above to give two esters in the ratio 1:5. Those were separated using the UCON column (140°, 150 ml/min).

The minor product was identified as *trans*-2,3-dimethyl-*syn*-carbethoxymethylenecyclobutane (**16**): ir 2950, etc., 1715, 1673, 1453, 1365, 1335, 1326, 1260, 1235, 1217, 1181, 1115, 1093, 1032, 960, 860, and 830 cm^{-1}; nmr (220 MHz) δ 1.16 (d, J = 7 Hz, 3 H), 1.24, triplet (J = 7 Hz) superimposed on a doublet (J = 7 Hz) at 1.28, total of 6 H, 1.90–2.10 (m, 1 H), 2.12–2.25 (symmetrical multiplet centered at 2.18, 1 H), 2.80–3.05 (m, 2 H), 4.05 (q, J = 7 Hz, 2 H), and 5.48 (3-line multiplet with 2-Hz separation of lines, 1 H); *m/e* 168.1146 (calcd for $C_{10}H_{16}O_2$, 168.1150).

The major product was identified as *trans*-2,3-dimethyl-*anti*-carbethoxymethylenecyclobutane (**17**): ir 2950, etc., 1710, 1160, 1445, 1363, 1330, 1300, 1258, 1238, 1132, 1150, 1112, 1090, 1035, 950, and 847 cm^{-1}; nmr (220 MHz) δ 1.13 (d, J = 7 Hz), 1.24 (t, J = 7 Hz), total of 9 H, 1.94 (5-line multiplet with 7-Hz spacing of lines, 1 H), 2.35–2.60 (m, 2 H), 3.17–3.33 (symmetrical multiplet centered at 3.25, apparently a d of d of t with 17, 8.5, and 3-Hz spacing of lines, 1 H), 4.05 (q, J = 7 Hz, 2 H), and 5.45 (4-line multiplet with 2-Hz spacing of lines, 1 H); *m/e* 168.1146 (calcd for $C_{10}H_{16}O_2$, 168.1150).

***cis*-2,3-Dimethyl-*syn*- and -*anti*-carbethoxymethylenecyclobutane (18 and 19).** *cis*-2,3-Dimethylcyclobutanone (100 mg) was added to the sodium salt of triethylphosphonium acetate as described above to give two esters in the ratio 1:4 which were separated using the UCON column (140°, 150 ml/min).

The minor product was identified as *cis*-2,3-dimethyl-*syn*-carbethoxycyclobutane: ir 2963, etc., 1712, 1670, 1460, 1440, 1422, 1363, 1331, 1291, 1259, 1240, 1212, 1182, 1122, 1095, 1050, 1032, 1012, 960, 945, 878, and 831 cm^{-1}; nmr (220 MHz) δ 1.05 (d, J = 7 Hz, 3 H), 1.18 (d, J = 7 Hz), 1.25 (t, J = 7 Hz), total of 6 H, 2.35–2.65 (m, 2 H), 2.70–2.90 (m, 1 H), 3.40 (m, 1 H), 4.05 (q, J = 7 Hz, 2 H), and 5.44 (4-line multiplet with 2-Hz spacing, 1 H); *m/e* 168.1146 (calcd for $C_{10}H_{16}O_2$, 168.1150).

The major product was identified as *cis*-2,3-dimethyl-*anti*-carbethoxymethylenecyclobutane (**19**): ir 2955, etc., 1713, 1668, 1450, 1440, 1363, 1333, 1258, 1242, 1218, 1185, 1135, 1098, 1032, 945, and 848 cm^{-1}; nmr (220 MHz) δ 1.02, doublet, superimposed on a doublet at 1.03, total of 6 H, 1.24 (t, J = 7 Hz, 3 H), 2.40–2.70 (m, 2 H), 3.00–3.20 (multiplet which appears to be a q of d with 9- and 2.5-Hz spacing between lines, 2 H), 4.05 (q, J = 7 Hz, 2 H), and 5.48 (4-line multiplet with 2-Hz spacing, 1 H); *m/e* 168.1163 (calcd for $C_{10}H_{16}O_2$, 168.1150).

Preparative Pyrolysis of *trans*-2-Methyl-*distal*-4-methylcarbethoxyspiropentane (26). The ester (80 μl) was sealed in a neutralized 50-ml Pyrex tube and heated at 290° for 1 hr. After this time 90% of the starting material had disappeared; two major products were formed in the ratio 56:44. These were isolated by preparative glpc using the UCON column (160°, 150 ml/min).

The major component was identified from its spectral characteristics as *trans*-2,4-dimethylcarbethoxymethylenecyclobutane (**28**): ir 2960, etc., 1720, 1670, 1452, 1370, 1337, 1292, 1262, 1238, 1207, 1185, 1143, 1105, 1040, and 850 cm^{-1}; nmr (220 MHz) δ 1.13 (d, J = 7 Hz, 3 H), 1.24 (t, J = 7 Hz), and 1.30 (d, J = 7 Hz), total of 6 H, 1.82 (m, 2 H), 3.18 (broad multiplet, 1 H), 3.38 (broad multiplet, 1 H), 4.06 (q, J = 7 Hz, 2 H), and 5.44 (unsymmetrical triplet, J = 2 Hz, 1 H); *m/e* 168.1143 (calcd for $C_{10}H_{16}O_2$, 168.1150).

The minor component was identified as *cis*-2,4-dimethylcarbethoxymethylenecyclobutane (**27**): ir 2955, etc., 1717, 1667, 1440, 1363, 1332, 1278, 1260, 1222, 1178, 1095, 1030, and 847 cm^{-1}; nmr (220 MHz) δ 1.20 (d, J = 7 Hz), 1.25 (t, J = 7 Hz), and 1.31 (d, J = 7 Hz) total of 9 H, 1.78 (m, 1 H), 2.46 (q of m, J = 10 Hz, 1 H), 2.87 (broad multiplet, 1 H), 3.27 (broad multiplet, 1 H), 4.06 (q, J = 7 Hz, 2 H), and 5.48 (unsymmetrical triplet, J = 2 Hz, 1 H); *m/e* 168.1156 (calcd for $C_{10}H_{16}O_2$, 168.1150).

Pyrolysis of *cis*- and *trans*-4,5-Dimethylcarbethoxyspiropentane (29 and 21) in Solvent. Pyrolyses were carried out in sealed, neutralized 2-mm Pyrex capillary tubing; xylene was used as an internal standard (see Table IV).

Table IV. Solvent Pyrolysis of **20** and **21** at 290° for 1 hr

Compound	Solvent	% reaction
20	Benzene	58 ± 3
20	Acetonitrile	71 ± 3
21	Benzene	36 ± 3
21	Acetonitrile	40 ± 3

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Cycloamyloses as Enzyme Models. The Decarboxylation of Phenylcyanoacetate Anions

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Abstract: Decarboxylation rate constants in aqueous solution were determined for eight ortho-, meta-, and para-substituted phenylcyanoacetate anions and for the 2-phenyl-2-cyanopropionate and 6-nitrobenzisoxazole-3-carboxylate anions in the presence and in the absence of cycloheptaamylose. The decarboxylation rate constants of four para-substituted phenylcyanoacetate anions were determined in ethanol. In addition, 4-chlorophenylcyanoacetate decarboxylation was examined in methanol, 2-propanol, dioxane, and aqueous 2-propanol and the pH–rate profile was determined for the aqueous reaction in the presence and absence of cycloheptaamylose. Activation parameters were determined for the aqueous, cycloheptaamylose-accelerated, and aqueous 2-propanol decarboxylations of the 4-chlorophenylcyanoacetate anion. All data were consistent with cycloheptaamylose catalysis arising from the solvation change experienced by the carboxylate anion on transfer from an aqueous environment to the cycloheptaamylose complex. The interrelationships among structure, binding constant, and reactivity for the cycloheptaamylose-accelerated reactions were examined and implications for binding and reactivity in enzymatic systems were discussed.

Apolar interactions are considered a major driving force for the binding of small molecules to proteins.[2,3] Supporting evidence includes, for example, the correlation between binding free energy and surface

(1) National Institutes of Health Postdoctoral Fellow, 1969–1971.

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area for aromatic competitive inhibitors of α-chymotrypsin[4] and the correlation between the binding free energy of *N*-acetyl-L-amino acid methyl esters to α-chymotrypsin and the free energy of transfer from water to ethanol of the corresponding amino acids.[5] The binding phenomenon is generally assumed to result in a solvation change for the bound molecule. However, it has been difficult to determine the interrelations among solvation change, structure, binding constants, and reactivity.

Many spectroscopic techniques have been used to estimate the polarity at protein binding sites.[6-9] However, it is possible to correlate binding site polarity, binding constant, and reactivity in only the most qualitative terms. To further investigate these relationships, a reaction was necessary that was (1) unimolecular, (2) highly solvent dependent, (3) devoid of complicating catalysis, and (4) easily monitored. A catalyst was needed that had (1) well-defined binding sites, (2) a known binding stoichiometry, (3) a predominantly apolar binding character, and (4) minimal covalent or electrostatic catalytic effects on the reaction chosen.

The reaction which best met the imposed requirements was the decarboxylation of carboxylic acid anions. Acid anion decarboxylation is unimolecular,[10] the rate constants are extremely solvent dependent,[11,12] the reaction is free from acid and base catalysis except in highly activated systems,[10] and the reaction can be monitored by a wide variety of techniques.

The catalyst chosen was cycloheptaamylose. Cycloheptaamylose consists of seven α-1,4-linked glucoside units and is shaped much like an open-bottomed basket with seven primary hydroxyl groups around the basket bottom and 14 secondary hydroxyl groups around the basket top. This catalyst was chosen to take advantage of the ability to form soluble inclusion compounds,[13] the known structure and stereochemistry,[14] the absence of multiple binding sites, and the absence of a variety of potential catalytic functional groups. The potential involvement of the hydroxyl groups in catalytic action has resulted in a postulated bifunctional catalytic mechanism for cycloamylose-accelerated decarboxylation.[15] Therefore, since hydroxyl participation would limit the objectives of this investigation, the mechanism of catalytic action must be established.

Experimental Section

General. Cycloheptaamylose was obtained from Corn Products Co. and was purified as described previously.[16] Aqueous solutions were made with double-distilled water and reagent grade buffers. Methanol was Allied Chemical absolute; ethanol was commercial absolute grade distilled from magnesium ethoxide;[17] 2-propanol was Merck spectrograde; and dioxane was Mallinckrodt spectrograde. The maximum water impurity present in all organic solvents was 0.05%. Triethylamine was purified by treatment with phenyl isocyanate and distillation.[18] pH measurements were made using a Radiometer 4c pH meter.

Acids. All phenylcyanoacetic acids were prepared by basic hydrolysis of the corresponding ethyl esters. Ethyl phenylcyanoacetate was obtained from Aldrich Chemical Co. Ethyl 2-, 3-, and 4-methylphenylcyanoacetates were prepared according to Hessler[19] from the appropriate phenylacetonitrile (Aldrich Chemical Co.), sodium, and diethyl carbonate in ether. Ethyl 4-methoxy-, 2-chloro-, 4-chloro-, and 4-bromophenylcyanoacetates were prepared from the appropriate phenylacetonitrile (Aldrich Chemical Co.), sodium ethoxide, and diethyl carbonate in toluene.[20] Ethyl 2-phenyl-2-cyanopropionate was prepared by methylation of ethyl phenylcyanoacetate.[21]

The esters were hydrolyzed by stirring with 25 ml of aqueous NaOH per g of ester at room temperature for 1–4 hr. The alkaline solution was extracted with ether, cooled in an ice bath, and acidified with hydrochloric acid to the Congo red indicator endpoint. The product was extracted into ether which was dried over $MgSO_4$, filtered, and evaporated to dryness. The solid product was recrystallized from benzene–petroleum ether.

The following acids could be compared to previous preparations: phenylcyanoacetic acid, mp 89–90° dec (lit.[19] 92° dec); 2-phenyl-2-cyanopropionic acid, mp 99–100° dec (lit.[15b] 98–99° dec); 2-methylphenylcyanoacetic acid, mp 123–124° dec (lit.[22] 122–123° dec); 3-methylphenylcyanoacetic acid, mp 88–89° dec (lit.[22] 86.5–87.5° dec); 4-methylphenylcyanoacetic acid, mp 120–121° dec (lit.[22] 113–114° dec); 2-chlorophenylcyanoacetic acid, mp 105–106° dec (lit.[22] 105–106° dec); and 4-chlorophenylcyanoacetic acid, mp 101–102° dec (lit.[22] 100–101° dec).

4-Methoxyphenylcyanoacetic acid exhibited a melting point with decomposition of 86–86.5°.

Anal. Calcd for $C_{10}H_9NO_3$: C, 62.82; H, 4.74; N, 7.33. Found: C, 62.77; H, 4.63; N, 7.30.

4-Bromophenylcyanoacetate had a melting point with decomposition of 101–102°.

Anal. Calcd for $C_9H_6BrNO_2$: C, 45.03; H, 2.52; Br, 33.28; N, 5.84. Found: C, 44.78; H, 2.40; Br, 33.38; N, 5.78.

Methyl 6-nitrobenzisoxazole-3-carboxylate was prepared from methyl 2,4-dinitrophenylacetate, isoamyl nitrite, and sodium methoxide in methanol according to Borsche.[23] The ester had mp 130° (lit.[23] 130–131). The ester was hydrolyzed in 80% sulfuric acid according to Lindemann and Cissee.[24] 6-Nitrobenzisoxazole-3-carboxylic acid exhibited a melting point with decomposition of 179–180° (lit.[24] 189–190°).

Reaction Kinetics. Rates of decarboxylation were followed spectrophotometrically using a Gilford 220 spectrophotometer with Beckman DU optics or a Cary Model 14 recording spectrophotometer. For phenylcyanoacetate anion decarboxylations, the disappearance of the anion was followed at 230 ± 5 nm where the $\Delta\epsilon$ values between reactants and products were 10^3 to 10^4. 6-Nitrobenzisoxazole-3-carboxylate decomposition was monitored by following the appearance of the 2-cyano-5-nitrophenoxide ion at 402 nm (ϵ at 60°, 2.4×10^3). Near room temperatures, temperature control was maintained at ±0.1° with a Wilkens-Anderson Lo-Temp circulating bath. Higher temperatures were maintained at ±0.2° by use of a Sargent thermonitor controlled-temperature bath with an external circulating pump. All rates were determined using 1-cm glass stoppered silica cells containing 3 ml of solution which had been equilibrated at the reaction temperature for 1 hr.

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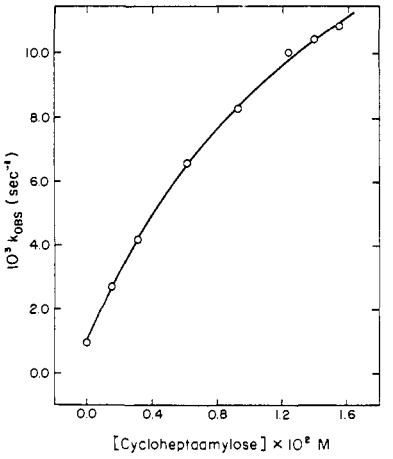

Figure 1. Rate of 4-chlorophenylcyanoacetate anion decarboxylation as a function of increasing cycloheptaamylose concentration at 60.4°, pH 8.6 Tris buffer, ionic strength = 0.1.

Reactions were initiated by the addition of 25 μl of an aqueous or ethanolic stock solution of the acid (0.10 to 0.15 *M*) on the flattened tip of a glass stirring rod through a hole in the cell compartment cover after removal of the cuvette stopper. For reactions with $t_{1/2} > 10$ min, the cuvette stopper was replaced immediately following reaction initiation; for shorter $t_{1/2}$, the stopper was replaced after 15 min. Pseudo-first-order rate constants were evaluated from plots of log $(A_\infty - A_t)$ or log $(A_t - A_\infty)$ *vs.* time where A_∞ was the absorbance after ten half-lives or from Guggenheim plots[25] in cases where reaction rates were very slow.

Determination of Maximum Rate Constants and Dissociation Constants for Cycloheptaamylose-Catalyzed Reactions. Rate constants, k_c, for fully complexed acid anions and the complex dissociation constants, K_s, were determined, assuming Scheme I

Scheme I

$$S + C \underset{K_s}{\rightleftharpoons} C\text{-}S \xrightarrow{k_c} P + C + CO_2$$
$$\downarrow k_{un}$$
$$P + CO_2$$

where S is the anion which can decarboxylate to give product P either spontaneously with rate constant, k_{un}, or following complexation with cycloheptaamylose, C, with rate constant, k_c. With [C] > 10[S] and following the derivation of Colter, *et al.*,[26] an expression for the observed rate constant, k_{obsd}, can be derived. Two forms of the resultant expression are analogous with the well-known Eadie[27] and Lineweaver-Burk[28] expressions for treatment of enzyme kinetic data and are presented in eq 1 and 2. Therefore k_c can be obtained from the intercepts, $k_c - k_{un}$ and $1/(k_c - k_{un})$,

$$k_{obsd} - k_{un} = (k_c - k_{un}) - K_s\frac{(k_{obsd} - k_{un})}{[C]} \quad (1)$$

$$1/(k_{obsd} - k_{un}) = 1/(k_c - k_{un}) + K_s/(k_c - k_{un})[C] \quad (2)$$

respectively, of Eadie or Lineweaver-Burk plots and K_s from the slopes.

Rate constants were obtained using initial acid anion concentrations of *ca.* 10^{-4} *M* and five–ten different concentrations of cycloheptaamylose varying from 10^{-3} to 10^{-2} *M*. Slopes, intercepts, and standard deviations were calculated by a computerized least-squares treatment.

Activation Parameters. Arrhenius activation energies, activation entropies, and free energies of activation were calculated in the usual way[29] from computer-calculated least-squares treatment of plots of log *k vs.* 1/absolute temperature. The thermodynamic parameters for the complex dissociation constant K_s were calculated as before.[30]

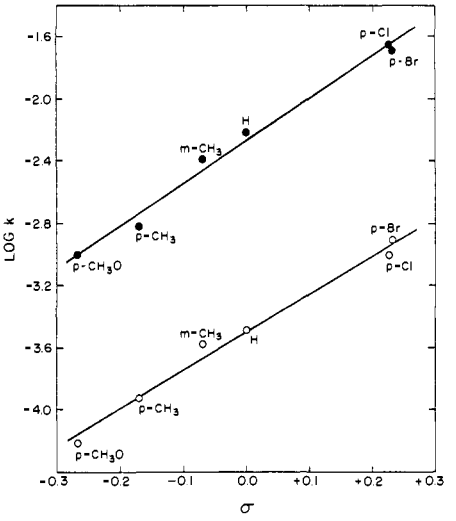

Figure 2. Hammett plots for the cycloheptaamylose-catalyzed (●) and spontaneous aqueous decarboxylation (○) of phenylcyanoacetate anions at 60.4°, pH 8.6 Tris buffer, ionic strength = 0.1. The lines drawn correspond to ρ values of +2.72 for cycloheptaamylose-catalyzed decarboxylation and +2.44 for spontaneous decarboxylation.

Results

Cycloheptaamylose accelerated the decarboxylation rates of all the carboxylate anions examined. However, the rate acceleration was not a linear function of the cycloheptaamylose concentration, as is shown in Figure 1 for the decarboxylation of the 4-chlorophenylcyanoacetate anion. The curve is characteristic of enzyme reactions which follow Michaelis–Menten kinetics[31] and may be linearized by plotting the change in observed rate constant against a function of the cycloheptaamylose concentration if Scheme I (Experimental Section) is assumed. The constants, spontaneous decarboxylation rate, maximum cycloheptaamylose-accelerated rate, and cycloheptaamylose–anion dissociation constant obtained by this treatment are compiled in Table I for eight phenylcyanoacetate anions, the 2-phenyl-2-cyanopropionate and 6-nitrobenzisoxazole-3-carboxylate anions.

An examination of Table I indicates that strong substituent effects exist for both the spontaneous and cycloheptaamylose-catalyzed decarboxylation rates of phenylcyanoacetate anions. The meta- and para-substituted compounds were used to create the Hammett plots[32] shown in Figure 2. The excellence of the Hammett correlation is demonstrated by the correlation coefficient of 0.994 found for each plot. Hammett ρ values of +2.44 and +2.72 were calculated for the spontaneous and cycloheptaamylose-accelerated reactions, respectively.

The Hammett correlation also was examined in absolute ethanol with triethylamine added to ensure ionization of the carboxylic acids. Tripling the triethylamine concentration had no effect upon rates of decarboxylation. The Hammett correlation yielded a ρ of +3.75.

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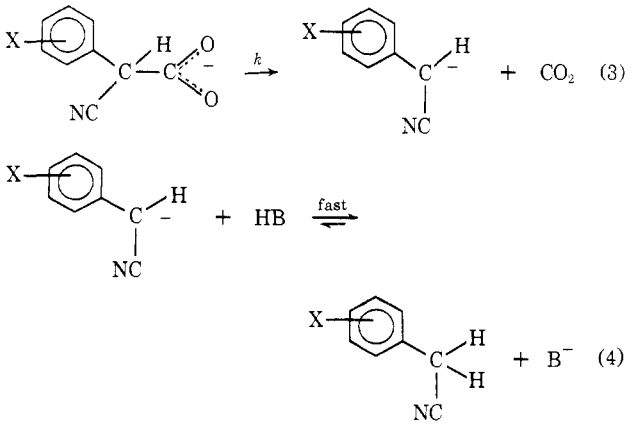
Table I. Rate Constants for the Spontaneous and Cycloheptaamylose-Catalyzed Decarboxylation of Carboxylate Anions

Anion	T, °C	10^3k_{un},[a] sec^{-1}	10^3k_c,[b,c] sec^{-1}	10^3K_s, M[b,c]	k_c/k_{un}	R[d]
			Aqueous Solutions[e]			
Phenylcyanoacetate						
4-CH_3O	60.4	0.0614	0.979 ± 0.052	17.6 ± 1.4	15.9	0.982
4-CH_3	60.4	0.119	1.51 ± 0.08	15.7 ± 1.4	12.7	0.982
3-CH_3	60.4	0.262	4.13 ± 0.26	37.3 ± 3.0	15.8	0.991
2-CH_3	60.4	0.374	4.48 ± 1.09[f]	67.8 ± 16.6[f]	12.0	0.998[f]
H	60.4	0.323	6.03 ± 0.71	39.5 ± 5.9	18.7	0.930
4-Cl	60.4	0.963	22.4 ± 0.6	17.6 ± 0.7	23.3	0.996
4-Cl	45.4	0.0996	3.32 ± 0.25	12.5 ± 1.6	33.3	0.976
4-Cl	35.4	0.0211	0.932 ± 0.045	10.6 ± 0.9	44.2	0.989
2-Cl	60.4	4.87	96.4 ± 6.7	29.8 ± 2.7	19.8	0.979
2-Cl	45.4	0.597	22.7 ± 1.9	31.9 ± 3.6	38.0	0.982
4-Br	60.4	1.21	20.1 ± 0.7	8.54 ± 0.55	16.6	0.990
2-Phenyl-2-cyanopropionate						
	60.4	0.111	0.615 ± 0.037	9.12 ± 1.15	5.5	0.963
	60.0	0.087[g]		8.2[h]	2.8[g]	
6-Nitrobenzisoxazole-3-carboxylate						
	60.4	0.735	2.55 ± 0.04	16.0 ± 0.5	3.5	0.999
			Absolute Ethanol[i]			
Phenylcyanoacetate						
4-CH_3O	25.0	0.0602				
4-CH_3	25.0	0.175				
H	25.0	0.584				
4-Cl	25.0	4.76				
			57.7% by Weight 2-Propanol[e]			
4-Chlorophenylcyanoacetate						
	60.4	24.4				
	45.4	3.95				
	35.4	1.07				
			62.6% by Weight 2-Propanol[e]			
4-Chlorophenylcyanoacetate						
	60.4	28.9				
	45.4	4.62				
	35.4	1.31				
			74.0% by Weight 2-Propanol[e]			
4-Chlorophenylcyanoacetate						
	60.4	42.4				
	45.4	6.78				
	35.4	1.79				

[a] Average of two–five determinations. Maximum deviation, ±2%. [b] Calculated from a least-squares treatment of eq 1 (Experimental Section) except where indicated. [Cycloheptaamylose] = 10^{-3} to 1.5×10^{-2} M. [c] Errors are standard deviations. [d] Correlation coefficient for the plot used to calculate k_c and K_s. [e] pH 8.6 Tris buffer; ionic strength = 0.1; [anion] = 10^{-4} M. [f] Equation 2 (Experimental Section) used. [g] Reference 15b. [h] Reference 15c. [i] [Acid] = 10^{-4} M, 3.6×10^{-3} M triethylamine added to ensure ionization.

This ρ value cannot be compared directly with the ρ values obtained from Figure 2 since different temperatures, 25.0° for ethanolic and 60.4° for aqueous solutions, were employed. However, these values are consistent with the generalization that ρ values become more positive as the solvent dielectric constant is decreased.[32] Also, these ρ values are consistent with the



accepted mechanism of thermal decarboxylation[10] (eq 3 and 4), a rate-determining heterolytic cleavage of the carbon–carboxyl carbon bond, a process favored by electron-withdrawing substituents.

If, instead of specific rate constants, the maximum cycloheptaamylose acceleration, k_c/k_{un}, is examined for phenylcyanoacetate decarboxylation as a function of phenyl substituent (Table I), a very small effect is observed. Therefore, the maximum cycloheptaamylose acceleration exhibits little or no specificity as to size, polarity, or position of the substituent.

An examination of the phenylcyanoacetate–cycloheptaamylose dissociation constants listed in Table I indicates a lack of dependence on the electron-withdrawing power of the phenyl substituent, K_s(4-CH_3) ≈ K_s(4-Cl). However, there is an obvious specificity as to both the size, K_s(H) > K_s(4-Cl) > K_s(4-Br), and position, K_s(2-CH_3) > K_s(3-CH_3) > K_s(4-CH_3), of the phenyl substituent.

When the α position of the phenylcyanoacetate anion is methylated to give the 2-phenyl-2-cyanopropionate anion or the seemingly similar 6-nitrobenzisoxazole-3-carboxylate anion is prepared, a definite decrease in cycloheptaamylose effectiveness is observed (Table I). The α-methyl substituent effect is due apparently only to electronic effects, since both the spontaneous decarboxylation rate constant and the maximum cyclo-

Table II. Solvent Effect on Acid Anion Decarboxylation

Solvent	4-Chlorophenylcyanoacetate k_{obsd}, sec^{-1} [a]	4-Chlorophenylcyanoacetate k_{obsd}/k_{H_2O}	6-Nitrobenzisoxazole-3-carboxylate k_{obsd}, sec^{-1} [b]	6-Nitrobenzisoxazole-3-carboxylate k_{obsd}/k_{H_2O}
Water	3.49×10^{-6} [c]	1.0	7.3×10^{-6}	1.0
Cycloheptaamylose		23.3 [d]		3.5 [d]
Methanol	1.42×10^{-3}	407	2.5×10^{-4}	34.0
Ethanol	4.76×10^{-3}	1360	1.0×10^{-3}	140.0
2-Propanol	9.19×10^{-3}	2630		
Dioxane	9.76×10^{-3}	2800		

[a] $T = 25.0°$, average of three determinations. Organic solvents contained 3.6×10^{-3} M triethylamine to generate anion. [b] $T = 30°$, data from ref 12. [c] Calculated from activation parameters (Table III). [d] Ratio at 60.4° (Table I).

heptaamylose acceleration, k_c/k_{un}, are decreased threefold, even though the dissociation constant for the cycloheptaamylose complex is fourfold better.

A direct comparison between 6-nitrobenzisoxazole-3-carboxylate and phenylcyanoacetate decarboxylation rates is difficult due to the structure differences involved. However, 6-nitrobenzisoxazole-3-carboxylate decarboxylation is known to be accelerated in the presence of micelles[33] or in nonaqueous solvents.[12] Therefore, the relative solvent effects for 4-chlorophenylcyanoacetate and 6-nitrobenzisoxazole-3-carboxylate were determined (Table II). The relative decarboxylation rates of 4-chlorophenylcyanoacetate in methanol and ethanol are seen to be about ten times greater than the corresponding 6-nitrobenzisoxazole-3-carboxylate rates, a factor similar to the relative cycloheptaamylose accelerations.

The effect of 2-propanol was examined in greater detail for spontaneous and cycloheptaamylose-accelerated 4-chlorophenylcyanoacetate decarboxylation. The results are shown in Figure 3. The spontaneous reaction rate rapidly increases with increasing 2-propanol concentration, but cycloheptaamylose accelerated rate is seen to be equivalent to the decarboxylation rate in ~55% 2-propanol.

To test the similarity between cycloheptaamylose-accelerated decarboxylation and decarboxylation rates in mixed 2-propanol–water solutions, the temperature dependence was determined for 4-chlorophenylcyanoacetate decarboxylation in water, in the presence of cycloheptaamylose, and in three 2-propanol–water solutions. The results are summarized in Table I. A large temperature dependence is noted for both the decarboxylation rates and accelerations due to the presence of either cycloheptaamylose or 2-propanol. These data were used to calculate the activation parameters presented in Table III. Significantly, activation energies and entropies for the cycloheptaamylose and mixed solvent reactions are nearly identical. Also it is clear that the rate enhancement caused by cycloheptaamylose or 2-propanol is due to a greatly decreased activation energy, while the activation entropy partially negates the activation energy change.

The data in Table I were also used to calculate thermodynamic parameters for K_s. The standard enthalpy, 4.2 ± 0.4, and the standard entropy, 4.5 ± 1.4 (the errors are standard deviations), calculated are consistent with values normally associated with cycloamylose complex formation.[30]

The pH dependencies for aqueous and cycloheptaamylose-accelerated 4-chlorophenylcyanoacetate decarboxylation rates were determined and are summarized in Table IV. The rate parameters examined, k_{un}, k_c, and K_s, are seen to be independent of pH over

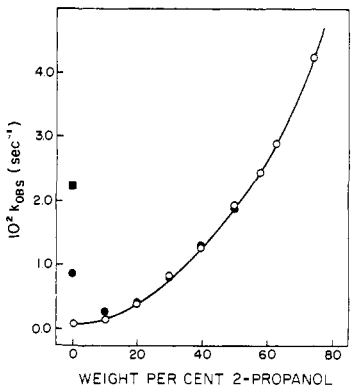


Figure 3. The rate constant for 4-chlorophenylcyanoacetate anion decarboxylation in the absence (○) and presence (●) of 10^{-2} M cycloheptaamylose as a function of 2-propanol concentration; 60.4°, 0.075 M Tris, 0.025 M Tris·HCl, 0.075 M KCl. ■ is the rate constant for decarboxylation of the anion–cycloheptaamylose complex.

Table III. Activation Parameters for 4-Chlorophenylcyanoacetate Decarboxylation

Solvent	E_a, kcal/mol[a]	$\Delta S^{\ddagger}$, eu[b,c]	$\Delta F^{\ddagger}$, kcal/mol[b]
Water	31.3 ± 0.5	19 ± 2	25.0 ± 1.1
Cycloheptaamylose[d]	26.1 ± 0.6	10 ± 2	22.5 ± 1.2
57.7 wt % 2-propanol	25.6 ± 0.1	8.6 ± 0.1	22.4 ± 0.2
62.6 wt% 2-propanol	25.3 ± 0.3	8.2 ± 1.0	22.3 ± 0.6
74.0 wt% 2-propanol	25.9 ± 0.1	10.6 ± 0.2	22.1 ± 0.2

[a] Errors are standard deviations. [b] Evaluated at 60.4°. [c] Errors calculated from standard deviation for y intercept of Arrhenius plot. [d] k_c.

Table IV. pH–Rate Data for p-Chlorophenylcyanoacetate Decarboxylation at 60.4°

pH[a]	$10^3 k_{un}$, sec^{-1}	$10^3 k_c$, sec^{-1} [b]	$10^3 K_s$, M[b]	R[c]
3.97	0.858	23.4 ± 2.5	20.4 ± 3.2	0.966
5.01	1.00	22.6 ± 2.0	16.3 ± 1.9	0.980
6.82	0.941	19.7 ± 1.1	13.0 ± 1.2	0.987
8.63	0.963	22.4 ± 0.6	17.6 ± 0.7	0.996
9.96	0.960	21.6 ± 2.0	15.0 ± 2.2	0.969
11.5[d]	0.891	22.2 ± 2.0	23.2 ± 2.9	0.977

[a] Measured at 25°; buffer salts are formate, acetate, phosphate, Tris, carbonate, hydroxide; $\mu = 0.1$. [b] Evaluated using eq 1, Experimental Section. Errors are standard deviations. [c] Correlation coefficient for plot used to determine k_c and K_s. [d] pH varied from 11.7 to 11.3 as cycloheptaamylose concentration was increased from 1.5×10^{-3} M to 1.5×10^{-2} M.

(33) C. A. Bunton and M. J. Minch, *Tetrahedron Lett.*, 3881 (1970).

the entire range, pH 4–12, examined. Extensions of the pH–rate profiles to more acidic solutions were not feasible due to the acid instability of both cycloheptaamylose[34] and the decarboxylation product, 4-chlorophenylacetonitrile. The instability of the decarboxylation product to acid was determined by simultaneous runs with 4-chlorophenylcyanoacetic acid and 4-chlorophenylacetonitrile in 0.1 *N* hydrochloric acid. The ultraviolet absorption change due to the nitrile reaction was substantially larger than that due to the acid reaction, which prevented accurate separation and evaluation of decarboxylation rates. The absence of an effect attributable to 4-chlorophenylcyanoacetic acid ionization in the pH region studied was not unexpected, since phenylcyanoacetic acid is known to have a pK_a of 1.88.[35]

Discussion

The Mechanism of Cycloheptaamylose-Accelerated Decarboxylation. Cycloheptaamylose accelerated the decarboxylation rates of all the acid anions examined (Table I). The kinetic data are consistent with the mechanisms of eq 3 and 4 and Scheme I, simultaneous decarboxylation of the anion and complexation with cycloheptaamylose followed by decarboxylation, These data are also compatible with a previous study of cycloamylose-accelerated decarboxylation.[15] However, the conclusions drawn by the two studies are quite different.

From their study of cycloamylose-accelerated decarboxylation, Cramer and Kampe favored a mechanism for the catalytic action of cycloamylose involving a general base-catalyzed nucleophilic attack by a cycloamylose oxygen at the carboxyl carbon of an acid anion assisted in β-keto and α-cyano acid anion decarboxylations by hydrogen bonding between the keto or cyano group and a cycloamylose hydroxyl group.[36] This mechanism was based on the following: (1) the known basicity of cycloamyloses as exemplified in diarylpyrophosphate hydrolysis[37] and phenyl ester cleavage;[16] (2) the observed substrate specificity; and (3) the large favorable decrease in activation energy for the cycloamylose reaction relative to the spontaneous aqueous reaction and the corresponding unfavorable decrease in the entropy of activation.

The proposed mechanism predicts the observation of general base catalysis. However, the pH–rate profile of 4-chlorophenylcyanoacetate decarboxylation (Table IV) shows an independence from spontaneous and cycloheptaamylose-catalyzed decarboxylations to buffer and pH from pH 4 to 12, a region which includes the pH used in the previous study.[15b] Therefore, there is negligible general base catalysis in cycloamylose-catalyzed decarboxylation.

From the study of β-keto, trihaloacetic, and α-cyano acid anion decarboxylations, the prior investigation found specificity for cycloamylose catalysis.[15] However, the experimental procedure involved an invariance of both anion and cycloamylose concentration, so it was not possible to obtain the cycloamylose catalytic constants and the binding constants. We have also observed a catalytic specificity for anion structure. However, by separation of k_c and K_s, the nature of the specificity could be examined further. The specificity in k_c/k_{un} is not dependent upon a fit of substrate and catalyst as is found for reactions involving covalent cycloamylose participation. For example, in cyclohexaamylose-accelerated phenyl ester cleavage, k_c/k_{un} for 2-, 3-, and 4-methylphenyl acetate at pH 10.6 and 25° are 19, 95, and 3.6,[16] respectively. In the cycloheptaamylose-catalyzed 2-, 3-, and 4-methylphenylcyanoacetate decarboxylations, k_c/k_{un} are identical within experimental error.

The small increase in k_c/k_{un} with substituent electron-withdrawing ability in phenylcyanoacetate decarboxylation is consistent with the solvent variation of Hammett ρ values in 6-nitrobenzisoxazole-3-carboxylate decarboxylation.[12] The relative k_c/k_{un} values for 4-chlorophenylcyanoacetate and 6-nitrobenzisoxazole-3-carboxylate decarboxylations are consistent with the relative solvent effects. Finally, the activation parameters for decarboxylation of the cycloheptaamylose complex and in 50–70% 2-propanol–water are identical within experimental error. Similarly, Thomson[22] has found that activation parameters for decarboxylation in 60–80% dioxane correspond to the activation parameters reported by Cramer and Kampe.[15] Therefore, it can be concluded that the catalysis of decarboxylation by cycloheptaamylose is solely a result of a milieu change occurring on complex formation, *i.e.*, the microsolvent[38] effect of cycloheptaamylose.

Cycloheptaamylose binding specificity was observed for gross structural changes and for substituent size and location, but notably less binding specificity was based on substituent electronegativity. This is consistent with previous studies of cycloamylose binding.[13–16] However, the relationship between binding and catalysis for decarboxylation enables the following conclusions to be drawn. (1) Since decarboxylation rates of the cycloheptaamylose–anion complexes are independent of the respective binding constants, catalytic and binding mechanisms are totally independent phenomena. (2) Since all the cycloheptaamylose accelerations can be correlated assuming the same solvent effect, all the complexed anions must have identical local environments. (3) Therefore, if binding occurs by inclusion of the substrate phenyl group in the cycloheptaamylose cavity,[13–16] then, due

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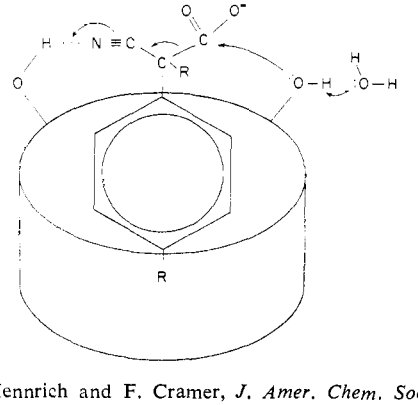
(36) The decarboxylation mechanism postulated by ref 15 is more easily drawn than described and the figure is included here for the sake of clarity.



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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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