Table I. Maximum Rate Accelerations and Binding Constants in the Cycloamylose-Catalyzed Hydrolysis of Phenyl Acetates[a,b,d-f]

Acetate	Hydroxide ion rate,[g] $k_u \times 10^4$, sec^{-1}	Cyclohexaamylose: $k_c \times 10^2$, sec^{-1}	Cyclohexaamylose: k_c/k_u	Cyclohexaamylose: $K_d \times 10^3$, M	Cycloheptaamylose: $k_c \times 10^2$, sec^{-1}	Cycloheptaamylose: k_c/k_u	Cycloheptaamylose: $K_d \times 10^3$, M
Phenyl	8.04	2.5	32	27	...	...	...
o-Tolyl	3.84	0.75	20	21	...	...	...
m-Tolyl	6.96	7.2	103	21	...	...	...
p-Tolyl	6.64	0.26	4	14	...	...	...
3,5-Dimethylphenyl	5.80	12	200	16(13)[c]	5.0	84	9.0
m-*t*-Butylphenyl	4.90	13	260	2.0	12.2	249	<0.5
p-*t*-Butylphenyl	6.07	0.075	1.2	7.7	...	...	...
m-Chlorophenyl	19.1	26	~140	~6(4.8)[c]	4.5	23	3.9
m-Nitrophenyl	46.4				42.6	90	7.3
p-Nitrophenyl	69.4				6.9	8.9	6.4

[a] In pH 10.6 carbonate buffer, I = 0.2, 0.5% acetonitrile–water, 25.0 ± 0.2°; ester ~10^{-4} M. [b] Six to eight cycloamylose concentrations from 0.001 to 0.02 M were used for each experiment. [c] Determined by direct measurement of binding constant using ultraviolet spectrophotometry at pH 2.2 in aqueous solution at 25°: 3,5-dimethylphenyl acetate, 235 mμ; *m*-chlorophenyl acetate, 230 and 235 mμ. [d] These data are calculated on the basis of a 1:1 complex, which, however, has not as yet been rigorously proven. [e] The spectra of the products corresponded to the spectra of the phenols. [f] The *meta–para* specificity is largely lost in reactions with cyclooctaamylose. [g] The first-order rate constants were independent of initial ester concentration.

= 0.978), the Hammett plot of the acceleration of these reactions by the cycloamyloses shows an unprecedented scatter. These results indicate that the cycloamylose accelerations are dependent on steric factors. This stereochemical preference is most strikingly seen in the cyclohexaamylose-catalyzed reactions of *m*- and *p*-*t*-butylphenyl acetates: the ratio of the maximum cyclohexaamylose rate to the uncatalyzed rate for the *meta* isomer is 260-fold while it is 1.2-fold for the *para* isomer.

The stereochemical cause of these differential rate accelerations must lie in the stereochemistry of the complexes formed during reaction. There appears to be an approximate linear relationship between the logarithm of the binding constant and the molecular volume of the guest (as measured by the parachor). For the various *meta–para* pairs, however, the binding constant is only slightly dependent on structure, there being no simple relationship between rate acceleration and strength of binding—*only one between rate acceleration and stereochemistry of binding.*

An X-ray diffraction analysis of cyclohexaamylose[8] shows a torus consisting of six α-D-(+)-glucose units in their normal C1 conformation, with the hydroxyl groups forming crowns around the top and bottom of the torus. Corey–Pauling–Koltun models[9] reproduce this structure well and delimit an internal diameter of ~5 A. Models of the cyclohexaamylose complexes of *p*-*t*-butylphenyl and *m*-*t*-butylphenyl acetate, constructed on the assumption of maximum hydrocarbon interaction of the guest molecule in the void of the host, orient the plane of the phenyl ring parallel to the axis of the cavity. This description is borne out by the spectrum of *p*-*t*-butylphenol in the presence of cyclohexaamylose which resembles its spectrum in dioxane, an unoriented analog of the cavity.

Molecular models of the complexes indicate that the *secondary hydroxyl groups of the host are much closer to the carbonyl group of m-t-butylphenyl acetate than they are to the carbonyl group of p-t-butylphenyl acetate.* Thus, if the stereochemical features of the rate of accelerations are the result of a chemical interaction it must involve the interaction of the ester with the hydroxyl groups of the cycloamylose. The following communication[10] shows that the accelerations are indeed the result of such an interaction. The relative amount of this interaction is seen to be determined by the stereochemistry of the fit between guest and host in these relatively rigid systems. Thus the relative rates of reactions of this series are controlled by the stereospecific complexing postulated in the "lock and key" theory of enzymatic catalysis proposed by Fischer.[11]

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The Mechanism of the Cycloamylose-Catalyzed Reactions of Phenyl Esters. A Model for Chymotrypsin Catalyses[1]

Sir:

Cycloamyloses accelerate the liberation of phenols from phenyl esters, exhibiting a stereospecificity which may result from an interaction of the hydroxyl groups of the host with the ester linkage of the guest in an inclusion complex of the two.[2] The present communication indicates that a chemical interaction does take place, involving a hydroxyl group of the cycloamylose acting as a nucleophile.

The reactions of several phenyl benzoates were examined here in order to investigate the fate of the acid portion of the ester in addition to the phenol portion. In the cyclohexaamylose- and cycloheptaamylose-catalyzed hydrolysis of *m*-chlorophenyl, *m*-nitrophenyl, and *m*-*t*-butylphenyl benzoates (Table I), two rate

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Table I. Rate Constants of the Cycloamylose-Catalyzed Hydrolysis of Some Aryl Benzoates[a]

Benzoate	Alkaline hydrolysis	Rate constants (sec^{-1}) × 10^4 — 0.01 M cyclohexaamylose — 290 or 390 mμ	0.01 M cyclohexaamylose — 245 mμ	0.01 M cycloheptaamylose — 290 or 390 mμ	0.01 M cycloheptaamylose — 245 mμ
m-Nitrophenyl	15.4	1400	4.6	250	3.3
m-Chlorophenyl	5.5	390	4.6	22	2.7
m-*t*-Butylphenyl	1.2	140[b]	4.4		

[a] pH 10.6, 25°, 0.5% (v/v) acetonitrile-water solution, $I = 0.2$, carbonate buffer, [ester] = 2.5×10^{-5} *M*. [b] This rate constant is independent of cyclohexaamylose concentration from 0.765 to 1.32×10^{-2} *M*.

processes are seen spectrophotometrically, one at 390 or 290 mμ, corresponding to the liberation of the phenoxide ion, and the other at 245 mμ, corresponding to the liberation of benzoate ion, the former rate constant greatly exceeding the latter. The first rate constant is dependent on cycloamylose concentration but the second is not, over a 100-fold range. The rate constants for the liberation of benzoate ion from these esters are identical whereas the rate constants for the liberation of the phenol differ by a factor of 3.5–10. These results indicate the formation and decomposition of a common benzoylcycloamylose intermediate. Indeed, gel filtration of a typical reaction mixture using Sephadex G-10 permitted the isolation (in yields of >50%) of benzoylcyclohexaamylose, whose ultraviolet spectrum (λ_{max} 233, 274, and 280 (sh) mμ (H_2O)) is practically superimposable on that of ethyl benzoate. Benzoylcyclohexaamylose hydrolyzes at pH 10.6 with a rate constant of 4.6×10^{-4} sec^{-1} giving a product whose spectrum corresponds to that of benzoate ion.

Information about the mechanism of the cycloamylose reactions includes: (1) a complex between ester and cycloamylose is formed;[2] (2) an acylcycloamylose is formed; (3) this intermediate is hydrolyzed in a subsequent step. These data lead to eq 1 as the pathway for the reaction, which includes two rates steps: (1) nucleophilic attack on the ester by cycloamylose anion to produce acylcycloamylose and phenoxide ion and (2) reaction of acylcycloamylose with hydroxide ion to produce carboxylate ion and regenerate the cycloamylose catalyst. Both steps k_2 and k_3 show dependence on a basic

$$\begin{array}{ccc} \text{CA–OH} + \text{RCO}_2\text{C}_6\text{H}_5 & \rightleftharpoons & \text{CA–OH}\cdot\text{RCO}_2\text{C}_6\text{H}_5 \\ K_{CA}\uparrow\downarrow & & K_{CA}\uparrow\downarrow \\ \text{H}^+ + \text{CA–O}^- + \text{RCO}_2\text{C}_6\text{H}_5 & \rightleftharpoons & \text{H}^+ + \text{CA–O}^-\cdot\text{RCO}_2\text{C}_6\text{H}_5 \xrightarrow{k_2} \end{array}$$

$$\text{CA—OCR} \xrightarrow{k_3} \text{CA—OH} + \text{RCO}_2^- + \text{OC}_6\text{H}_5^- \qquad (1)$$

group of $pK_a \sim 12$, presumably an alkoxide ion of the cyclohexaamylose of $pK_a \sim 12$. In the k_2 step, alkoxide ion would be the catalytically active species while in the k_3 step the presence of alkoxide ion would inhibit the hydroxide ion reaction electrostatically.

The rate constant for the hydrolysis of benzoylcyclohexaamylose, probably an ester of a secondary alcohol, is approximately 200 times faster than the rate constant for the hydrolysis of isopropyl benzoate at pH 10.6. This difference in rate constants is partially due to the difference in pK_a values of the leaving groups of the two esters (12 *vs.* 16), which accounts for approximately a tenfold difference.[3] The remainder may indicate intramolecular catalysis by neighboring hydroxyl groups.

The over-all hydrolysis of *m*-*t*-butylphenyl benzoate is catalyzed by the cycloamyloses (Table I). However, although the liberation of the phenol from *m*-chlorophenyl and *m*-nitrophenyl benzoates is accelerated by the cycloamyloses, the over-all hydrolysis is not.

Catalysis of ester hydrolysis both by α-chymotrypsin[4] and by cycloamyloses[2] proceeds *via* eq 1, and thus it is of interest to compare these two catalytic systems. The binding constants for the two catalyst systems are quite similar, as has been pointed out before with respect to ethyl N-acetyl-L-tyrosinate.[5] The maximal kinetic specificity in cycloamylose catalysis is 200-fold[2] while it is 10,000-fold in chymotrypsin catalysis (k_3 values of *p*-nitrophenyl N-acetyl-D- and -L-tryptophanate). At pH 8 the rate constants k_2 and k_3 for the α-chymotrypsin catalysis are much larger than for the cyclohexaamylose catalysis. In the α-chymotrypsin-catalyzed hydrolysis of *p*-nitrophenyl acetate, deacylation (k_3) is rate determining while in the hydrolysis of N-acetyl-L-tryptophanamide, acylation (k_2) is rate-determining.[4] Likewise in the cycloheptaamylose-catalyzed hydrolysis of *m*-*t*-butylphenyl acetate deacylation is rate determining, while in the hydrolysis of *p*-*t*-butylphenyl acetate, acylation is rate-determining. The relative magnitude of the binding constants is independent of the relative magnitude of the rate enhancements in both cycloamylose and α-chymotrypsin catalyses. This independence of binding constant and rate constant may be explained on the basis that the binding constant reflects the strength of binding whereas the catalytic rate constant reflects the stereochemistry of binding. This conclusion is stereochemically definable in the cycloamylose system, and a similar picture may hold in the α-chymotrypsin system.

Thus, cycloheptaamylose catalysis appears to mirror chymotrypsin catalysis in a qualitative and even semiquantitative way, with one major exception: chymotrypsin contains in addition to a hydroxyl group at the active site which acts as nucleophile, a catalytic group of $pK_a \sim 7$, presumably an imidazole group, which acts as a general acid–base catalyst in each step of the catalysis.

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