upon cooling in the refrigerator: mass spectrum M^+ calcd for $C_{11}H_{12}O_2$ 176.0837, found 176.0837.

Reaction of Triene 1 with 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD, 5). A 10-mL flask, provided with a magnetic stirbar and a 10-mL pressure-equalizing dropping funnel, was charged with 1 mmol of triene **1** in 3 mL of dry CH_2Cl_2. The solution was cooled to 0 °C, and a solution of 1 mmol of freshly sublimed PTAD in 5 mL of CH_2Cl_2 was added dropwise while the solution was cooled and stirred magnetically. The red color disappeared almost instantaneously upon addition. The resulting pale yellow solution was concentrated under reduced pressure and the residue purified by preparative TLC (silica gel, 30% ethyl acetate–petroleum ether, 40:60) to give the adduct (**12**) in 82% yield as colorless crystals. Recrystallization from ethanol gave colorless needles: mass spectrum M^+ calcd for $C_{19}H_{17}N_3O_2$ 319.1321, found 319.1314.

Reaction of Triene 1 with Diethyl Azodicarboxylate (6). A solution of 0.5 mmol each of **1** and diethyl azodicarboxylate in 3 mL of benzene was placed into a constricted glass tube, sealed under nitrogen, and heated in an oven at 130 °C for 6 h. After the tube was cooled to room temperature, the solvent was removed under reduced pressure (ca. 20 °C at 15 torr), and the residue was purified by preparative TLC (silica gel, CH_2Cl_2) to give the adduct (**13**) in 68% yield as a colorless oil.

Addition of Chlorosulfonyl Isocyanate (CSI, 7) to 1. Into a 25-mL flask, provided with a stir bar and a rubber septum, was placed 1 mmol of triene **1** in 10 mL of dry CH_2Cl_2. An atmosphere of dry nitrogen was maintained through a stainless steel needle, and the mixture was cooled to −10 °C. A solution of 1.1 mmol of CSI in 5 mL of dry CH_2Cl_2 was added through a syringe within 15 min. After the solution was stirred at −10 °C for 0.5 h, it was poured into a mixture of 2 g of $NaHCO_3$ and 700 mg of Na_2SO_3 in 25 mL of water. After the solution was stirred for 1 h at room temperature, the two phases were separated, and the water layer was extracted with two 20-mL portions each of CH_2Cl_2 and ether. The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure (20 °C, 15 torr). The residue was purified by preparative TLC (silica gel, ether–methanol, 95:5) to give the β-lactam (**14**) in 84% yield as a colorless oil: mass spectrum M^+ calcd for $C_{12}H_{13}NO$ 187.0997, Found 187.0996.

Dichloroketene Addition to 1. A 25-mL flask equipped with a reflux condenser, magnetic stirrer, addition funnel, and nitrogen inlet was flame-dried under nitrogen. The cool flask was then charged with 144 mg (1.0 mmol) of triene **1**, 72 mg (1.1 mmol) of freshly activated zinc,[10] and 15 mL of dry ether. The suspension was stirred under nitrogen while a solution of 117 μL (190 mg, 1.04 mmol) of Cl_3CCOCl and 96 μL (161 mg, 1.05 mmol) of $POCl_3$ (distilled from K_2CO_3) in 10 mL of dry ether was added dropwise (ca. 10 min). The progress of the reaction was monitored by TLC and appeared to be complete after 4 h. After the mixture was refluxed for 28 h, the reaction suspension was filtered through Celite, and the remaining zinc was washed with 30 mL of ether. Solvent removal under reduced pressure yielded a purple oil that was purified by preparative TLC on silica gel, eluting with 15% ethyl acetate–petroleum ether, to give 139 mg (55%) of the dichloroketene adduct **15** as colorless crystals and 38 mg of recovered triene **1**: mass spectrum M^+ calcd for $C_{13}H_{12}Cl_2O$ 254.0265, Found 254.0264.

Epoxidation of 1. A 25-mL flask was charged with a solution of 0.55 mmol of triene **1** in 2 mL of dry CH_2Cl_2. To this was added a solution of 0.62 mmol of *m*-chloroperbenzoic acid in 3 mL of dry CH_2Cl_2 from a 5-mL pressure-equalizing dropping funnel, while the reaction mixture was stirred magnetically and maintained at −20 °C. After the mixture was stirred for 0.5 h at −20 °C, the precipitated *m*-chlorobenzoic acid was removed by filtration and washed with 20 mL of cold CH_2Cl_2. The filtrate was washed twice with 20-mL portions of saturated $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. The solvent was removed at reduced pressure and the residue subjected to preparative TLC to afford the epoxide (**19**) in 24% yield as a colorless liquid.

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Role of Cyclohexaamylose C-3 Hydroxyls in Catalytic Hydrolysis

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Abstract: Both the static and dynamic aspects of the sodium 4-nitrophenolate and the sodium 2,6-dimethyl-4-nitrophenolate/dodecakis-2,6-*O*-methylcyclohexaamylose complexes are investigated. The geometries of these complexes are determined on the basis of intermolecular homonuclear nuclear Overhauser enhancements and chemical shift analysis while the dynamic parameters of complexation, i.e., rotational correlation times and coupling constants, are calculated from $^{13}C[^1H]$ T_1 measurements. Comparison of these results with analogous measurements of the unmethylated cyclohexaamylose inclusion complexes of these same substrates indicates that the substrates are prevented from equivalent penetration of the methylated derivative. This casts some doubt upon the premise that the cyclohexaamylose 3-hydroxyls are inherently unreactive and therefore uninvolved in cyclohexaamylose-catalyzed hydrolyses.

In recent years, the cycloamyloses have received substantial attention as enzyme active site models.[1,2] These cyclic oligosaccharides have been shown to complex a variety of guest molecules in their hydrophobic interiors[3,4] and in some cases catalyze the reaction of the guest molecule.[5,6] Major research efforts involving these systems have focused on the chemical modification of the cycloamylose cavity with the goal of improving their catalytic abilities and/or expanding the scope of reactions that they catalyze.[7,8] In all of the cycloamylose-catalyzed hydrolyses of guest molecules, it has been assumed that the cyclo-

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amylose C-2 hydroxyls, not the C-3 hydroxyls,[9,10] are active. This assumption is based on two lines of evidence: (1) under basic conditions, the C-2 hydroxyls are substantially more reactive with electrophilic reagents than the C-3 hydroxyls,[11] suggesting they have a lower pK_a, and (2) when cycloheptaamylose and cyclohexaamylose are exhaustively 2,6-*O*-methylated,[33] they lose their hydrolytic activity under conditions in which they were previously active.[9]

With respect to the first line of evidence, early X-ray studies[12,13] suggested the C-3 hydroxyl hydrogens were hydrogen bonded to the C-2 hydroxyl oxygens. This was later substantiated by ^1H NMR studies in Me_2SO.[14] Such a hydrogen-bonding scheme is certainly copacetic with the idea that the C-2 hydroxyl has the more acidic proton. However, it must be emphasized that the forces responsible for the crystal structure of the cycloamylose are not the same as those in solution and, furthermore, that the hydrogen-bonding network of the cycloamyloses in Me_2SO might well be different in water. Thus, conclusions regarding the relative values of the C-2 and C-3 hydroxyl pK_as based on these measurements are not necessarily valid in aqueous solution. In fact, Laufer et al. have now shown that both these hydroxyls have approximately equal pK_a values in aqueous solution.[15]

However, the fact that the 2,6-per-*O*-methylcycloamyloses lose their hydrolytic activity still seemed strong evidence in support of the fact that the C-2 hydroxyls were active in hydrolysis. It occurred to us that an investigation of the structure of dodecakis-2,6-*O*-methylcyclohexaamylose–substrate complexes in aqueous solution might shed some light on this apparent anomaly.

In an earlier study,[16,17] we were able to evaluate both the position and dynamic coupling of sodium 4-nitrophenolate (PNP) and sodium 2,6-dimethyl-4-nitrophenolate (2,6-PNP) upon binding to cyclohexaamylose (CD). The location of the substrate in the cavity was determined by using ^1H homonuclear Overhauser effects while the host–guest coupling parameters were determined by measuring the $^{13}C[^1H]T_1$ values of the host and guest molecules in the free and bound states. The results of these experiments indicated that 2,6-dimethyl-4-nitrophenolate coupled with the cyclohexaamylose cavity twice as effectively as the 4-nitrophenolate anion. Furthermore, rotation of the methyl groups of 2,6-dimethyl-4-nitrophenolate about the methyl aromatic carbon–carbon single bond was slowed by cyclohexaamylose complexation. We attributed this reduced rotation of the methyl groups to contact between the methyl groups and the "rim" of the cycloamylose cavity. This proposed interaction is in keeping with the tighter coupling observed between the 2,6-dimethylated substrate and the cycloamylose cavity.

The fact that we were able to make these measurements encouraged us to use these same techniques in considering the effect of methylating the cyclohexaamylose C-6 and C-2 hydroxyls on the disposition and coupling of these same substrates. If sodium 4-nitrophenolate and 2,6-dimethyl-4-nitrophenolate penetrated the 2,6-O-methylated cyclohexaamylose cavity to the same degree as in the unmethylated case, we expected them to couple more tightly to this derivative than to the parent oligosaccharide due to increased "rim" interaction. In analogy to the previous case, we further expected the 2,6-dimethyl-4-nitrophenolate to couple more tightly to the methylated oligosaccharide than the 4-nitrophenolate.

Earlier rate studies[18] and our initial dynamic coupling experiments[17] indicated that these substrates should rotate many times before leaving the cycloamylose cavity. For example, the off rate of the sodium 4-nitrophenolate/cyclohexaamylose complex is $3.1 \times 10^4\ s^{-1}$ while the rotation rate of that substrate in the cavity is $1.7 \times 10^{10}\ s.^{-1}$ Thus, the PNP molecule can rotate $\approx 10^6$ times in the CD cavity before the complex breaks up. Clearly, the substrate does not just rapidly move in and out of the cavity but resides in the cavity sufficiently long to "sense" steric inhibition, i.e., to fall into the "slots" provided by the methyls. In this paper we demonstrate that neither sodium 4-nitrophenolate nor sodium 2,6-dimethyl-4-nitrophenolate penetrate the dodecakis-2,6-*O*-methylcyclohexaamylose (DMCD) cavity as deeply as they do the cyclohexaamylose cavity. This finding strongly suggests that arguments for the singular involvement of the C-2 hydroxyls in the hydrolyses of the nitrophenylacetates based on the lack of catalytic activity of dodecakis-2,6-*O*-methylcyclohexaamylose are not strictly correct.

Experimental Section

Materials. The cyclohexaamylose, 4-nitrophenol, 2,6-dimethyl-4-nitrophenol, and deuterium oxide (99.8%) were obtained from Aldrich Chemicals Co. 4-Nitrophenol was crystallized from chloroform while 2,6-dimethyl-4-nitrophenol was purified by high-vacuum sublimation. Dodecakis-2,6-*O*-methylcyclohexaamylose was prepared by the method of Boger et al.[19] and crystallized from chloroform–petroleum ether.

Determination of the Sodium 2,6-Dimethyl-4-nitrophenolate/Dodecakis-2,6-*O*-methylcyclohexaamylose Binding Constant by Visible Spectroscopy. The change in absorbance at 470, 440, 410, and 380 nm of sodium 2,6-dimethyl-4-nitrophenolate was measured as a function of increasing dodecakis-2,6-*O*-methylcyclohexaamylose concentration with a Cary 219 recording spectrophotometer with the cell compartment thermostated at 25.0 ± 0.5 °C. All solutions were prepared in phosphate buffer, pH 11.0, $I = 0.5$.

The data were treated according to a modified Hildebrand–Benesi equation[20] by plotting $[C_0]/\Delta A + [S_0]\Delta\epsilon^2$ vs. $[C_0] + [S_0]$ to obtain a straight line with slope equal to $1/S_0\Delta\epsilon$ and intercept equal to $K_D/[S_0]\Delta\epsilon$. Initially, $[C_0]/\Delta A$ is plotted vs. $[C_0] + [S_0]$ (analogous to a Hildebrand–Benesi plot) to give an approximate value for $\Delta\epsilon$. Successive approximations to the actual value of K_D and $\Delta\epsilon$ are obtained by performing the plot indicated above until the value of $\Delta\epsilon$ used in generating the $\Delta A/[S_0]\Delta\epsilon^2$ form is sufficiently close to the actual value of $\Delta\epsilon$ calculated from the slope of the line. Only K_D values with high correlation coefficients (>0.98) were accepted.

Sample Preparation for NMR Experiments. For ^1H NMR studies, the hydroxyl protons of dodecakis-2,6-*O*-methylcyclohexaamylose were exchanged for deuterium by lyophilizing a solution of 1 g of the carbohydrate in 50 mL of D_2O (99.8%). This was done 3 times to minimize the HOD in the final sample. Sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate were prepared from the corresponding phenols by dissolving the phenol in slightly less than 1 equiv of aqueous NaOH, lyophilizing this solution, and continuously extracting the residue with $CHCl_3$ to remove the excess phenol. Buffer solutions were prepared with anhydrous Na_3PO_4 in D_2O and pD adjusted with deuteriophosphoric acid. The final pD value, 11.00 ± 0.02, was obtained by adding 0.40 to the pH meter reading[21] made by using an electrode which had been standardized with pH 10.00 ± 0.01 and pH 7.00 ± 0.01 buffers in H_2O and then rinsed with D_2O. The ionic strength of all sample solutions was 0.5. Samples for both ^{13}C and ^1H NMR measurements had total volumes of 5 mL.

Determination of Proton-Decoupled ^{13}C Spin–Lattice Relaxation Times. Proton-decoupled ^{13}C Fourier transform NMR spectra of the complexes (0.05 M in substrate and 0.05 M in DMCD) were obtained at 25.2 MHz on a Varian XL-100 spectrometer equipped with a Nicolet NT 440 frequency synthesizer with 12-mm sample tubes; the temperature was maintained at 25.0 ± 0.5 °C. The spectrum of the free dodecakis-2,6-*O*-methylcyclohexaamylose (0.05 M) was obtained on a JEOL FX-

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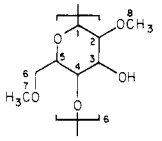
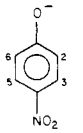
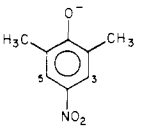
Table I. ^{13}C Proton-Decoupled Spin-Lattice Relaxation Times for Both Free and Bound Dodecakis-2,6-*O*-methylcyclohexaamylose (DMCD), Sodium 4-Nitrophenolate (I), and Sodium 2,6-Dimethyl-4-nitrophenolate (II)

	concn, M	C_1	C_4	C_2	C_3	C_6	C_5	$C_{7,8}$	$\langle T_1\rangle_{1-5}$
	0.05	0.076	0.078	0.078	0.087	0.054	0.070	0.47, 0.52	0.075
DMCD, I	0.05, 0.05	0.084	0.089	0.095	0.090	0.058	0.073	0.47, 0.52	0.083
DMCD, II	0.05, 0.05	0.080	0.080	0.094	0.081	0.055	0.081	0.51, 0.55	0.078
	concn, M	2,6	3,5						$\langle T_1\rangle_{2-6,3-5}$
	0.1	4.67[a]	4.93[a]						4.80
I, DMCD	0.05, 0.05	0.482	0.461						0.472
	concn, M	3,5	2,6-CH_3						
	0.1	1.93[a]	4.19[a]						
II, DMCD	0.05, 0.05	0.205	0.778						

[a] Taken from ref 17.

100 spectrometer operating at 25.00 MHz in a 10-mm sample tube with the temperature maintained at 23 ± 1 °C. In both cases, the fast inversion recovery method (FIRFT)[22] was used to measure the ^{13}C spin–lattice relaxation times, employing a pulse sequence of $(\pi - \tau - \pi/2 - w)_n$ where w, the waiting time between pulses, was $\leq 5\ T_1$. The spin–lattice relaxation time, T_1, was then determined from a least-squares fit of signal intensities and τ values to the expression $M_\tau = M_\infty[1 - (2\exp(-\tau/T_1))]$, where M_τ is the magnetization measured at the time τ.[23]

Determination of ^1H Homonuclear Nuclear Overhauser Enhancements (NOEs). The ^1H homonuclear nuclear Overhauser enhancements (NOEs) were determined on a Varian XL-100 FT spectrometer at 25.0 ± 0.5 °C. Both the substrate and DMCD concentration was 0.05 M, corresponding to 94.8% sodium 4-nitrophenolate and 93.9% sodium 2,6-dimethyl-4-nitrophenolate bound. Each NOE is reported as the percentage enhancement in the integrated intensity of the observed resonance when the decoupling frequency was first set to a vacant region in the spectrum and then applied at the frequency to be irradiated.

Sodium 4-Nitrophenolate and Sodium 2,6-Dimethyl-4-nitrophenolate Induced Chemical Shift Changes in the ^1H NMR of Dodecakis-2,6-*O*-methylcyclohexaamylose. ^1H chemical shift measurements were made at 100.1 MHz on a JEOL FX-100 spectrometer at 25 ± 1 °C. The dodecakis-2,6-*O*-methylcyclohexaamylose concentration was held constant at 0.013 M and the sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate concentrations were varied from 0.006 to 0.030 M in two separate series of experiments. Chemical shift changes were measured relative to a 0.10 M sodium acetate sample contained in a coaxial insert.

^1H chemical shift measurements were also made on a 220.02 MHz Varian spectrometer at 25 ± 1 °C. The DMCD concentration was held constant at 8.5×10^{-3} M and sodium 4-nitrophenolate varied vetween 2.4×10^{-3} M and 9.9×10^{-2} M. Chemical shifts were again measured relative to external 0.10 M sodium acetate contained in a coaxial insert.

Determination of Sample Viscosities. The viscosities of the samples used in the ^{13}C [^1H]T_1 experiments were determined with a calibrated Cannon-Finske viscometer. The viscosities were calculated from the relationship $\eta = Bt\rho$ where B is a constant for the viscometer, t is the flow time for sample between the two calibration marks, and ρ is the density of the solution. The temperature of the viscometer and solutions was maintained at 25.0 ± 0.5 °C.

Results

Dodecakis-2,6-*O*-methylcyclohexaamylose Binding of Sodium 2,6-Dimethyl-4-nitrophenolate. The changes in the visible spectra of sodium 2,6-dimethyl-4-nitrophenolate, measured as a function of increasing DMCD, suggested strong substrate binding. The complex showed isosbestic points at 420 nm and 453 nm, indicating a 1:1 complex. A plot of the data in the form of $[C_0][S_0]/\Delta A$ vs. $[C_0] + [S_0]$ showed excellent straight-line fits pointing to an A + B ⇄ AB equilibrium model. The sodium 2,6-dimethyl-4-nitrophenolate/dodecakis-2,6-*O*-methylcyclohexaamylose complex in pH 11.00 ± 0.02 phosphate buffer, I = 0.5 at 25 °C, has a K_D of 1.48 ± 0.22 × 10^{-4} M.

^{13}C Spin–Lattice Relaxation Times for Unbound Dodecakis-2,6-*O*-methylcyclohexaamylose, Sodium 4-Nitrophenolate, and Sodium 2,6-Dimethyl-4-nitrophenolate. The observed spin–lattice relaxation times for dodecakis-2,6-*O*-methylcyclohexaamylose, sodium 4-nitrophenolate, and sodium 2,6-dimethyl-4-nitrophenolate in phosphate buffer, pD 11.0, I = 0.5, are listed in Table I. The DMCD resonances are listed in order of increasing field, i.e., $C_1 < C_4 < C_2 < C_3 < C_6 < C_5 < C_7, C_8$. The assignments are according to Boger[19] et al. Since their assignments were made on DMCD in chloroform while our measurements were in aqueous solutions, some differences in signal assignments may be possible. However, all the singly protonated carbons (C_1–C_5) have T_1s within experimental error of one another, as has been observed previously[17,24] for cyclohexaamylose. This is attributed to the relative rigidity of the cavity allowing little conformational freedom in the glucose ring carbons. Thus, these relaxation times, being essentially equivalent, were averaged together as $\langle T_1\rangle_{1-5}$, rendering the absolute assignments unnecessary. As for the remaining assignments, C_6 is distinguishable from C_1–C_5, since it has two attached protons and, subsequently, a shorter relaxation time. Finally, the 2-*O* and 6-*O*-methyl carbons are easily distinguishable from the other resonances because of their much longer relaxation times, a result of the added spin-rotation relaxation mechanism available to methyl groups. No attempt was made to differentiate the 2-*O*- from the 6-*O*-methyls since no relaxation differences were observed between them. The resonance assignments and relaxation times for uncomplexed sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate were previously determined[17] and are included in Table I for purposes of comparison.

The observed relaxation times were all viscosity corrected and appear in Table II as $(T_1)^{\eta}_{obsd}$. This was necessary since different

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Table II. Viscosity-Corrected Spin-Lattice Relaxation Times and Rotational Correlation Times for Both Free and Bound Dodecakis-2,6-*O*-methylcyclohexaamylose (DMCD) and the Sodium Nitrophenolates (I and II)

compd	concn, M	T_1obsd, s	$T_1{}^v$obsd, s	$T_1{}^v$free, s	$T_1{}^v$complex, s	$\tau_c{}^v$, ps
		DMCD Overall				
DMCD	0.05	0.075		0.091		540
DMCD, I	0.05, 0.05	0.083	0.100		0.101	490
DMCD, II	0.05, 0.05	0.078	0.093		0.091	530
		Substrate Overall				
I	0.1	4.80[a]		4.68[a]		11
I, DMCD	0.05, 0.05	0.472	0.570		0.545	90
II	0.1	1.93[a]		1.84[a]		27
II, DMCD	0.05, 0.05	0.205	0.245		0.232	200
		Substrate Methyl Groups				
II	0.1	4.19[a]		4.01[a]		4
II, DMCD	0.05, 0.05	0.778	0.928		0.885	18

[a] Taken from ref 17.

viscosities in different solutions affect the measured T_1s by influencing the overall molecular reorientation rates and make comparisons among them invalid.[23]

Complexation-Induced Changes in ^{13}C Spin–Lattice Relaxation Times of Dodecakis-2,6-*O*-methylcyclohexaamylose, Sodium 4-Nitrophenolate, and Sodium 2,6-Dimethyl-4-nitrophenolate. The measured ^{13}C spin–lattice relaxation times for free DMCD and the sodium 4-nitrophenolate/DMCD and sodium 2,6-dimethyl-4-nitrophenolate/DMCD complexes are listed in Table II along with their viscosity-corrected values. The observed relaxation times for both DMCD and the substrates are some complex function of the actual relaxation times for free and complexed species and the rate constants for association and dissociation of the complex. However, when the rate constant for dissociation is small compared to the correlation times (τ_c) for the complexed species, the relaxation times may be described by[24]

$$(T_1)^{-1}_{obsd} = \alpha(T_1)^{-1}_{complex} + [1 - \alpha](T_1)^{-1}_{free} \qquad (1)$$

where α is the fraction of substrate or DMCD bound. Temperature-jump measurements of the dissociation rates for a variety of cycloamylose complexes reveal a variety of rates depending upon the substrate.[18] However, in all cases, dissociation is slow compared to the observed reorientation rates for cycloamylose and the complexes; thus, this relation holds in these systems.

With the dissociation constants determined by visible spectroscopy for the DMCD/sodium 4-nitrophenolate[15] and DMCD/sodium 2,6-dimethyl-4-nitrophenolate complexes, the substrates under the experimental conditions imposed were 95% and 94% bound, respectively. Therefore, using the values of $(T_1)^v_{free}$ from Table II in eq 1 and $(T_1)^v_{obsd}$, the viscosity-corrected T_1 values for DMCD and the substrates at 100% bound were calculated, and these are included in Table II as $(T_1)^v$ complex.

Examination of these values indicates that the general trends in complexation-induced changes in the relaxation times of both the modified cyclodextrin and the substrates are consistent with those observed previously.[17] The T_1 values for C_1–C_5 for DMCD are relatively unaffected by complexation and are within experimental error of those for free DMCD. Similarly, the relaxation times for the 2-*O* and 6-*O*-methyl groups are unchanged. However, the relaxation times for the ring carbons of sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate are substantially decreased. This is consistent with an increase in the overall correlation times (τ_c) for the substrate molecules in the presence of DMCD.

Rotational Correlation Times. Measurements of the NOEs of protonated carbons indicate that dipole–dipole interactions are the predominant ^{13}C relaxation mechanism except for the special case of methyl groups, where spin rotation is important.[26] The intermolecular dipole–dipole relaxation of a nucleus, I_1 (I = spin $^1/_2$), separated by a distance r from a nucleus, I_2, may be described by

$$(T)^{-1} = 3\gamma_1{}^2\gamma_2\hbar^2 I_2(I_2 + 1)r_{1,2}{}^{-6}\tau_c \qquad (2)$$

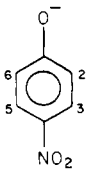
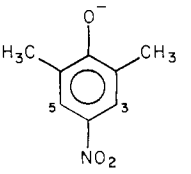
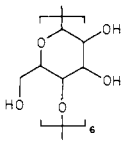
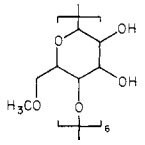
where γ_1 and γ_2 are the gyromagnetic ratios for the respective nuclei and τ_c is the overall molecular correlation time.[27] With the $(T_1)^v_{free}$ values of Table II and C–H bond length of 1.1 ± 0.2 Å, the viscosity-corrected rotational correlation time ($\tau_c{}^v$) was calculated from eq 2 for DMCD. This value is reported in Table II along with those previously reported for free sodium 4-nitrophenolate. Similarly, the $(T_1)^v_{complex}$ values of Table II were used to calculate the corrected correlation times for DMCD and the substrates upon complex formation.

Inspection of the rotational correlation times for free DMCD and complexed DMCD indicates that the overall reorientation of the molecule is relatively unaffected by complexation. Such behavior has been previously observed[17,24] and is a reflection of the small perturbation felt by the large oligosaccharide (M_r > 1100) upon inclusion of the much smaller substrates. The guest molecules, on the other hand, experience substantial increases in their overall reorientation times. The correlation time for sodium 4-nitrophenolate increases from 11 ps (τ_c free) to 90 ps (τ_c bound) upon complexation. Similarly, τ_c for sodium 2,6-dimethyl-4-nitrophenolate increases from 27 ps to 200 ps upon binding. Only an upper limit for the correlation times of the methyl groups in sodium 2,6-dimethyl-4-nitrophenolate may be obtained due to the spin-rotation contribution to the relaxation times. However, it is clear that the effective correlation times for these methyl groups increase by a factor of 4.5 on complexation with DMCD. These observations are consistent with hindered reorientation due to interaction with the cavity upon inclusion of the substrates within DMCD.

It has been suggested that a dynamic coupling coefficient, ξ, can be used to describe complex formation along with the usual thermodynamic association constant.[24] This parameter is defined as the ratio of the substrate correlation time to that of the host correlation time. Therefore, a tightly coupled complex will have a value of 1, where the substrate motion is the same as the host. Where there is little coupling between the host and guest, the coupling coefficient will be less than 1 with some mimimum theoretical value.[28] The coupling coefficients for the binding of sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate with DMCD were calculated and are included in Table III. The ξ value of 0.19 for the sodium 4-nitrophenolate/DMCD complex indicates weak coupling of the substrate upon complexation; the substrate is still able to rotate fairly freely. On the other hand, the coefficent of 0.38 for the sodium 2,6-dimethyl-4-nitrophenolate/DMCD complex indicates a greater coupling between the guest and host.

(25) R. J. Bergeron and M. P. Meeley, *Bioorg. Chem.*, **5**, 197 (1976).
(26) G. C. Levy, J. D. Cargioli, and F. A. Anet, *J. Am. Chem. Soc.*, **95**, 1527 (1973).
(27) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect", Academic Press, New York, 1971.
(28) C. H. Brevard, J. P. Kintzinger, and J. M. Lehn, *Tetrahedron*, **28**, 2447 (1972).

Table III. Dissociation Constants (K_D), Dynamic Coupling Constants (ξ), and Substrate Nuclear Overhauser Enhancements (NOEs) for the Binding of Sodium 4-Nitrophenolate and Sodium 2,6-Dimethyl-4-nitrophenolate to Cyclohexaamylose and Dodecakis-2,6-*O*-methylcyclohexaamylose

							
			% NOE				% NOE
host	K_D, M	ξ	2,6	3,5	K_D, M	ξ	3,5
	$(3.7 \pm 0.02) \times 10^{-4}$ [a]	0.14[b]	1[b]	34[b]	$(1.96 \pm 0.53) \times 10^{-4}$ [c]	0.41[b]	22[b]
	$(1.4 \pm 0.2) \times 10^{-4}$ [c]	0.19	16	17	$(1.48 \pm 0.22) \times 10^{-4}$	0.38	15

[a] Taken from ref 16. [b] Taken from ref 17. [c] Taken from ref 25.

^{1}H NMR Changes in Dodecakis-2,6-*O*-methylcyclohexaamylose Induced by Complexation with Sodium 4-Nitrophenolate and Sodium 2,6-Dimethyl-4-nitrophenolate. The ^{1}H NMR spectrum of DMCD has been previously described.[29] Upon inclusion of an aromatic guest into the cavity of DMCD, any protons of DMCD which are situated within the aromatic π cloud of the substrate will be shielded and their resonances moved upfield[30] with respect to the uncomplexed DMCD. When a 0.013 M solution of DMCD was titrated with increasing concentrations of substrate, the 2-*O*-methyl resonance of DMCD was observed to shift upfield with respect to external sodium acetate. The observed shifts, measured at 100 MHz, were 3.7 Hz for the DMCD/sodium 4-nitrophenolate complex and 4.2 Hz for the DMCD/sodium 2,6-dimethyl-4-nitrophenolate complex (shifts extrapolated to 100% bound).

For both complexes, the anomeric protons of DMCD were observed to also shift upfield, 4.6 Hz and greater than 4 Hz for the sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate complexes, respectively. The exact measurements were rendered difficult due to the close proximity of the residual HOD signal at 100 MHz. The only other resolvable resonance, that of the 6-*O*-methyl group of DMCD, was shifted downfield slightly in both complexes, i.e., 1.4 Hz when bound to sodium 4-nitrophenolate and 1.0 Hz when bound with sodium 2,6-dimethyl-4-nitrophenolate.

Measurements of the DMCD (8.5×10^{-3}M)/sodium-4-nitrophenolate [(2.4×10^{-3}M)–(9.9×10^{-2}M)] complex at 220.02 MHz gave somewhat more detailed information about the structure of the complex. At this field, the DMCD interior protons (H-3 and H-5) are resolved, and H-3 was observed to shift 11 Hz upfield when 57% bound by substrate; H-5 was shifted little, if at all. The DMCD anomeric proton resonance was observed to shift upfield with increasing sodium-4-nitrophenolate concentration. However, this shift continued even after the concentration of substrate was increased beyond that of DMCD. When the substrate to DMCD molar ratio was increased to 11.6 (corresponding to a large molar excess of substrate and DMCD completely bound), an upfield shift of 19.4 Hz of the anomeric proton was measured. Therefore this movement must be attributed to some mechanism other than substrate binding. Similar, nonbinding-induced changes in the ^{1}H NMR of cyclohexaamylose have previously been observed in solution with sodium 3,5-dimethyl-4-nitrophenolate and were attributed to substrate-induced medium effects.[16]

Intermolecular Homonuclear ^{1}H NOEs for Sodium 2,6-Dimethyl-4-nitrophenolate and Sodium 4-Nitrophenolate/Dodecakis-2,6-*O*-methylcyclohexaamylose Complexes. ^{1}H homonuclear nuclear Overhauser experiments were performed on the DMCD/sodium 4-nitrophenolate complex (0.05 M substrate, 0.05 M DMCD corresponding to 95% substrate bound) and on the DMCD/sodium 2,6-dimethyl-4-nitrophenolate complex (0.05 M substrate, 0.05 M DMCD corresponding to 94% substrate bound). The 2,6-methine and 3,5-methine protons of sodium 4-nitrophenolate showed a 16 ± 2% enhancement and 17 ± 2% enhancement, respectively, on irradiation of the DMCD proton resonances. Similarly, the 3,5-methine protons of sodium 2,6-dimethyl-4-nitrophenolate exhibited a 15 ± 2% enhancement in the complex. The numbers are corrected to show the enhancement expected when the substrate is 100% bound.

Since the proton resonances of DMCD are poorly resolved at 100 MHz, it was not possible to selectively decouple individual signals. H-3 and H-5 on the interior of the cavity and H-4 on the exterior of the cavity in addition to the H-6 methylenes and the 2-*O*- and 6-*O*-methyl group protons all fall under one broad envelope in the spectrum. The observed NOEs therefore contain potential contributions from all these protons.

Discussion

Geometry of the Complexes. Examination of the aromatic substrate induced chemical shifts in the ^{1}H NMR spectrum of the cycloamyloses provides a convenient method for determining the direction of substrate penetration into the cavity. An upfield shift of the cycloamylose H-3 and/or H-5 resonance upon inclusion of an aromatic substrate has been attributed to π shielding by the guest.[30] For example, if the cycloamylose H-3 resonance is shifted while H-5 is not affected, clearly the substrate[15] is entering from the wide C-2,C-3 hydroxyl face of the cavity (Figure 1a). Similarly, if H-5 shifts upfield while H-3 is unaffected, substrate penetration probably is occurring from the narrow, primary hydroxyl face (Figure 1b). Shifting of both resonances is not so easily interpreted and could result either from very deep substrate penetration or from nonspecific penetration where the substrate enters the cavity from both faces. While the H-3 and H-5 resonances of DMCD were not resolvable at 100 MHz and therefore could not be followed, the 2-*O*- and 6-*O*-methyl groups were easily observed. In both the sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate/DMCD complexes, the 2-*O*-methyl groups were observed to shift upfield. This shift is clearly con-

(29) B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, *Tetrahedron*, **24**, 803 (1968).
(30) P. V. Demarco and A. L. Thakker, *J. Chem. Soc., Chem. Commun.* 2 (1970).

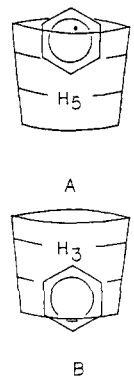


Figure 1. Possible modes of substrate binding in cycloamylose (A) penetration from the C-2, C-3 secondary hydroxyl face, (B) penetration from the C-6 primary hydroxyl face.

sistent with penetration from the wide face of the torus-shaped cavity.

The resonance corresponding to the anomeric proton (H-1) of DMCD was also observed to shift upfield. However, the proximity of this signal to residual HOD in the sample rendered measurements difficult at 100 MHz. For more accurate examination of this behavior, the experiment was repeated at 220 MHz for the DMCD/sodium 4-nitrophenolate complex. If this phenomenon were, indeed, complexation induced, the change in chemical shift should reach a maximum when DMCD is completely saturated, and subsequent addition of substrate would cause no further shift. Clearly, this is not the case, since the anomeric proton continued to shift upfield as the substrate to DMCD molar ratio was increased to well beyond the point of complete DMCD saturation, i.e., [substrate]/[DMCD] > 11. This nonsaturability is likely the result of some non-binding-induced phenomenon such as a substrate-induced change in the bulk solvent structure.

Finally, the cause of the small downfield shifts of the DMCD 6-*O*-methyl groups (~1 Hz at 100 MHz) upon complexation is not clear. However, the direction of this shift clearly is not consistent with inclusion of the substrate from the narrow, primary hydroxyl face of the DMCD cavity.

The complexation-induced shifts of H-3 and H-5, on the interior of the DMCD cavity, are sensitive indicators of substrate position. Easily resolved at 220 MHz, the multiplet associated with H-3 was seen to shift substantially upfield upon inclusion of the substrate while H-5 remained effectively unchanged. This is entirely consistent with inclusion of the substrate in the DMCD cavity to the extent that H-3 is located within the diamagnetic shielding zone of sodium 4-nitrophenolate but H-5 is not. While chemical shift data provide a good picture of substrate orientation in the cavity, i.e., whether penetration is from the wide or narrow face of the DMCD cavity, more precise configurational information is available from the nuclear Overhauser enhancement data.

Intermolecular NOEs are manifested as a through-space interaction involving nuclei that are not coupled by any other relaxation mechanism. The requirements for the observation of an NOE are that the two nuclei be close to one another (r^{-6} dependence) and that they remain in close proximity long enough that cross relaxation may take place to a measurable extent.[27] The application of intermolecular NOEs to cycloamylose complexation already has been extensively discussed.[17,31] For example, the 15% NOE observed for the meta protons of sodium 2,6-dimethyl-4-nitrophenolate when in the bound state indicates close proximity of the substrate to the interior protons of DMCD. However, since the DMCD H-3, H-5, and H-6 methylenes along with the 2-*O*- and 6-*O*-methyl group proton resonances all overlap into one broad envelope at 100 MHz, selective irradiation of individual signals was impossible. Therefore, the substrate NOEs measured here contain potential contributions from all these DMCD protons. However, this seemingly complex situation may be greatly simplified by considering the following observations. From the direction and depth of substrate penetration discussed above (as indicated by the DMCD H-3 chemical shift changes at 220 MHz) and by analogy with similar systems, it seems clear that the major NOE contribution to the substrate meta protons comes from H-3 of DMCD. For example, in an earlier NOE study[31] at 250 MHz, selective saturation of cyclohexaamylose H-3 and H-5 resonances was possible. The results placed the meta protons of guest sodium 4-nitrophenolate in intimate contact with cyclohexaamylose H-3 protons, while little or no interaction was observed between the host H-5 protons and the guest. In addition, since the substrate only partially penetrates the cavity from the wide face of the DMCD cavity, it is not in close proximity to DMCD H-6 methylenes or 6-*O*-methyl groups. Because of the r^{-6} dependence between the observed and relaxing nuclei, the NOE rapidly falls off as the distance between these hydrogens increases. Consequently, NOE contributions from these protons, if any at all, will be negligible. Therefore, the magnitude of the NOE may be rationalized in terms of the proximity of the substrate meta protons to the cycloamylose H-3 protons. When, in a previous study, sodium 2,6-dimethyl-4-nitrophenolate was complexed with cyclohexaamylose, a 22% NOE was observed in the substrate meta protons.[17] Thus, the smaller NOE observed here upon introduction of methyl groups to 2,3-hydroxyl face of the cavity can be interpreted as a decrease in the depth of substrate penetration. Clearly, these methyl groups are providing an effective barrier to maximum substrate penetration.

The same behavior is observed for sodium 4-nitrophenolate upon complexation. The presence of an NOE in the ortho protons, which is not observed upon inclusion in the unmodified cyclohexaamylose cavity, probably results from the 2-*O*-methyl groups of DMCD (Table III). However, the 17% NOE observed in the meta protons for sodium 4-nitrophenolate upon complexation with DMCD is substantially decreased from the 34% observed for this substrate when included in the unmodified cyclohexaamylose cavity.[17] Again, the introduction of methyl groups at the wide 2,3-hydroxyl face of the cavity appears to effectively hinder maximum substrate penetration.

Dynamic Coupling. The low ξ values observed for both the sodium 4-nitrophenolate/DMCD and sodium 2,6-dimethyl-4-nitrophenolate/DMCD complexes examined here indicate that the complexes are of a rather "loose" nature. Especially in the case of sodium 4-nitrophenolate (ξ = 0.16), the substrate does not appear to be very tightly coupled to the DMCD cavity. The presence of the substrate methyl groups in the sodium 2,6-dimethyl-4-nitrophenolate improves coupling to DMCD (ξ = 0.38) to some extent, but the improvement is not substantial. The increase in rotational correlation times for the substrate methyl groups upon complexation suggests that they are in close contact with the face of the DMCD cavity. However, when these coupling parameters are compared to those for the sodium 4-nitrophenolate/cyclohexaamylose complex (ξ = 0.14) and sodium 2,6-dimethyl-4-nitrophenolate/cyclohexaamylose complex (ξ = 0.41),[17] it appears that both substrates interact to about the same extent with both the methylated and unmodified cavities. In the case of sodium 2,6-dimethyl-4-nitrophenolate, penetration of the cavity such that the substrate methyl groups were in close proximity to the DMCD 3-hydroxyl would require that the substrate was, in fact, "slipping" between the DMCD 2-*O*-methyl groups. In this case, a much tighter coupling (as reflected in a ξ value approaching 1) would be expected than that observed. Furthermore, a decrease in NOEs experienced by the substrate's meta protons is inconsistent with this type of penetration.

With respect to the catalytic activity of the 3-hydroxyls in cyclohexaamylose, the fact that 2,6-*O*-methylation results in a loss of cycloamylose activity in the deacylation of the 3- and 4-nitrophenylacetates is easily understood. The introduction of methyl groups at the catalytic face of the cavity simply prevents access by the substrate to the catalytic sites. Both the NOE data and dynamic coupling of sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate to the methylated cyclohexaamylose clearly indicate that the substrates are not penetrating the cavity as deeply as in the case of the unmodified cavity. If the geometry

(31) R. J. Bergeron and R. Rowan, *Bioorg. Chem.*, **5**, 425 (1976).

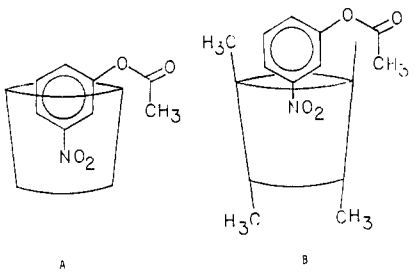


Figure 2. Estimated depth of penetration of 3-nitrophenyl acetate into (A) cyclohexaamylose and (B) dodecakis-2,6-*O*-methylcyclohexaamylose.

of binding of the nitrophenylacetates themselves is similar to that examined here, then the acyl carbonyls would be too far from the DMCD 3-hydroxyls to react with them (Figure 2).

Finally, it is interesting to speculate about the correlations (or lack of) among the thermodynamic binding constants, substrate NOEs, and coupling coefficients, ξ, for cycloamylose substrate binding. Inspection of Table III shows that there is a decrease in K_D through the series of complexes: sodium 4-nitrophenolate/cyclohexaamylose > sodium 2,6-dimethyl-4-nitrophenolate/cyclohexaamylose > sodium 4-nitrophenolate/DMCD $\simeq$ sodium 2,6-dimethyl-4-nitrophenolate/DMCD. Such a sequence suggests that as the hydrophobic character of the substrate and/or host is increased, the binding is increased. However, as the NOEs reflect substrate penetration, this order is reversed; i.e., the magnitude of the binding constant does not correlate directly with the degree of insertion. In contrast, the coupling coefficients show no discernible trends. Thus, the dynamic aspects of molecular association, at least in these complexes, are substantially different from the picture implied by examination of the binding constants. Particularly in the case of the sodium 2,6-dimethyl-4-nitrophenolate/DMCD complex, while the possibility that the substrate methyls could slip between the DMCD rim methyls, drawing the substrate more deeply into the cavity and thereby increasing dispersion interactions between guest and host exists, this does not appear to be occurring here.

Conclusions

For some time, arguments that the 2- and 3-hydroxyls of cycloamylose differ greatly in their degree of involvement in catalytic deacylation have been made. However, in light of our findings, the basis of such arguments must be carefully reconsidered. The alleged difference in catalytic ability between these two hydroxyl groups was based on two lines of evidence: a substantial difference between their respective pK_as implied by a difference in their reactivity with electrophiles under basic conditions, and the loss of catalytic activity on methylation of the 2- and 6-hydroxyls.

With respect to the first of these two suggestions, Laufer has recently shown that the difference in pK_as is virtually nonexistent.[15] Furthermore, Breslow has isolated mixtures of acylcycloamylose intermediates that, based on coupling constants and chemical shift analyses, appear to be substituted at both the 2- and 3-positions.[32] While these findings suggest that the 2- and 3-hydroxyls do not differ substantially in catalytic ability, the curious loss of catalytic activity in 2,6-O-permethylated cycloamylose derivatives remained to be explained. Others have suggested that this loss of activity could not be attributed to steric effects, i.e., the 2-*O*-methyl groups did not hinder the substrate's access to the remaining 3-hydroxyl, and thus concluded the 3-hydroxyl was simply unreactive. However, such suggestions were based solely upon examination of molecular models.[9] In contrast, as a result of our NOE, chemical shift, and dynamic coupling measurements of sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate/dodecakis-2,6-*O*-methylcyclohexaamylose complexes, we conclude that methylation of cyclohexaamylose does, indeed, provide an effective barrier for access to the remaining 3-hydroxyl. Comparison of these parameters to those obtained for the same substrates' binding to cyclohexaamylose clearly shows that the substrates are prevented from equivalent penetration of the cycloamylose cavity when the 2-hydroxyls are methylated. Thus, this result alone appears to be sufficient to explain Bender's original observations.

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Registry No. I, 824-78-2; II, 63831-48-1; DMCD, 51166-72-4; cyclohexaamylose, 10016-20-3.

(32) R. Breslow, M. F. Czarniecki, J. Emert, and H. Hamaguchi, *J. Am. Chem. Soc.*, **102**, 762 (1980).

(33) The di-*O*-methylated cyclodextrin was first described by J. Staerk and H. Schlenk. These authors suggested that the unsubstituted hydroxyls are those at C-2 (J. Staerk and H. Schlenk, 149th National Metting of the American Chemical Society, Detroit, Spring, 1965, Abstract 11c). This assignment has since been shown to be incorrect. The methylation occurs at C-2 and C-6, leaving the C-3 hydroxyls unsubstituted.[29]

Cycloaddition of Substituted Allenes with 1,1-Dichloro-2,2-difluoroethene. A Model for the Two-Step, Diradical-Intermediate Cycloaddition of Allenes

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Abstract: The stereoselectivities, chemoselectivities, relative reactivities, and kinetic isotope effects have been determined in the cycloaddition reactions of substituted allenes with 1,1-dichloro-2,2-difluoroethene (1122). The stereoselectivity observed about the exocyclic double bond in the cycloadducts is governed by steric interactions generated in the formation of the stereosiomeric diradical intermediates **13** and **16**, the rate of formation of the latter decreasing with increasing size of R. The chemoselectivity is also determined by steric interactions generated in the transition states for ring closure, favoring closure to the least substituted end of the allyl radical as the degree of substitution and size of the alkyl groups increase. The relative reactivity sequence for the substituted allenes is mono- < 1,1-dialkyl- < 1,1,3-trialkylallene. Within the mono- and 1,1-dialkylallene series, the relative reactivities decrease as the size of the alkyl groups increases. The observed relative reactivity sequence is discussed in terms of steric factors and changes in the energies of the π MO's. The trends discovered in the present study provide an excellent model for comparison with other cycloaddition reactions of allenes whose mechanisms are still in question.

Recent investigations in our laboratories have focused on the possible mechanisms of allene cycloaddition and cyclodimerization reactions.[1] The results of a theoretical study suggested that a $[{}_{\pi}2_s + ({}_{\pi}2_s + {}_{\pi}2_s)]$ concerted process, which involves interactions

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