Reactions of Diaryl Carbonates and Methylphosphonates with Cycloamyloses

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Abstract: Rates of substituted phenol release from diphenyl and bis(*p*-nitrophenyl) carbonate (I and II, respectively) and from diphenyl, bis(*p*-nitrophenyl), and bis(*m*-nitrophenyl) methylphosphonate (III, IV, and V, respectively) are accelerated in the presence of cycloamyloses. The reactions of I and II were studied in the presence of cycloheptaamylose, while III–V were studied in the presence of cyclohexa- and cycloheptaamylose. pH values of reaction solutions were between *ca.* 5.6 and 11.0 and depended on the substrate and whether a rate–pH profile was being examined. Michaelis–Menten kinetics are observed. The substrates rapidly bind to the cycloamylose. For I–III and V with cycloheptaamylose and III with cyclohexaamylose, 2 mol of substituted phenol is released in first-order processes after binding. The step releasing the first mole of substituted phenol (k_c, Schemes I–III) is rate determining. It is due to nucleophilic attack by a secondary cycloamylose hydroxyl group in its anionic form. This is followed by a rapid reaction, releasing the second phenolic moiety. The latter process is an intramolecular reaction of a second adjacent hydroxyl group on the cycloamylose, forming a cyclic cycloamylose carbonate or methylphosphonate. A neutral monomodified derivative, presumed to be a cyclic cycloamylose methylphosphonate, has been isolated from the reaction of cycloheptaamylose with III. Corrected for hydrolysis, the reactions of IV in the presence of cycloheptaamylose and IV and V with cyclohexaamylose release less than 2 mol of substituted phenol in a first-order process. This can be interpreted as a general base reaction of water, catalyzed by the cycloamylose anion, effectively competing with the nucleophilic reaction of cycloamylose anion (Scheme IV). The reaction of IV in the presence of cycloheptaamylose at pH 5.6 was also examined. The reaction is modestly accelerated and 1 mol of *p*-nitrophenol is released. In this case the cycloamylose acts as a binding site. Species from the bulk solution react with the included substrate. The reactions of V and IV with cycloamylose in basic solution display a meta to para stereospecific rate acceleration analogous to meta- and para-substituted phenyl acetates. The rate acceleration of the intramolecular cyclization reaction for IV at pH 9.86, as compared to an intermolecular hydrolysis, must be at least 10^3–10^4 *M*.

It is generally agreed that the close proximity of reacting groups in a single molecule often leads to a large rate enhancement as compared to the bimolecular reaction of these groups in separate molecules.[2-5] Such intramolecular reactions are often compared to catalysis by enzymes where the substrate and enzyme active site are brought together by sometimes powerful binding forces. Acyl and phosphoryl esters containing a hydroxyl group α to the esteratic site often react with intramolecular hydroxyl participation. Thus the rate of acetyl migration from the 2′- to the 3′-hydroxyl group in *O*-acetyluridine is 3.5×10^4 faster than its hydrolysis.[3,6] *cis*-Tetrahydrofuran-4-ol 3-phenyl phosphate reacts in aqueous solution to yield exclusively *cis*-tetrahydrofuran 3,4-cyclic phosphate under hydrolytic conditions.[7] The *cis*-4-methoxy ester is stable under the same conditions.[7]

Investigations in these and other laboratories have shown that cycloamyloses (also called cyclodextrins) are good models for certain enzyme-catalyzed reactions.[8-17] Cycloamyloses are cyclic α-1,4-linked D-(+)-glucose oligomers; the glucose units exist in the chair conformation. Polymers with six, seven, and eight glucose residues have been most useful as enzyme models. Cycloamylose molecules are doughnut-shaped with primary hydroxyl groups located on one side of the torus and the secondary hydroxyl groups on the other side of the torus.[12,18] The interior cavity of the ring is hydrophobic in nature and binds hydrophobic portions of certain molecules, usually forming 1:1 complexes in aqueous solution. Details of the X-ray structure of cyclohexaamylose have been published.[19] Cycloamyloses exhibit complex formation, saturation, stereospecific rate accelerations, enantiomeric specificity, and competitive inhibitions[12] and are said to demonstrate the lock and key theory of enzyme activity.[12]

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The hydrolysis of diaryl carbonates and methylphosphonates in the presence of cycloamyloses yielded further information about the nature of such intramolecular reactions. Of additional interest is the manner in which organophosphorus esters behave in the presence of cycloamyloses as compared to carbonyl esters in the presence of cycloamyloses.

Experimental Section

Materials. Diphenyl methylphosphonate,[20] bis(*p*-nitrophenyl) methylphosphonate, and bis(*p*-nitrophenyl) carbonate[21] were synthesized by previously reported techniques. Bis(*m*-nitrophenyl) methylphosphonate (mp 72–73°) was prepared using the method for the *p*-nitro derivative.[20] Diphenyl carbonate was an Aldrich Chemical Co. product. Substrates were recrystallized from appropriate solvents[20,21] to sharp melting points, often higher than literature values. Nmr spectra of all substrates were consistent with their structural characteristics. Cyclohexa- and cycloheptaamylose were obtained from CPC International Corp.[22] and purified by the method of French.[23] Acetonitrile was Mallinckrodt Nanograde. All other materials were reagent grade and used without further purification. Nmr measurements were made on a Varian Model T-60 instrument, and pH values were determined on a Radiometer Model 26 meter. Lyophilizations were performed on a Virtis research freeze-dryer. Analyses were performed by Micro-Tech Laboratories, Skokie, Ill.

Spectra. Ultraviolet and visible spectra were recorded on a Cary-14 spectrophotometer. pK_a values at 25° of 7.15, 8.38, and 10.00 were taken for *p*-nitrophenol, *m*-nitrophenol, and phenol, respectively.[20,24] Absorbance maxima and extinction coefficients in aqueous buffer solutions are as follows:[20,25] *p*-nitrophenol, 317 nm (9500); *m*-nitrophenol, 330 nm (1960); phenol, 271 nm (1510); *p*-nitrophenolate anion, 400 nm (18200); *m*-nitrophenolate anion 392 nm (1510); phenolate anion, 286 nm (2600); and *p*-nitrophenyl methylphosphonate (*p*-NPMP), 288 nm (9560).[26] Phenol, phenolate anion, and *p*-NPMP showed no spectral changes in the presence of cyclohexa- and cycloheptaamylose even though appreciable binding is known to occur.[11,12] *p*-Nitrophenolate and *m*-nitrophenolate ions show absorption maxima shifted to longer wavelength in cyclohexaamylose[11,12] with isosbestic points (due to 1:1 complex formation) at 398 and 403.5 nm, respectively. *p*-Nitrophenoxide in cycloheptaamylose yielded an isosbestic point at 398 nm; *m*-nitrophenoxide showed little spectral shift. Absorbance values of phenols after substrate reaction are defined as follows. A_1 refers to the observed absorbance upon release of 1 mol of substituted phenol. A_2 is the absorbance on release of 2 mol of substituted phenol. $A_{\infty(max)}$ is the observed infinity value at saturation in the presence of cycloamylose; its value will represent the release of 1 or 2 mol of substituted phenol or between 1 and 2 mol. $A_{\infty(max)}/A_1$ is equal to the number of moles of substituted phenol released.

Kinetics. Reactions were performed by following the rate of appearance of substituted phenols or phenolate anions in the absence and presence of cycloamyloses on a Cary-14 spectrophotometer equipped with 0–1 and 0–0.1 slide-wires. Substrate concentrations depended on the extinction coefficient of substituted phenol or its anion and on substrate solubility. The solubility of bis(*p*-nitrophenyl) carbonate, for example, is $\leq 3 \times 10^{-6}$ *M* in I = 0.2 *M* carbonate buffer. In certain cases substrate disappearance and methylphosphonate monoanion formation were followed. EDTA (10^{-4} *M*) was added to all solutions. Buffer preparations and techniques of kinetic experiments were as described.[12,26] First-order kinetic constants were calculated using the usual first-order expression for a reaction to completion.[27] For some slow reactions, first-order rate constants were calculated using computer-calculated least-squares Guggenheim[28] or Kézdy plots.[29]

Calculation of Dissociation and Maximum Rate Constants. Cycloamylose concentrations were always at least tenfold greater than that of the substrate for these determinations. Calculations were made based on Scheme I,[13,15] where S is the carbonate or methylphosphonate substrate, C is the cycloamylose, S·C is the cycloamylose–substrate complex, K_D is the dissociation constant of the 1:1 cycloamylose–substrate complex, P and S_3 are the products of substrate hydrolysis, CS_1 and CS_2 are products of cycloamylose reaction with the substrate, k_{un} is the uncatalyzed pseudo-first-order rate constant, and k_c denotes the first-order rate constant for all processes occurring within the cycloamylose cavity.

Scheme I

$$S + C \underset{}{\overset{K_D}{\rightleftharpoons}} S\cdot C \xrightarrow{k_c} P + CS_1 \xrightarrow{\text{fast}} P + CS_2$$
$$\downarrow k_{un}$$
$$P + S_3$$

According to the method of Colter, *et al.*,[30] eq 1 can be derived.

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

When a 1:1 stoichiometry exists between reactants and products and when [C] ≫ [S], k_{obsd} is the pseudo-first-order rate constant in the presence of varying cycloamylose concentrations. Equation 1 takes into account that a fraction of S is bound in S·C, so that unity minus this fraction multiplied by k_{un} is used instead of k_{un} itself.[30] For the case in Scheme I, 2 mol of product P is produced/mole of S consumed. Equation 2 is then applicable. Equations 1 and 2 are

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

of the same form as the Lineweaver–Burk equation[31] and can be transformed into the form of Eadie[32] (eq 3), which is statistically

$$k_{obsd} - k_{un} = -(K_D/[C])(k_{obsd} - k_{un}) + (2k_c - k_{un}) \quad (3)$$

preferable. Plots of the form $(k_{obsd} - k_{un})$ *vs.* $(k_{obsd} - k_{un})/[C]$ yield K_D as the slope and $(2k_c - k_{un})$ as the intercept.[33] Data were calculated in this investigation from a computer-analyzed least-squares fit of Eadie plots.

In Scheme IV (see Results) two products are formed from the cycloamylose–substrate complex. One path produces 2 mol of phenol, while the second path yields 1 mol of phenol per mole of substrate. In this case eq 4 is applicable for parallel first-order reactions, where $k_c(obsd)$ is the constant determined from an Eadie plot (eq 3) and k_1 and k_2 are the rate constants for each reaction occurring in the complex.

$$k_c(obsd) = 2k_1 + k_2 \quad (4)$$

Determination of Reaction Products. Cycloheptaamylose was dissolved in ~100 ml, I = 0.05 *M*, pH 10.20, carbonate buffer. Bis(diphenyl) methylphosphonate (*b*-PMP) dissolved in 2 ml of acetonitrile was added. Final concentrations of cycloheptaamylose and *b*-PMP were 1.0×10^{-3} *M* and 1.2×10^{-3} *M*, respectively. The reaction mixture was stirred at room temperature for 2.5 hr and then neutralized to pH ≅7 with 1 *M* HCl. Three drops of reagent grade benzene were added to the complex with cycloheptaamylose species, thereby preventing subsequent complexations. The resulting solution was passed once through a column containing Amberlite MB-3 mixed-bed indicating ion-exchange

(20) E. J. Behrman, M. J. Biallas, H. J. Brass, J. O. Edwards, and M. Isaks, *J. Org. Chem.*, **35**, 3063 (1970).
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(33) An important point must be noted. The intercept of this Eadie plot gives the quantity $2k_c - k_{un}$ (or $k_c - k_{un}$ if 1 mol of product being followed kinetically is released). k_{un} must be added to $2k_c$ (or k_c) to determine the correct catalytic constants. Many authors have seemingly neglected this point. The error is small if the ratio k_c/k_{un} is large, but the error can be large if the ratio is small.

Table I. Reactions of Diaryl Carbonates and Phosphonates in the Presence of Cycloamyloses[a,b]

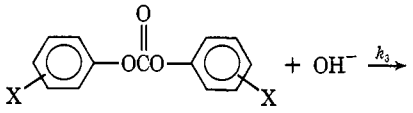
Substrate[c]	pH	$k_{un} \times 10^3$, sec^{-1}	k_c(obsd) $\times 10^3$[d,e] sec^{-1}	k_c(obsd)/k_{un}	$K_D \times 10^3$, M^{-1} [d,f]
		Cycloheptaamylose			
$CO(OPh)_2$ (I)	10.41[g]	1.64	3.77 ± 0.23	2.30	7.25 ± 1.30
$CO(OPh\text{-}p\text{-}NO_2)_2$ (II)	9.06–9.09[h]	4.71	35.1 ± 4.7	7.45	15.4 ± 6.4
$CH_3PO(OPh)_2$ (III)	10.79[g]	0.345	5.61 ± 0.32	16.0	1.43 ± 0.18
$CH_3PO(OPh\text{-}p\text{-}NO_2)_2$ (IV)	9.86[g]	8.54	159 ± 4	18.6	4.64 ± 0.15
	9.80[i]	5.31	139 ± 3	26.2	4.80 ± 0.16
	9.26[g]	3.78	43.3 ± 1.6	11.5	3.94 ± 0.24
	8.41[j]	1.03	9.90 ± 0.29	9.61	7.67 ± 0.40
	7.63[k]	0.887	2.46 ± 0.03	2.77	3.81 ± 0.21
$CH_3PO(OPh\text{-}m\text{-}NO_2)_2$ (V)	9.86[g]	2.85	118 ± 4	41.4	3.45 ± 0.19
		Cyclohexaamylose			
$CH_3PO(OPh)_2$	10.75[g]	0.313	11.0 ± 1.0	35.1	37.7 ± 4.6
$CH_3PO(OPh\text{-}p\text{-}NO_2)_2$	9.83[g]	8.30	69.2 ± 2.6	8.35	31.2 ± 1.9
$CH_3PO(OPh\text{-}m\text{-}NO_2)_2$	9.83[g]	2.84	185 ± 31	66.1	94.9 ± 21.3
	9.23[g]	1.13	55.6 ± 6.6	49.2	128 ± 18

[a] Kinetic parameters for cycloamylose catalysis were calculated by the method of Eadie: G. S. Eadie, *J. Biol. Chem.*, **146**, 85 (1942). [b] Temperature = 25.5°. [c] Substrate concentrations $\cong 2 \times 10^{-4}$–2×10^{-6} *M*, depending on substrate solubility and on the extinction coefficient of the phenol product species. [d] Errors are standard deviations. [e] For the cases where 2 mol of phenol are released k_c(obsd) is equivalent to $2k_c$ in eq 2. Where less than 2 mol of phenol is released, corrected for hydrolysis, k_c(obsd) is equivalent to $2k_1 + k_2$, eq 4. [f] Not statistically corrected. [g] Ionic strength, I, equals 0.2 *M* carbonate buffer. [h] 90% H_2O–10% CH_3CN, v/v, 0.1 *M* KH_2PO_4 used due to low substrate solubility. [i] I = 0.2 *M* consisting of 0.1 *M* carbonate ion plus 0.1 *M* KCl. [j] I = 0.2 *M* Tris buffer. [k] I = 0.2 *M* phosphate ion.

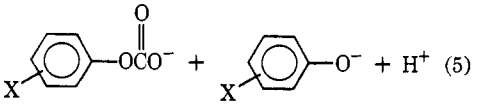
resin. The column was rinsed once with water. The eluate was chloride free, as determined by a silver nitrate test, and was lyophilized overnight; 21.5 mg of product was isolated, which represents an 18% yield. When analyzed this product contained 1.90% phosphorus. Modification of cycloheptaamylose by one substrate molecule to form a cyclic cycloheptaamylose methylphosphonate theoretically yields 2.58% phosphorus.

Results

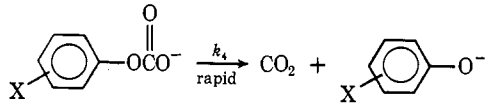
Substrate Hydrolysis. Diaryl carbonates hydrolyze in alkaline buffer solution *via* a two-step process (eq 5 and 6), the first of which is rate-determining.[34–36]



I, X = H
II, X = *p*-NO_2



(5)



(6)

Carbon dioxide will react with water and the carbonic acid produced will ionize to bicarbonate or carbonate, depending on pH. In addition to reaction with hydroxide ion (shown for illustrative purposes) or water at low pH values, substrate hydrolysis is sometimes accompanied by marked buffer catalysis. The general rate law for the disappearance of reactant is given in eq 7, where k_w, k_{OH^-}, and k_B[37] are, respectively, rate

$$\text{rate} = k_w[H_2O][S] + k_{OH^-}[OH^-][S] + \sum k_B[B][S] \quad (7)$$

constants for the reactions of water, hydroxide ion, and other reacting bases in solution.

(34) T. H. Fife and D. M. McMahon, *J. Org. Chem.*, **35**, 3699 (1970).
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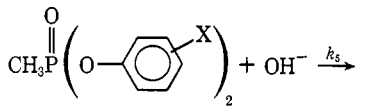
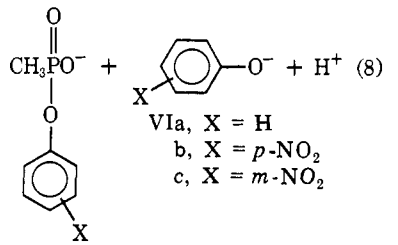
(36) Hydrolysis of dialkyl carbonates occurs with the second step being rate determining: G. D. Cooper and B. Williams, *J. Org. Chem.*, **27**, 3717 (1962).
(37) k_B refers to general base catalysis, where B assists in water attack.

Diaryl methylphosphonates hydrolyze according to eq 8, producing a phosphonate monoanion which is



III, X = H
IV, X = *p*-NO_2
V, X = *m*-NO_2



(8)

VIa, X = H
b, X = *p*-NO_2
c, X = *m*-NO_2

stable under the experimental conditions in this investigation.[20,26] Hydrogen ion produced will be neutralized by the buffers in solution. The general rate law in eq 7 obtains,[38] except where buffer components act as nucleophiles.[39]

Reactions in the Presence of Cycloamyloses. Data for the reactions of diaryl carbonates and diaryl methylphosphonates in the presence of cyclohexa- and cycloheptaamylose are given in Table I. In the absence of cycloamyloses, 2 mol of substituted phenol is released in the reactions of carbonates, while 1 mol of phenol is released *via* methylphosphonate reactions.[38,39] Data in Table I were determined with cycloamylose concentration always in excess over that of the substrate so that pseudo-first-order kinetics obtained.

Diaryl Carbonates. The rate of substituted phenol

(38) The reactions of diaryl carbonates in the presence of phosphate and carbonate buffers presumably take place by the kinetic pathways in eq 7. Reaction due to buffer components (Table I) likely is general base,[34] while the reactions of hydroxide ion and water may follow either nucleophilic or general base pathways. The reactions of diaryl methylphosphonates with phosphate and carbonate species as well as hydroxide ion and water can be nucleophilic and/or general base in nature.[26]
(39) IV reacts with phosphate dianion (and probably carbonate species) by a nucleophilic mechanism.[26] The intermediate formed decomposes to VI rapidly.

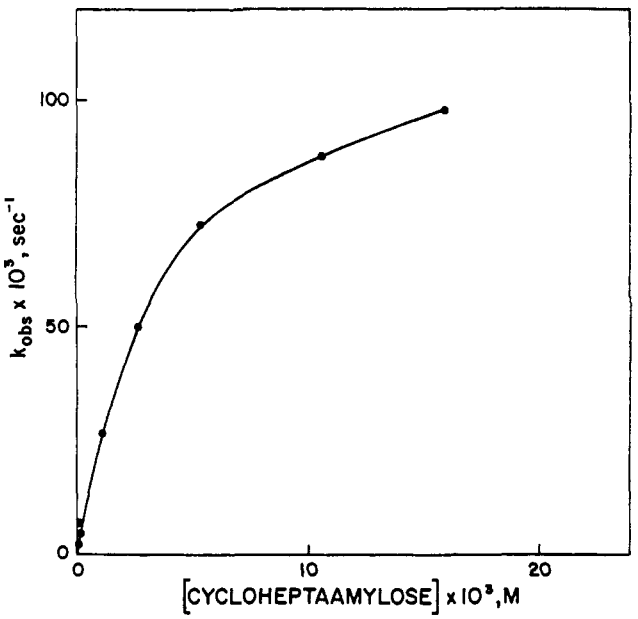
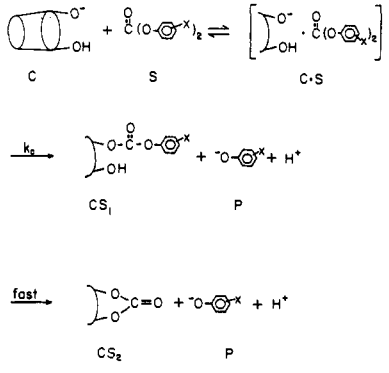


Figure 1. Pseudo-first-order rate constant for release of *m*-nitrophenol from bis(*m*-nitrophenyl) methylphosphonate at pH 9.86 plotted as a function of added cycloheptaamylose; data of Table I.

release from diphenyl carbonate (I) and bis(*p*-nitrophenyl) carbonate (II) was accelerated in the presence of cycloheptaamylose. II was studied with 10% added acetonitrile to increase substrate solubility. Two moles of phenol or *p*-nitrophenol was always released in a first-order process. Reaction in the cycloheptaamylose cavity which leads to production of the first mole of substituted phenol is rate determining. In a subsequent rapid step the second phenolic moiety is released. Scheme II (as well as Scheme I) is consistent with the

Scheme II

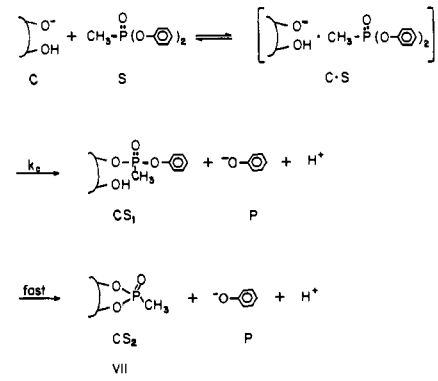


data. P is the released phenol, CS_1 is the first product of the cycloheptaamylose–substrate complex, and CS_2 is the product of the fast reaction of CS_1, releasing the second mole of phenol. k_c(obsd) in Table I is then equal to $2k_c$ and must be divided by 2 to obtain k_c. k_c(obsd)/k_{un} must also be divided by 2 to calculate a statistically corrected maximum rate acceleration. In Table I dissociation constants, K_D, have not been statistically corrected. Since I and II contain two identical aromatic moieties, each of which may bind in the cycloamylose cavity, the observed K_D values must be multiplied by 2 to obtain statistically corrected dissociation constants.

The magnitude of the maximal rate accelerations and binding constants of I and II is similar to the values reported for the reactions of substituted phenyl acetates in the presence of cycloamyloses.[12] Added acetonitrile probably increases the K_D value of II.[12] Qualitative experiments (data not shown) reveal that k_c(obsd) for I and II displays approximately a first-order dependence on hydroxide ion concentration at pH values between 9 and 11. The cycloamylose in Scheme II is shown in its reactive anionic form, acting as a nucleophilic reagent.[12] The values of K_D (Table I) are for binding into unionized plus ionized cycloamylose.

Diaryl Methylphosphonates. The rates of substituted phenol release from diphenyl methylphosphonate (**III**), bis(*p*-nitrophenyl) methylphosphonate (**IV**), and bis(*m*-nitrophenyl) methylphosphonate (V) were also accelerated in the presence of cycloamyloses. The reactions of III and V with cycloheptaamylose present and of III in the presence of cyclohexaamylose yielded 2 mol of phenol or *m*-nitrophenol in a first-order process. Comments about statistically corrected k_c(obsd) and K_D values made for diaryl carbonate reactions are applicable to these cases. Scheme III shows the proba-

Scheme III



ble mechanistic pathway which is analogous to the diaryl carbonate reactions. Figure 1 shows the saturation phenomenon, predicted from Scheme I, on measuring pseudo-first-order rate constants for *m*-nitrophenoxide formation from V, with increasing cycloheptaamylose concentration. k_c(obsd) and K_D values in Table I were calculated from such behavior.

Corrected for hydrolysis, the reactions of IV in the presence of cycloheptaamylose and of IV and V in the presence of cyclohexaamylose released less than 2 mol of substituted phenol in a first-order reaction. A detailed study of the reactions of IV in the presence of cycloheptaamylose was undertaken. In the first series of kinetic experiments at pH 9.86 (Table I), approximately 1.6 mol of *p*-nitrophenoxide ion (*p*-NP) was released near saturation, even though k_c(obsd) was nearly 20-fold greater than k_{un}. Further data are given in Table II. $A_{\infty(max)}/A_1$ remains constant and is independent of pH, except for the pH 7.63 value where $A_{\infty(max)}/A_1$ decreases slightly. Data at pH 8.41 (Table I) could not be interpreted in this manner since the Tris buffer reacts with IV, likely by a nucleophilic pathway,[26] releasing more than 1 mol of *p*-NP.

Data for the reaction of IV in the absence and presence of cycloheptaamylose at pH $\cong$ 5.6 are given in Table III. The difference in the hydrolysis rate constants in acetate and phosphate buffers is due to the different reactivities of the buffer species.[26] Slightly less than a twofold increase in the rate of *p*-nitrophenol

Table II. Reactions of Diaryl Methylphosphonates Releasing Less than 2 Mol of Phenol in the Presence of Cycloamyloses

Substrate[a]	Cycloamylose	pH	A_1	$A_{\infty(max)}$	$A_{\infty(max)}/A_1$	$2k_1 \times 10^2$, sec^{-1}	$k_2 \times 10^2$, sec^{-1}
IV	Hepta	10.41	0.471[b]	0.767[b]	1.63	*c*	*c*
	Hepta	9.86	0.462[b]	0.750[b]	1.62	9.9	6.0
	Hepta	9.80	0.462[b]	0.750[b]	1.62	8.7	5.2
	Hepta	9.26	0.472[b]	0.755[b]	1.60	2.5	1.8
	Hepta	7.63	0.360[b]	0.530[b]	1.47	1.2	1.3
IV	Hexa	9.83	0.530[b]	0.700[b]	1.32	2.2	4.7
V	Hexa	9.83	0.419[d]	0.680[d]	1.62	12	7.0
	Hexa	9.23	0.395[d]	0.620[d]	1.57	3.2	2.3

[a] Initial substrate concentration is constant throughout a series of determinations at each pH value. [b] At 398 nm. [c] Reaction too fast to determine rate constants. [d] At 403.5 nm.

Table III. Reaction of *b*-*p*-NPMP in the Presence of Cycloheptaamylose at Low pH

[Cycloheptaamylose], *M*	pH	$k_{obsd} \times 10^4$, sec^{-1}
0	5.57[a]	1.57
1.53×10^{-2}	5.57[a]	2.60
1.02×10^{-2}	5.58[a]	2.57
0	5.60[b]	0.529
1.57×10^{-2}	5.62[b]	1.07
1.05×10^{-2}	5.62[b]	0.99

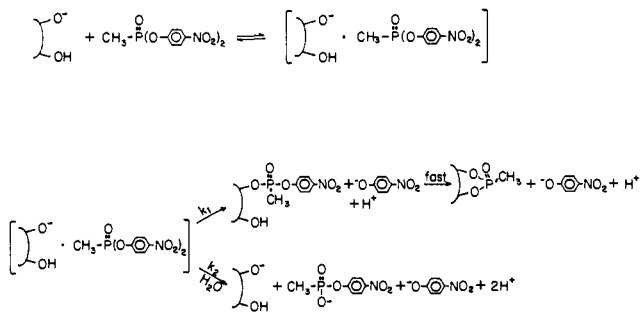
[a] I = 0.2 *M* phosphate. [b] I = 0.2 *M* acetate.

production is observed in the presence of ~0.15 and 0.10 *M* cycloheptaamylose. The fact that the observed first-order rate constants in each buffer are only slightly changed on varying cycloheptaamylose concentration indicates good binding of the substrate to the cycloheptaamylose cavity. The rate of reaction of bound substrate must be only slightly accelerated over substrate reaction in the buffer solution. For the reaction given in Table III, only 1 mol of *p*-nitrophenol is produced. An additional reaction path must obtain at pH 5.6 rather than at higher pH values.

A rate–pH profile for the data of IV in Table I is given in Figure 2; k_c(obsd) values are used. The line drawn has a unit slope. The k_c(obsd) values at the three highest pH values fall on the line. Hence, at high pH, processes inside the complex appear to depend on the fraction of cycloheptaamylose in an ionized form. The point of lowest pH (5.6) falls considerably above the line. The point at pH 7.63 (and possibly the Tris point at pH 8.41) falls slightly above the line. These data further indicate a change of reaction mechanism on decreasing pH.

Scheme IV can possibly account for the reactions of

Scheme IV



IV in the presence of cycloheptaamylose at high pH. Once bound in the cavity, IV can react by two pathways having rate constants k_1 and k_2. The first occurs *via*

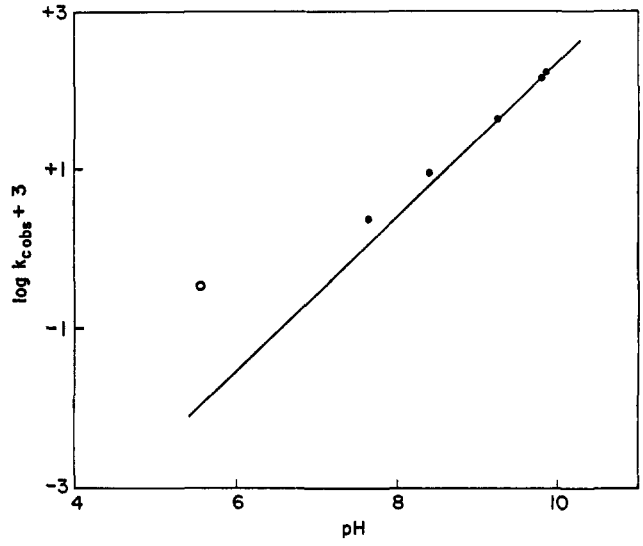


Figure 2. Plot of log k_c(obsd) against pH for the reactions of bis(*p*-nitrophenyl) methylphosphonate in the presence of cycloheptaamylose; data of Table I.

nucleophilic substitution at phosphorus by the cycloheptaamylose anion. This releases 2 mol of *p*-NP and is identical with the path proposed in Scheme III. In the second path (k_2) the cycloheptaamylose anion acts as a general base catalyst for the attack of water. *p*-Nitrophenyl methylphosphonate (*p*-NPMP), a stable product, and 1 mol of *p*-NP are produced. Such a mechanism is consistent with the data in Table II; the fraction of the second mole of *p*-NP released should be pH independent.[40] The mechanism consistent with the data at low pH is one where the cycloheptaamylose acts as a binding site. Buffer species, water, and in part hydroxide ion from the bulk solution can react with bound substrate producing *p*-NPMP.[26] Another possibility is the reaction of neutral cycloheptaamylose with IV.

In the absence of cycloheptaamylose, 1 mol of *p*-nitrophenyl methylphosphonate is produced from IV and can be quantitatively measured spectrophotometrically.[26] Data presented in Table IV show that at pH 9.26 with cycloheptaamylose present *p*-NPMP can be detected after reaction. The amount observed is approximately equal to the quantity of the second mole of *p*-nitrophenol that is not released. The absorbances in Table IV are infinity values after reaction.

(40) The release of *p*-NP from *p*-NPMP is only slightly accelerated in the presence of cycloheptaamylose: H. J. Brass and M. L. Bender, unpublished results.

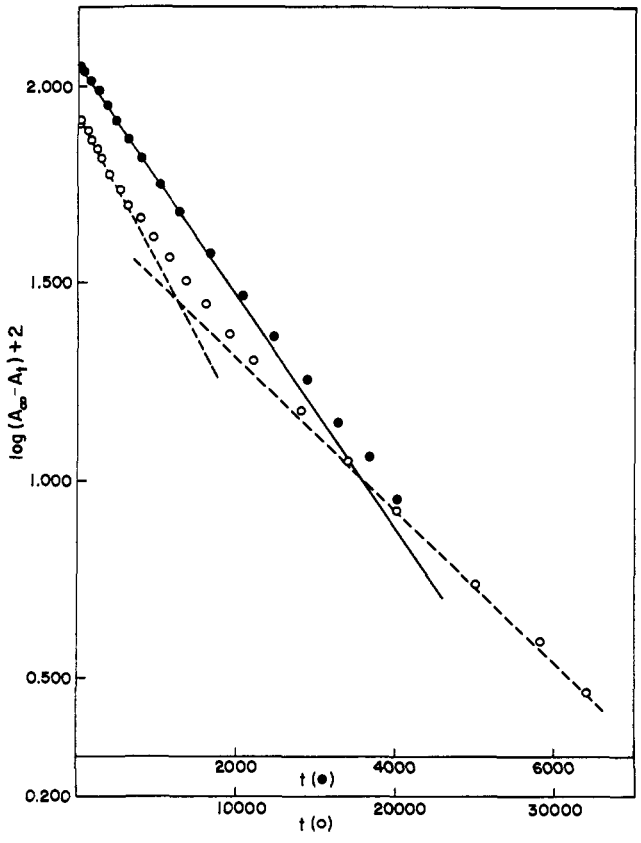


Figure 3. First-order kinetic plot (●) for the reaction of diphenyl methylphosphonate (III) in the presence of cycloheptaamylose; [cycloheptaamylose] = 9.49×10^{-4} *M*, [III] = 3.35×10^{-4} *M*, experimental conditions given in Table VI. Same plot (○) [cycloheptaamylose] = 9.49×10^{-5} *M*, [III] = 3.35×10^{-4} *M*.

Table IV. Spectral Data for the Reactions of IV in the Presence of Cycloheptaamylose[a]

[Cycloheptaamylose], *M*	A_∞ (400 nm)[b]	A_∞ (290 nm)	[*p*-Nitrophenolate] $\times 10^5$, *M* unreleased	[*p*-NPMP] $\times 10^5$, *M*
1.59×10^{-2}	0.730	0.140	1.18	1.47
1.06×10^{-2}	0.710	0.156	1.29	1.64
5.29×10^{-3}	0.676	0.162	1.47	1.71
2.65×10^{-3}	0.665	0.171	1.53	1.80
1.56×10^{-3}	0.645	0.188	1.64	1.98
5.19×10^{-4}	0.611	0.200	1.83	2.11
2.60×10^{-4}	0.554	0.212	2.14	2.23
0	0.472	0.250	2.59	2.61

[a] [*b*-*p*-NPMP] = 2.58×10^{-5} *M*; I = 0.2 *M* carbonate, pH = 9.26. [b] A_t for release of 1 mol of *p*-nitrophenolate = 0.472.

The concentrations of *p*-NP and *p*-NPMP are based on the extinction coefficients of these species. In the presence of cyclohexa- and cycloheptaamylose, we observed an absorbance maximum at *ca.* 270 nm due to the *p*-NP anion. A shoulder of this absorbance occurs at 290 nm, superimposed on the *p*-NPMP absorbance. *p*-NPMP concentrations reported in Table IV are uncorrected for *p*-NP absorbance and thus are slightly higher than the concentrations of unreleased *p*-NP. We feel that the data in Table IV are completely consistent with Scheme IV.

The pathways in Scheme IV can be analyzed as two parallel first-order reactions.[41] The observed rate constant at any cycloheptaamylose concentration corrected for hydrolysis is given in eq 4. Spectrophotometrically the disappearance of IV as well as the appearance of *p*-NPMP and *p*-NP can be followed. Data are given in Table V. Almost all of the substrate

$$k_o(\text{obsd}) = 2k_1 + k_2 \quad (4)$$

Table V. *b*-*p*-NPMP–Cycloheptaamylose Reactions Followed by Substrate Disappearance and Product Appearance[a]

Species followed	λ_{obsd}, nm	k_{obsd}, sec^{-1}
IV	270[b]	1.08×10^{-1}
VI	320[c]	1.06×10^{-1}
p-NP	398[b]	1.11×10^{-1}

[a] [*b*-*p*-NPMP] = 2.62×10^{-5} *M*; [cycloheptaamylose] = 1.06×10^{-2} *M*; pH 9.82, I = 0.2 *M* carbonate buffer. [b] Followed on 0–1 absorbance slide wire. [c] Followed on 0–0.1 absorbance slide wire.

is bound in the cavity at the cycloheptaamylose concentration employed. Consistent with parallel first-order reactions, the rate constants for substrate disappearance and formation of products are identical.[42] For each species followed, 10 μl of substrate solution was injected into the buffer–cycloheptaamylose solution and the reaction followed to completion. A second 10-μl aliquot of substrate was then added and the reaction observed. Identical rate constants obtained, indicating no product inhibition.

Using the k_c(obsd) values for IV in Table I, eq 4, and the infinity values where cycloheptaamylose is saturated by IV, values of $2k_1$ and k_2 were calculated and are reported in Table II. Scheme IV presumably obtains for IV and V in the presence of cyclohexaamylose and the data can be treated in the same fashion; $2k_1$ and k_2 values are also given in Table II.

Reaction in the Presence of Excess Substrate. If Schemes II–IV are correct, neglecting substrate hydrolysis, one cycloamylose molecule should be modified per substrate molecule. This is true for substrates releasing 2 mol of phenol and when cycloamylose concentration is equal to or greater than that of the substrate. The modified cycloamylose should react differently than the unmodified polymer. The data in Table VI and Figure 3 show this to be the case. Diphenyl methylphosphonate (**III**) was chosen as substrate for reaction with cycloheptaamylose due to its

Table VI. Reactions of III in the Presence of Cycloheptaamylose[a,b]

[Cycloheptaamylose] $\times 10^4$, *M*	$k'_{obsd} \times 10^4$, sec^{-1}	$k''_{obsd} \times 10^5$, sec^{-1}
9.49	6.95	
3.16	3.60[c]	
0.949	1.63[d]	8.80[d]
0.949	1.86[d]	9.15[d]

[a] [III] = 3.35×10^{-4} *M*, I = 0.05 *M* carbonate, 0.15 *M* KCl, pH 10.19; measured at 286 nm. [b] $k_{un} = 8.90 \times 10^{-5}$, sec^{-1}. [c] First-order kinetic plot is curved. Rate constant from first 20% of reaction. [d] First-order kinetic plot is linear during the earlier and latter portions of the reaction (Figure 3). Rate constants k'_{obsd} and k''_{obsd} are for each linear segment.

(41) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, Wiley, New York, N. Y., 1965, pp 160–162.

(42) A. J. Kirby and W. P. Jencks, *J. Amer. Chem. Soc.*, 87, 3209 (1965).

high solubility, favorable binding constant with cycloheptaamylose, and high $k_c(\text{obsd})/k_{un}$ value. Buffer concentration was low to reduce the value of k_{un}.

When the cycloheptaamylose concentration was threefold greater than III, an eightfold rate enhancement of phenol release occurs. The reaction approximates first-order kinetics (Figure 3). At equal concentrations of III and cycloamylose, an approximate rate constant is calculated. When the concentration of III is fourfold greater than cycloamylose, a first-order kinetic analysis approximates two first-order reactions, a faster one followed by one that is slower (Figure 3). The rate constant for the slower reaction is identical to that of the uncatalyzed reaction.

Reaction Products. Modification of cycloheptaamylose by III was performed (Experimental Section). Only 18% of the cycloheptaamylose material was isolated, presumably due to its partial retention on the ion exchange column. The product isolated contained 73% of the phosphorus expected on the basis of monomodification of one cycloheptaamylose (structure VII). Using a mixed bed ion exchange column, only the neutral cyclic phosphonate VII could have been isolated. Table VI indicated that ~25% of the substrate should have proceeded through an uncatalyzed path, and thus the maximal theoretical value of the phosphorus modification is 75%. The 73% value based on modification of one cycloheptaamylose is then reasonable.

Discussion

The reactions of diphenyl carbonate (I) and bis(*p*-nitrophenyl) carbonate (II) in the absence and presence of cycloamyloses must proceed *via* carbonyl carbon–oxygen bond cleavage. Diphenyl methylphosphonate (III), bis(*p*-nitrophenyl) methylphosphonate (IV), and bis(*m*-nitrophenyl) methylphosphonate (V) analogously must proceed by phosphorus–oxygen bond cleavage. Attack at aromatic carbon can be excluded, since such attack would yield smaller rate constants than those found in this investigation.[26,43]

The rates of substituted phenol release from diaryl carbonates (I and II) are accelerated in the presence of cycloheptaamylose. Substituted phenol release is also accelerated from diaryl methylphosphonates (III–V) in the presence of cyclohexa- and cycloheptaamylose. Substrate–cycloamylose complex formation occurs, which is analyzed based on a 1:1 association (Scheme I). Saturation kinetics (Figure 1) and stereospecific rate accelerations are exhibited. At high pH the cycloamylose molecule reacts, *via* its ionized form, with bound substrate. The reactions reported in this investigation are examples where cycloamyloses participate covalently.[17,44] Initial reactions of cycloamyloses and substrates after binding are similar to the reactions of phenyl esters,[12,13,45] penicillin derivatives,[14] and organophosphorus esters.[10,16]

From saturation phenomena (Scheme I, Figure 1), rate constants, $k_c(\text{obsd})$, are determined. Maximal rate accelerations can be calculated by dividing $k_c(\text{obsd})$ by k_{un}, the pseudo-first-order rate constant for reaction of the substrate in the absence of cycloamylose. Care must be taken in interpreting these values. At a given pH established by a buffer and constant ionic strength, $k_c(\text{obsd})$ is a constant (Table I). It reflects the reactivity of the ionized cycloamylose molecule with bound substrate.[46] When hydroxide ion is the kinetically important reactive species, k_{un} is also constant, independent of the concentration of buffer species. Upon varying pH, $k_c(\text{obsd})/k_{un}$ will be constant as long as the cycloamylose ion and hydroxide ion continue to be the reactive species; this is only true if the pH is much less than the pK_a values of water and cycloamylose.

The situation is more complex when the buffer species reacts with unbound substrate. At constant pH and ionic strength, upon varying buffer concentration, k_{un} and thus k_c/k_{un} will be proportional to this change.

For IV (Table I) at pH 9.86, I = 0.2 *M* carbonate, $k_c(\text{obsd})/k_{un}$ is 18.6. At pH 9.80, I = 0.1 *M* carbonate (plus 0.1 *M* KCl), $k_c(\text{obsd})/k_{un}$ increases to 26.2. This difference is due to the decrease in the value of k_{un}. Furthermore at constant ionic strength but with varying pH, k_{un} (and $k_c(\text{obsd})/k_{un}$) will be a complex function, depending on the pK_a of the buffer and the reactivities of two (or more) buffer species, each having a different state of ionization. As pH is decreased to 9.26, $k_c(\text{obsd})$ for the reaction of IV decreases in a first-order fashion with respect to hydroxide ion concentration. The decrease in k_{un} is less than first order and, as a result, $k_c(\text{obsd})/k_{un}$ is 11.5.

Diaryl Carbonates. The values of $k_c(\text{obsd})$, $k_c(\text{obsd})/k_{un}$, and K_D for the reactions of I and II are of the same magnitude as the rate and binding constants for phenyl acetates in the presence of cycloheptaamylose. Constants determined for I and II are for the initial acylation of bound substrate, which is the rate-determining k_c step in Scheme II. Of interest are the reactions of carbonates (and phosphonates to be discussed below) after this initial acylation. Corey–Pauling–Koltun models show that a hydroxyl group, either on the same glucose residue to which the carbonate CS_1 is bound or on an adjacent glucose residue, is in position to attack the carbonyl center, releasing the second mole of substituted phenol and producing a cyclic cycloheptaamylose carbonate. These hydroxyl groups are appropriately located for attack, whether the aromatic ring is within the cycloamylose cavity or resides in the bulk solution. Such an intramolecular reaction is expected to be rapid. The release of the second mole of phenol or *p*-nitrophenol from I and II at a rate faster than the initial acylation supports the mechanism proposed in Scheme II. The synthesis of nucleoside carbonates has been demonstrated.[47,48] Further support for Scheme II is derived from the product analysis, showing that monomodification of cycloheptaamylose by III (see below) apparently yields a cyclic cycloamylose methylphosphonate VII.

The reactions of II in the presence of α-chymotrypsin

(43) H. J. Brass, J. O. Edwards, and M. J. Biallas, *ibid.*, **92**, 4675 (1970).
(44) A likely exception is the reaction of IV at pH ~5.6.
(45) K. Flohr, R. M. Paton, and E. T. Kaiser, *Chem. Commun.*, 1621 (1971).
(46) This assumes that processes within the cycloamylose cavity attributable to reactions by the substrate with species from the bulk solution are minor.
(47) R. L. Letsinger and K. K. Ogilvie, *J. Org. Chem.*, **32**, 296 (1967).
(48) J. R. Tittensor, *J. Chem. Soc. C*, 2656 (1971).

have been reported.[49,50] After binding of the substrate to the enzyme, a rapid reaction occurs, liberating 1 mol of *p*-nitrophenol. This corresponds to acylation of the enzyme. Subsequently, the second mole of *p*-nitrophenol is released in a slower "aging" reaction probably *via* attack of a functional group in one of the enzyme side chains. The histidine-57 imidazole likely acts as a general base in both the aging and subsequent reactivation processes.[49] In the case of the cycloamylose, a hydroxyl group is able to participate in a rapid intramolecular reaction after initial acylation. Thus, acylation is the slow step in the cycloamylose cases; it is the fast step for α-chymotrypsin.

Similar intramolecular reactions have been previously reported. One example was given in the introduction, involving the acetyl migration of a ribonucleoside derivative.[3,6] Acetyl migrations in aminoacyl ribonucleotides from the 2′ to the 3′ position are faster than hydrolysis by 10^5–10^6 near neutral pH. This large rate increase must be due to the proximity of the neighboring hydroxyl group.[3] The case of aryl cycloamylose carbonates is analogous. The adjacent hydroxyl moiety is in a position to displace the labile aryl function.

Diaryl Methylphosphonates. Simple Cases. The reactions of III and V in the presence of cycloheptaamylose and of III in the presence of cyclohexaamylose are comparable to the diaryl carbonate cases. The mechanism proposed in Scheme III is analogous to that offered in Scheme II. (The reaction of III in Scheme III is used for illustrative purposes.) It is similar to the mechanism postulated by Cramer, *et al.*,[10b] for the hydrolysis of diaryl pyrophosphates in the presence of calcium ions, catalyzed by cycloamyloses. According to the Cramer mechanism, after substrate binding a phosphorylation of the cycloamylose occurs, yielding a monophenyl phosphate and a cycloamylose-phenyl phosphate diester. This reaction is rate determining. In a subsequent rapid reaction, phenol is displaced from the diester by attack of an adjacent hydroxyl group of the cycloamylose.[10b,17] This step yields a cyclic phosphate ester.

Hydrolysis of III–V in buffer (eq 8) yields the stable aryl methylphosphonate anions (VIa–c) and 1 mol of substituted phenol. Two moles of substituted phenol produced in the presence of cycloamylose implies the formation of a neutral ester in the initial rate-determining phosphorylation step. The rapid release of the second mole of substituted phenol is consistent with a facile intramolecular phosphorylation, producing VII.

The reaction of III in the presence of cycloheptaamylose, under conditions ranging from cycloheptaamylose in excess to substrate in excess, yields further mechanistic information (Table VI, Figure 3). The approximate linearity of the first-order kinetic plot with cycloheptaamylose in excess is expected on the basis of Scheme I. With substrate in excess two consecutive first-order reactions obtain. The slow second reaction becomes kinetically important after the consumption of substrate, corresponding to a monomodification of the cycloheptaamylose. The rate constant for the first reaction is twofold greater than the uncatalyzed rate constant, further implying modification of the cycloheptaamylose.

The rate constant of the slower second reaction equals the uncatalyzed rate constant. Two interpretations are possible. The reactivity of the modified cycloheptaamylose may be less than the reactivity of reagents from bulk solution. Another possibility is that III, which is present in excess, does not bind well into the monomodified cycloamylose. This would prevent a substantial amount of substrate III from being included into the monomodified cycloamylose at any time in solution. The contribution to the overall rate of reaction by the modified species would then be small.

The isolation of an apparently monomodified cycloheptaamylose from reaction with III is convincing proof of the existence of VII and thus the mechanism leading to its formation. The species isolated must be neutral since an ionic molecule would not have been eluted from the chromatographic column. Similar isolations of cycloamyloses modified by the addition of a phosphorus function have been reported.[10b,16]

Experiments in our laboratories show that when IV is allowed to react in slightly alkaline solution in the presence of α-chymotrypsin, biphasic behavior is observed.[51] Two moles of *p*-nitrophenol are released, with the step leading to the production of the second mole being slower than the initial phosphorylation. This observation is analogous to the reaction of II in the presence of α-chymotrypsin.[49] The reaction of IV in the presence of acetylcholinesterase also shows biphasic behavior with 2 mol of *p*-nitrophenol being released.[52] Presumably a neighboring group in the phosphorylated enzyme cannot react, rapidly leading to production of the second mole of *p*-nitrophenol.

The formation of cyclic phosphonate VII is analogous to the first step of ribonuclease action.[53,54] Binding of substrate to the enzyme forces the reactive groups to be so oriented that cyclic phosphate formation is facile. In aryl cycloamylose methylphosphonates, the reactive groups are fixed by covalent binding so that a rapid intramolecular reaction is possible. The magnitude of the rate accelerations observed in the formation of VII is probably comparable to the formation of cyclic phosphates from nucleoside phosphate diesters.[3]

Complex Cases. The reactions of IV in the presence of cycloheptaamylose and of IV and V in the presence of cyclohexaamylose release between 1 and 2 mol of substituted phenol in a first-order process. The data of Table II show that the number of moles of *p*-nitrophenol released from IV, $A_{\infty(\max)}/A_1$, is pH independent from pH 10.41 to approximately pH 8. $A_{\infty(\max)}/A_1$ is pH independent for the reaction of V in the presence of cyclohexaamylose over a limited pH range.

Several mechanisms appear possible. After binding, two types of cycloamylose hydroxyl groups may be phosphorylated. In one case a rapid intramolecular reaction occurs, leading to a cyclic methylphosphonate and a second mole of phenol. In the other a stable neutral aryl cycloamylose methylphosphonate is formed. The data in Table IV show that this interpretation is

(49) (a) M. L. Bender and F. C. Wedler, *J. Amer. Chem. Soc.*, **94**, 2101 (1972); (b) M. L. Bender and F. C. Wedler, *Biochem. Biophys. Res. Commun.*, **47**, 820 (1972).
(50) T. H. Fife, J. E. C. Hutchins, and D. M. McMahon, *J. Amer. Chem. Soc.*, **94**, 1316 (1972).
(51) H. J. Brass and M. L. Bender, unpublished results.
(52) J. W. Hovanec and C. N. Lieske, *Biochemistry*, 11, 1051 (1972).
(53) D. A. Usher, *Proc. Nat. Acad. Sci. U. S.*, **62**, 661 (1969).
(54) Reference 5, p 523.

not possible. After the reaction of IV in the presence of cycloheptaamylose, *p*-nitrophenyl methylphosphonate VIb, *an anion*, is detected. The concentration of VIb is always equal to the amount of the second mole of *p*-nitrophenol that is unreleased.

Another possibility is direct attack by hydroxide ion on the bound substrate in competition with phosphorylation by cycloamylose. Such attack would yield VIb from IV and explain $A_{\infty(max)}/A_1$ being pH independent. This interpretation seems unlikely, though it cannot be disproved. The second-order rate constant for the reaction of hydroxide ion with IV in the absence of cycloamylose is 35 M^{-1} sec^{-1}.[26] At pH 9.86 the pseudo-first-order rate constant for hydroxide reaction is calculated to be 2.5×10^{-3}, sec^{-1}. At pH 9.86, k_c(obsd) for the reaction of IV in the presence of cycloheptaamylose is 1.59×10^{-1}, sec^{-1}. The rate of hydroxide reaction with IV would have to increase nearly 100-fold in the cycloheptaamylose cavity, as compared to reaction with IV in bulk solution, in order to compete effectively with the reaction of the cycloheptaamylose anion. Such a rate increase seems improbable.

The mechanism most likely operative is given in Scheme IV. After inclusion, a general base reaction of water with IV catalyzed by the cycloamylose anion competes with direct nucleophilic attack. This mechanism is postulated to obtain for the reactions of IV and V (at pH values above approximately 8), where less than 2 mol of substituted phenol are released, catalyzed by cycloamylose. IV has been shown to be susceptible to the general base attack of water catalyzed by amines.[26]

General base and nucleophilic reactions of included substrate can be treated as parallel pseudo-first-order reactions.[55] Consistent with such treatment, the disappearance of IV, the appearance of VIb, and the appearance of *p*-nitrophenol have identical rate constants at high cycloheptaamylose concentration (Table V).

A possible explanation for those cases where competitive general base and nucleophilic mechanisms obtain can be offered, based on the precise way in which the substrate binds to the cycloamylose. Subtle differences in the orientation of the substrate in the cycloamylose cavity can determine the reaction mechanism. Such precise orientation of a substrate upon complexation is thought to lead to the high specificity of enzyme-catalyzed reactions. The possible presence of competing general base and nucleophilic reactions of cycloamylose anions is analogous to the mechanisms postulated in the hydrolysis of aspirin.[56]

The reaction of IV at pH 5.6 in the presence of cycloheptaamylose yields only 1 mol of *p*-nitrophenol. A small acceleration in the rate of *p*-nitrophenol release is observed and substrate binding is apparent (Table III). Upon lowering the pH, the fraction of cycloheptaamylose existing in its ionized form is reduced. We feel that at low pH the importance of pathways proceeding *via* the cycloamylose anion is also reduced. These pathways are superseded by one where reagents from the bulk solution react with the bound substrate.

For the cases where less than 2 mol of substituted phenol are released, k_c(obsd) is the sum of two parallel reactions (eq 9). k_c(obsd) values for these cases are directly comparable to k_c(obsd) values when 2 mol of phenol are liberated.

The data in Table VII show that the meta to para stereospecific rate acceleration shown by nitro-substituted phenyl acetates[12] is also displayed by diaryl methylphosphonates IV and V. This represents the first time that a stereospecific rate acceleration has been demonstrated for an organophosphorus ester. The rate acceleration by V as compared to IV is not as large as that shown by *m*- and *p*-nitrophenyl acetates. It is reasonable that a tetrahedral phosphorus molecule will not bind to the cycloamylose in the same way as the planar carbonyl substrate. Differences in the magnitude of the stereospecific rate accelerations are reasonable.

Table VII. Comparison of Stereospecific Rate Accelerations between Phenyl Acetates and Diaryl Methylphosphonates

Substrate[a]	k_c(obsd)/k_{un}	$K_D \times 10^3$ M^{-1} [b]
Cyclohexaamylose		
Phenyl acetate[c]	27	22
p-Nitrophenyl acetate[c]	3.4	12
m-Nitrophenol acetate[d]	300	19
III[e,f]	35.1	37.7
IV[e,g]	8.35	31.2
V[e,g]	66.1	94.9
Cycloheptaamylose		
Phenyl acetate	*h*	*h*
p-Nitrophenyl acetate[c]	6.34	6.1
m-Nitrophenyl acetate[c]	44.4	8.0
III[e,f]	16.0	1.43
IV[e,g]	18.6	4.64
V[e,g]	41.4	3.45

[a] Data for phenyl acetates from ref 12a. [b] Error limits not given since comparisons are being made. [c] In pH 10.60 carbonate buffer, I = 0.2 *M*, 25°. [d] In pH 10.01 carbonate buffer, I = 0.2 *M*, 25°. [e] Data in this investigation. [f] In pH 10.8 carbonate buffer, I = 0.2 *M*. [g] In pH 9.83 (hexa) or 9.86 (hepta) carbonate buffer, I = 0.2 *M*. [h] Data not available.

One can estimate the acceleration of the intramolecular phosphorylation of *p*-nitrophenyl cycloheptaamylose methylphosphonate (k_1 path, Scheme IV). The first-order rate for the reaction of hydroxide ion with isopropyl *p*-nitrophenyl methylphosphonate at pH 9.86 is 1.4×10^{-5}, sec^{-1}.[16b] The minimum rate constant for the step leading to the production of the second mole of *p*-nitrophenol from IV at pH 9.86 is 5.0×10^{-2}, sec^{-1} ($2k_1/2$, Table II). The minimum rate acceleration for the intramolecular phosphorylation is 10^3–10^4 *M*. It is probably greater than this value.

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(55) The reaction of bound substrate with hydroxide ion and cycloamylose anion can also be treated as parallel reactions.

(56) Reference 4, p 7.