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Ester Hydrolyses Catalyzed by Modified Cyclodextrins[1]

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Cyclohexaamylose-*N*-(*N*,*N*′-dimethylaminoethyl)acetohydroxamic acid and cyclohexaamylose-*N*-(4-imidazolemethyl)acetohydroxamic acid were synthesized. Their catalytic power is greater than that of cyclohexaamylose or of cyclohexaamylose-*N*-methylhydroxamic acid. In addition, optical selectivity was exhibited in the hydrolysis of D- and L-acetylphenylalanine *p*(*m*)-nitrophenyl esters by cyclohexaamylose-*N*-(*N*,*N*′-dimethylethyl)acetohydroxamic acid, and cycloheptaamylose.

INTRODUCTION

Cyclodextrin has been shown to form inclusion complexes with a number of molecules and ions (*1*). For this reason, cyclodextrins have been used as simple enzyme models. For instance, the effect of cyclodextrin on the alkaline hydrolysis of phenyl esters displayed enzyme-like kinetic behavior such as saturation, nonproductive binding and competitive inhibition (*2*).

However, there were two limitations to this model system as compared to the enzyme system. One is that the deacylation rate of the acyl-cyclodextrin intermediate is very small, less than the rate of alkaline hydrolysis of the ester. The other is that the rate constant for the enzyme reaction is maximal in the neutral pH region, whereas the rate constant for the cyclodextrin-catalyzed reaction is maximal at pH 13.

Recently the *N*-alkylhydroxamate ion was shown to be an efficient nucleophilic catalyst in the neutral pH region and shown to have the property of high nucleophilicity toward ester substrates with exceptional lability of the intermediate on the reaction pathway leading to the formation of product and regeneration of the catalyst (*3*). *N*,*N*′-dimethylaminoethylhydroxamic acid was also found to be a very effective catalyst for the overall process because the *N*,*N*′-dimethylaminoethyl group assists the deacylation step intramolecularly to regenerate the catalyst much faster than other *N*-alkylhydroxamic acids (*3*, *4*).

In addition, asymmetric catalysis in the cyclodextrin-catalyzed hydrolysis of mandelic acid esters was observed through the formation of a cyclodextrin-ester complex, although the catalysis was negative (*5*). Moreover, several optical resolutions of racemic sulfoxides were reported (*6*). These results indicate the existence of an asymmetric inclusion complex. Stereospecificity was also observed in the formation of an inclusion

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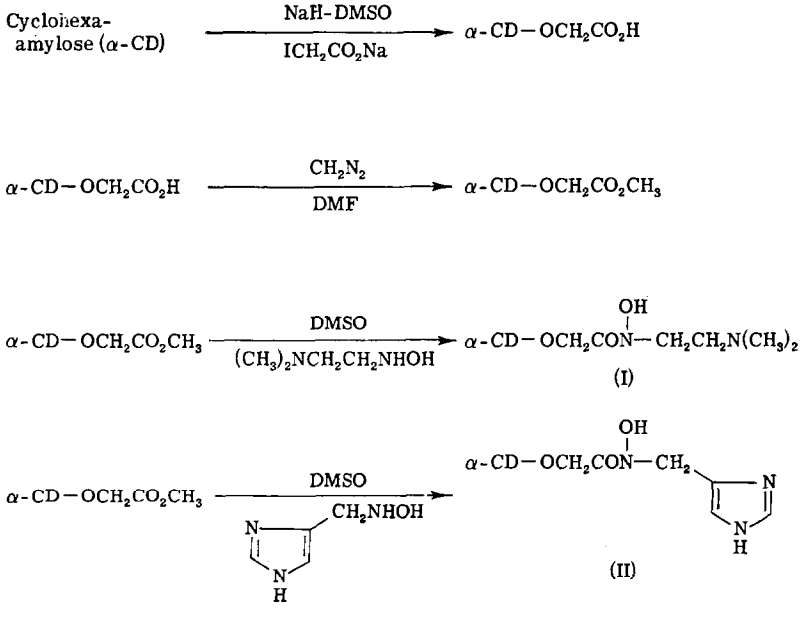
complex with the enantiomers of isopropyl *S*-2-dimethylaminoethyl methylphosphonothioate (*7a*). Stereospecificity has been observed in the α-cyclodextrin-catalyzed hydrolysis of the phosphorus esters, isopropyl methylphosphonofluoridate, isopropyl *p*-nitrophenyl methylphosphonate, and isopropyl *S*-2-dimethylaminoethyl methylphosphonothioate (*7b*). Therefore, it appeared possible that stereospecific (optically specific) positive catalysis might be observed with a carboxylic ester if the reactivity of the inclusion complex could be increased by introducing a second functionality having high nucleophilicity, such as a substituted hydroxamic acid, or by changing the geometry of the inclusion complex. Consequently, this modified cyclodextrin may really display enzyme-like behavior by utilizing the proximity effect, the orientation effect, fast acylation, fast deacylation in the neutral pH region, and optical selectivity. The aim of this paper is to design such enzyme-like catalysts which cause stereoselective and large rate accelerations in the overall process in the neutral pH region.

RESULTS AND DISCUSSION

*Syntheses of Cyclohexaamylose-*N*-Substituted-Acetohydroxamic Acids*

Two cyclohexaamylose derivatives were synthesized. The general synthetic scheme is shown below.

Scheme I



The detailed synthetic procedures are described in the experimental section. The purities of **I** and **II** are determined to be 81 and 63%, respectively by potentiometric titration after purification on a Sephadex column.

Hydrolyses of p-*Nitrophenyl Acetate* (*PNPA*), m-*Nitrophenyl Acetate* (*MNPA*) *and* p-*Nitrophenyl Thiolacetate* (*PNTA*) *in the Presence of* **I**, **II** *and Cyclohexaamylose* (*CA*)

To investigate the effect of modified cyclohexaamyloses on ester hydrolysis, **I**, **II**, and CA were reacted with PNTA, PNPA, and MNPA at pH 7.80. Pseudo-first-order rate constants for these reactions are summarized in Table 1. A plot of k_{obs} versus [**I**] is shown in Fig. 1 for PNTA. As this figure shows, k_{obs} shows a linear dependence on [**I**] and Eq. [1],

$$k_{obs} = k_I[\mathbf{I}] + k^0_{obs}, \qquad [1]$$

describes k_{obs} as a function of [**I**]. The second-order rate constant, k_I, was calculated from the slope of the plot in Fig. 1. The second-order rate constants, k_I and k_{II} for PNPA, PNTA, and MNPA, were obtained in the same way and are summarized in Table 2.

TABLE 1

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE REACTIONS OF PNTA WITH I AND II[a]

[I] × 10^4 *M*	k_{obs} × 10^3 sec^{-1}	[II] × 10^4 *M*	k_{obs} × 10^3 sec^{-1}
4.10	4.46	3.15	1.83
3.40	3.77	1.80	1.20
2.56	1.99	0	0.024
1.64	1.02		
0.66	0.18		
0	0.024		

[a] [PNTA] = 5×10^{-6} *M*, $I = 0.20$, pH 7.80 phosphate buffer, $T = 25.5 \pm 0.3$°C.

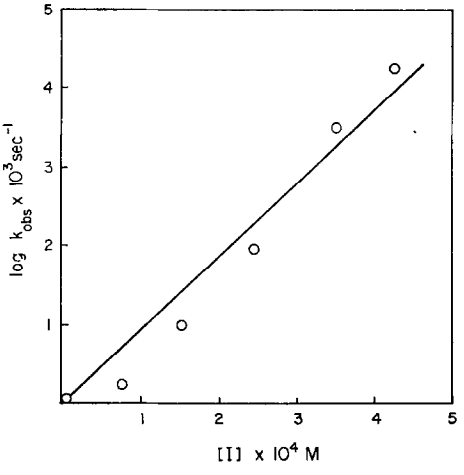


FIG. 1. A plot of the liberation of *p*-nitrophenol from *p*-nitrophenyl thiolacetate (k_{obs}) as a function f [I]; conditions indicated in Table 1.

TABLE 2

SECOND-ORDER RATE CONSTANTS FOR THE REACTIONS OF THREE ESTERS (PNPA, PNTA AND MNPA) WITH I, II AND CA

	k_{II} (M^{-1} sec^{-1})	k_{II}/k_{CA}	k_I (M^{-1} sec^{-1})	k_I/k_{CA}	k_{CA} (M^{-1} sec^{-1})
PNTA	6.10	1525	11.10	2775	0.004
PNPA	3.0	1500	5.0	2500	0.002
MNPA	—	—	1.2	240	0.05

I and II react much faster than unsubstituted cyclohexaamylose with PNTA at this pH. However, I reacts with MNPA only 240 times faster than cyclohexaamylose does. This is the opposite of the stereospecificity observed for cyclohexaamylose-catalyzed hydrolysis which reacts where the *m*-substituted phenyl ester reacts faster than the *p*-substituted ester (*2a*). However, it is the same stereochemistry as that observed in cyclohexaamylose-*N*-methylacetohydroxamic acid-catalyzed reactions (*5*) and can be explained by the stereochemistry of the inclusion complex that exists in a favorable geometrical fit of the carbonyl group of the *para* isomer to the hydroxamate ion.

PNTA reacts with I and II only 2–2.2 times faster than PNPA does. An -SR group is at least 250 times a better leaving group than the -OR group (*8*). Therefore, the thiol ester should react much faster if the leaving group is eliminated in the same step. This result may indicate indirectly that carbon–oxygen and carbon–sulfur bond fission is not the rate-determining step and consequently suggests the existence of an intermediate (tetrahedral intermediate). This same argument has been used in enzyme-catalyzed reactions (*9*).

Hydrolyses of D- *and* L-*Acetylphenylalanine* p- *and* m-*Nitrophenyl Ester* (*APANP*) *in the Presence of* I, II, *and Cycloheptaamylose* (*CHA*)

To investigate the stereochemical selectivity of cyclodextrin and modified cyclodextrins toward ester substrates, I, II, and CHA were reacted with D- and L-*p*-APANP and *m*-APANP. Pseudo-first-order rate constants k_{obs} showed a linear dependence on the catalyst concentration. Second-order rate constants were calculated from Eq. [1] and are shown in Table 3. CHA inhibited the reactions of D- and L-*p*-APANP, whereas

TABLE 3

SECOND-ORDER RATE CONSTANTS FOR THE REACTIONS OF *p*(*m*)-PNPAL WITH I, II, AND CHA[a]

Ester	k_{CHA} (M^{-1} sec^{-1})	L/D	I M^{-1} sec^{-1}	L/D	II M^{-1} sec^{-1}	L/D
m-D-PNPAL	3.7×10^{-2}	2.0	1.23	0.65	—	—
m-L-PNPAL	7.7×10^{-2}		0.80		—	—
p-D-PNPAL	Inhibition		—		17.0	1.20
p-L-PNPAL	Inhibition		—		20.0	

[a] pH = 7.80, $I = 0.20$ phosphate buffer, $T = 25.5 \pm 0.3$°C.

II accelerated the reaction of both optical isomers and optical selectivity was also observed. By introducing the *N*-imidazolemethyl-acetohydroxamic acid group into cyclohexaamylose, the reactivity of the complex was increased and positive catalysis was observed. That is, *p*-L-APANP reacts with **II** 1.2 times faster than *p*-D-PNPAL. On the other hand, *m*-D- and L-APANP is catalyzed by CHA and positive catalysis was observed. CHA reacts with the L-isomer two times faster than D-isomer. However, **I** reacts with D-isomer 1.5 times faster than L-isomer. This is the opposite of the optical specificity observed in the reactions of CHA with these compounds. This result indicates the reactivity of the complex can also be increased by changing the geometry of the substrate. The above results imply that the optical specificity comes from asymmetric complex formation (asymmetric binding), but the reactivity of the complex that determines the catalyzed reaction is positive or negative depending solely on the geometry of binding.

pH Dependence of the Reaction of PNPA with **I** *and* **II**

I and **II** were reacted at different pH's with PNPA. The values of k_{obs} obtained at different pH values are recorded in Table 4 and plots of $\log k_{obs}$ versus pH are shown in Figs. 2 and 3. With **II**, the pH–$\log k_{obs}$ profile shows two maxima which indicates

TABLE 4

pH DEPENDENCE OF THE REACTIONS OF **I** AND **II** WITH PNPA[a]

pH	$k_{Iobs} \times 10^3$ (sec^{-1})	$k_{IIobs} \times 10^3$ (sec^{-1})
7.10	0.64	0.70
7.80	1.04	1.84
8.30	0.79	3.55
8.75	0.97	—
9.20	1.34	6.82

[a] [PNPA] = 5×10^{-6} *M*, [**I**], [**II**] = 5×10^{-4} *M*. $I = 0.2$, $T = 25.50$°C, phosphate or borate buffer.

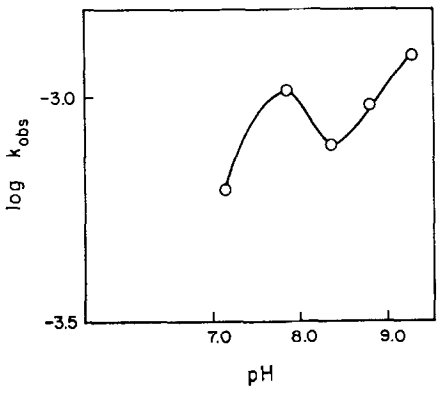


FIG. 2. A plot of the pH dependence of the acylation of **I** by PNPA; conditions indicated in Table 4.

the existence of two catalytically active groups, an imidazole group and a hydroxamic acid hydroxyl group, whereas only one catalytically active group is observed in **I** (the hydroxamate ion). These results show that a nucleophilic or a general basic mechanism operates in acylation.

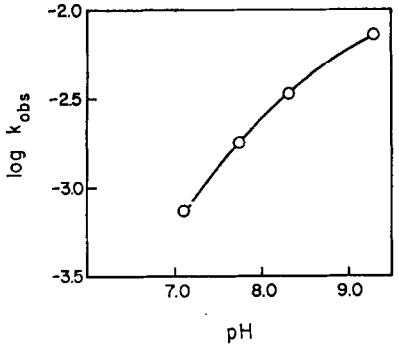


FIG. 3. A plot of the pH dependence of the acylation of II by PNPA; conditions indicated in Table 4.

Acylation of **I** *with Acetic Anhydride and Deacylation of Acyl-***I**

The acylation of the hydroxyl group in the hydroxamic acid moiety of **I** and the deacylation of acetyl-**I** were studied at pH 7.80 using acetic anhydride as substrate. Twenty milliliters of 5.0×10^{-3} *M* acetic anhydride solution in acetonitrile was added to 3 ml of a 1×10^{-4} *M* solution of **I** in pH 7.80 phosphate buffer. The final acetic anhydride concentration was 1×10^{-4} *M*. The reaction was followed at 260 nm. The absorbance dropped rapidly and then increased. These absorbance changes correspond to rapid acylation followed by deacylation of acetyl-**I**. The pseudo-first-order rate constant of deacylation, k_3, was 1.12×10^{-2} sec^{-1} at pH 7.80. The pseudo-first-order rate constant for the alkaline hydrolysis of benzoyl-cyclohexaamylose at pH 7.80 is 4.6×10^{-7} sec^{-1} (extrapolated) (*2b*). Although acetyl derivatives are in general about tenfold faster than benzoyl derivatives because of resonance, some other factor must account for the approximately 10^5-fold difference in rates. This marked lability of acetyl-**I** can be ascribed to intramolecular general base catalysis as seen in the hydrolysis of *N,O*-diacetyl-*N,N'*-dimethylethylhydroxylamine (*4*). Thus, **I** is a true catalyst for it is regenerated at neutral pH.

EXPERIMENTAL

Purification of cyclohexaamylose (*10*). The Corn Products Co. material, which was used in this investigation, was contaminated with cycloheptaamylose; this impurity was removed as follows according to the method of D. French. Cycloheptaamylose was precipitated by adding bromobenzene to cyclohexaamylose in aqueous solution. The precipitate was filtered, and, from the filtrate, cyclohexaamylose was precipitated as a complex by adding tetrachloroethane. The crystalline complex was air dried, and tetrachloroethane was boiled off with water. The resulting aqueous solution was treated with one-half volume of *n*-propyl alcohol and allowed to cool at room tem-

perature. The crystalline propanol complex was collected and recrystallized from water, followed by drying *in vacuo* for 10 hr at 80°C. The purity was judged by paper chromatography (butanol:DMF:water, 2:1:1) using 1% alcoholic iodine as a developing reagent.

Preparation of N,N'-*dimethylaminoacetaldoxime* (*4*). *N,N'*-dimethylaminoacetaldehyde diethyl acetal (100 g, 0.62 mole) was dissolved in 350 ml of concentrated HCl with cooling and kept in a refrigerator for 2 days. The resulting slightly yellow solution was evaporated under reduced pressure to give *N,N*-dimethylacetaldehyde as a yellow oily residue. To this aldehyde, a solution of potassium hydroxide (56 g, 1.0 mole) and hydroxylamine hydrochloride (68 g, 0.98 mole) dissolved in 60 ml of water was added slowly, cooling in an ice–salt bath. The resulting solution was allowed to stand at room temperature for 3 hr and kept in the refrigerator for 1 day. The solution was saturated with potassium carbonate and extracted with 300 ml of ether. The ether extract was dried over magnesium sulfate and filtered, and then the ether was evaporated to give a brownish oily residue which on distillation gave *N,N'*-dimethylaminoacetaldoxime (60 g) as a white solid; bp, 100°C (11 mm Hg).

Preparation of 2-(*N,N'-dimethylamino*)*ethyl hydroxylamine. N,N'*-dimethylaminoacetaldoxime (9.0 g, 0.088 mole) was dissolved in 50 ml of anhydrous tetrahydrofuran and placed in a three-necked flask. Nitrogen gas was introduced to this system. A constant nitrogen stream was maintained with cooling of the flask to 2°C in an ice bath. Borane (200 ml) in tetrahydrofuran (0.1 mole) was added to this solution dropwise from a syringe such that the temperature remained below 5°C. After the addition of borane, the solution was stirred at 5°C for half an hour and for 3 hr at room temperature. The solvent was removed *in vacuo* and the resulting residue was cooled to 0°C in an ice bath, followed by the addition of 50 ml of 1 *N* HCl and 25 ml of 2 *N* HCl at such a rate that the temperature did not exceed 5°C. The resulting reaction mixture was refluxed for 1 hr, made basic to pH 12 with 10 *N* NaOH solution and extracted five times with 200-ml portions of ether. The combined ether extracts were dried over magnesium sulfate and evaporated to give a colorless liquid (7.00 g). A tlc analysis of this product showed one major spot and two minor spots. An attempt to distill this liquid at room temperature or 60°C under 0.2 mm Hg led to decomposition. Column chromatography on silica gel also led to decomposition. This compound was therefore used for the synthesis of cyclohexaamylose derivatives without further purification; it reduced Fehling's solution strongly.

*Preparation of cyclohexaamylose-*N-(N,N'-*dimethylaminoethyl*)*acetohydroxamic acid* (**I**). *1. Carboxymethylcyclohexaamylose* (*5*). Sodium hydride, 0.41 g, (55% purity) was placed in a 100-ml round-bottom flask which was flushed with nitrogen gas. The mineral oil was removed by washing with two 10-ml portions of *n*-hexane. Then 20 ml of dry dimethyl sulfoxide (DMSO) was added and the solution was maintained at 80°C in an oil bath for 2 hr. To the resulting pale green solution, cyclohexaamylose (8.4 g, 86.64 mmoles in dry DMSO, 30 ml) and sodium iodoacetate (0.37 g 1.75 mmoles), were added. After heating at 55°C for 2 days in an oil bath under a nitrogen atmosphere, the resulting brownish solution was acidified to pH 2 by adding 1 *N* HCl solution. This reaction mixture was then poured into 100 ml of acetone to give a precipitate of cyclohexaamylose. The acetone was decanted, the precipitate was air dried, dissolved in a few milliliters of water and lyophilized to give a slightly brown cottonlike solid. The

ir spectrum of this solid showed a weak absorption at 5.75 μ due to the carbonyl group. This solid was dissolved in double-distilled water and chromatographed on a Sephadex G-10 column (70 × 2.5 cm), eluting with double-distilled water. Fractions of 25 ml were collected, and each fraction was tested for cyclohexaamylose and sodium chloride using a 1 % alcoholic iodine solution and an aqueous silver nitrate solution, respectively. Sodium chloride-free cyclohexaamylose fractions were collected and lyophilized to give a cyclohexaamylose mixture (6.20 g) whose ir spectrum showed a 5.75-μ carbonyl absorption. This cyclohexaamylose mixture was then dissolved in 15 ml of double-distilled water and the pH of this solution was adjusted to 6.0 by adding 1 *N* NaOH solution. This solution was chromatographed on a Sephadex column (A-25-DEAE). This column was first eluted with 500 ml of distilled water to remove unreacted cyclohexaamylose, followed by 0.5 *N* formic acid to elute carboxymethylcyclohexaamylose. Fractions of 25 ml were collected and individual fractions were lyophilized. Lyophilizing of fraction 7 gave a white crystalline solid (600 mg) whose ir spectrum showed the same spectrum as that of the parent cyclohexaamylose except for a strong absorption at 5.75 μ due to the carbonyl group.

2. Preparation of carboxymethylcyclohexaamylose methyl ester (4). Carboxymethylcyclohexaamylose (500 mg) was dissolved in 20 ml of dry DMF (dried over CaH_2) and cooled to 0°C in an ice bath. To this solution, 5 ml of a diazomethane solution in ether was added dropwise. After nitrogen evolution ceased, the solution was pale yellow. The excess diazomethane was destroyed by adding acetic acid until a colorless solution was obtained. The resulting solution was poured into 100 ml of anhydrous ether with the formation of a white colloidal precipitate. After centrifuging the suspension and decanting the organic solvent, a white precipitate was obtained and air dried. This white precipitate was dissolved in 10 ml of water and lyophilized to give 480 mg of white leaflets. The ir spectrum showed a strong carbonyl absorption at 5.70 μ.

3. Cyclohexaamylose-N-(N,N′-*dimethylaminoethyl*)*acetohydroxamic acid* (I). Carboxymethylcyclohexaamylose methyl ester (480 mg, 0.45 mmole, dried in a drying pistol for 10 hr at 80°C) was dissolved in 20 ml of anhydrous DMSO under a nitrogen atmosphere. To this solution, 2-(*N*,*N*′-dimethylaminoethyl)hydroxylamine (1.04 g, 10 mmoles) was added at room temperature. The resulting solution was heated to 70°C in an oil bath and the reaction checked by adding 5 % $FeCl_3$ in 0.1 *N* HCl solution to 20-μl aliquots of the reaction mixture in 1 ml of water (hydroxamic acid forms a complex with Fe^{3+}; hydroxamic acid formation was followed by measuring the absorbance at 540 nm). The solution turned dark brown after 48 hr. The reaction mixture was poured into 100 ml of acetone. The precipitate was collected by decantation, air dried and dissolved in 10 ml of distilled water. This solution was passed through a Sephadex G-10 column (65 × 2.5 cm). The column was eluted with double-distilled water and 25-ml fractions were collected. Twenty fractions were collected and individual fractions were tested for hydroxamic acid ($FeCl_3$ assay) (*12*) and sodium chloride (silver nitrate). Fractions 5–8 contained hydroxamic acid and were free of sodium chloride. Fractions 5–8 were lyophilized to give a white crystalline product. The ir spectrum of fraction 6 had a weak absorption at 5.75 μ which is due to the –COOH or $-COOCH_3$ group. Fraction 6 (210 mg) was dissolved in 2 ml of water and chromatographed on a Sephadex A-25-DEAE column (1 × 20 cm), cooled externally

with water. Distilled water was eluted at 7°C; 15-ml fractions were collected. Hydroxamic acid was also found in fractions 2 and 3. These fractions were lyophilized to give 60 and 20 mg of white crystals, respectively. The ir spectrum of fraction 3 still showed a weak absorption at 5.75 μ. However, the ir spectrum of fraction 2 had no absorption at 5.75 μ but a strong absorption at 6.05 μ which is due to hydroxamic acid. After drying fraction 2 at 80°C for 10 hr, 2.20 mg of this fraction was dissolved in 30 ml of double-distilled water and titrated potentiometrically with 0.1 *N* NaOH solution. One inflection point was observed corresponding to 82% purity of the hydroxamic acid. *Anal.* Calcd for $C_{42}H_{72}O_{32}N_2$ (MW = 1117): C, 45.16; H, 6.45; N, 2.51; calcd for $C_{42}H_{72}O_{32}N_2 \cdot 6H_2O$ (MW = 1225): C, 41.17; H, 6.86; N, 2.58. Found: C, 39.58; H, 6.12; N, 2.58. The sample was dried at 80°C for 10 hr before sending it for analysis and weighed in air by Micro-Tech Labs. The hydroxamic acid derivative is very hygroscopic which partially accounts for the discrepancy in analysis.

Preparation of 4-hydroxymethylimidazole (13). To 222 g (1 mole) of basic cupric carbonate in a 5-liter round flask were added 1.5 liters of distilled water and 720 g (800 ml, 12 moles) of 28% NH_3. The bulk of the cupric carbonate was brought into solution by swirling. Then 112 g of formaldehyde (100 ml, 1.3 moles) and 90 g (0.475 mole) of commercial 95% fructose were added. The solution was well mixed and placed on a steam bath under a fume hood. After 30 min of heating with occasional shaking, a moderate current of air was bubbled through the solution; heating was continued for 2 hr longer. This mixture was then chilled in an ice bath for 3 hr, and the olive-brown precipitate of the copper complex of the imidazole derivative was filtered. The product was washed with about 500 ml of cold water, suspended in 1 liter of water while moist and rendered just acid to litmus by the addition of concentrated HCl (400 ml). Hydrogen sulfide was passed into the suspension with frequent shaking until precipitation of the copper was complete. The precipitate was filtered and extracted with 500 ml of hot water in two or three portions. The clear, light-brown to reddish-brown filtrate and washings were added with stirring. Heating was continued until solution was complete. The greenish-yellow plates, which separated as the solution was cooled to room temperature, were filtered, washed three times with 150-ml portions of water and air dried. The filtrate and first washings were combined and heated, 10 g of picric acid was added and the mixture cooled and filtered. This process was repeated using 10 g of picric acid until the air-dried picrate fraction so obtained melted below 195°C. All fractions were combined and recrystallized from water. On cooling, yellow crystals were deposited, filtered, washed and air dried. One-hundred milliliters of 37% HCl, 250 ml of water and 500 ml of benzene were placed in a 2-liter flask which was then immersed in a water bath maintained at 80°C. Pure picrate, 100 g, was added to this mixture and the flask was shaken thoroughly until the picrate dissolved. The benzene layer was decanted and the aqueous layer was then extracted five times with 300-ml portions of benzene, treated with about 3 g of Norit and filtered. The clear, pale yellow filtrate was evaporated under reduced pressure. The resulting crystals were taken up in 30 ml of absolute alcohol. Ether, 100 ml, was added and the mixture was kept in a refrigerator to give colorless crystals (37 g); mp, 107–108°C.

Preparation of 4-formylimidazole (14). Twenty grams of hydroxymethylimidazole and 38 ml of HNO_3 ($d = 1.38$) were mixed and kept in a covered dish on the steam bath until the evolution of brown fumes was almost complete. The cover was removed and

the liquid was evaporated under reduced pressure to give a pale yellow crystalline mass. This was dissolved in a warm concentrated solution of sodium carbonate and allowed to stand at room temperature to give 11 g of formylimidazole. After removing the crystals, the filtrate was acidified faintly to methyl orange with HCl and kept at room temperature to give imidazolecarboxylic acid (2.1 g).

Preparation of 4-imidazoleformaldoxime (15). Formylimidazole (2.0 g), hydroxylamine hydrochloride (1.4 g) and sodium carbonate (1.0 g) were dissolved in 40 ml of water and allowed to stand at room temperature overnight. The resulting solution was evaporated to 20 ml and cooled in an ice bath; colorless crystals separated (2.1 g). After recrystallization from absolute alcohol, colorless prisms were obtained; mp, 183–184°C, yield, 1.50 g.

Preparation of 4-imidazolemethylhydroxylamine. 4-Imidazole-formaldoxime (3.1 g, 0.028 mole) was dissolved in 50 ml of dry THF. This solution was placed in a 100-ml three-necked flask and the system was purged with nitrogen. The flask was cooled to 0°C in an ice bath. To this solution, 100 ml of borane in THF (equivalent to 0.05 mole of diborane) was added slowly from a syringe and stirred magnetically under a nitrogen atmosphere such that the temperature remained below 5°C. The resulting solution was stirred further for 30 min at 5°C and then 1 *N* HCl (25 ml) was added dropwise such that the temperature did not exceed 25°C. The resulting reaction mixture was stirred for 1 hr at room temperature and then refluxed for 1 hr. The reaction was checked with Fehling's solution for the formation of hydroxylamine and decomposition of an intermediate. This reaction mixture reduced Fehling's solution strongly. The reaction mixture was made basic by adding a 1 *N* NaOH solution at 0°C. The two-phase mixture was extracted three times with 250-ml portions of ether. The ether layer was evaporated under reduced pressure but none of the imidazole derivative was obtained. The basic aqueous layer was acidified with concentrated HCl, and to this solution 15 g of picric acid was added. The mixture was heated until a clear solution was obtained. On cooling to room temperature, pale yellow plates separated and were collected by filtration to give a picrate (5.2 g). This picrate was added to the mixture of 50 g of 37% HCl, 50 ml of water and 100 ml of benzene. This mixture was shaken thoroughly until the picrate dissolved and the benzene layer was discarded. The aqueous layer was extracted three times with 50 ml of benzene, treated with Norit and filtered. The clear filtrate was evaporated almost to dryness and 10 ml of water was added and lyophilization was attempted. However, a pale yellow solid rather than crystals was obtained (2.3 g). Recrystallization of this yellow solid from absolute alcohol–ether was unsuccessful. The aqueous solution was positive to Fehling's test. The nmr spectrum of this crude solid showed an imidazole ring proton. This compound was used for the synthesis of **II** without further purification.

*Preparation of cyclohexaamylose-*N*-(4-imidazolemethyl)acetohydroxamic acid* (**II**). 4-Imidazolemethylhydroxylamine dihydrochloride (1.20 g) was dissolved in 10 ml of dry DMSO and cooled to 0°C. Then 0.81 g (15 mmole) of sodium methoxide was added and the precipitated sodium chloride was removed by filtration. After adding the solution of carboxymethylcyclohexaamylose methyl ester (400 mg, 0.41 mmole) in 30 ml of dry DMSO, the solution was heated to 70°C under a nitrogen atmosphere and maintained for 72 hr. Hydroxamic acid formation was followed by measuring the absorbance at 540 nm. After cooling to room temperature, 300 ml of acetone was added.

The precipitate formed was collected by filtration, dissolved again in 10 ml of water and passed through a Sephadex G-10 column (2.5 × 70 cm). The column was eluted with distilled water. Fractions of 25 ml were collected and each fraction was checked for hydroxamic acid by $FeCl_3$ assay. Fractions 5 and 6 contained hydroxamic acid and were free of sodium chloride. These fractions were lyophilized to yield light brown solids. The ir spectrum of fraction 5 (120 mg) showed no carbonyl absorption due to COOH or $COOCH_3$ but a hydroxamic acid amide absorption at 6.10 μ. The uv spectrum of fraction 5 (aqueous solution) showed no absorption maximum and had a broad absorption in the region 210–230 nm due to the imidazole group. The purity of this compound was determined by potentiometric titration. A 2.65-mg portion of fraction 5 was dissolved in 3 ml of water and titrated with 0.01 *N* NaOH solution. One inflection point was observed around pH 8.10 and the purity was 63.2%. Then this fraction was chromatographed on a Sephadex A-25-DEAE column (1 × 20 cm) and eluted with water at 6°C, cooling the column externally with water. Each 10-ml fraction was checked for hydroxamic acid ($FeCl_3$ assay). Fractions 3 and 4 contained hydroxamic acid and were lyophilized to give white solids of 30 mg each (total yield, 60 mg). The purity of this compound was also determined by potentiometric titration but no change was observed. *Anal.* Calcd for $C_{42}H_{67}O_{32}N_3$ (MW = 1126): C, 44.80; H, 5.95; N, 3.72. Calcd for $C_{42}H_{67}O_{32}N_3 \cdot 10H_2O$ (MW = 1306): C, 38.68; H, 6.66; N, 3.21. Found: C, 37.11; H, 5.75; N, 3.00. This compound is also very hygroscopic as is **I**. The discrepancy in the analysis is probably due to this factor.

Preparation of p-*nitrophenyl thiolacetate* (*16*). To a solution of 15.7 g of *p*-chloronitrobenzene in 25 ml of boiling alcohol, an alcoholic solution of Na_2S_2 (prepared from 17.5 g of $Na_2S \cdot 9H_2O$ and 2.3 g of sulfur) was added portionwise over a period of 10 min. Then an alcoholic solution of 4.0 g of NaOH was added dropwise over a period of 10 min as the reaction mixture was boiled under reflux. The mixture was cooled and then poured onto 100 g of ice and 150 ml of water. A precipitate was removed by filtration, the filtrate was acidified with concentrated HCl and *p*-nitrothiophenol was collected by filtration and washed with 100 ml of water. The crude phenol was dissolved n 15 ml of alcohol. After addition of 4 g of NaOH in 50 ml of water, the solution was filtered and *p*-nitrothiophenol was reprecipitated from the filtrate by addition of concd HCl. The phenol was filtered, washed with water and dried to give 4.5 g of *p*-nitrothiophenol, mp, 75°C. *p*-Nitrothiophenol (2.7 g, 0.017 mole) was dissolved in 20 ml of pyridine. To this solution, acetyl chloride (2.0 g, 0.025 mole) was added dropwise with cooling in an ice bath over a 10-min period. The resulting solution was poured into ice water (50 ml) and the precipitate was filtered to give a pale yellow solid (2.50 g). This crude ester was dissolved in absolute alcohol and treated with Norit. Three recrystallizations from absolute alcohol gave 1.0 g of *p*-nitrophenylthiolacetate as colorless needles, mp, 82–83°C (lit. (*9*) mp, 82.3–82.6°C).

Preparation of N-*acetyl*-D- *and* L-*phenylalanine* p-*nitrophenyl ester* (*17*). Two grams of D-phenylalanine was dissolved in 4.8 ml of 2 *N* NaOH solution. This solution was cooled to 0°C in an ice bath and eight additions of 4.0 ml of 2 *N* NaOH and 0.4 ml of acetic anhydride were made at intervals of 2 min, the mixture being continually stirred magnetically. After the solution had been allowed to stand at room temperature, 12.3 ml of 6 *N* H_2SO_4 was added and the solution was cooled to 0°C. Colorless crystals were obtained (1.80 g). These crystals were recrystallized from water, mp, 172°C. The

N-acetyl-D-phenylalanine (1.0 g) and *p*-nitrophenol (0.67 g) thus obtained were dissolved in 40 ml of dry ethyl acetate and cooled to 0°C. To this solution, 1.0 g of *N,N*-dicyclohexylcarbodiimide was added with vigorous stirring; this mixture was kept for 1 hr at 0°C. After the solution was allowed to stand at room temperature, the dicyclohexylurea was filtered and washed with 30 ml of cold ethyl acetate. The combined filtrate was evaporated to dryness at 20°C and the residual solid was recrystallized from absolute alcohol to give colorless needles (1.20 g), mp, 163–164°C; $[\alpha]_D^{25} = 82.7$ ($c = 1.10$ in CH_3CN). The L-isomer was prepared by an analogous method; mp, 164–165°C; yield, 90%; $[\alpha]_D^{25} = -83.8$ ($c = 0.93$ in CH_3CN). The optical purity of these compounds was confirmed by kinetic analysis.

Preparation of p-*nitrophenyl and* m-*nitrophenyl acetate.* The phenol was dissolved in a large excess of acetic anhydride and refluxed for 10 hr. The resulting solution was poured into ice water, saturated with sodium bicarbonate and extracted with ether. The ether layer was dried over anhydrous Na_2SO_4 and evaporated to give crude crystalline acetate. The crude acetate was recrystallized from ethanol to give colorless needles; mp, 81–82°C for *p*-nitrophenyl acetate (lit. (*9*) mp, 79.5–80°C); mp, 55–56°C for *m*-nitrophenyl acetate (lit. (*4*) mp, 55–56°C).

Kinetic measurements. The reactions were carried out by following the release of phenol spectrophotometrically in a Cary Model 14 recording spectrophotometer. The reaction medium, 3.00 ml of pH 7.80 phosphate buffer, was placed in a 1-cm silica cell and equilibrated at 25°C in the Cary cell compartment. The reaction was initiated by adding 20 μl of stock solution of ester in acetonitrile using a plastic stirring rod. Pseudo-first-order rate constants, k_{obs}, were obtained from the following first-order equation:

$$\log(A_\infty - A_t) = -(0.4343)\, k_{obs} \cdot t + \log(A_\infty - A_0). \qquad [2]$$

Plots of $\log(A_\infty - A_t)$ versus t were linear over at least three half-lives. The rate constants were the average of two or three determinations which agreed within 3–4%. For slow reactions, pseudo-first-order rate constants were determined by method of Kezdy without following the reaction to completion according to the following equation:

$$A_t = (A_t + \Delta)\, e^{\Delta} \cdot k_{obs} + A_\infty(1 - e^{\Delta} \cdot k_{obs}). \qquad [3]$$

A plot of A versus $A_t + \Delta$ gives a slope $e^{\Delta} \cdot k_{obs}$ and $k_{obs} = 2.303 \log(\text{slope}/\Delta)$.

TABLE 5

ABSORBANCE OF I[a]

pH	Absorbance	ε
11.10	0.120	2000[b]
10.20	0.125	2080
9.20	0.095	1580
8.70	0.084	1400
8.20	0.061	1016
7.10	0.057	950[c]
4.50	0.040	660

[a] $I = 0.2$, borate buffer, $T = 25$°C.
[b] ε_I.
[c] ε_P.

Determination of pK_a *value of* **I**. Spectra of 6.0×10^{-5} *M* solutions of **I** at different pH's were recorded. The absorbances at 260 nm are shown in Table 5. From these data the p*K* value was calculated at each pH from the following equation and averaged. $pK_a = 8.75 \pm 0.20$.

$$pK_a = pH + \log(\varepsilon_I - \varepsilon)/(\varepsilon - \varepsilon_P). \quad [4]$$

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