Enantioselectivity Effects in the Hydrolytic Cleavage of Activated Substrates with α- and β-Cyclodextrins

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The kinetics of hydrolytic cleavage of the enantiomers of *p*- and *m*-nitrophenyl (Np) phenylacetate esters of general structure $PhCR^1R^2–CO_2–Np$ where R^1,R^2 = H,Me [(**1**),(**2**)], H,OMe [(**3**),(**4**)], or CF_3,OMe [(**5**),(**6**)], and of the carbonates PhCHMe–OCO_2–*p*-Np (**7**) and n-C_6H_{13}–CHMe–OCO_2–*p*-Np (**8**), have been measured in the presence of α- and β-cyclodextrins. The rates of intracomplex cleavage were found to be larger for the *R*- than for the *S*-enantiomers in almost all cases; the enantioselectivity factors range from unity to *ca.* 19 depending on the relative size of the pairs of substituents R^1 and R^2. The effects have been interpreted with the help of molecular models.

Cyclodextrins[1] (CDs) have attracted great attention as enzyme mimics because of their ability to bind substrates in their cavities. Within the complex, reaction may occur with one of the cyclodextrin hydroxy groups. The cleavage of activated esters is the most widely investigated of such reactions, and the mode of action has been clearly established by Bender and his co-workers.[2] The accelerations resulting from binding of common esters are generally modest. In only a few cases, such as those investigated by Breslow and his co-workers[3] using *p*-nitrophenyl esters of ferrocenyl derivatives as substrates, very large accelerations of magnitude similar to those observed for enzymic reactions were reported.

In accord with the chiral environment provided by the CD cavity, stereoselective reactions were found to occur for complexed substrates.[1a,4] In particular, enantioselectivities in the hydrolytic cleavage of complexed carboxylic esters were observed in a number of cases, but these were not studied in detail. Relatively large effects have been reported by Kaiser and his co-workers[5] in the hydrolysis of enantiomeric *p*-nitrophenyl 2,2,5,5-tetramethyl-1-oxopyrrolidine-3-carboxylates in the presence of α-CD and, more recently, by Daffe and Fastrez[6] in the ring opening of substituted oxazolones complexed with either α- or β-CD. The largest enantioselectivity factor (greater than 60) so far reported was observed by Breslow and his co-workers[3c] in the hydrolytic cleavage of the *p*-nitrophenyl ester of (*E*)-3-(carboxymethylene)-1,2-ferrocenocyclopentene with β-CD. On the other hand, Kitaura and Bender,[7] Yamada,[8] and Okhubo,[9] and their co-workers, who investigated enantioselectivity effects in the hydrolysis of amino acid esters in the presence of natural or modified cyclodextrins, reported moderate effects. A rationale for the enantioselectivity has seldom been offered;[3b,6] subtle differences in the modes of insertion of enantiomeric substrates are involved, and molecular models are often of little help.

We report here a kinetic analysis of the hydrolytic cleavage of structurally related pairs of enantiomeric nitrophenyl carboxylic esters (**1**)—(**6**) and of the carbonates (**7**) and (**8**) in the presence of α- and β-CDs in aqueous sodium carbonate buffers. A preliminary account of the effects observed for the ester (**3**) and the carbonate (**8**) has been published.[10]

$PhCR^1R^2–CO_2–L$

(**1**) R^1 = H, R^2 = Me, L = *p*-Np
(**2**) R^1 = H, R^2 = Me, L = *m*-Np
(**3**) R^1 = H, R^2 = OMe, L = *p*-Np
(**4**) R^1 = H, R^2 = OMe, L = *m*-Np
(**5**) R^1 = CF_3, R^2 = OMe, L = *p*-Np
(**6**) R^1 = CF_3, R^2 = OMe, L = *m*-Np

PhCHMe–OCO_2–*p*-Np (**7**)

n-C_6H_{13}–CH(Me)–OCO_2–*p*-Np (**8**)

Np = nitrophenyl (*para* or *meta*)

Results

The esters and carbonates investigated were obtained from the enantiomers of the corresponding acids or alcohols by standard methods which do not involve racemization.

The kinetic measurements were carried out by following the appearance of the nitrophenol, and the observed first-order rate constants, k_ψ, were determined in the absence (k_{un}) and in the presence of α- or β-cyclodextrins, using 6 to 12 different CD concentrations from 1×10^{-4} to 1×10^{-2} M ([CD] > 10 × [substrate]). From the k_ψ values the following parameters were evaluated as described in refs. 2 and 11: k_c, the first-order rate constant for the cleavage of fully bound substrates, and K_d, the dissociation constant of the CD–substrate complex (assuming 1:1 stoicheiometry). The K_d values are generally affected by a rather large error, particularly in cases where the kinetic effects are small and the rate–concentration profiles are, therefore, rather flat. In a few cases, these values could not be estimated with a reasonable degree of confidence. Measurements were carried out with aqueous 20mM-sodium carbonate buffers containing added CH_3CN (1% v/v), mostly at pH 10.5 and 25 °C. In a few cases, data were obtained at different pH values in the range 9.5—11.4 without noticeable changes[10] in k_c/k_{un} and K_d.

Table 1 shows the kinetic parameters. For each substrate, k_c/k_{un} is an indication of the acceleration due to CD.[2,3] For each pair of enantiomers, the enantioselectivity factor is expressed (*a*) as the ratio of the k_c values for the two enantiomers, which is a straightforward indication, in agreement with k_c/k_{un}, of the (maximum) difference in rate for the complexed substrates; (*b*) as the ratio of the (k_c/K_d) values, *i.e.* the second-order rate constants for the reaction between the substrate and the CD. This is a more rigorous and also more frequently used method of expressing enantioselectivity, although it is often affected by a large error, including that in K_d, as already discussed.

Discussion

The following observations can be made from the data in Table 1. (*a*) The rate ratios, k_c/k_{un}, for intracomplex cleavage relative to simple hydrolysis are rather modest for the *p*-nitrophenyl esters and for the carbonate (**7**), but larger for the *m*-nitrophenyl esters (as usually observed;[2,3] referred to as the '*meta*' effect) and for the carbonate (**8**). (*b*) Enantioselectivities are larger with β-CD than with α-CD, except in the case of the carbonate (**8**). (*c*) Enantioselectivity effects do not substantially depend on the different strengths of binding of the two enantiomers, which are mostly similar within estimated error. Binding enantioselectivities are generally reported as small or zero.[3,5,6] (*d*) The relative sizes of the pairs of substituents R^1 and R^2 at the chiral

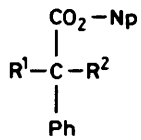
Table 1. Kinetic parameters for the hydrolytic[a] cleavage of substrates in the presence of α- and β-CDs

		α-CD				β-CD			
Substrate	Configuration	$\frac{k_c}{k_{un}}$[b]	$10^3\ K_d$/mol dm^{-3}	$\frac{(k_c)_R}{(k_c)_S}$	$\frac{(k_c/K_d)_R}{(k_c/K_d)_S}$	$\frac{k_c}{k_{un}}$[b]	$10^3\ K_d$/mol dm^{-3}	$\frac{(k_c)_R}{(k_c)_S}$	$\frac{(k_c/K_d)_R}{(k_c/K_d)_S}$
(1)	*R*(−)	1.9	1.5 ± 0.8	1.2	1.6	8.3	1.7 ± 0.3	9.5	
	S(+)	1.7	2.0 ± 0.7			0.9	—		
(2)	*R*(−)	175	6.6 ± 1.2	8.7	9.3	77.5	3.7 ± 0.4	15.5	19.0
	S(+)	20	7.1 ± 1.5			5.0	4.5 ± 1.0		
(3)	*R*(−)	3.0	9.1 ± 2.5	1.2	1.1	14.1	3.0 ± 0.7	7.9	12.0
	S(+)	2.5	8.7 ± 3.0			1.8	4.5 ± 2.0		
(4)	*R*(−)	150[c]	9.5 ± 0.9	2.3	2.9	70.5[c]	2.4 ± 0.2	5.4	4.1
	S(+)	66[c]	12.0 ± 1.1			13.1[c]	1.8 ± 0.3		
(5)	*R*(−)	*d*				1.2	5.5 ± 2.0	3.0	
	S(+)	*d*				0.4			
(6)	*R*(−)	2.3	5.0 ± 1.5	0.8	0.9	1.8	5.2 ± 1.9	0.8	1.2
	S(+)	3.0	5.7 ± 1.8			2.3	8.1 ± 2.1		
(7)	*R*(+)	19.0	7.1 ± 0.9	5.3	3.8	3.0	2.1 ± 0.5	0.6	0.5
	S(−)	3.6	5.1 ± 1.2			5.2	1.6 ± 0.5		
(8)	*R*(−)	240	2.5 ± 0.2	6.9	12.3	125.0	4.4 ± 0.3	5.7	4.0
	S(+)	35	4.5 ± 0.5			22.0	3.3 ± 0.4		

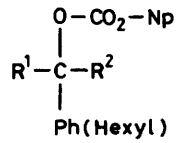
[a] At pH 10.5 (unless otherwise specified). [b] $10^3\ k_{un}$/s^{-1}; (1) 2.5; (2) 2.2; (3) 28; (4) 1.9 (pH 9.5); (5) 3.7; (6) 1.7; (7) 0.75; (8) 0.065. [c] At pH 9.5. [d] Small rate increases were observed, describing atypical rate–[CD] profiles.

carbon atom exert a marked effect on the rate differences between enantiomers. Enantioselectivity effects decrease in the series of esters (**1**)—(**6**) with increasing bulk of R^1,R^2 (H,Me > H,OMe > CF_3,OMe).

As shown in Table 1, the faster-complexed enantiomer has (fortuitously) the *R*-configuration, with the exception of the carbonate (**7**) with β-CD (a small effect) and of the ester (**6**), for which enantioselectivity is virtually absent. Interestingly, however, in each case the configuration of the *R*-enantiomer of the esters can be represented by the Fischer projection formula (**9**) and that of the carbonates by the analogous formula (**10**) where R^2 is bulkier than R^1 (Me or OMe *versus* H, or OMe *versus* CF_3).

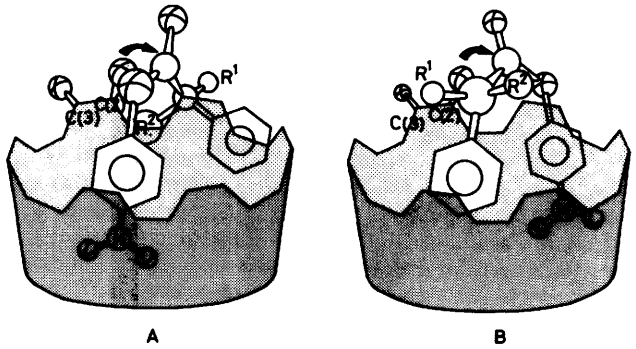


(**9**)



(**10**)

Molecular models indicate two likely modes of insertion of the substrates in the CD cavity: mode A, with the nitrophenyl inside, and mode B, with the phenyl [n-hexyl in the case of (**8**)] group inside. Moreover, whereas mode A is allowed in all cases, mode B is not fully allowed in the case of the substrates (**3**)—(**6**) with α-CD, owing to interference by the groups R^2 and R^1. Table 1 reveals that in the case of the esters (**1**)—(**4**) the accelerations k_c/k_{un} and the magnitude of the '*meta*' effect observed with α-CD are of the magnitude observed for simple nitrophenyl esters such as the acetate, for which a mode A type of insertion is established;[2] with β-CD, a less pronounced '*meta*' effect may indicate that whereas mode A still prevails, mode B can compete for inclusion in the larger cavity. On the other hand, the much larger than usual (for similar substrates[11]) values of k_c/k_{un} observed for the carbonate (**8**) with both α- and β-CD indicate a prevailing mode B insertion. Evidence of alkyl insertion in the case of long-chain nitrophenyl alkanoates has been recently



A B

Figure. Schematic representation of inclusion complexes following insertion modes A and B (see text) of the *R*-enantiomer of the esters. In the case of the carbonates, the complexes are analogous, provided an oxygen atom is inserted between the carbonyl group and the chiral carbon atom of the substrate and, in the case of (**8**), the phenyl group is replaced by an n-hexyl residue. The arrows indicate the nucleophilic interaction between the C-2 oxido group of the CD and the carbonyl carbon atom of the substrate

reported.[12,13] The effects observed for the esters (**5**) and (**6**), including the '*meta*' effect, are small: models show that whatever the mode and extent of insertion, intracomplex nucleophilic attack is made difficult by the presence of the two bulky groups R^1 and R^2. Furthermore, the rate data obtained for the esters (**5**) with α-CD could indicate a complexation stoicheiometry other than 1:1 at higher CD concentrations.

Molecular models indicate that an important role in determining enantioselectivity is likely to be played by the space between two sugar residues, as pointed out by Breslow and his co-workers[3b] and by Daffe and Fastrez.[6] If we assume, as is generally indicated and strongly suggested by recent results obtained in our laboratory,[14] that the C-2 and not the C-3 hydroxy groups (in ionized form) are the nucleophiles attacking the carbonyl carbons of the substrates,[1a,2,15] it appears that the *R*-enantiomers with *both* mode A and mode B insertions are better suited than the *S*-enantiomers for a juxtaposition of the reactive sites. This may occur, as shown in the Figure, while

Table 2. Analytical data for the esters (1), (2), and (4)—(6) and the carbonate (7)

Compound	Yield (%)	Found (%) [Required] C	H	N	$[\alpha]_D^{25}(c)^a$
(*S*)-(1)[b]	95	66.1	4.85	5.15	117° (10)
(*R*)-(1)[b]	94	66.15	4.7	5.15	−112° (10)
(*S*)-(2)	83	66.25	4.7	5.1	97° (7)
(*R*)-(2)	82	66.2	4.9	5.1	−90° (3)
($C_{15}H_{13}NO_4$)		[66.4]	[4.8]	[5.15]	
(*S*)-(4)	70	62.35	4.4	4.65	98° (10)[c]
(*R*)-(4)	75	62.4	4.75	4.8	−98° (10)[c]
($C_{15}H_{13}NO_5$)		[62.7]	[4.55]	[4.85]	
(*S*)-(5)	53	54.4	3.5	4.10	52° (20)
(*R*)-(5)	59	54.5	3.65	3.9	−51° (23)
(*S*)-(6)	73	54.1	3.5	3.9	45° (20)
(*R*)-(6)	75	54.3	3.3	3.85	−43° (17)
($C_{16}H_{12}F_3NO_5$)		[54.1]	[3.4]	[3.95]	
(*S*)-(7)	62	62.2	4.35	4.7	−115° (15)
(*R*)-(7)	58	62.45	4.7	4.65	+114° (15)
($C_{15}H_{13}NO_5$)		[62.7]	[4.55]	[4.85]	

[a] Concentration *c* in mg ml^{-1}; in $CHCl_3$, unless otherwise stated. [b] M.p. 75—76 °C. Other compounds were viscous oils. [c] In CH_3OH.

allowing sufficient penetration of the aryl residue and providing, in the case of mode A insertion, space to accommodate conveniently the phenyl [hexyl in the case of (8)] and the larger R^2 groups in close proximity to the external wall. In the case of mode B, room would be available for the bulky leaving group between glucose residues.

Molecular models cannot offer conclusive but only suggestive support for this explanation. Unfortunately, published studies in this area involve systems which are not structurally [3,6] related to the ones investigated here, or with unknown absolute configurations;[5,8] thus our rationale cannot be further substantiated. However, the present experimental facts are coherent for a number of related structures: complexes such as those in the Figure react faster than those with the enantiomeric substrates, and the enantioselectivity depends on the relative and absolute sizes of the substituents R^1 and R^2.

Experimental

The enantiomers of α-methoxyphenylacetic acid were obtained from the corresponding isomers of mandelic acid as described previously.[16,17] Optically active (>98% optically pure) carboxylic acids and alcohols, precursors of the esters and carbonates, respectively, were commercial products. The enantiomers of *p*-nitrophenyl α-methoxyphenylacetate (3) and *p*-nitrophenyl 1-methylheptyl carbonate (8) were obtained as described before.[16,17]

(+)-(*S*)-*p*-Nitrophenyl α-phenylpropionate and its (−)-(*R*)-isomer (1), (+)-(*S*)-*m*-nitrophenyl α-phenylpropionate and its (−)-(*R*)-isomer* (2), (+)-(S)-p-*nitrophenyl α-methoxy-α-trifluoromethylphenylacetate* and its (−)-(R)-*isomer* (5), and (+)-(S)-m-*nitrophenyl α-methoxy-α-trifluoromethylphenylacetate* and its (−)-(R)-*isomer* (6) were obtained from the corresponding acids of the same rotation sign, by the following procedure. The acid (3 mmol) was dissolved in thionyl chloride and after 2 days [at room temperature in the case of esters (1) and (2), and at 60 °C in the case of esters (5) and (6)] the excess of $SOCl_2$ was removed under reduced pressure. To the residue, toluene (30—40 ml) was added together with the nitrophenol (4 mmol) and pyridine (3—4 ml), and the mixture was kept for 6 h at room temperature [esters (1) and (2)] or for 2 h at 60 °C [esters (5) and (6)]. The solvent was then removed, diethyl ether was added to the residue, and the filtered ethereal solution was evaporated to dryness. Chromatography of the crude materials on a silica gel column [9:1 toluene–ethyl acetate for (1) and (2); 20:1 chloroform–methanol for (5) and (6)] afforded the products.

(+)-(S)-m-*Nitrophenyl α-methoxyphenylacetate* (4). (+)-(*S*)-α-Methoxyphenylacetic acid (0.83 g, 5 mmol) and oxalyl chloride (3.2 g, 25 mmol) were kept at room temperature for 2 h. Unchanged oxalyl chloride was then evaporated off and a solution of *m*-nitrophenol (0.77 g, 5.5 mmol) and pyridine (5 ml) in toluene (80 ml) was added to the residue. The solution was kept for 2 h at 60 °C, then evaporated, and the residue was chromatographed on a silica gel column (20:1 chloroform–methanol as eluant).

The (−)-(R)-*isomer* was similarly obtained from the (+)-(*R*)-acid.

(−)-(S)-p-*Nitrophenyl 1-phenylethyl carbonate* (7). To a solution of (−)-(*S*)-1-phenylethyl alcohol (0.4 g, 3.3 mmol) and pyridine (0.27 g, 3.3 mmol) in chloroform, a solution of *p*-nitrophenyl chloroformate in chloroform was added dropwise, in a dry atmosphere. After 1 h at room temperature, the solvent was removed and the residue chromatographed on a silica gel column (4:1 n-hexane–diethyl ether).

The (+)-(R)-*isomer* was similarly obtained from the (+)-(*R*)-alcohol.

Physical characteristics of the esters (1), (2), and (4)—(6) and of the carbonate (7) are summarized in Table 2.

Kinetic Measurements.—The general procedure has been described.[12] The appearance of *p*-nitrophenol was monitored at 400 nm and that of *m*-nitrophenol at 388 nm, using a Perkin-Elmer Lambda 5 or a Varian Cary 219 spectrophotometer equipped with a thermostatically controlled cell holder.

* Racemic (1) and (2) have been described;[18] the enantiomers of the ester (1) have been investigated[19] but their preparation and properties, to our knowledge, have not been reported.

Acknowledgements

We thank Dr. P. Bertin for his participation, Mr. E. Castiglione for technical assistance, and the Ministry of Public Education (Italy) for financial support.

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Received 3*rd April* 1986; *Paper* 6/667