Cyclodextrin-catalysed Hydrolysis of Oxazol-5(4*H*)-ones. Enantioselectivity of the Acid–Base and Ring-opening Reactions

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The kinetics of hydrolysis of three oxazol-5(4*H*)-ones bearing hydrophobic substituents on the positions 2 and 4 have been determined in the presence of α-, β-, and γ-cyclodextrin. The macrocycles catalyse both the deprotonation–reprotonation equilibrium on the asymmetric carbon and the ring opening. For this second reaction, both a nucleophilic mechanism with formation of an acylcyclodextrin and general base catalysis by the macrocycle appear to be operative. β-Cyclodextrin is a better catalyst than the other two, the effect of the macro-ring size on the reactivity being more important with the 2-phenyloxazolones. The enantioselectivity is low for the binding and for the catalysis of the acid–base reaction but larger for the ring opening. With the 2-phenyloxazolones, the L-isomer reacts faster in the ring-cleavage reaction with all three cyclodextrins. On the other hand, with the 4-benzyl-2-methyloxazolone, β- and γ-cyclodextrins favour the D-isomer; there is no stereoselectivity with α-cyclodextrin. The absence of solvent isotope effect on the enantioselectivity indicates that hydrogen bonding or proton transfer are not involved in chiral recognition. The effect of the cyclodextrins on the reactivity and stereoselectivity is interpreted with the help of molecular models, assuming reasonable binding modes.

A considerable effort has been devoted in recent years to the investigation of the properties of the cyclodextrins because they represent interesting, albeit crude, enzyme models.[1] An understanding of their properties could pave the way for the design of better synthetic hydrophobic host molecules with enzyme-like properties. Despite the amount of information already available, some questions of interest have hardly been dealt with, especially in relation to the reactions of optically active substrates. In particular, little is known on the relation between the size of the macro-ring of the cyclodextrin and the catalysis and specificity or stereospecificity. When this work was begun, no study had appeared on the relationship between substrate structure and stereoselectivity with an effort to explain the enantioselectivity.[2]

In the course of our investigation of the resolution of oxazolones by hydrolysis in the presence of chiral catalysts,[3] we realized that this system was worth a more detailed study. With these substrates, two reactions take place at the same time, a deprotonation–reprotonation equilibrium and a ring cleavage. This gives a chance to observe the effect of the cyclodextrins on these two very different processes taking place in competition.

The following questions could then be asked. Would the cyclodextrins catalyse the acid–base reaction as well as the ring cleavage and to what extent would these reactions be enantioselective? Would there be a systematic difference between α-, β-, and γ-cyclodextrin in relation to catalysis?

Three oxazolones were chosen for detailed study with hydrophobic groups in the positions 2 and/or 4 to try to control the binding modes and observe a relationship between substrate structure and reactivity or stereospecificity. Furthermore, an attempt was made to determine the origin of the enantioselectivity by measuring the solvent isotope effect on the most enantioselective reaction.

Experimental

Materials.—The racemic oxazolones were prepared as described previously.[3] The optically active oxazolones were prepared by the method of Chen *et al.*[4] and characterized by i.r., ^{1}H, and ^{13}C n.m.r. spectroscopy. L-4-Benzyl-2-phenyloxazolone had m.p. 88—90 °C, $[\alpha]_D^{25}$ −68.4° (*c* 0.25, dioxan) (lit.,[5] 88—89 °C; −71.2°). D-4-Benzyl-2-phenyloxazolone had m.p. 88—89 °C, $[\alpha]_D^{25}$ +69.1° (*c* 0.25, dioxan). L-4-Benzyl-2-methyloxazolone was a viscous oil, $[\alpha]_D^{25}$ −123.7° (*c* 0.67, dioxan) (lit.,[6] −133°). D-4-Benzyl-2-methyloxazolone had $[\alpha]_D^{25}$ +129.6° (*c* 1.2, dioxan). L-4-Methyl-2-phenyloxazolone was a viscous oil at room temperature, $[\alpha]_D^{25}$ −72.5° (*c* 0.25, dioxan). D-4-Methyl-2-phenyloxazolone had $[\alpha]_D^{25}$ +73.2° (*c* 0.1, dioxan). The optical purity of this compound, previously described only as a racemic mixture, was determined by reaction with hydrazine[5] and comparison of the optical activity of the product with that of a sample of *N*-benzoyl-L-alanylhydrazide, m.p. 144—145 °C (lit., 143—145 °C), $[\alpha]_D^{25}$ +18.5° (*c* 1.75, H_2O) prepared by the method of Bergmann and Zervas.[8]

The optical rotations were measured on a Perkin-Elmer 241 polarimeter.

The oxazolones were stored at −78 °C at which temperature they are all crystalline. They can be kept at that temperature for more than one month without significant racemization.

β- and γ-cyclodextrin were obtained from Aldrich, α-cyclodextrin from Aldrich or Serva; they were used without further purification after checking their purity.[9] Deuterium oxide, 99.8 atom %, was bought from Aldrich.

Methods.—The kinetics of the reactions are measured by following the change in absorbance at the $\lambda_{max.}$ of the enolate. The fast reactions (enolate plus product formation) were followed on a Durrum stopped flow apparatus by mixing the oxazolone in water at pH 4 (non-buffered solution made up from water and a stock solution of oxazolone in CH_3CN kept in ice) with the cyclodextrin in buffer. The slow reactions (enolate disappearance) were measured on a Cary 16 spectrophotometer by adding oxazolone in acetonitrile (30 μl) to cyclodextrin (3.0 ml) in the buffer.

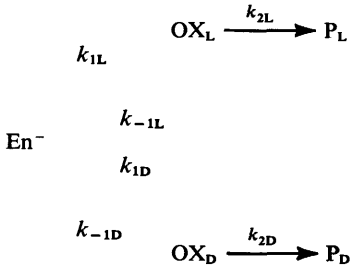
Kinetic Treatment.—The differential equations corresponding to Scheme 1 are (1)—(3).

$$d[OX_L]/dt = -(k_{1L} + k_{2L})[OX_L] + k_{-1L}[En^-] \quad (1)$$

$$d[OX_D]/dt = -(k_{1D} + k_{2D})[OX_D] + k_{-1D}[En^-] \quad (2)$$

$$d[En^-]/dt = k_{1L}[OX_L] + k_{1D}[OX_D] - (k_{-1L} + k_{-1D})[En^-] \quad (3)$$

Integrated solutions for $[OX_L]$, $[OX_D]$, and $[En^-]$ can be



Scheme 1.

worked out either by first deriving the homogeneous differential equations which are of the form (4) where x stands for $[OX_L]$, $[OX_D]$, or $[En^-]$ and equations (5)—(7), then solving,

$$d^3x/dt^3 + Ad^2x/dt^2 + Bdx/dt + C = 0 \quad (4)$$

$$A = (k_{1L} + k_{1D} + k_{-1L} + k_{-1D} + k_{2L} + k_{2D}) \quad (5)$$

$$B = k_{1L}(k_{-1D} + k_{2D}) + k_{1D}(k_{-1L} + k_{2L}) + (k_{2L} + k_{2D})(k_{-1L} + k_{-1D}) + k_{1L}k_{1D} + k_{2L}k_{2D} \quad (6)$$

$$C = k_{2L}k_{-1L}(k_{1D} + k_{2D}) + k_{2D}k_{-1D}(k_{1L} + k_{2L}) \quad (7)$$

or by the more elegant matrix method.[10] The general solution is given by equation (8) where the λ_t terms are the solutions of the algebraic equation (9). All the λ_t terms are positive and

$$x = C_1e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} + C_3e^{-\lambda_3 t} \quad (8)$$

$$\lambda^3 - A\lambda^2 + B\lambda - C = 0 \quad (9)$$

real but the Cardan formulae giving them in terms of A—C cannot be simplified so that the imaginary parts disappear. However, numerical examples for simulation purposes are handled easily by the trigonometric solution of the cubic equation.[11] The properties (10)—(12) of the solutions known from standard algebra are useful. The coefficients C_t are derived from the initial conditions. For $x = [En^-]$ equations (13)—(16) hold where the relationships (17)—(19) obtain.

$$\lambda_1 + \lambda_2 + \lambda_3 = A \quad (10)$$

$$\lambda_1\lambda_2 + \lambda_1\lambda_3 + \lambda_2\lambda_3 = B \quad (11)$$

$$\lambda_1\lambda_2\lambda_3 = C \quad (12)$$

$$C_1 = \{(\lambda_3 - \lambda_2)L + (\lambda_2^2 - \lambda_3^2)K\}/D \quad (13)$$

$$C_2 = \{(\lambda_1 - \lambda_3)L + (\lambda_3^2 - \lambda_1^2)K\}/D \quad (14)$$

$$C_3 = \{(\lambda_2 - \lambda_1)L + (\lambda_1^2 - \lambda_2^2)K\}/D \quad (15)$$

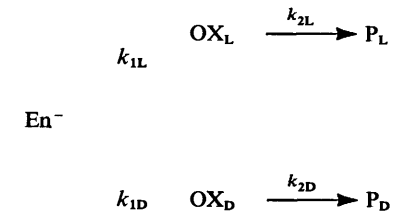
$$C_1 + C_2 + C_3 = 0 \quad (16)$$

$$K = k_{1L}[OX_L]_0 + k_{1D}[OX_D]_0 \quad (17)$$

$$L = k_{1L}(k_{1L} + k_{2L})[OX_L]_0 + k_{1D}(k_{1D} + k_{2D})[OX_D]_0 + (k_{-1L} + k_{-1D})(k_{1L}[OX_L]_0 + k_{1D}[OX_D]_0) \quad (18)$$

$$D = (\lambda_2 - \lambda_1)(\lambda_3 - \lambda_2)(\lambda_1 - \lambda_3) \quad (19)$$

According to these equations, the change in enolate concentration *versus* time should always be described by a sum of three exponentials even for optically active oxazolones.



Scheme 2.

Experimentally, it is difficult to find the three correct C_t,λ_t pairs to fit the absorbance change; detecting three exponentials is easy only when the three λ_t values differ significantly (*e.g.* by more than a factor of five) and no C_t is close to zero. It can be seen from realistic numerical examples that this condition will rarely be satisfied for the system studied.[12] Furthermore, the individual k_t values are not easily extracted from the C_t and λ_t parameters. Nevertheless, interesting chemical information can be obtained in the following way.

Approximate Kinetic Analysis with Racemic Oxazolones.—In practice, the experimentally observed change in enolate concentration during the hydrolysis of the racemic oxazolones in the presence of cyclodextrin can be described quite well by a sum of two exponentials [equation (20)]. The reason for this is

$$[En^-]_{obs} \cong C_1' e^{-\lambda_1' t} + C_2' e^{-\lambda_2' t} \quad (20)$$

$$k_{tm} = (k_{tL} + k_{tD})/2 \quad (21)$$

apparent when looking at the equations. Replacing the correct k_{1L}, k_{1D} . . . values by their mean [equation (21)] in the parameters A—C will not change their values very much if the ratios k_{tL}/k_{tD} are not too large: A does not change; B and C change only to the extent that the $(k_{tm})^2$ values are different from $k_{tL} . k_{tD}$. On the other hand, if the k_{tL} and k_{tD} terms in Scheme 1 are replaced by k_{tm}, the equations simplify; the relationships between the three λ_t terms and k_{tm} become (22)—(24) leading to $C_3 = 0$.

The solution for $[En^-]$ is given by equation (25). In summary, the sum of two exponentials (20) used to describe the actual data will be close to the artificial solution (25) based on the mean values of k_t. This method will be used to obtain approximate values of the mean k_t (calculated as explained in the preceding paper) in the pH range where no other simplification is possible and check the pH dependence of the reactions. Numerical examples with k_{tL}/k_{tD} values similar to those found by the analysis with the optically active oxazolones at high pH have been used to test the validity of the method and the k_{tm} values calculated by approximate analysis of the artificially generated curves (sum of three exponentials) are well within 50% of the true means.[12]

$$\lambda_3 = k_{1m} + k_{2m} \quad (22)$$

$$\lambda_1 + \lambda_2 = k_{1m} + k_{2m} + 2k_{-1m} \quad (23)$$

$$\lambda_1\lambda_2 = 2k_{-1m}k_{2m} \quad (24)$$

$$[En^-] = C_{1m} e^{-\lambda_{1m} t} + C_{2m} e^{-\lambda_{2m} t} \quad (25)$$

Hydrolysis of Optically Active Oxazolones at High pH.—At high pH, where k_{1L}, k_{1D}, k_{2D}, and k_{2L} are much larger than k_{-1L} and k_{-1D}, the initial stage of the reaction is described by Scheme 2.

Each enantiomer is transformed into a mixture of enolate and product with rate constants given by equations (26) and

$$\lambda_1 = k_{1L} + k_{2L} \quad (26)$$

$$\lambda_2 = k_{1D} + k_{2D} \quad (27)$$

(27) and enolate concentrations extrapolated to $t = 0$ from the slow step by (28) and (29).

$$[En^-]_{L,t=0} = k_{1L}/(k_{1L} + k_{2L})[OX_L]_0 \quad (28)$$

$$[En^-]_{D,t=0} = k_{1D}/(k_{1D} + k_{2D})[OX_D]_0 \quad (29)$$

The rate of enolate disappearance is given by equation (30).

$$\lambda_3 = \frac{\lambda_1\lambda_2\lambda_3}{\lambda_1\lambda_2} = \frac{C}{(k_{1L} + k_{2L})(k_{1D} + k_{2D})} = \frac{k_{-1L}k_{2L}}{(k_{1L} + k_{2L})} + \frac{k_{-1D}k_{2D}}{(k_{1D} + k_{2D})} \quad (30)$$

The k_1 and k_2 values are obtained from λ_1 or λ_2 and extrapolated enolate concentrations as explained in the preceding paper.

The λ_1 and λ_2 values are measured as a function of the cyclodextrin concentration and follow the saturation curves of equation (31) where λ_{obs}, λ_0, and λ_{CD} are the observed rate

$$\lambda_{obs} = \frac{\lambda_0 K_{diss} + \lambda_{CD}[CD]}{K_{diss} + [CD]} \quad (31)$$

constants, their value in the absence of cyclodextrin, or at infinite concentration and K_{diss} is the dissociation constant of the oxazolone–cyclodextrin complex; they are obtained by curve-fitting methods.

From Scheme 3 a relationship can be derived between the pK_a values or k_1/k_{-1} ratios at constant pH and the binding constants [equation (32)]. The ratio k_{-1L}/k_{-1D} is thus obtained.

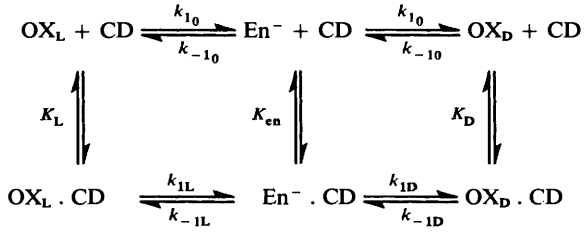
$$\frac{k_{1L}/k_{-1L}}{k_{1D}/k_{-1D}} = \frac{K_L}{K_D} \quad (32)$$

This value in combination with (30) leads to the values of k_{-1L} and k_{-1D}.

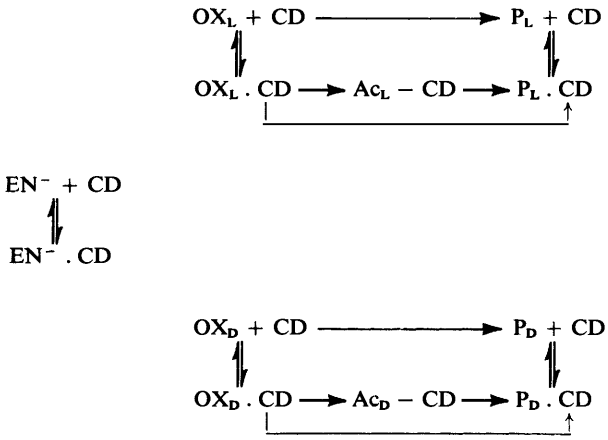
Results

The general kinetic scheme for the hydrolysis of oxazolones in the presence of cyclodextrins is rather complex (Scheme 4). Each enantiomer and the enolate can exist free in solution or complexed by the macrocycle; the ring-opening reaction can occur by two mechanisms, direct hydrolysis or acylation of the cyclodextrin followed by deacylation.[3] Furthermore, the acid–base reactions and the ring-opening reaction can be catalysed by hydroxide ions or buffers. Despite this complexity, the essential features of the mechanism can be demonstrated by measuring the kinetics of the reactions under a variety of experimental conditions. All the reactions are followed by monitoring the absorbance change at the $\lambda_{max.}$ of the enolate as in the previous paper.

A few experiments were first performed with the racemic oxazolones. (1) The rate of the slow step (enolate disappearance) has been measured as a function of the cyclodextrin concentration. Typical results are given in Figure 1: the observed rate constants follow saturation curves; with β-CD, there is always catalysis; with α-CD, there is catalysis at low pH and apparent inhibition at high pH. The complexation of



Scheme 3.



Scheme 4.

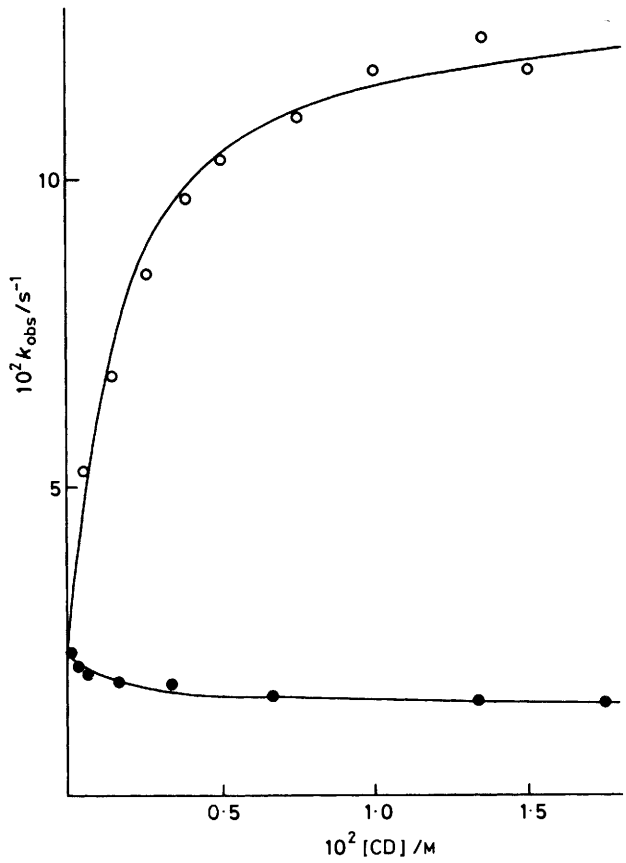


Figure 1. Effect of the α- and β-cyclodextrins (CD) on the rates of enolate disappearance with 4-methyl-2-phenyloxazolone at 25 °C and pH 9.16 in borate buffer: ●, α-CD; ○, β-CD

Table 1. Competitive inhibition by *p*-chlorobenzoic acid on the effect of the cyclodextrins on the hydrolysis of 4-benzyl-2-phenyloxazolone [a]

[Cyclodextrin]/M	[*p*-ClC_6H_4-COOH]/M	k_{obs}/s^{-1}	k_{calc} [b]$/s^{-1}$
α, 7.4×10^{-3}		4.62×10^{-3}	4.60×10^{-3}
α, 7.4×10^{-3}	1.1×10^{-2}	3.51×10^{-3}	3.56×10^{-3}
β, 7.0×10^{-4}		8.30×10^{-3}	8.20×10^{-3}
β, 7.0×10^{-4}	9.2×10^{-3}	5.50×10^{-3}	5.28×10^{-3}

[a] pH 7.86, ionic strength 0.1, $[OX]_0$ 5.5×10^{-5}M. [b] Calculated from the following constants: $k_0 = 2.63 \times 10^{-3}$ s^{-1}. With α-CD: k_{CD} 7.79×10^{-3} s^{-1}, K_D 1.19×10^{-2}M, K_I 6.0×10^{-3}M (ref. 13); with β-CD: k_{CD} 1.39×10^{-2} s^{-1}, K_D 7.19×10^{-4}M, K_I 4.0×10^{-3}M (ref. 13).

Table 2. Catalytic rate constants of hydrolysis of racemic 4-benzyl-2-phenyloxazolone in the presence of α- or β-cyclodextrin [a]

Cyclodextrins	Catalyst	k_1/ l mol^{-1} s^{-1}	k_{-1}/ l mol^{-1} s^{-1}	k_2/ l mol^{-1} s^{-1}
α [c]	$H_2PO_4^-$–HPO_4^{2-}	2.7 (1.3)	15 (0.2)	0.04 (1.6)
	H_3BO_3–$B(OH)_4^-$	1.9 (1.1)	0.02 (0.08)	1.2 (2.3)
	HCO_3^-–CO_3^{2-}	50 (2.3)	0.08 (0.08)	31 (6.8)
	H_2O–OH^-	4.4 × 10^4	0.012 [b]	2.6 × 10^4
β [d]	$H_2PO_4^-$–HPO_4^{2-}	1.3 (0.6)	45 (0.6)	0.1 (4.0)
	H_3BO_3–$B(OH)_4^-$	0.4 (0.23)	0.5 (2.0)	0.8 (1.5)
	HCO_3^-–CO_3^{2-}	8.5 (0.4)	0.14 (0.13)	2.0 (0.44)
	H_2O–OH^-	3.7 × 10^4	0.12 [b]	3.2 × 10^4

[a] The numbers in parentheses are the ratios between the catalytic rate constants in the presence and absence of CD. [b] In s^{-1}. [c] [α-CD] 1.25×10^{-2}M (or 2.0×10^{-2}M in carbonate buffer) 25% (or 33%) saturation for the OX_L and OX_D, 96% saturation for $E^{\cdot\cdot-}$. [d] [β-CD] 7.5×10^{-3}M, 88% saturation for OX_L and OX_D, 91% saturation for En^-.

the oxazolone can be competitively inhibited by *p*-chlorobenzoic acid. The observed rate constants in the presence of inhibitor can be recalculated from the parameters determined in this work (k_{CD} and K_{diss}) from curves similar to Figure 1 and the K_I values from the literature [13] using equation (33). The

$$k_{obs} = \frac{k_{CD}[CD] + k_0K_{diss}(1 + [I]/K_I)}{[CD] + K_{diss}(1 + [I]/K_I)} \qquad (33)$$

data are shown in Table 1.

(2) The change in enolate concentration (increase and decrease) as a function of time was then followed at constant cyclodextrin concentration and variable pH and buffer concentration. The data are treated according to the approximate kinetic analysis described in the Experimental section. The results obtained with 4-benzyl-2-phenyloxazolone are collected in Table 2. A pH profile is shown in Figure 2. Because of the intrinsic limitations of that analysis, the accuracy on the $k_{1m} = (k_{1L} + k_{1D})/2$ values is rather low. Nevertheless, it is clear that the deprotonation (k_1) and ring-opening reactions (k_2) are hydroxide ion and buffer catalysed; the reprotonation is essentially pH independent above pH 8 as it was in the absence of cyclodextrins. These results lead to the determination of the pH range where the condition: k_1 and $k_2 \gg k_{-1}$, necessary for the simplification of the kinetics, is fulfilled.

A full kinetic analysis was applied to the reactions between the optically active oxazolones and the three cyclodextrins at constant high pH and fixed low buffer concentration. The change in enolate concentration can now be described by a sum of two exponentials. Representative results are shown in Figure 3. The rate constants of the fast step ($\lambda_1 = k_1 + k_2$)

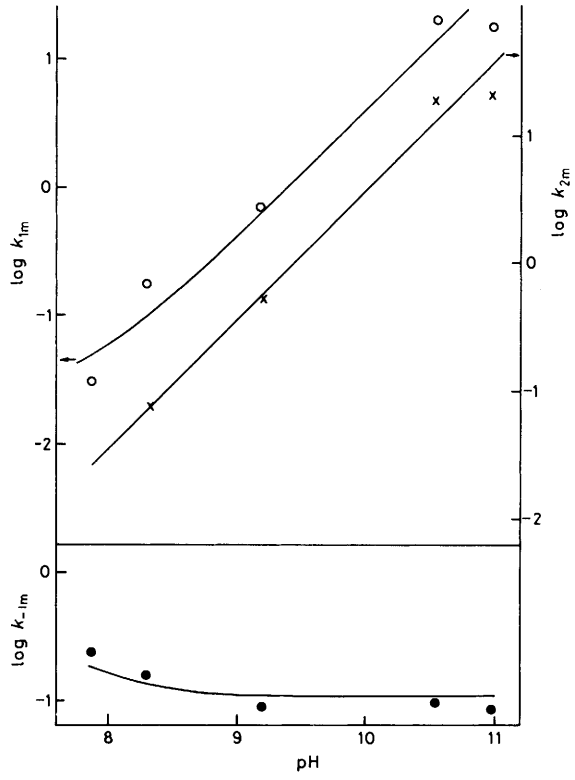


Figure 2. pH profile for the mean rate constants of deprotonation (○), reprotonation (●), and ring opening (×) of the 4-benzyl-2-phenyloxazolone at 25 °C in the presence of cyclodextrin

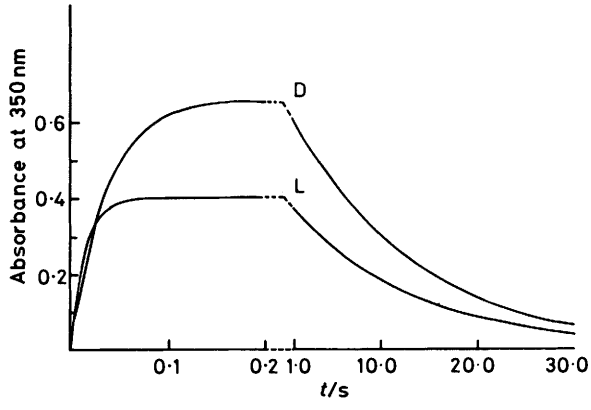


Figure 3. Change in absorbance due to the enolate of L- and D-4-methyl-2-phenyloxazolone (5×10^{-3}M) as a function of time at pH 11.05 in the presence of 7.5×10^{-3}M-β-cyclodextrin

and the maximum enolate concentration $[EN^-]_{max.} \cong k_1/(k_1 + k_2)$] can be different for both enantiomers; the rate constants of the slow step are obviously identical. The observed rate constants of the fast step (λ_1) are plotted as a function of the cyclodextrin concentration (Figure 4). The saturation curves are described by equation (31) from which the binding constants of the neutral oxazolones are obtained. They are calculated by curve-fitting methods and given in Table 3. For both L- and D-4-benzyl-2-methyloxazolone with α-cyclodextrin and for all the oxazolones with γ-cyclodextrin, the plots of λ *versus* cyclodextrin concentration do not deviate sufficiently from linearity even at the highest concentration used (4×10^{-2}M for α-CD and 10^{-2}M for γ-CD) to calculate a binding constant.

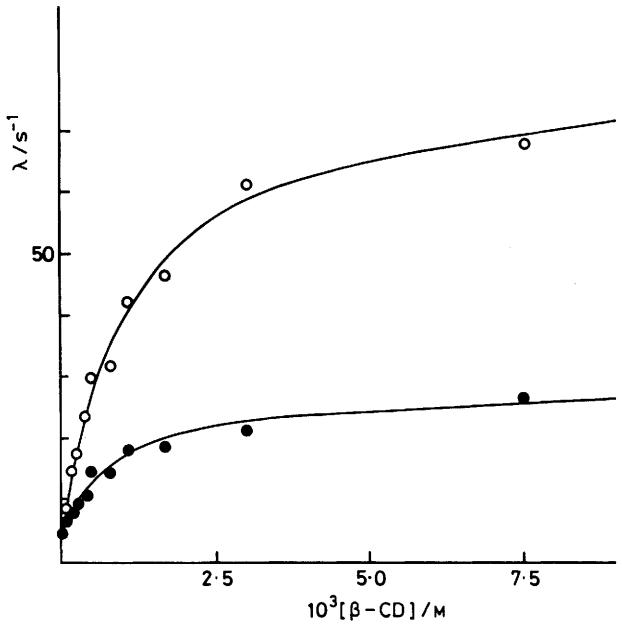


Figure 4. Effect of the β-cyclodextrin concentration on the rate of the fast step (enolate increase: $\lambda = k_1 + k_2$) for the 4-benzyl-2 phenyloxazolone at pH 11.05 and 25 °C: L, ○; D, ●

Table 3. Binding constants of the oxazolones to the cyclodextrins [a]

Substrate	Cyclo-dextrin	$K_{ox.L}$/ mol l^{-1}	$K_{ox.D}$/ mol l^{-1}	K_{En-}/ mol l^{-1}
4-Benzyl-2-phenyl-OX	α	4.0×10^{-2}	4.25×10^{-2}	9.1×10^{-4}
	β	1.14×10^{-3}	8.9×10^{-4}	7.9×10^{-4}
4-Methyl-2-phenyl-OX	α	1.37×10^{-2}	1.38×10^{-2}	3.95×10^{-4}
	β	5.3×10^{-3}	4.7×10^{-3}	9.6×10^{-4}
4-Benzyl-2-methyl-OX	α	$>4 \times 10^{-2}$	$>4 \times 10^{-2}$	*b*
	β	5.0×10^{-3}	3.6×10^{-3}	8.9×10^{-3}

[a] K_{diss} from γ-cyclodextrin: $>10^{-2}$M. [b] $K_{En-} = K_{ox.L} = K_{ox.D}$ because $pK_{a_0} = pK_{aL} = pK_{aD}$.

Table 4. Cyclodextrin-catalysed hydrolysis of L- and D-4-benzyl-2-phenyloxazolone [a]

Cyclo-dextrin	Substrate	k_1/ s^{-1}	k_{-1}/s^{-1}	pK_a	k_2/ s^{-1}	% Acyl-ation [b]
α	L	71	6.4×10^{-3}	7.0	66	54 [c]
	D	87	7.3×10^{-3}	7.0	28	
β	L	36	1.1×10^{-1}	8.5	43	77 [d]
	D	21	7.9×10^{-2}	8.6	7.2	
γ	L	8.3	8.9×10^{-2} [e]	9.1	9.6	Not
	D	7.0	7.5×10^{-2} [e]	9.1	2.7	measured
	L or D	3.2	1.3×10^{-2}	8.7	1.3	

[a] At pH 11.05, in 1.5×10^{-2}M-phosphate buffer, 25 °C; k_i given for α- and β-cyclodextrin are the values calculated at saturation; the k_i values for γ-cyclodextrin are the values measured at 10^{-2}M-cyclodextrin. [b] Mean value for L- and D-form measured by pH-stat and by hydroxamate assay (see ref. 3) at pH 7.86. [c] 51% at pH 8.5, 50% at pH 9.5. [d] 69% at pH 8.5, 67% at pH 9.5. [e] The k_{-1} values for the γ-cyclodextrin are calculated with the assumption that $K_{ox.L} = K_{ox.D}$.

From these data, the k_i values in the absence of cyclodextrin and at saturation (or at the highest cyclodextrin concentration used in the absence of saturation) are calculated as described in the Experimental section. They are given in Tables 4—6. These Tables have been organized to facilitate comparisons between the different oxazolones with the same cyclodextrin or different cyclodextrins with the same oxazolone.

Table 5. Cyclodextrin-catalysed hydrolysis of L- and D-4-methyl-2-phenyloxazolone [a]

Cyclo-dextrin	Substrate	k_1/ s^{-1}	k_{-1}/s^{-1}	pK_a	k_2/ s^{-1}	% Acyl-ation [b]
α	L	76	2.5×10^{-2}	7.6	65	44
	D	117	3.8×10^{-2}	7.6	20	
β	L	81	1.4×10^{-1}	8.3	147	81
	D	47	1.0×10^{-1}	8.35	11.2	
γ	L	11	6.4×10^{-2} [c]	8.8	25	Not
	D	12	6.8×10^{-2} [c]	8.8	5.8	measured
	L or D	2.5	2.6×10^{-2}	9.0	1.3	

[a] See note *a* of Table 4. [b] Measured by pH-stat at pH 7.86. [c] See note *e* of Table 4.

Table 6. Cyclodextrin-catalysed hydrolysis of L- and D-4-benzyl-2-methyloxazolone [a]

Cyclodextrin	Substrate	k_1/ s^{-1}	k_{-1}/ s^{-1}	pK_a	k_2/ s^{-1}	% Acyl-ation [b]
α	L	23	0.66 [c]	10.2	56	47
	D	23	0.66 [c]	10.2	52	
β	L	6.7	0.34	10.5	15.4	48
	D	6.0	0.46	10.6	25.4	
γ	L	10	0.4 [c]	10.3	21	Not
	D	8	0.3	10.3	37	measured
	L or D	6.2	0.19	10.2	4.6	

[a] Measured at pH 11.75 in 1.25×10^{-2}M-phosphate buffer at 25 °C. The k_i values given for the α- and β-cyclodextrin are the values calculated at saturation; the k_i values for the γ-cyclodextrin are the values measured at 10^{-2}M-cyclodextrin concentration (see text). [b] Measured by pH-stat at pH 7.86. [c] See note *e* of Table 4.

Table 7. Solvent isotope effect on the enantioselectivity of the β-cyclodextrin-catalysed hydrolysis of 4-methyl-2-phenyloxazolones [a]

Solvent	L/D	k_1/s^{-1}	k_{1L}/k_{1D}	k_2/s^{-1}	k_{2L}/k_{2D}
H_2O [b]	L	40.2 ± 3.1	1.55 ± 0.13	66.5 ± 5.2	9.6 ± 0.9
	D	25.9 ± 0.2		6.9 ± 0.1	
D_2O [c]	L	52.7 ± 3.4	1.35 ± 0.19	87.1 ± 5.6	8.4 ± 1.2
	D	39.0 ± 3.0		10.4 ± 0.8	

[a] [β-CD] 7.5×10^{-3}M. [b] In 0.014M-phosphate buffer, HPO_4^{2-}/PO_4^{3-} 5.1; pH 10.97; [OH^-] 9.3×10^{-4}M. [c] In 0.014M-phosphate buffer, DPO_4^{2-}/PO_4^{3-} 5.1; pD 11.71; [OD^-] 6.9×10^{-4}M, from $pK_{w.D_2O}$ 14.87 (A. K. Covington, R. A. Robinson, and R. G. Bates, *J. Phys. Chem.*, 1966, **70**, 3820).

The solvent isotope effect was measured with the most enantioselective system, β-cyclodextrin and the 4-methyl-2-phenyloxazolone at a single cyclodextrin concentration. The results are given in Table 7.

Discussion

The three oxazolones form inclusion complexes with the cyclodextrins as most hydrophobic substrates do; this is shown by the saturation curves and the competitive inhibition of the kinetics. There is no evidence in the saturation curves for a 2 : 1 stoicheiometry even for the largest substrate, 4-benzyl-2-phenyloxazolone. The binding is always stronger in β-cyclodextrin than in α- or γ-cyclodextrin. The α-cyclodextrin is too small to permit a full inclusion that could take advantage of all the possible favourable contacts, γ-cyclodextrin is too large for the substrate to fill the cavity. Very few

Table 8. Second-order rate constants and enantioselectivities for the cleavage of oxazolone rings by cyclodextrins (CD) [a]

		α-CD		β-CD		γ-CD	
Oxazolone	L/D	$(k_2/K_D)_{L \text{ or } D}$/ l mol^{-1} s^{-1}	$\frac{(k_2/K_D)_L}{(k_2/K_D)_D}$	$(k_2/K_D)_{L \text{ or } D}$/ l mol^{-1} s^{-1}	$\frac{(k_2/K_D)_L}{(k_2/K_D)_D}$	$(k_1/K_D)_{L \text{ or } D}$/ l mol^{-1} s^{-1}	$\frac{(k_2/K_D)_L}{(k_2/K_D)_D}$
4-Benzyl-2-phenyl-OX	L	1.65×10^3	2.5	3.77×10^4	4.65	8.30×10^2	5.9
	D	0.66×10^3		0.81×10^4		1.40×10^2	
4-Methyl-2-phenyl-OX	L	4.75×10^3	3.3	2.77×10^4	11.6	2.37×10^3	5.3
	D	1.45×10^3		2.38×10^3		0.45×10^3	
4-Benzyl-2-methyl-OX	L	2.60×10^2	1.05	5.90×10^2	0.43	3.10×10^2	0.72
	D	2.45×10^2		13.5×10^2		4.3×10^2	
2,4-Dimethyl-OX	Racemic	1.40×10^3 [c]	0.33 [d]	1.78×10^3 [c]	1.35 [d]		

[a] At pH 11.05. [b] Corrected to pH 11.05 from the data at pH 11.75. [c] Mean k_2/K_D, corrected to pH 11.05 from the data obtained at pH 7.86 with the racemic oxazolone; in the absence of cyclodextrin, this oxazolone is 7.5 times more reactive than 4-benzyl-2-methyl-OX. [d] Overall enantioselectivity obtained from product analysis (ref. 3).

data are available on comparisons of binding strength for the three cyclodextrins; with the nitrophenyl esters of adamantane-1-carboxylic acid, the binding was also found to be better to the β-cyclodextrin than to the other two hosts.[14]

The enolates of the 2-phenyloxazolones bind more strongly than the neutral substrates, the difference in K_{diss} being largest for α-cyclodextrin. Increased binding of delocalized anions has been observed with other acid–base pairs, especially phenols; it is a consequence of the increased polarizability of the anion which is favourable to the van der Waals interaction forces.

The chiral recognition on binding is quite low. The largest ratio of binding constants is 1.4. Larger differences in K_{diss} between enantiomers have been reported: 6.7 for isopropyl methyl phosphonofluoridate to α-cyclodextrin [15] or 3.7 for a nitroxide derived from 3-methylcyclohexanone to β-cyclodextrin.[16] In these molecules, the most hydrophobic part is itself chiral. When the portion of the substrate being included is more symmetrical as in the ferrocenyl derivatives of Breslow [2] or with all the *m*-nitrophenyl esters studied,[17–19] the K_D/K_L ratio remains below 2.5. In general, the cylindrical binding cavity of the cyclodextrin is too symmetrical itself to induce large enantioselectivities on binding.

In the complexes, the oxazolones remain largely accessible to the species in solution as shown by the fact that the catalytic constants of the buffers are generally of the same order of magnitude as those in the absence of cyclodextrin. The only rate constants that are systematically lower are those of reprotonation of the enolate complexed by α-cyclodextrin. Although the binding of the enolate is as strong to β-CD as to α-CD, the larger macro-ring does not shield the substrate as efficiently.

To our knowledge, there is so far no information on the catalysis of acid–base reactions by cyclodextrins. In fact, the cyclodextrins can be quite effective at promoting the deprotonation with catalytic factors up to 70. The $k_{1\,.\,CD}/k_{1_0}$ ratio is pH independent between pH 9 and 11. This reaction clearly involves a deprotonated hydroxy-function of the cyclodextrin. There should be a levelling off above the pK_a of the macrocycle (12.2).[20] $k_{1\,.\,11m}$ (above pH 13, too fast to measure) calculated for the deprotonation of 4-benzyl-2-phenyloxazolone by the α- and β-cyclodextrin anions varies between 3×10^2 and 2.6×10^3 s^{-1}. The rate constant of the deprotonation by the phosphate buffer, a base of approximately the same pK_a, is 1.65×10^2 l mol^{-1} s^{-1} (see preceding paper). The ratio between these rate constants gives an indication of the effective molarity of the oxyanion in the substrate–macrocycle complex. It lies between 1 and 50M. These values are typical for intramolecular catalysis of acid–base reactions, particularly enolizations.[21]

The enantioselectivity on the deprotonation or reprotonation reactions is never large, the largest ratio being *ca.* 2. In this respect, the cyclodextrins are very poor models of enzymes. However, larger differences on the rates of either deprotonation or reprotonation should be found in more favourable cases: when the difference in binding constants between enantiomers is large, there must by definition be a large enantioselectivity for at least one of these rates.

The effectiveness of the catalysis on the ring-opening reaction is in the range observed for the cleavage of the carboxylic acid derivatives.[2] Two mechanisms have been demonstrated for the cyclodextrin catalysis of the hydrolysis of esters, nucleophilic catalysis with formation of an acylcyclodextrin analogous to that of serine proteases [22] and a general base catalysis.[23] Both mechanisms appear to be operating concurrently with the oxazolones as shown by the fact that the maximum percentage of acylcyclodextrin detectable (Tables 4—6) is never close to 100% and is pH independent. The contribution of the general base catalysis is especially important with the α-cyclodextrin.

The enantioselectivity on the ring-opening reaction is larger than on the deprotonation reaction, the ratio k_{2L}/k_{2D} varying between 0.3 and 13. Higher stereoselectivities in cyclodextrin-catalysed reactions have been observed by other authors: with organophosphates where the centre of chirality is the atom being attacked,[15] and with the ferrocenyl derivatives of Breslow,[2] especially designed to show up a large enantioselectivity.

It is of interest to observe the relationship between enantioselectivity and reactivity, on the one hand, and host size and substrate structure, on the other hand. But before doing that, we should first consider the possible modes of binding for the three substrates. It is very likely that in the preferred binding mode, the most hydrophobic part of the molecule, the phenyl ring, will penetrate the cavity. There is thus one binding mode for 4-methyl-2-phenyl- and 4-benzyl-2-methyloxazolone but there are two possible binding modes for 4-benzyl-2-phenyloxazolone. Now, there are more similarities of behaviour between the two oxazolones bearing a phenyl in position 2 than with the third substrate, for catalysis or inhibition of the acid–base and ring-opening reactions, for pK_a shifts on complexation, and for enantioselectivity. Accordingly, one must conclude that, if there are effectively two binding modes for 4-benzyl-2-phenyloxazolone (this cannot be excluded from a consideration of the binding constants) the one with the 2-phenyl in the cavity contributes more to the reactivity.

The effect of the host and substrate structure on the catalytic efficiency is best discussed in terms of k_{CD}/k_D as recommended for enzymes [24] (Table 8); this quantity measures the difference in free energy between the free species in solution and the

bound transition state; as such, it takes into account the contribution of the binding to the decrease in the free energy of the transition state. With the 2-phenyloxazolones, the reactivity is rather sensitive to the size of the host. This arises from the mechanism of the reaction: to acylate the cyclodextrins, the substrates cannot remain parallel to the axis of the cavity, they have to lie on the wall. In this position, the phenyl group is still well within the cavity of β-cyclodextrin, but there is hardly enough room in α-cyclodextrin and γ-cyclodextrin is too large for the binding to contribute as much as with β-cyclodextrin. This explains why the reactivity is highest with β-cyclodextrin * and why the percentage of acylation is lower with the α-cyclodextrin than with the β-cyclodextrin.

The reactivity of 4-benzyl-2-methyloxazolone is lower than that of the other oxazolones, it is less sensitive to the cyclodextrin size, and the percentage of acylation remains low. The lower reactivity is the result of both a poor binding and a poor catalysis in the complex. Because of the presence of the CH_2 group connecting the bound phenyl and the heterocycle, the binding contributes less to the positioning of the reactive groups; the additional degree of freedom in the complex is deleterious to the catalysis. During the acylation, there is a steric interaction between the CH of the asymmetric carbon and the wall of the cyclodextrin; this may also contribute to the decrease in the reactivity and in the percentage of acylation. Because of the additional flexibility of the substrate, the size of the macro-ring is less important.

There is no straightforward and general relationship between the cyclodextrin size or its catalytic efficiency and the enantioselectivity. In his work on the cyclodextrin-catalysed hydrolysis of the 3-carboxy-2,2,5,5-tetramethylpyrrolidinyl-1-oxyl nitrophenyl esters, Kaiser had observed a much higher enantiomeric selectivity with α- than with β-cyclodextrin and had tentatively concluded that this might reflect a general trend, because D/L specificity is derived from tight binding.[17] The data reported here do not support this conclusion. With the oxazolones, β-cyclodextrin is the best catalyst in terms of the k_{CD}/K_D ratio and it appears to be generally the most enantioselective both for the deprotonation and the ring-opening reaction, but there is one exception; the ring-opening reaction of 4-benzyl-2-phenyloxazolone catalysed by γ-cyclodextrin is more enantioselective than with β-cyclodextrin. There might be a very rough tendency for the larger substrates to show the highest stereospecificity with the largest macro-ring, the smallest substrate being more stereospecific with α-cyclodextrin. This tendency is partially explained at least for the 2-phenyloxazolones as being due to the duality of mechanism: with α-cyclodextrin, <60% of the reaction goes through the nucleophilic pathway and general base catalysis is probably much less stereoselective.

In the catalysis by inclusion compounds; it is in principle easier to rationalize the enantioselectivity of reactions in terms of a three-point attachment model. The hydrophobic binding hole is the first point; the reacting atom of the cyclodextrin the second; the third point can be either attractive or repulsive, namely a hydrogen bond to a hydroxy-group or a steric interaction with any group of the host. If we apply this to the oxazolones on the hypothesis that the third point is a hydrogen bond, the recognition points would be defined as in Figure 5.

Furthermore, the imino-nitrogen is converted into an amido-nitrogen by the ring-opening reaction and consequently it takes up a proton. If the protonation occurs before or in the rate-limiting step and a hydroxy-group of the cyclodextrin is the donor, differences in the k_{2L}/k_{2D} ratios in H_2O and D_2O are expected. The solvent isotope effect has been measured for the reaction between the enantiomers of 4-methyl-2-phenyloxazolone and β-cyclodextrin. The k_1/k_2 ratio for the L- and D-isomer, easily measured with accuracy, are the same in H_2O and D_2O. The differences for the k_{1L}/k_{1D} and k_{2L}/k_{2D} ratios between H_2O and D_2O remain within experimental error. Moreover, after correction for the difference in hydroxide or deuterioxide concentration, the k_1 and k_2 values show an inverse isotope effect reflecting the difference in pK_a of the cyclodextrin hydroxy-groups in the two solvents but also showing that no proton transfer occurs in the rate-limiting step. All these elements strongly suggest that hydrogen bonding or proton transfer are not the main factors involved in chiral recognition. The enantioselectivity should then be explained in terms of steric interactions.

The enantioselectivities on the deprotonation and reprotonation reactions are low, presumably because these reac-

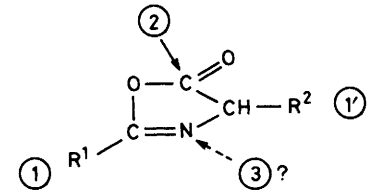


Figure 5. 1, the more hydrophobic of the two substituents R^1 and R^2, 2, carbonyl group 3, imino-nitrogen

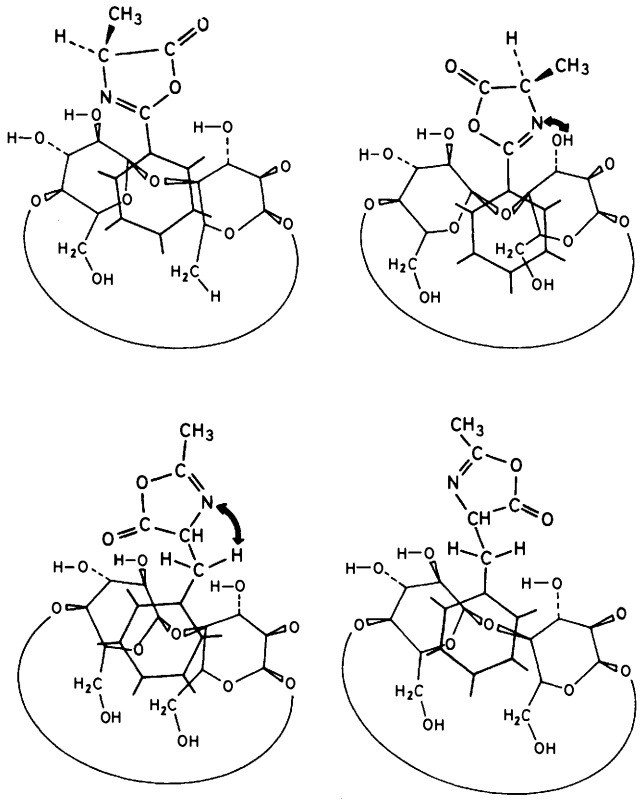


Figure 6. Schematic drawing showing the interactions between the 2-phenyloxazolones or 4-benzyl-2-methyloxazolone and the glucose units during acylation of the host. The models suggest that there is a more unfavourable steric interaction with the D-isomer of the 2-phenyloxazolones and the L-isomer of 4-benzyl-2-methyloxazolone

* In terms of k_{CD}, α-cyclodextrin appears to be as reactive as β-cyclodextrin; this overshadows the fact that with this host, the binding is as poor in the transition state as in the complex.

tions do not require a tight transition state; consequently the preference for the L- or D-isomer remains difficult to rationalize, even when considering the binding modes.

The trends on the ring-opening reactions are easier to understand. For the 2-phenyloxazolones, the preference of the cyclodextrins for the L-isomer can be explained by an inspection of molecular models: if the 2-phenyl group penetrates the cavity first and the side chain on the asymmetric carbon is arranged to point away from the wall for both isomers to permit an interaction between the C=O and the nucleophilic 2-hydroxy-group of the cyclodextrin,[25] there is more unfavourable steric interaction between the next glucose unit and the oxazolone ring in the D- than in the L-isomer (Figure 6). The lower enantioselectivity observed with 4-benzyl-2-phenyloxazolone may result from the contribution of the other binding mode.

When 4-benzyl-2-methyloxazolone binds to cyclodextrin, the extended conformation of the substrate is favoured. The lower reactivity of the L-isomer with the β- and γ-cyclodextrin is again explained by a larger steric interaction between the rest of the oxazolone ring and the next glucose unit when the carbonyl group is approached by the nucleophilic hydroxy-group (Figure 6). However, the lack of enantioselectivity with α-cyclodextrin remains unexplained.

Conclusions.—The following conclusions arise from this study. (1) The cyclodextrins are able to catalyse acid–base reactions as efficiently as the well documented cleavage of carboxy-derivatives; however, the enantioselectivity of these reactions remains low. (2) To observe reasonable enantioselectivity, a covalent interaction is required between the macrocycle and the substrate as shown by the fact that the enantioselectivity is higher for the ring-opening reaction than for the enolization reaction and that it is higher when the percentage of acylation is higher. (3) The effect of the cyclodextrin size on the D/L stereoselectivity is much less dramatic than suggested in previous studies; there is some relationship between reactivity and enantioselectivity and it might be of interest to match the size of the host with that of the substrate. (4) The direction of the enantioselectivity can be interpreted by assuming reasonable binding modes. This success is not limited to the oxazolone–cyclodextrin system.[2] To the extent that binding modes can be predicted from a consideration of the relative binding abilities (hydrophobicity, polarizability . . .) of various portions of the molecules, the direction of enantioselectivity becomes predictable.

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