temperature for 3 days. The reaction mixture was filtered, diluted with water, and extracted with ether. The ether extract was removed to leave 525 mg of a clear, colorless oil. This material was separated into three components by preparative thin layer chromatography on silica gel G. Fraction 1, 17 mg (3%), was not further investigated. Component 2, 251 mg (48%), was a transparent, colorless oil which was determined to be **8** from its infrared and nmr spectra. Component 3, 145 mg (28%), was a white solid which proved to be **7**.

Determination of the Acetolysis Rate of 28. The rate of acetolysis was followed spectrophotometrically by measuring the decrease in absorbance of **28** (λ_{max} 261 mμ) with time. A stock solution of 25 ml of acetic acid which was 1.50×10^{-3} *M* in **28** and 1.50×10^{-3} *M* in anhydrous sodium acetate was prepared, *ca.* 3 ml of the solution placed in a uv cell and allowed to equilibrate to the temperature inside the sample chamber of the Beckman DU spectrophotometer (**29**). The reference cell was filled with pure acetic acid. Table I gives the measurements which were taken in a sample run. From the data in Table I, a plot of $-\ln (D - D_\infty)$ *vs.* time gave a straight line the slope of which gave $k = 2.03$ hr^{-1}. The average value of k obtained from three runs was 1.99 hr^{-1}.

Acetolysis of 3β-Methoxy-D-norandrostanyl 16α-*p*-Toluenesulfonate (31). A solution of 695 mg (1.56 mmoles) of **31** in 0.5 *M* potassium acetate–acetic acid (50 ml) was stirred at 120° under nitrogen for 3 days. The reaction mixture was diluted with water and extracted with ether. The ethereal solution was washed with 10% sodium bicarbonate, then with water, dried ($MgSO_4$), filtered, and the ether removed to leave 490 mg of a slightly yellow oil. After column chromatography of this material on Florisil, two fractions were obtained. Fraction 1 was 125 mg (25%) of a clear, slightly colored oil which showed a complex number of absorptions in the nmr. This material appeared to be a mixture of several compounds and was not further investigated. Fraction 2 was 292 mg (60%) of a clear, colorless oil, **18** which was found to be identical with the acetate obtained by treating **17** with acetic anhydride in pyridine: infrared (CCl_4) 1740 (C=O), 1260, 1245 (ester C—O), and 1100 cm^{-1} (OCH_3); nmr (CCl_4) τ 4.80 (s, 1, olefinic *H*), 5.00 (m, 1, C*H*OAc), 6.70 (s, 3, O*CH*$_3$), 6.90 (m, 1, C_3-*H*), 8.02 (s, 3, CO*CH*$_3$), 8.80 (d, J = 6 cps, 3, CH-*CH*$_3$), 9.15 (s, 3, C_{10}-*CH*$_3$), and the remaining ring protons at 7.7–9.0; mass spectrum (70 eV) *m/e* (relative intensity) 334 (2), 319 (1), 302 (1), 292 (1), 290 (1), 274 (100), 259 (4), 242 (10), 227 (6), 213 (2), 202 (3), 187 (3), 173 (2), 159 (3), 148 (30), 134 (25), 119 (6), 107 (30), 91 (11), 81 (10), 79 (14), 71 (6), 68 (9), 55 (6), 43 (40), and 29 (2).

Table I

Time, sec × 10^2	D	$D - D_\infty$	$-\ln (D - D_\infty)$
0	1.333	0.356	1.033
1.25	1.317	0.340	1.079
2.14	1.298	0.321	1.136
3.25	1.287	0.310	1.171
3.86	1.273	0.296	1.217
4.53	1.263	0.285	1.255
6.08	1.245	0.268	1.317
8.61	1.216	0.239	1.431
1.294	1.173	0.196	1.630
1.882	1.123	0.146	1.924
2.602	1.077	0.100	2.302
3.657	1.033	0.056	2.882
4.904	1.003	0.026	3.650
5.008	1.000	0.023	3.772
5.620	0.990	0.013	4.343
	0.977		

Cycloamyloses as Enzyme Models. Effects of Inclusion Complex Formation on Intramolecular Participation

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Abstract: Cycloheptaamylose forms complexes with mono-*p*-carboxyphenyl esters of 3-substituted glutaric acids and markedly depresses the rates of intramolecular carboxylate ion attack. However, cycloheptaamylose does not form any covalent intermediates in the reaction; it is involved only as a binding site. The sensitivity of the rate depressions to added cycloheptaamylose depends primarily upon the reactivities of the complexes. The 3-methyl, 3,3-dimethyl, and 3-isopropyl glutarate esters form complexes which show little or no reactivity compared to the free esters. The complex of the 3-phenyl glutarate ester, however, is only slightly less reactive than the free 3-phenyl glutarate ester. There appears to be no relationship between the relative reactivities of the free glutarate esters and those of the complexed esters. Consequently, the specificity of binding in these complexes can induce large changes in overall relative reactivity. This model study supports the idea that specificity in enzymic reactions may result from the geometry of binding the substrates to the enzymes, rather than resulting from the catalytic reactions themselves.

A major problem in enzymology is to explain the specificities observed in enzymic reactions. A number of hypotheses have been proposed[3] to account for specificity, but an evaluation of the importance of each of these ideas is difficult. The experimental evidence which model studies furnish in support or rejection of these various proposals involves only kinetic data. These data are very useful for discussing ideas about mechanisms of enzymic reactions, but they seldom contribute to an understanding of specificity. The main problem is to separate the study of binding from the catalytic reactions themselves in order to evaluate each factor independently.

Cycloamyloses have been utilized as enzyme models because of their ability to form inclusion complexes.[4-6]

(1) National Institutes of Health Postdoctoral Fellow, 1967–1969.

(2) National Institutes of Health Predoctoral Fellow, 1964–1967.

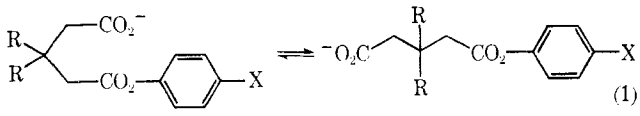
(3) A number of reviews are available which explore these proposals: D. E. Koshland, Jr., and K. E. Heet, *Ann. Rev. Biochemistry*, **37**, 359 (1968); W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill Book Co., Inc., New York, N. Y., 1969, p 282; M. L. Bender and F. J. Kezdy, *Ann. Rev. Biochemistry*, **34**, 49 (1965).

(4) (a) For a recent review, see F. Cramer and H. Hettle, *Naturwissenschaften*, **54**, 625 (1967); also see (b) D. French, *Advan. Carbohydrate Chem.*, **12**, 189 (1957); (c) J. A. Thoma and L. Stewart in "Starch:

These complexes can be used as models of enzyme–substrate complexes. For example, recently it was reported from this laboratory[7] that the effects of cycloamyloses on the rates of phenoxide release from substituted phenyl acetates and benzoates resemble certain enzymic catalyses in several ways: reactivity is more sensitive to the position of the substituent than to its electronic nature, with *m*-substituted esters showing greater reactivity than *p*-substituted esters; several aspects of the kinetics are similar to enzyme kinetics, including saturation, competitive inhibition, and nonproductive binding. Accelerations of up to 300-fold have been observed for phenoxide release. Hennrich and Cramer[8] have shown that cycloamyloses also accelerate the decomposition of diaryl phosphates by factors up to 200. In both of these studies, the cycloamyloses are involved covalently with resultant formation of acyl-cycloamylose intermediates.

The present model study was initiated to help in examining the problem of specificity. Since cycloamyloses are rigid molecules which can be used as binding sites in the formation of inclusion complexes, they appeared to offer a convenient tool to separate the study of specificity of binding from the catalytic reaction. In particular, we have studied the effects of inclusion-complex formation on some intramolecular reactions in which binding was not expected to be followed by covalent participation of the cycloamylose. It was anticipated that such studies might give some insight into the relationship between the specificity of reactions and the geometry of binding.

The main systems examined in this study were mono-*p*-carboxyphenyl esters (pCP esters) of 3-substituted glutaric acids. Bruice and Bradbury have shown[9] that the pH-independent rates of intramolecular carboxylate ion attack in mono esters of 3-substituted glutaric acids are sensitive to the size of the 3-substituent. This sensitivity was interpreted as a measure of changes in the ground-state distribution of reactive and nonreactive conformers as the 3-substituents were varied (eq 1). The main questions of the present



(1)

study are: do the cycloamyloses complex preferentially with one conformer and thus change the ground-state distribution, and is there specificity in the binding which depends upon the geometry of the complexes?

Experimental Section

Cyclohexaamylose and cycloheptaamylose were purified as described previously.[7] All reaction rates were determined in aqueous solutions made from double-distilled water and reagent grade buffers. Solutions of esters were prepared fresh in acetonitrile (Mallinckrodt, Nanograde). pH measurements were made on Radiometer 4c or Corning Model 12 pH meters.

The pCP esters of 3-substituted glutaric acids were prepared by treating the corresponding anhydrides with the disodium or dipotassium salts of *p*-hydroxybenzoic acid, using a procedure similar to that of Bruice and Pandit.[10] 3-Methyl and 3-isopropylglutaric acids and 3,3-dimethylglutaric anhydride are commercially available (Aldrich Chemical Co.). 3-Phenylglutaric acid was prepared by the method of Altschul, Bernstein, and Cohen.[11]

Mono-*p*-carboxyphenyl ester of 3,3-dimethylglutaric acid showed mp at 153–154°. *Anal.* Calcd for $C_{14}H_{16}O_6$: C, 59.99; H, 5.75. Found: C, 59.98; H, 5.74. Mono-pCP ester of 3-methylglutaric acid exhibited mp at 167.8–168.5°. *Anal.* Calcd for $C_{13}H_{14}O_6$: C, 58.65; H, 5.29. Found: C, 58.77; H, 5.32. Mono-pCP ester of 3-phenylglutaric acid had a mp of 167.5–169°. *Anal.* Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.77; H, 5.07. Mono-pCP ester of 3-isopropylglutaric acid showed mp of 134–135°. *Anal.* Calcd for $C_{15}H_{18}O_6$: C, 61.22; H, 6.16. Found: C, 61.32; H, 6.06.

o-Hydroxy-*cis*-cinnamic acid and 2-hydroxy-5-nitro-*cis*-cinnamic acid were prepared by basic hydrolysis of coumarin and 6-nitrocoumarin, respectively, in the dark. Stock solutions of the acids were prepared in mild alkaline solutions and were used directly in studies of the effects of cycloamyloses on the lactonization reactions in acid. *o*-Hydroxymethylbenzoic acid was similarly prepared from phthalide. 6-Nitrocoumarin was synthesized from coumarin using the procedure of Morgan and Micklethwait,[12] mp 181–182° (lit.[13] mp 185°).

Methyl *o*-formylbenzoate was made by methylation of the acid, according to the procedure of Auwers and Heinze,[14] bp 141–144° (14 mm) (lit.[15] bp 136–138° (13 mm)). Methyl-5-nitro-2-formylbenzoate was prepared according to the procedure of Wegscheider and Dubrav,[16] mp 84.5–85° (lit.[16] mp 85–86°). *o*-Acetoxybenzaldehyde was prepared by acetylation of salicylaldehyde giving product mp 39–40° (lit.[17] mp 36–37°).

Reaction Kinetics. Rates of hydrolysis were determined spectrophotometrically using either a Gilford 220 with Beckman DU optics, or a Cary Model 14 recording spectrophotometer. Temperature control was maintained ±0.1° with Wilkens-Anderson Lo-Temp circulating baths. All rates were run in 1-cm silica cells containing 3 ml of solution. Reactions were initiated by adding 20 μl of the stock solutions of the esters in acetonitrile on the tip of a flattened stirring rod. Pseudo-first-order rate constants were computer calculated from the integrated first-order rate equation, or from Guggenheim[18] plots in cases where the rates were very slow. Correlation coefficients of 0.999 were considered acceptable for the least-squares plots.

Kinetic Determination of the Dissociation Constants. The dissociation constants, K_{diss}, for inclusion complexes formed between cycloheptaamylose and the mono-pCP esters of 3-substituted glutaric acids were determined kinetically at temperatures between 20 and 60°. Rates were measured in pH 9.4 buffers, [buffer] = 0.0865 *M*, μ = 0.2 (adjusted with KCl), by following release of the *p*-carboxyphenoxide anion at 280 mμ. Generally, for each ester and at each temperature, 2–4 runs were made at each of 5–7 concentrations of cycloheptaamylose, in the range 10^{-3}–10^{-2} *M*. Buffers were equilibrated for at least 30 min before initiation of the reactions. The initial ester concentration was *ca.* 10^{-4} *M*. The values for K_{diss} were obtained as follows: assuming Scheme I where substrate (S) can directly give product (P) by rate constant k_{un}, or can complex with cycloheptaamylose (C) to form an inclusion com-

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(5) Cycloamyloses and cyclic α-1,4-linked poly-D-(+)-glucose molecules; the 6-unit polymer is named cyclohexaamylose (also called α-cyclodextrin), and the 7-unit polymer is named cycloheptaamylose (or β-cyclodextrin). These are commercially available from Corn Products Co.

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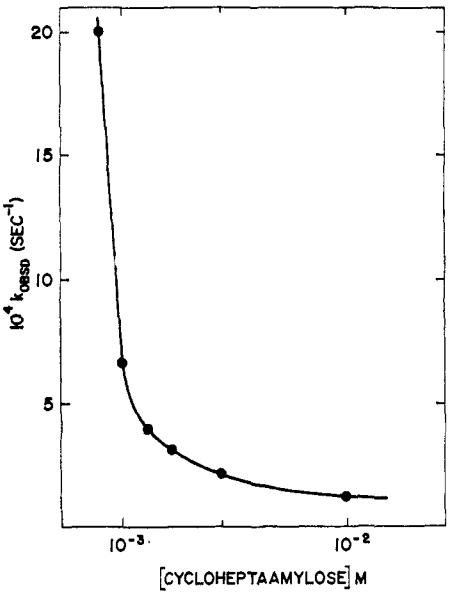


Figure 1. Rate of hydrolysis of the mono-pCP ester of 3,3-dimethylglutaric acid as a function of increasing cycloheptaamylose concentration; 30°, pH 9.4 carbonate buffer, $\mu = 0.2$.

Scheme I

$$\begin{array}{l} S + C \underset{K_{diss}}{\rightleftharpoons} C\text{–}S \xrightarrow[k_c]{} P + C \\ \quad\downarrow k_{un} \\ \quad P \end{array}$$

plex which can go to product by rate constant k_c, it can be shown that the experimentally observed rate constant, k_{obsd}, has the following form

$$k_{obsd} = \left[\frac{k_{un}}{1 + \dfrac{[C]}{K_{diss}}} + \frac{k_c[C]}{K_{diss}\left(1 + \dfrac{[C]}{K_{diss}}\right)}\right]$$

Rearrangement gives the following equation which is similar to

$$[k_{obsd} - k_{un}] = -K_{diss}\left[\frac{k_{obsd} - k_{un}}{[C]}\right] + k_c - k_{un}$$

that previously[7] used. Computer calculated least-squares slopes from plots of $[k_{obsd} - k_{un}]$ against $[k_{obsd} - k_{un}]/[C]$ give the dissociation constants $-K_{diss}$. The rate constants of the complexes, k_c, are obtained from the intercepts of these plots. These are only approximate values for k_c because of the extrapolations involved. The precision of the data increase as $1/K_{diss}$ and k_{un}/k_c increase.

Determination of the Thermodynamic Parameters for Complex Formation. Computer-calculated least-squares slopes of plots of log K_{diss} against reciprocal absolute temperature were used to determine the temperature dependence of the dissociation constants for the complexes between cycloheptaamylose and the mono-pCP esters. From the van't Hoff relationship, $d[\ln K]/(d(1/T) = -\Delta H°/R$, and from the assumption of negligible heat capacity differences between reactants and products,[7] the enthalpy change $\Delta H° = -R$(slope) and the entropy change $\Delta S° = R$(intercept). The error limits for $\Delta H°$ were determined from the standard deviations of the slopes, while the error limits for $\Delta S°$ were calculated from the extremes of the errors in the slopes.

Results

Effects of Cycloheptaamylose on Hydrolysis of Mono-pCP Esters of 3-Substituted Glutaric Acids. Cycloheptaamylose markedly depresses the rates of intramolecular carboxylate ion attack in mono-pCP esters of 3-substituted glutaric acids. However, the rate decrease is not a linear function of the cycloheptaamylose concentration, as shown in Figure 1 for the mono-pCP ester of 3,3-dimethylglutaric acid. A hyperbolic curve characteristic of enzyme reactions which follow Michaelis–Menten kinetics is observed with one difference; the curve for enzyme systems approaches a maximum value, V_{max}, when all of the enzyme is saturated with substrate,[19] while the cycloheptaamylose complexes with these mono-pCP esters approach minimum values, V_{min}, when all of the ester is in the complexed state. The rates are pH-independent below pH *ca.* 10 both in the absence and presence of cycloheptaamylose. Consequently, only the intramolecular carboxylate ion attack is occurring. Cycloheptaamylose is involved only as a binding site; it does not affect the reaction mechanism by forming covalent intermediates.

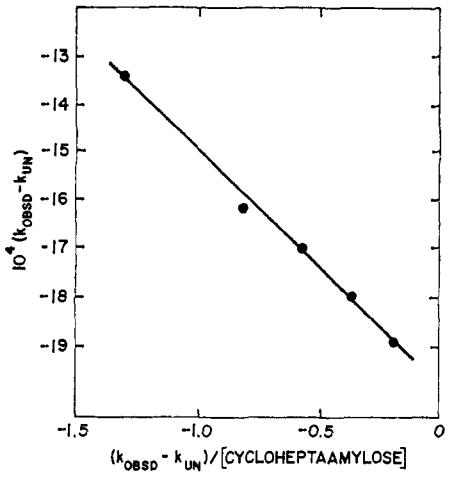


Figure 2. Linear plot of the rate data of Figure 1; $(k_{obsd} - k_{un})$ is the difference in rate in presence and absence of added cycloheptaamylose. This linear relationship is derived from Scheme I, Experimental Section.

The curve of Figure 1 is converted into a straight line by plotting the change in k_{obsd} against a function of the cycloheptaamylose concentration according to Scheme I (Experimental Section). This is shown in Figure 2 for the mono-pCP ester of 3,3-dimethylglutaric acid. The rate constants in the absence of cycloheptaamylose, k_{un}, for several mono-pCP esters are given in Table I along with the rate constants, k_c, of the complexed esters. For the 3,3-dimethyl and 3-methyl esters, k_c is very small compared to k_{un} (expressed as $k_c/k_{un} \times 100$ in Table I). Because of the extrapolations involved in obtaining k_c, the values of k_c for these two esters are, within experimental error, close to zero. The values of Table I will be considered real for purposes of discussion. The reactivity of the complex with the 3-isopropyl ester is somewhat greater than the reactivities of the 3,3-dimethyl and 3-methyl esters, although k_c is much less than k_{un} for this ester also. In contrast with these results, the 3-phenyl ester shows a rather small difference in reactivity between the free ester and the complexed ester.

(19) The Michaelis–Menten scheme in its simplest form is represented as

$$E + S \underset{}{\overset{K}{\rightleftharpoons}} E\cdot S \xrightarrow{k_{cat}} P + E;\quad V = \frac{V_{max}[S]}{K_m + [S]}$$

where V = rate; V_{max} = maximum rate when all of the enzyme E is saturated with substrate S; K_m = Michaelis–Menten constant related to the dissociation constant K.

Table I. Rates of Hydrolysis of Mono-*p*-carboxyphenyl Esters of 3-Substituted Glutaric Acids

3-Substituent	Temp, °C	$10^4 k_{un}$, sec^{-1} [a]	$10^4 k_c$, sec^{-1} [b]	k_c/k_{un} × 100, [c] %	$10^4 K_{diss}$, M
3,3-Dimethyl	20.0	8.33	0.18	(2.2)	2.4
	30.0	20.1	0.40	(2.0)	4.6
	40.0	46.6	2.3	(4.9)	5.7
	50.0	133	4.0	(3.0)	7.1
	60.0	315	7.0	(2.2)	10.3
3-Isopropyl	30.0	21.6	1.5	(7.0)	14.5
	40.0	51.4	4.2	(8.1)	19.7
	50.0	113	14.8	(13.1)	21.2
	60.0	255	27.0	(10.6)	33.0
3-Phenyl	30.0	1.96	0.47	(24.0)	13.1
	40.0	5.41	1.69	(31.3)	17.8
	50.0	15.6	4.5	(28.8)	24.5
	60.0	36.2	14.5	(40.0)	27.8
3-Methyl	30.0	4.54	0.04	(0.88)	32.8
	40.0	11.8	−0.5	(−4.2)	46.4
	50.0	28.1	0.2	(0.71)	47.9
	60.0	65.9	2.3	(3.5)	56.7

[a] k_{un} is the pseudo-first-order rate constant, pH 9.4, in absence of cycloheptaamylose. [b] k_c is the rate constant for complexed ester. These are approximate values because lengthy extrapolations are required if K_{diss} is large (see Experimental Section). $V_{min} = k_c$ [complex]. [c] $k_c/k_{un} \times 100$ represents the approximate per cent reactivity remaining when the ester is fully complexed.

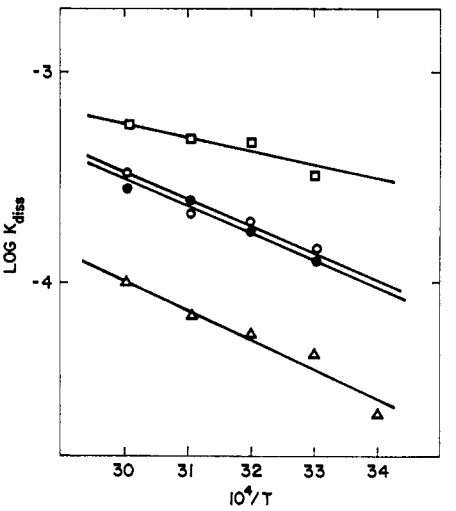


Figure 3. Plot of log K_{diss} against reciprocal absolute temperature for the mono-pCP esters of three-substituted glutaric acids: □ for the 3-methyl ester; ○ for the 3-isopropyl ester; ● for the 3-phenyl ester; and △ for the 3,3-dimethyl ester.

Temperature Effects on the Dissociation Constants of Cycloheptaamylose Complexes with Mono-pCP Esters of 3-Substituted Glutaric Acids. Kinetically determined dissociation constants were calculated for the cycloheptaamylose complexes with mono-pCP esters of 3-substituted glutaric acids at temperatures between 20 and 60°. The results are given in Table I.

Discussion

Geometry of Binding in the Cycloheptaamylose Complexes of Mono-pCP Esters of 3-Substituted Glutaric Acids. Cycloheptaamylose depresses the rates of intramolecular carboxylate ion attack for all of the mono-pCP esters of 3-substituted glutaric acids examined; Table I. However, there is no covalent involvement by cycloheptaamylose; it merely binds the esters. If one assumes that a reactive conformation (eq 1) of these esters will go to product at a rate independent of the presence of cycloheptaamylose, that is, assume that the transition state energy for intramolecular attack is essentially the same for both a free ester and its cycloheptaamylose complex, then from Scheme II it can be shown that for all of the mono-pCP esters examined, K_2 must be greater than K_4. In this scheme, U and R represent unreactive and reactive ester conformations, respectively, both of which can complex

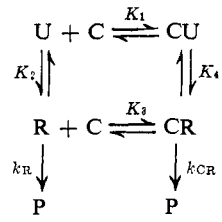
Table II. Thermodynamic Parameters for Cycloheptaamylose Inclusion Complex Formation

Substrate	$\Delta H°$, kcal/mole	$T\Delta S°$, kcal/mole[a]	$\Delta G°$, kcal/mole[a]
Mono-*p*-carboxyphenyl Esters of 3-substituted glutaric acids			
3,3-Dimethyl	−6.6 ± 0.8	−1.8 ± 1.2	−4.8
3-Isopropyl	−5.1 ± 1.0	−1.2 ± 0.9	−3.9
3-Phenyl	−5.2 ± 0.6	−1.2 ± 0.6	−4.0
3-Methyl	−3.4 ± 0.8	0 ± 0.6	−3.4
Substituted phenyl acetates[b]			
m-Chloro	−1 ± 1	2.4 ± 0.9	−3.4
m-Ethyl	−4.6 ± 0.7	−0.9 ± 0.6	−3.7
3,4,5-Trimethyl	−2.5 ± 0.7	0.6 ± 0.9	−3.1
p-Nitrophenoxide[c]	−7.2	−2.7	−4.5

[a] 298°K; $\Delta G° = \Delta H° - T\Delta S°$ at 298°K. [b] Reference 7. [c] Reference 8; determined spectrophotometrically.

The temperature effects on the dissociation constants determined by plotting log K_{diss} against reciprocal absolute temperature are shown in Figure 3. For all of the esters examined, there is a significant temperature dependence for K_{diss}. The thermodynamic parameters for formation of these inclusion complexes are given in Table II along with available data on the formation of cycloheptaamylose inclusion complexes with other compounds. Significantly, the cycloheptaamylose complexes with mono-pCP esters of 3-substituted glutaric acids all show sizable enthalpic contributions to binding. These $\Delta H°$ values are much larger than the experimental errors involved in their determination. However, when these thermodynamic parameters are compared with those for the other compounds in Table II, there is observed a complete spectrum from primarily enthalpic to primarily entropic driving forces for inclusion complex formation. The free energy of binding can result from either $\Delta H°$ and/or $\Delta S°$.

Scheme II



with cycloheptaamylose, C. The assumption is that $k_R \simeq k_{CR}$. The conclusion that $K_2 > K_4$ (where K_2 = [R]/[U], and K_4 = [CR]/[CU]) follows from the experimental observation that cycloheptaamylose depresses the rates of reaction for all of the mono-pCP esters. The extent of the rate depression depends upon K_4. If K_4 is large, the completely complexed ester is reactive, which is the case with the 3-phenyl ester. Conversely, if K_4 is small, the reactivity of the complexed ester is low as with the 3,3-dimethyl and 3-methyl esters. Consequently, cycloheptaamylose affects reactivity in these mono-pCP esters by preferentially complexing unreactive conformations, thereby lowering the energy of the ground-state distribution of the conformers. The extent of this lowering depends upon the 3-substituents of these esters.

Although the actual geometry of these complexes cannot be determined from consideration of Scheme II, several factors strongly support preferences. The data in Table III show that cycloamyloses do not form strong complexes if charged groups are placed inside of the cycloamylose cavity. Although a direct comparison between the dissociation constants of the mono-pCP ester complexes and the other complexes in Table III may not be made because different cycloamyloses were used, a rough comparison is possible. Previous studies[7] have shown that the dissociation constants for substituted phenyl acetate complexes with cycloheptaamylose are 2–10 times smaller than with cyclohexaamylose. The range for K_{diss} in Table III is much larger than these factors. From consideration of these values

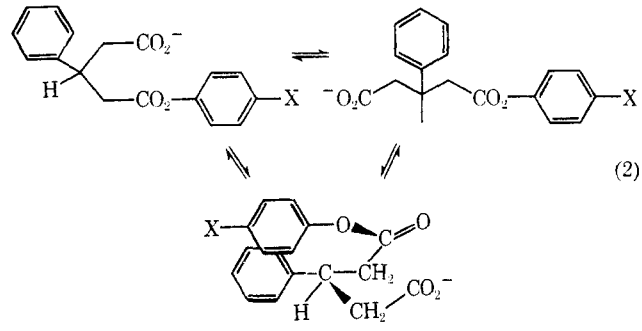
Table III. Dissociation Constants of Cycloamylose Complexes with Charged Substrates

Substrate	Cycloamylose	$10^4 K_{diss}$
Propionate[a]	Hexa	5700
Isobutyrate[a]	Hexa	2200
Benzoate[a]	Hexa	810
p-Phenylbenzoate[a]	Hexa	170
p-Carboxyphenyl esters		
Acetate[a]	Hexa	1500
2-Methylpropionate[a]	Hexa	120
3,3-Dimethylbutyrate[a]	Hexa	11
3,3-Dimethylglutarate[b]	Hepta	4.6
3-Isopropylglutarate[b]	Hepta	14.5
3-Phenylglutarate[b]	Hepta	13.1
3-Methylglutarate[b]	Hepta	32.8

[a] Data from ref 7 at 25° using cyclohexaamylose. [b] Data from this study at 30° using cycloheptaamylose.

of K_{diss}, it seems reasonable to conclude that the geometry of the cycloheptaamylose complexes with these mono-pCP esters of three-substituted glutaric acids does not place the *p*-carboxyphenyl group inside of the cavity, based upon the good binding which is observed.

In their study of the effects of 3-substituents upon the reactivities of monoesters of glutaric acids, Bruice and Bradbury[9] suggested that the 3-substituents affect the distribution between extended, unreactive conformers and coiled, reactive conformers (eq 1). However, they observed that the 3-phenyl ester was less reactive than one would predict from the steric size of the phenyl group; that is, the phenyl substituent did not increase the population of the reactive conformers as much as was anticipated. These authors suggested that the small effect of a 3-phenyl substituent was due to an apolar interaction between this group and the aromatic leaving group, producing a conformation of lessened reactivity compared to the coiled, reactive conformation (eq 2). This explanation adequately explains the results of this study. Examination of space-filling molecular models[20] suggests that cycloheptaamylose can form tight inclusion complexes with the extended conformations of the mono-pCP esters unless the 3-substituent is too large as is the case with the phenyl group. In these complexes with the extended conformations the charged carboxylate groups protrude from the top and the bottom of the cavity, and the rest of the mono-pCP ester is inside of the cavity. Examination of these molecular models also suggests that putting the 3-phenyl ester into a conformation which allows aromatic overlap (as in eq 2) produces a structure which can fit partly into the cycloheptaamylose cavity. Added support for this conclusion results when cyclohexaamylose is used instead of cycloheptaamylose. The smaller cavity of cyclohexaamylose is unable to accommodate, even partially, this conformation involving aromatic overlap of the 3-phenyl group and the leaving group. Significantly, the reactivity of the mono-pCP ester of 3-phenylglutaric acid is completely insensitive to cyclohexaamylose. Yet, cyclohexaamylose depresses the rates of the 3,3-dimethyl and 3-methyl esters as expected from consideration of the molecular models. Consequently, although definite conclusions about the geometry of the complexes are speculative, there is consistency between the tentative conclusions drawn here and the results of Bruice and Bradbury.



(2)

Specificity in the Reactivities of the Cycloheptaamylose–Mono-pCP Ester Complexes. The geometry of binding in the cycloheptaamylose complexes of the mono-pCP esters of 3-substituted glutaric acids determines the reactivities of these complexes. Since the geometry of binding is dependent upon the 3-substituents, the reactivities of the complexes show specificity. In Table IV the relative rates of the free and complexed esters are given along with their ratios.

(20) Corey–Pauling–Koltun scale molecular models, W. L. Koltun, *Biopolymers*, 3, 665 (1965).

These ratios, k_{un}/k_c, give approximate values of the extent to which the rate is depressed by cycloheptaamylose. For the 3,3-dimethyl and 3-methyl esters which have little or no reactivities in the complexes, these ratios are large, while the ratio is small for the 3-phenyl which is quite reactive in the complex. The relative rates of the complexed esters appear to be independent of the relative rates of the free esters. Consequently, the specificity resulting from complexing the esters with cycloheptaamylose can induce large changes in the overall relative reactivities and can, in fact, completely change the order, as shown in Table IV.

Table IV. Effects of Inclusion Complex Formation upon Relative Reactivities

Mono-pCP esters of 3-substituted glutaric acids	$10^4 k_{un}$, sec^{-1}, 30°	rel k_{un}, 30°	k_{un}/k_c[a]	rel k_c,[b] 30°
3,3-Dimethyl	20.1	10.2	≥ 36	≤ 1
3-Isopropyl	21.6	11.0	10	3.5
3-Phenyl	1.96	1.0	3.2	1
3-Methyl	4.54	2.2	≥ 50	≤ 0.2

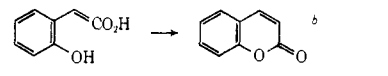
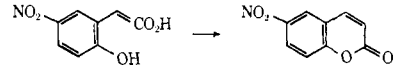
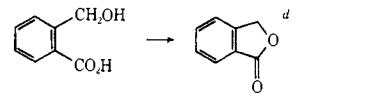
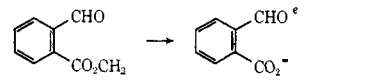
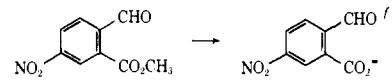
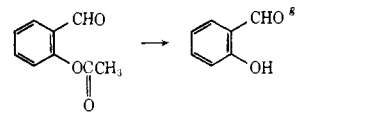
[a] The values of k_c were calculated by averaging the per cent reactivities of the complexes, Table I. These calculated values for k_c at 30° were used instead of the experimental values of Table I in order to minimize the errors in k_c, especially for the 3-methyl ester. [b] The signs $\geq$ and $\leq$ for the 3,3-dimethyl and 3-methyl esters reflect the experimental uncertainty in how much reactivity remains when these esters form complexes with cycloheptaamylose.

From the work of Bruice and Bradbury,[9] it is apparent that the relative reactivities of the free monoglutarate esters reflect the influence of the 3-substituents upon raising of the ground-state levels of the conformer distributions. The data from the cycloheptaamylose complexes of the glutarate esters in the present study reflect the influence of 3-substituents upon lowering of the ground-state distributions. Therefore the specificity observed in these complexes can be called a "negative specificity."

The importance of the observation of this specificity is that it results entirely from the geometry of complex formation. This model study supports the idea that specificity in enzymic reactions may result from the geometry of binding the substrates to the enzymes, rather than resulting from the catalytic reactions themselves.

Effects of Cycloamyloses on Other Intramolecular Reactions. The data in the study of mono-pCP esters of 3-substituted glutaric acids suggest formation of inclusion complexes in which the ground-state distribution of conformers is shifted toward the unreactive or less reactive side (eq 1). It should in principle be possible to trap out more reactive conformations preferentially and thereby increase the reaction rate relative to the rate in absence of cycloamylose. In Table V several reactions are given where added cycloheptaamylose and cyclohexaamylose had no significant effects, positive or negative. It was anticipated that proper geometry of binding might enhance the intramolecular rates of reaction by rotating the reacting groups into geometries more favorable for reaction. For example, it is known[21] that the *ortho*–formyl group tremendously accelerates the rate of hydrolysis of methylbenzoate, and that this reaction is enhanced further[22] if the ester is forced out of the plane of the ring by adding substituents in the other *ortho* position. Apparently cycloamyloses do not perform this function even when a nitro substituent is present to enhance binding.[23] Nevertheless, it should be possible to find conditions where reactions are enhanced by binding. Presumably highly flexible systems will be better disposed for such study, judging from the results with the flexible glutarate esters where the rate changes, although negative, are very large.

Table V. Effects of Cyclohexaamylose and Cycloheptaamylose on Some Intramolecular Reactions[a]

Reaction	pH	$10^4 k_0$ sec^{-1}	k_α/k_0	k_β/k_0
	4.6	3.57	1.05	0.95
	3.1	2.24	0.86	0.79
	1.2	4.04	1.12	0.67
	9.1	230	1.00	0.54
	7.6	185	1.08	0.75
	8.1	170		0.93

[a] k_α/k_0 refers to ratio of rate constants in presence and absence of 10^{-2} *M* cyclohexaamylose; similarly k_β/k_0 refers to cycloheptaamylose. All reactions at 25°, all buffers adjusted to $\mu = 0.2$. [b] Acetate buffer. [c] Formate buffer. [d] HCl; J. F. Bunnett and C. F. Hauser, *J. Amer. Chem. Soc.*, **87**, 2214 (1965). [e] Carbonate buffer; M. L. Bender, J. A. Reinstein, M. S. Silver, and R. Mikulak, *J. Amer. Chem. Soc.*, **87**, 4545 (1965). [f] Phosphate buffer. [g] Phosphate buffer; L. Holleck, G. A. Melkonian, and S. B. Rao, *Naturwissenschaften*, **45**, 438 (1958).

Thermodynamics of Inclusion Complex Formation. There is considerable interest at present concerning the origin of the driving forces for apolar interactions, especially in proteins. Two major models currently under discussion are the "hydrophobic" model of Kauzmann[24] and the "hydrotactic" model of Klotz.[25] The hydrophobic model considers apolar interactions in aqueous media to be the extension of the solution process; the driving force is entropic from release of some of the water which is structured around each separated apolar group. The hydrotactic model is based on the idea of clathrate hydrate formation, wherein water is reoriented into the so-called "iceberg"

(21) See Table V, footnote *e*.

(22) M. S. Newman and A. L. Leegwater, *J. Amer. Chem. Soc.*, 90, 4410 (1968).

(23) It is assumed that binding occurs here by analogy with similar systems where binding does occur (ref 7).

(24) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).

(25) I. M. Klotz, *Science*, **128**, 815 (1958); *Fed. Proc. Suppl.*, **15**, 24 (1965).

structure.[26] It is known that formation of clathrate hydrates results from a favorable enthalpy which is sufficient to counteract an unfavorable entropy.[27] A number of temperature studies on K_{diss} of apolar complexes have been made. For example, Wagner and coworkers[28] conducted temperature studies on the kinetically determined K_{diss} for the interactions of long-chain N-acylhistidine derivatives with *p*-nitrophenyl-N-dodecyl-N,N-dimethylammonioethyl carbonate ion. They reported temperature independence for K_{diss} from which they concluded that their data supported the hydrophobic model. The data in Table II can be used to support both models if one looks simply at the $\Delta H°$ and $\Delta S°$ values. They show that both entropic and enthalpic contributions to binding are important in cycloamylose complexes. To the extent that these cycloamylose complexes are able to simulate enzyme–substrate complexes, it appears worthwhile to be aware of the possibility that enzyme–substrate complex formation may also result from both entropic and enthalpic contributions to the free energy of binding. It should also be recognized that other factors such as van der Waals–London type forces may be involved in complex formation. Present theory does not allow a simple interpretation of the thermodynamic data in Table II.

(26) H. Frank and M. Evans, *J. Chem. Phys.*, **13**, 507 (1945); H. Frank and W.-Y. Wen, *Discussions Faraday Soc.*, **24**, 133 (1957).
(27) I. M. Klotz, "Horizons in Biochemistry," M. Kasha and B. Pullman, Ed., Academic Press, New York, N. Y., 1962, pp 523–550.
(28) R. G. Shorenstein, C. S. Pratt, C.-J. Hsu, and T. E. Wagner, *J. Amer. Chem. Soc.*, **90**, 6199 (1968).

Conclusion

Several observations have been made in this study of cycloheptaamylose complexes with mono-pCP esters of 3-substituted glutaric acids.

1. The rates in all cases are slower when cycloheptaamylose is present, due to preferential complexing of the unreactive or less reactive conformers which exist in solution.
2. The reactivity of the complexes depends upon the geometry of binding the esters to cycloheptaamylose. The specificity of this binding depends upon the 3-substituents.
3. This specificity of binding can be called a "negative specificity" because it results from lowering the ground-state energies of the esters.
4. There is no relationship between the relative reactivities of the free esters and the relative reactivities of the complexed esters. Consequently, the specificity of binding in the complexes can induce large changes in the overall relative reactivities of these esters.

This model study supports the idea that specificity in enzymic reactions may result from the geometry of binding the substrates to the enzymes, rather than resulting from the catalytic reactions themselves.

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Coenzyme B_{12} and Coenzyme B_{12} Model Compounds in the Catalysis of the Dehydration of Glycols[1]

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Abstract: Synthesis and reactions of 5′-deoxyadenosylcobaloximes are described. The model compounds of coenzyme B_{12} undergo Co–C bond cleavage with acids, cyanide ion, or on light irradiation just like coenzyme B_{12} itself. However, they are also alkali labile, yielding the cobaloxime(I) nucleophiles and β-elimination products of the adenosyl moiety. A similar Co–C bond cleavage reaction of coenzyme B_{12} was unknown previously but is shown to occur in strong alkali. The coenzyme B_{12} model compounds are only weak inhibitors in coenzyme B_{12} dependent enzymes and have no coenzyme activity. The mechanisms proposed for the action of coenzyme B_{12} in the diol dehydratase of Aerobacter aerogenes are discussed in the light of the new experimental evidence and with particular reference to the factors influencing the reactivity of 2-hydroxyethylcobalamin and -cobinamide derivatives previously assumed to be actual or functional intermediates in the enzymatic reaction. A new interpretation of the substrate–coenzyme hydrogen exchange in the glycol dehydratase reaction is given which is shown to be consistent with mechanistic postulates derived on the basis of model experiments. This leads to the formulation of a mechanism of coenzyme B_{12} action in the dehydration of glycols whose individual steps are in agreement with the chemical properties and reactions of all constituents and reactants of the system.

Coenzyme B_{12} dependent enzymes achieve intriguing molecular transformations presumably by virtue of the unique catalytic activity of the cobalt ion in the corrin system.[3] In the free coenzyme the cobalt ion is six-coordinated, however, and as such not accessible for any substrate. It is therefore plausible to suggest

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(2) Petroleum Research Fund Predoctoral Fellow, 1968–1969, PRF-GF-62.

(3) See reviews by (a) H. A. Barker, *Biochem. J.*, **105**, 1 (1967); (b) F. Wagner, *Ann. Rev. Biochem.*, **35**, 405 (1966); (c) H. P. C. Hogenkamp, *ibid.*, **37**, 225 (1968); (d) K. Bernhauer, *et al.*, *Angew. Chem.*, **75**, 1145 (1963), and references cited therein.