Eadie, *J. Biol. Chem.*, **146**, 85 (1942).
(32) B. E. C. Banks, *J. Chem. Soc.*, 5043 (1961); 63 (1962).
(33) H. H. Jaffe and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," Wiley, New York, N. Y., 1962, p 178.
(34) F. Cramer, *Chem. Ber.*, **86**, 1576 (1953).

has been shown in both aqueous and nonpolar solvents.[16]

A complete treatment of the effect of cycloheptaamylose on decarboxylation necessitates the separation of the effects for un-ionized and ionized acid decarboxylation according to Scheme III. The constants

Scheme III



k_1, k_2, and K_A were obtained from the study of the spontaneous reaction (Table I). The constants k_{c1}, k_{c2}, and K_{cA} can be obtained from the pH dependence of the cycloheptaamylose–acid decarboxylation rate, k_c of Table II, and the dissociation constants K_{s1} and K_{s2} can be evaluated from the pH dependence of the K_s values in Table II.

Attempts were made to determine directly k_{c2} and K_{s2} values. However, rate enhancements in the experimentally accessible cycloheptaamylose concentration range (upper limit, 1.5×10^{-2} M at 25°[35]) were too small for accurate assessment by Eadie plots. The observed rate enhancements in 10^{-2} M cycloheptaamylose at pH 7 are listed in Table III.

Table III. Relative Effects of Cyclohexaamylose and Cycloheptaamylose on Benzoylacetic Acid Decarboxylation[a]

XC_6H_4-$COCH_2CO_2H$ X =	k_{obsd}/k_{un} 0.020 M cyclohexaamylose pH 3.0	0.020 M cyclohexaamylose pH 6.85	0.010 M cycloheptaamylose pH 3.0[c]	0.010 M cycloheptaamylose pH 6.85
4-CH_3	0.64	1.11	4.93	1.31
3-CH_3	0.84[b]		4.66	
H	0.94		4.34	1.20
4-Cl	0.62	1.26	3.51	1.60
3-Cl			4.16	1.36
4-NO_2			1.68	1.65

[a] T = 50.3°; ionic strength = 0.10; initial acid concentration = 10^{-4} M. [b] 0.030 M cyclohexaamylose. [c] Values calculated from data in Table II and eq 4.

A direct determination of the cycloheptaamylose–un-ionized acid decarboxylation rate constant was not possible due to the hydrolytic instability of cycloheptaamylose in acid solutions.[36] Therefore, k_{c1} and K_{cA} were obtained by fitting the observed pH dependence of log k_c to the normalized theoretical curve, eq 5, which is simply a rearrrangement of the normal inverted sigmoid relation (eq 3) assuming un-ionized acid decarboxylation is the only reaction effectively catalyzed. This technique is based on the method devised by Brubacher, *et al.*,[37] and is statistically more accurate than the normal reciprocal plots,[38] since each

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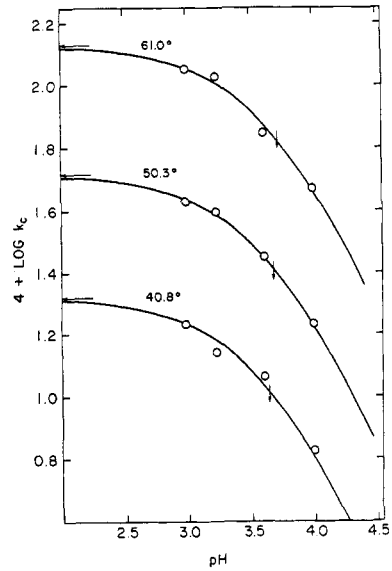


Figure 4. The pH–log rate profile for cycloheptaamylose-catalyzed 3′-chlorobenzoylacetic acid decarboxylation. The solid lines were obtained by fit of eq 5 to the experimental points. Horizontal arrows represent the y intercept, log k_{c1}, and vertical arrows are pK_{ca} values.

datum point has equal weight. The fit of the experimental points with the theoretical curve is shown in

$$\log \frac{k_{c1}}{k_c} - 1 = \text{pH} - pK_{ca} \qquad (5)$$

Figure 4 for 3′-chlorobenzoylacetic acid decarboxylation. The resultant k_{c1} and pK_{ca} values are listed in Table I.

The assumption that decarboxylation of un-ionized acid is the only reaction rate-determining k_c is partially justified by the fit of the data to the theoretical curve. However, a different approach is also available. The data at 50.3° for the reaction treated in this fashion having the smallest k_1/k_2 ratio, 3′-chlorobenzoylacetic acid decarboxylation, will be used. The determined pK_{ca} (3.68) implies that the bound acid is two-thirds ionized at the highest pH of the pH–rate profile, 3.99. In the presence of 10^{-2} M cycloheptaamylose, $k_{obsd} - k_1$ at pH 6.85, T = 50.3°, was determined to be 8.1×10^{-5} sec^{-1}. At two-thirds ionization the rate contribution due to anion decarboxylation is 5.4×10^{-5} sec^{-1}. This factor is only 5% of the observed rate, 1.08×10^{-3} sec^{-1}, for a 10^{-2} M cycloheptaamylose solution at pH 3.99 and is equal to the expected experimental error. At lower pH values or for reactions having larger k_1/k_2 ratios, even this contribution would be greatly reduced.

At pH 2.98 where the benzoylacetic acids are little ionized, decarboxylation was inhibited by addition of cyclohexaamylose. However, at pH 6.85 where benzoylacetic acids are totally ionized, decarboxylation was accelerated in the presence of cyclohexaamylose. The relative effects of cyclohexa- and cycloheptaamylose are compared in Table III at pH 3.0 and 6.85.

Activation Parameters. Data from Table I were employed to calculate activation energies and entropies for 4′-methyl-, 3′-chloro-, and benzoylacetic acid decarboxylation. These results and activation parameters for benzoylacetic acid decarboxylation in 50%

aqueous dioxane[23] are compiled in Table IV for the un-ionized acids and Table V for the anions. These

Table IV. Activation Parameters for Benzoylacetic Acid Decarboxylation and Rate Constants in Various Solvents

Phenyl substituent	$10^3 k_{50.3°}$, sec^{-1}	E_a, kcal mol^{-1} [a]	$\Delta S^{\pm}$, eu[b]
	Aqueous Solution, pH 1.0		
4-NO_2	1.066		
3-Cl	0.939	21.81 ± 0.01	−6.96 ± 0.04
H	0.950	22.8 ± 0.3	−3.8 ± 1.0
4-CH_3	0.967	22.1 ± 0.8	−6.2 ± 2.4
	Cycloheptaamylose Complex		
3-Cl	5.20	19.3 ± 0.1	−11.5 ± 0.2
H	5.90	20.7 ± 0.9	−6.8 ± 2.8
4-CH_3	6.04	19.9 ± 1.0	−9.2 ± 3.1
	50% Aqueous Dioxane[c]		
4-NO_2	1.65	21.3	−7.5[d]
H	2.17	21.6	−6.0[d]
4-CH_3O	2.52	21.3	−6.5[d]
	Benzene[e]		
3-NO_2	0.074[f]		
4-Cl	0.103		
H	0.256		
4-CH_3	0.388		
4-CH_3O	0.576		

[a] Error is standard deviation. [b] Evaluated at 50.3° except where indicated. Error calculated from standard deviation of y intercept of Arrhenius plot. [c] E_a and $\Delta S^{\pm}$ from ref 23 at [HCl] = 0.2 M, $k_{50.3°}$ extrapolated from data at 0.2 M HCl in ref 23. [d] Evaluated at 308.6°K, ref 23. [e] Data from ref 16, T = 50.2°. [f] Solvent toluene.

Table V. Activation Parameters for Benzoylacetate Anion Decarboxylation

Phenyl substituent	$k_{50.3°}$, sec^{-1}	E_a, kcal mol^{-1} [a]	$\Delta S^{\pm}$, eu[b]
	Aqueous Solution, pH 6.85		
4-NO_2	8.79×10^{-4}		
3-Cl	2.25×10^{-4}	26.4 ± 0.1	4.3 ± 0.2
H	7.40×10^{-5}	27.1 ± 0.2	4.4 ± 0.6
4-CH_3	3.59×10^{-5}	27.0 ± 1.1	2.6 ± 3.5
	50% Aqueous Dioxane[c]		
4-NO_2	1.49×10^{-2}	24.7	9.3[d]
H	9.33×10^{-4}	25.7	5.0[d]
4-CH_3O	2.49×10^{-4}	25.2	2.7[d]

[a] Error is standard deviation. [b] Evaluated at 50.3° unless indicated. Error based on standard deviation of y intercept of Arrhenius plot. [c] Data at 0.005 M NaOH from or extrapolated from values in ref 23. [d] Evaluated at 308.6°K, ref 23.

data indicate that the accelerations observed in either mixed solvents or cycloheptaamylose complex formation are a result of favorable changes in the activation energy. Activation entropies are slightly more negative for the cycloheptaamylose accelerated reaction of the acids but the lack of dependence upon reaction milieu is more striking.

The cycloheptaamylose binding constants (Table II) are much less pH dependent than the corresponding rate constants. This enables the approximation that the binding constant measured at pH 2.98 is the binding constant of the un-ionized substituted benzoylacetic acid. The temperature dependence of these constants was used to evaluate the standard enthalpies and entropies of binding (Table VI). These results are completely consistent with values normally associated with cycloamylose binding constants.[14]

Table VI. Thermodynamic Parameters for Cycloheptaamylose–Benzoylacetic Acid Dissociation Constants

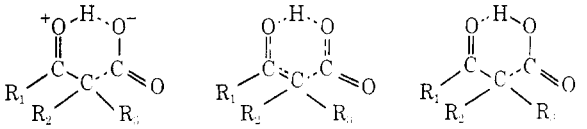
Phenyl substituent	$10^3 K_{s1}^{50.3°}$, M[a]	$\Delta H°$, kcal mol^{-1}	$\Delta S°$, eu[b]
H	9.77 ± 0.64	5.7 ± 1.3	8.6 ± 3.8
4-CH_3	4.65 ± 0.29	6.6 ± 0.4	9.8 ± 1.2
3-Cl	6.02 ± 0.14	5.2 ± 1.1	6.0 ± 3.3

[a] Data from Table I. [b] Errors are standard deviations.

Discussion

Benzoylacetic Acid Decarboxylation in Aqueous Solution. The normal mechanism for β-keto acid decarboxylation is well established and consists of a rate-determining loss of carbon dioxide and intramolecular proton transfer to form the corresponding enol, followed by a rapid tautomeric equilibration (Scheme I).[16,21,39–44] However, the nature of the transition state preceding loss of carbon dioxide is highly controversial.

Three transition states (structures I–III) have been proposed for β-keto acid decarboxylation. Structure I, which has never been clearly defined as a transition



I II III

state or intermediate, was postulated initially[45] and has been invoked recently to explain volumes of activation for β-keto acid decarboxylation.[46] Structure II, the concerted Westheimer mechanism,[47] has received wide support,[16,23,48,49] while structure III has been proposed only for the decarboxylations of bicyclic β-keto acids in which the acid moiety occupies a bridgehead position and where rate-determining enol formation would be in violation of Bredt's rule.[50] These three structures obviously represent a reactivity continuum from proton transfer preceding C–C bond cleavage to C–C bond cleavage without proton transfer. In accord with general theories of reactivity such as the Hammond postulate,[51] a continuous variation in transition state structure, dependent upon reactant and reaction conditions, should be expected. Similarities between structures I and II have received comment from several authors,[23,48,52,53] and this investigation

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of the aqueous decarboxylations of benzoylacetic acids, coupled with previous studies in aqueous dioxane[23] and benzene,[16] provides the evidence for this reactivity continuum.

In benzene solutions, benzoylacetic acids are predominantly in internally hydrogen-bonded conformations,[16] a nonpolar situation. Charge separation, *i.e.*, intramolecular proton transfer, is expected to be energetically unfavorable and should be accompanied by a very large negative Hammett ρ value. However, the species formed by proton transfer is a carboxylate anion and decarboxylation is greatly accelerated by transfer from aqueous to nonpolar solvents and is characterized by strong positive ρ values.[27,54] Therefore the maximum point on the decarboxylation reaction coordinate would be expected to be the point where the energy required for charge separation equals the decreased energy required for C–C bond cleavage.

Due to hydrogen bonding with the solvent, an internally hydrogen-bonding conformation is probably unimportant for benzoylacetic acids in aqueous solutions.[12b] This view is supported by the Taft σ^* value, +0.76 (calculated for the benzoyl moiety by extrapolation of the pK_a data in Table I and use of the literature values for aqueous carboxylic acid ionization[55]), a value considerably more positive than the corresponding Hammett para σ value, +0.459. In aqueous solution it is evident that internal proton transfer can occur only by substantial desolvation and conformational restriction of the benzoylacetic acids. Charge separation in the transition state would be stabilized by solvation. Therefore the maximum on the reaction coordinate should correspond to the least-polar structure.

Decarboxylation rate constants for benzoylacetic acids are presented in Table IV. The observed solvent effects, $k(H_2O) < k(50\%$ dioxane) $\gg k$(benzene), are typical of β-keto acid decarboxylation.[23,29] Hammett plots, constructed from these data, give ρ values of −1.0 in benzene (−1.5 if the *m*-nitro point is excluded), −0.16 in 50% dioxane, and +0.03 in water. These ρ values are consistent with significant charge separation in the transition state for decarboxylation in benzene and an essentially nonpolar transition state in water.

Additional support is obtained by a consideration of the deuterium isotope effects for decarboxylation. In benzene, isotope effects for 4′-methoxy-, 4′-chloro-, and benzoylacetic acids are k_H/k_D = 1.2–1.7,[16] values similar to the isotope effect, 1.45, expected for the equilibrium conversion HO → HO$^+$ at 50°.[56] The value for the 4′-CH_3 compound, 0.85, a compound of intermediate reactivity, seems completely anomalous. The isotope effect for 3′-nitrobenzoylacetic acid decarboxylation is 2.8 at 50° and increases with temperature. Increased substituent electron donating ability increases the basicity of the carbonyl oxygen easing charge formation and increases C–C bond stability requiring increased charge separation in the transition state. The isotope effect increase with temperature is simply a result of the greater activation energy for C–C bond cleavage than for proton transfer between oxygens and results in decreased proton transfer with increased temperature. A nonpolar transition state in aqueous solution requires a partially transferred proton and should be characterized by a substantial positive isotope effect. The isotope effect, k_{H_2O}/k_{D_2O}, for aqueous $EtO_2CCOCH_2CO_2H$ decarboxylation (the decarboxylation rates are very similar to those for benzoylacetic acids) is 3.1 at 30°,[44] definitely indicating the partially transferred proton required by a relatively nonpolar transition state.

This mechanistic description readily accounts for all available experimental data. C–C bond cleavage occurs in the rate-determining step, accounting for the observed carbon isotope effects. The more negative volumes of activation in solvents of lower polarity[46] are consistent with increased solvent electrostriction due to increased charge separation. Even the rate maxima observed in mixed aqueous solvents can be predicted from the relative effects of decreased ground state solvation, increased difficulty of charge separation, and the dependence of C–C bond stability upon charge separation.

Benzoylacetic Acid Decarboxylation in the Presence of Cycloamylose. Cycloheptaamylose accelerates the decarboxylations of all benzoylacetic acids examined over the entire pH range studied. Due to the pK_a shift upon complex formation and the differing accelerations of acid and anion decarboxylations, the cycloheptaamylose acceleration, k_c/k_{un}, should vary with pH. The data in Table II confirm this supposition. Using the pH dependency of k_c/k_{un}, the ratio of eq 2 for the spontaneous aqueous reaction and an analogous equation for the reaction of the cycloheptaamylose complex, the pH of maximum acceleration can be calculated by setting the derivative $d(k_c/k_{un})/d[H^+]$ equal to zero. Even neglecting acceleration of anionic decarboxylation, a somewhat complex quadratic, eq 6, is obtained. This equation

$$(k_1K_{ca} - k_1K_a + k_2K_a)[H^+]^2 + 2k_2K_aK_{ca}[H^+] + k_2K_a^2K_{ca} = 0 \quad (6)$$

is simply solved, but more important is the dependence upon the relative magnitudes of k_1 and k_2. As k_1/k_2 increases, eq 6 predicts an increase in the pH of maximum acceleration. Since the free energy of activation is larger for anionic decarboxylation than for acid decarboxylation, k_1/k_2 will decrease as temperature is increased and the pH of maximum acceleration will decrease. Also since $k_{c1}/k_1 > k_{c2}/k_2$, the maximum acceleration should increase as k_1/k_2 increases. This description is completely consistent with the data in Table II and explains both the pH dependence and much of the apparent acceleration specificity observed for cycloheptaamylose accelerations.

The cycloheptaamylose acceleration of carboxylate anion decarboxylation has been attributed to a microsolvent effect arising from substrate–cycloheptaamylose binding by inclusion of the substrate in the cycloamylose cavity.[27] This microsolvent effect is sufficient to account for the observed accelerations of benzoylacetate decarboxylation, since the cycloheptaamylose accelerations are much smaller than

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those obtained by transfer from aqueous solution to 50% dioxane (Table V). However, the cycloheptaamylose accelerations of un-ionized benzoylacetic acid decarboxylation cannot be explained solely on the basis of a microsolvent effect, since these accelerations are much larger than the accelerations obtained by transfer to partially aqueous or nonpolar solvents (Table IV and Figure 3). A reasonable explanation of the increased acceleration of benzoylacetic acid decarboxylation results from an induced conformational restriction in the substrate due to inclusion in the cycloheptaamylose cavity. Certainly the observed accelerations are close to the factor 5 that has been calculated as the expected acceleration for an intramolecular reaction resulting from the loss of a single internal rotation.[8]

Cycloheptaamylose acceleration by conformational restriction is supported by the effects of cyclohexaamylose on decarboxylation. In phosphate buffers where decarboxylation of the anion is prevalent, the relative effects of cyclohexa- and cycloheptaamylose are similar, especially when allowance is made for a generally poorer binding constant for cyclohexaamylose.[15] Therefore, decarboxylation rate constants of the cycloamylose–anion complexes are quite comparable and consistent with similar microsolvent effects for cyclohexa- and cycloheptaamylose.

At pH 3 where the un-ionized acid is the predominant species, cyclohexaamylose does not accelerate decarboxylation, whereas cycloheptaamylose does. As indicated by Table III, cyclohexaamylose actually inhibits decarboxylation. Preliminary experiments to determine the magnitude of cyclohexaamylose inhibition indicate complete inhibition of bound un-ionized acid decarboxylation but implicate some form of aggregation at high cyclohexaamylose concentrations. The nature of this aggregation is being investigated, but the important result for this discussion is that there was no evidence for any cyclohexaamylose acceleration. Since micro-solvent effects for the cyclohexa- and cycloheptaamylose cavities appear to be similar, cyclohexaamylose inhibition is only consistent with benzoylacetic acids binding in nonreactive conformations. Since the primary difference between cyclohexa- and cycloheptaamylose is the 2-Å difference in cavity diameter,[38] the nonreactive conformation could only result from the physical interaction of the cyclic internally hydrogen-bonded conformation with the "wall" of the cavity. This is an extremely large and significant reaction specificity and this mechanism may be very important for enzyme specificity.

The specificity or, more correctly, the absence of specificity for the cycloheptaamylose accelerations k_{c1}/k_1 in the decarboxylations of substituted benzoylacetic acids was surprising. The absence of a correlation between acceleration and binding constant was expected from previous studies of cycloamylose accelerations.[15,27] However, the greatly increased cycloamylose acceleration of phenol release from 3-substituted phenyl esters relative to the 4-substituted compounds was consistent with an increased proximity of the substrate carbonyl with the hydroxyl groups ringing the cycloamylose cavity.[15] A similarly increased proximity resulting in increased conformational restriction was expected for decarboxylations of benzoylacetic acids, but only a very small effect was observed (Table I). A possible explanation is that maximal acceleration resulting from loss of internal rotations[8] is achieved with 4′-substituted benzoylacetic acids, and the increased interaction of the 3′-substituted compounds results simply in looser binding. However, the 4.4×10^3 acceleration of acid-catalyzed lactonization of hydrocoumaric acid resulting from β-*gem*-dimethyl substitution[5] indicates that much greater effects than we achieved should be possible. Therefore, additional examples of cycloamylose-catalyzed intramolecular reactions are currently under investigation.

Acknowledgment. This research was supported by grants from the National Science Foundation.