Effect of Cyclodextrins on the Hydrolysis of Amides

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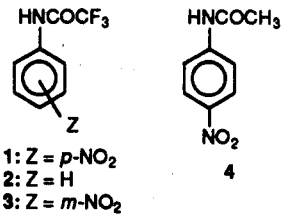
The hydrolysis of *p*-nitrotrifluoroacetanilide (1), trifluoroacetanilide (2), *m*-nitrotrifluoroacetanilide (3), and *p*-nitroacetanilide (4) was studied in the presence of cyclodextrins. The reactions of 1 are catalyzed by α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) and also by (hydroxypropyl)cyclodextrin (HPCD). The hydrolysis of 4 is catalyzed by β-CD. The reaction of 2 is inhibited by β-CD and so is the reaction of 3 by HPCD. Compounds 1 and 4 form two types of inclusion complexes with β-CD, a 1:1 and a 1:2 substrate:cyclodextrin complex. Both types of complexes react faster than the free substrate, and for 1 at pH = 7 all the catalysis is due to the reaction of the 1:2 complex since the 1:1 complex reacts at about the same rate as the free substrate. The results are explained in terms of two mechanisms: one which involves the acylation of CD by the amides and another which predominates at neutral pH where the two cyclodextrins complexing the substrate stabilize the transition state for water addition.

Cyclodextrins are cyclic oligomers of α-D-glucose which are produced by enzymatic degradation of starch. Compounds with six, seven, or eight glucose units are called α-, β-, and γ-cyclodextrins.[1] They have been shown to be good models for hydrolytic enzymes, and many studies have been done on the cyclodextrin-catalyzed hydrolysis of esters,[2-6] but much less attention has been given to the effect on the reaction of amides.[7-9] Here we report a comparative study of the effect of α- (α-CD), β- (β-CD), γ- (γ-CD) cyclodextrin and β-(hydroxypropyl)cyclodextrin (HPCD) on the hydrolysis of amides 1-4.

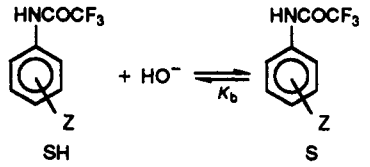


1: Z = *p*-NO_2
2: Z = H
3: Z = *m*-NO_2
4

In the hydrolysis of esters, important differences were found when the leaving group was changed to poorer leaving groups.[6] As the reaction takes place within an inclusion complex in which the phenyl group of the ester resides in the hydrophobic cavity of CD, the efficiency of the ester cleavage relative to that by hydroxide ion is generally greater for meta than for para substituents since the orientation of the former within the CD cavity has a geometry more suitable for acyl transfer.[3-5] It is surprising that in the case of *m*- and *p*-nitro-substituted trifluoacetanilides the para isomer is more strongly catalyzed than the meta isomer by α-CD.[7] Since in the reaction of amides either the addition of the nucleophile or the leaving group may be rate determining depending on the leaving group, the pH, and the buffer concentration,[10] the effect of cyclodextrin may be different. We considered it of interest to study the effect of CD on the hydrolysis of amides 1-4 under conditions where the rate-determining step of the mechanism in water solution is well established in order to find the factor which lead to catalysis or inhibition of the reactions.

Results

The hydrolysis rate of substrates 1-3 was measured as a function of pH in the range between pH 7 and 12 (Table I).[11] Since this compound ionizes according to eq 1,[12] giving



SH S (1)

the anion S which does not react further, the observed pseudo-first-order rate constant was corrected for the ionization of the substrates according to eq 2.

$$k_{corr} = k_{obs}\frac{K_b + [HO^-]}{K_b} = \frac{k_{obs}}{F} \qquad (2)$$

$$K_b = \frac{[SH][HO^-]}{[S]}$$

The corrected rate constant (k_{corr}) depends linearly on the concentration of HO^- for 1 and 3 in the range studied, whereas for 2 there is a curvilinear dependence (Figure 1). The mechanism of the hydrolysis of 1 has been studied, and it is known that above pH 9 the addition of HO^- is rate determining. The same is true for 3 at pH higher

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(7) Komiyama, M.; Bender, M. L. *J. Am. Chem. Soc.* **1977**, *99*, 8021.
(8) Mokimoto, S.; Suzuki, K.; Yashihiro, T. *J. Chem. Soc. Jpn.* **1984**, *57*, 175.
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(10) Komiyama, M.; Bender, M. L. *J. Am. Chem. Soc.* **1978**, *100*, 5977.
(11) Supplementary material.
(12) Stauffer, C. E. *J. Am. Chem. Soc.* **1972**, *94*, 7887.

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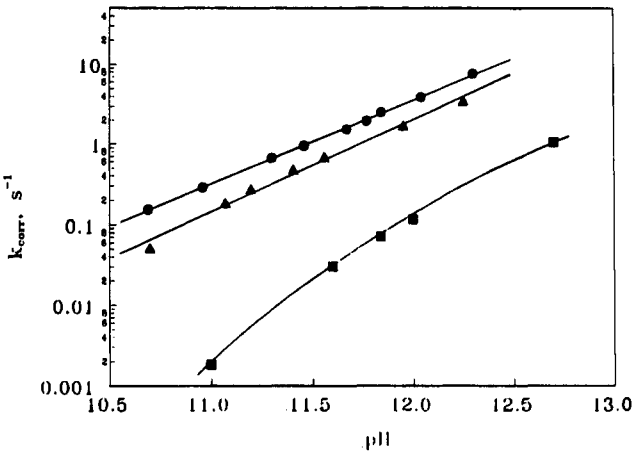

Figure 1. k_{corr} for 1, 2, and 3.

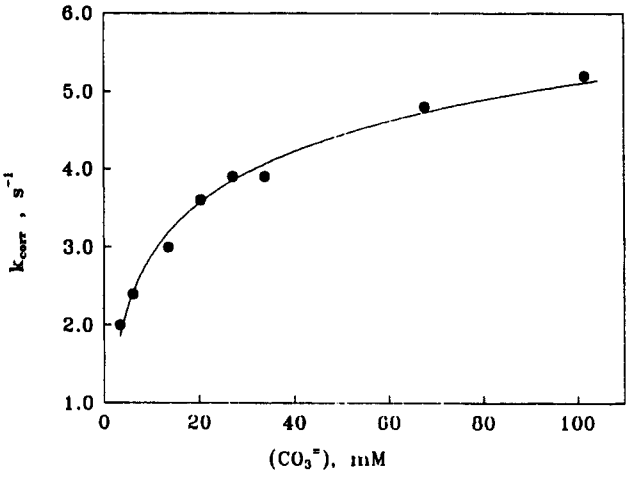

Figure 2. Buffer dependence for the hydrolysis of 3 at pH = 10.5.

than 10. On the other hand, for 2 the product-forming steps are fully rate determining at pH up to 12.[13] At pH 10, there is little buffer dependence for the hydrolysis of 1 and much stronger buffer dependence for the hydrolysis of 3 (Figure 2). These results are consistent with the fact that the leaving group expulsion rate should increase in the order *p*-nitroaniline > *m*-nitroaniline ≫ aniline.[14]

Effect of Cyclodextrins. The hydrolysis rate of substrate 1 was measured at pH 10 in the presence of several concentrations of α-, β-, and γ-CD and HPCD (Table II).[11] The effect of β-CD was studied also at pH 7, 8.5, and 12. At pH 10 the reaction was catalyzed by α- and β-CD and HPCD, but it was not affected by γ-CD or soluble starch. With α-CD and HPCD the observed rate constant showed a saturation effect, but with β-CD, a nearly linear dependence was observed at pH 10 and 12 (Figure 3 is representative). At pH 7 there is a slight upward curvature in the plot of k_{corr} vs CD and at pH 8.5 there is a small downward curvature (Figure 4).

The hydrolysis rate of substrate 2 was studied at pH 8, 10.9, and 12.7 in the presence of β-CD. At pH 8, the rate decreased as the CD concentration increased, and there is saturation effect. At pH 12 there was a small increase in rate which amounts for at most 25%.

The hydrolysis rate of substrate 3 was measured in the presence of β-CD and HPCD at pH 10. With β-CD the rate increases in an amount which is almost within experimental error. With HPCD the rate decreases showing saturation effect (Figure 5).

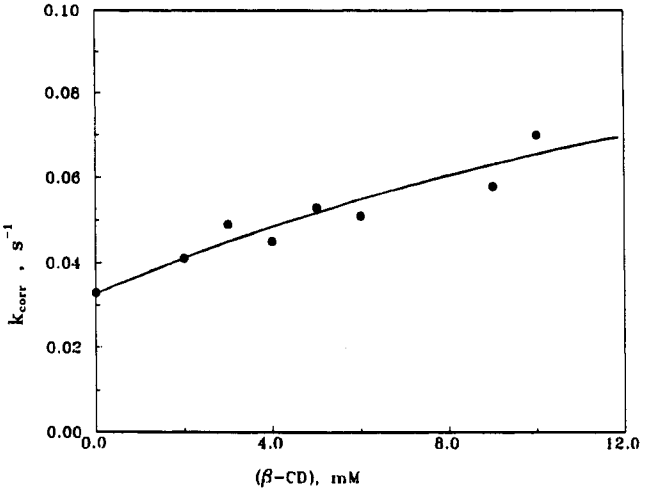

Figure 3. Effect of β-CD on the hydrolysis of 1 at pH = 10.

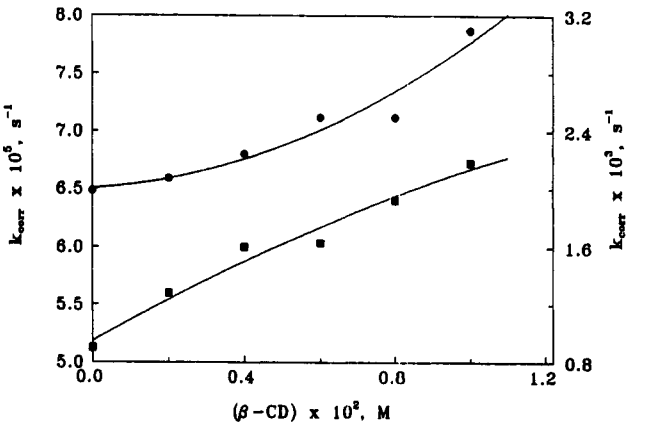

Figure 4. Effect of β-CD on the hydrolysis of 1 at pH 7 (left ordinate) and at pH = 8.5 (right ordinate). The curve was drawn using eq 11 and the data of Table III.

The effect of β-CD on the reaction of 4 was studied at pH 11.6 and 50 °C since this substrate is much less reactive than the others. This compound is less acidic than substrates 1–3 (pK_a = 13.8),[15] so the observed rate constant does not need to be corrected (F = 1). There was a linear increase in the rate when the concentration of CD was increased.

Association Equilibrium Constants. The addition of β-CD or HPCD produced a batochromic shift in the spectrum of 1. With β-CD it seems that there are two types of interactions at pH 10, since there is one isosbestic point with solutions with concentrations up to 4×10^{-1} M and then there is a different set of spectra corresponding to solutions having β-CD in the range $(4–10) \times 10^{-3}$ M (Figure 6). On the other hand, only one isosbestic point is observed with HPCD at pH 10. The spectrum of 1 and 4 at pH = 6 also changes in the presence of β-CD, but there is no isosbestic point. There is no change in the spectrum of 2 and 3 in the presence of β-CD.

For the sake of comparison, the effect of β-CD and HPCD on the spectrum of *m*-nitroaniline was determined and the equilibrium constant was calculated (see the Experimental Section). In both cases there is an isosbestic point.

(13) Mader, P. M. *J. Am. Chem. Soc.* **1965**, *87*, 3191.
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(15) Pollack, R. M.; Bender, M. L. *J. Am. Chem. Soc.* **1970**, *92*, 7190.

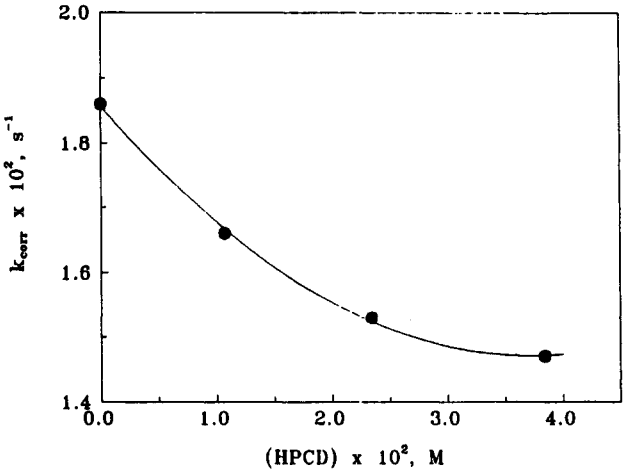


Figure 5. Effect of HPCD on the hydrolysis of 1 at pH = 10. The curve was drawn using eq 10 and the data of Table III.

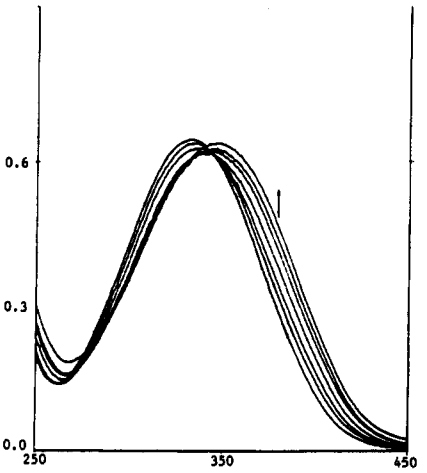
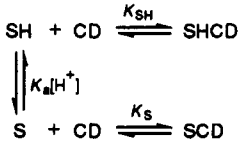
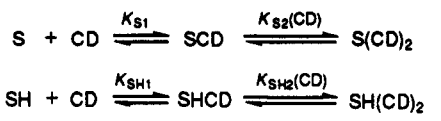


Figure 6. Effect of β-CD on the spectrum of 1 at pH = 10. $[1]_0$ = 4.2 × 10^{-5} M. [β-CD] = 0, 1, 2, 4, 6, 8, and 10 mM.

Scheme I



Scheme II



The change in the spectrum with CD indicates that there is some sort of interaction between the substrate and CD. Taking into account the fact that the substrates have a relatively low pK, we can consider Scheme I for the 1:1 interaction where SH and S represent the neutral and ionized substrates and SHCD and SCD the 1:1 complex between the substrate and the CD. At pH ≫ pK_a all the substrate is in the form of the anion; therefore only the species in the lower part of Scheme I contributes to the measured absorbance. The change in absorbance under these conditions is given by eq 3 where $K_1 = K_S$ and S_0 is the stoichiometric substrate concentration.

$$A = \frac{[(\epsilon_{SCD} - \epsilon_S)K_1[CD]][S_0]}{1 + K_1[CD]} + A_0 \qquad (3)$$

The data for substrate 1 with HPCD were adjusted to eq 3, and so K_S was determined (Table III). On the other hand, the data for 1 and β-CD at pH 10 or those at pH 6 where the predominant species is the neutral substrate SH could not be fitted to eq 3.

We then suggest that a second type of complex involving two CD units is formed (Scheme II). For this scheme the change in absorption is given by eq 4

$$A - A_0 = \frac{[(\epsilon_1 - \epsilon_0)K_1[CD] + (\epsilon_2 - \epsilon_0)K_1K_2[CD]^2][S_0]}{1 + K_1[CD] + K_1K_2[CD]^2} \qquad (4)$$

where K_1 and K_2 represent the equilibrium constant for the 1:1 and 1:2 substrate:cyclodextrin complex, respectively, with S at pH = 10 and with SH at pH = 6. A good fit of the data to eq 4 is obtained, and K_1 and K_2 are calculated by nonlinear adjustment (Table III).

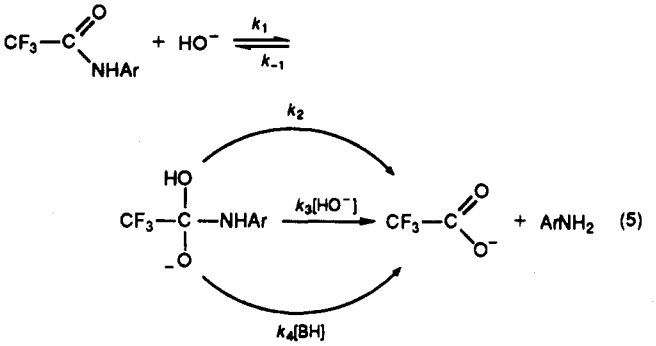
Table III. Calculated Values for the Rate and Equilibrium Constants[a]

substrate (CD)	k_0, s^{-1}	k_1, s^{-1}	k_2, s^{-1}	K_1, M^{-1}	K_2, M^{-1}	k_1/k_0	k_2/k_0
1 (α-CD)	3.4×10^{-2}	0.23 ± 0.01		50 ± 10[b]		6.7	
				59[c]			
				15[d]			
1 (β-CD)	3.35[e]	7.2 ± 0.4[e]	6.3 ± 0.5[e]	70 ± 4[f]	60 ± 13[f]	2.1 ± 0.1[e]	1.9 ± 0.2[e]
				100 ± 4[g]	16 ± 6[g]		
	3.4×10^{-2} [h]	$(11 \pm 2) \times 10^{-2}$ [h]	$(8 \pm 4) \times 10^{-2}$ [h]			3.2 ± 0.6[h]	2.3 ± 0.6[h]
	9×10^{-4} [i]	$(3.7 \pm 0.5) \times 10^{-3}$ [i]	$(2 \pm 1) \times 10^{-3}$ [i]			4.1 ± 0.6[i]	2.2 ± 0.1[i]
	6.3×10^{-5} [j]	$(6.4 \pm 0.6) \times 10^{-5}$ [j]	$(2.4 \pm 0.5) \times 10^{-4}$ [j]			1.0 ± 0.1[j]	3.8 ± 0.1[j]
1 (HPCD)[k]	3.4×10^{-2}	4.8×10^{-2} [k]		(233 ± 15)[g]		1.41[l]	
2 (β-CD)[p]	2.2×10^{-5}	$(4.6 \pm 0.8) \times 10^{-6}$		(87 ± 14)[a]		0.21 ± 0.04	
3 (HPCD)[h]	1.87×10^{-2}	1.1×10^{-2} [l]		28 ± 2[b]		0.59	
4 (β-CD)[m]	6.3×10^{-4}	$(8 \pm 1) \times 10^{-4}$	$(8 \pm 2) \times 10^{-3}$	(220 ± 4)[f]	(7 ± 2)[f]	1.3 ± 0.2	12.7 ± 0.3
p-nitrophenylacetate[n]	8.1×10^{-4}	1.1×10^{-2}		67		13.5	
m-nitrophenylacetate[o]	8.6×10^{-2}	5.3		83		62	

[a] The error limits in the measured rate constants are in all cases less than 5%. The errors for the calculated rate constants is that calculated for the program used to fit the data. [b] Value obtained from the kinetic data, corresponds to the association of S, K_{S1}. [c] Reported value determined spectrophotometrically at pH = 9.5, corresponds to K_{S1}. Bender, M. L.; Komiyama, M. *J. Am. Chem. Soc.* **1977**, *99*, 8021. [d] Spectrophotometric value determined at pH = 6 in the same reference as in note *b*, corresponds to K_{SH1}. [e] Data at pH = 12. [f] Value determined spectrophotometrically at pH = 6, corresponds K_{SH1} and K_{SH2}. [g] Value determined spectrophotometrically at pH = 10, corresponds K_{S1} and K_{S2}. [h] Data at pH = 10. [i] Data at pH = 8.5. [j] Data at pH = 7. [k] Value of the observed rate constant at the highest HPCD concentration. [l] Calculated assuming $K_{SH1} \approx K_{S1}$. [m] Data at 50 °C and pH = 11.7. [n] Data at pH = 9.88 taken from Barra, M.; de Rossi, R. H. *Can. J. Chem.* **1991**, *69*, 1124. [o] Data at pH = 11.7 from Tee, O. S.; Mazza, C.; Xian-Xian, D. *J. Org. Chem.* **1990**, *55*, 3603. [p] Data at pH = 8.

Discussion

The mechanism of the hydrolysis of amides can be described as in eq 5[10,16]



The observed rate constant for eq 5 is given by eq 6 which simplifies to eq 7 at zero buffer concentration provided that $k_2 \ll k_{-1}, k_3[HO^-]$.[12]

$$k_{corr} = \frac{k_1(k_2 + k_3[HO^-] + k_4[BH])[HO^-]}{k_{-1} + k_2 + k_3[HO^-] + k_4[BH]} \quad (6)$$

$$k_{corr} = \frac{k_1(k_2 + k_3[HO])[HO^-]}{k_{-1} + k_2 + k_3[HO^-]} \approx \frac{k_1k_3[HO^-]^2}{k_{-1} + k_3[HO^-]} \quad (7)$$

The rate constant for substrates 1 and 3 above pH 10.5 are linearly dependent on the HO^- concentration which indicates that $k_3[HO^-] \gg k_{-1}$ in this pH range and k_1 is the rate-determining step. These results are in good agreement with previous findings.[10,12,16] For substrate 3 at pH 10.5 there is buffer dependence of the observed rate constant (Figure 2) with the rate leveling off at buffer concentration above 0.15 M, indicating that under these conditions k_1 is the rate-determining step. Nonlinear adjustment of the data to eq 6 allows the calculation of k_{-1}/k_4. The value of k_1 obtained, namely 167 M^{-1} s^{-1}, is in good agreement with the value calculated from the slope of the plot of k_{corr} vs HO^-. In the case of substrate 2, no buffer dependence was observed at pH within 7 and 8 using $PO_4H_2^-/PO_4H^{2-}$ as buffer but up to pH 12 there was nonlinear dependence of the k_{corr} with HO^-, indicating that under all our experimental conditions the product-forming steps are partially rate determining although the k_4 step in eq 5 is not significant. Equation 7 can be rearranged to eq 8.

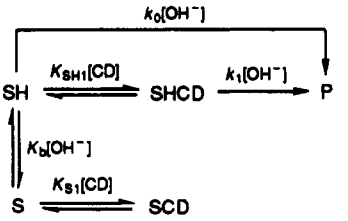
$$\frac{[HO^-]}{k_{corr}} = \frac{k_{-1}}{k_1k_3[HO^-]} + \frac{1}{k_1} \quad (8)$$

A plot (not shown) of the left-hand side of eq 8 vs $(HO^-)^{-1}$ is linear and from the intercept and slope, $k_1 = 50$ M^{-1} s^{-1} and $k_{-1}/k_3 = 2.5 \times 10^{-2}$ M are calculated.

There is spectroscopic evidence for the association of cyclodextrins with 1 and 4 but not for 2 and 3. The calculated equilibrium constants for 1 are smaller than the corresponding values for anilines. For instance, the K_{ASSOC} for 1 with β-CD is 70 M^{-1} whereas for *p*-nitroaniline is 260 M^{-1}.[17] Similar decrease is observed when the values compared are the association equilibrium constant for *p*-nitrophenylacetate (67 M^{-1})[18] and *p*-nitrophenol (130 M^{-1}).[19] This may be due to changes in the dipolar moment of the substrate, since this has been shown to be an important factor in the driving force for the association.[20]

If a similar decrease in the association occurred for substrate 3 with β-CD, still an equilibrium constant in the order of 30 M^{-1} would be expected considering that the corresponding value for *m*-nitroaniline is 60 M^{-1}. Therefore the lack of a noticeable effect on the rate can be due to the fact that they are only weakly associated with β-CD or more likely, that the reactivity of the associated substrate is about the same as that of the free substrate. The lack of effect due to γ-CD or soluble starch indicates that the observed kinetic effect is due to the formation of an inclusion complex with CD and not just to unspecific interactions. Therefore and for all the substrates, we may consider a minimum mechanism represented by Scheme III where the ionization of the substrate and the formation of 1:1 complex between the neutral (SH) and anionic (S) substrate is taken into account.

Scheme III



The observed rate constant for this mechanism, corrected for the ionization of the substrate as indicated in eq 2, is given by eq 9

$$k_{corr} = \frac{(k_1K_{SH1}[CD] + k_0)[HO^-]}{(1 - F)(1 + K_{S1}[CD]) + F(1 + K_{SH1}[CD])} \quad (9)$$

$$F = \frac{K_b}{K_b + HO^-}$$

which simplifies to eq 10 at $[HO^-] \gg K_b$ and $F \ll 1$, since K_{S1} and K_{SH1} are of the same order of magnitude (see Table III).

$$k_{corr} = \frac{(k_1K_{SH1}[CD] + k_0)[HO^-]}{1 + K_{S1}[CD]} \quad (10)$$

The data for substrate 1 catalyzed by α-CD fit to eq 10 and the values of k_1K_{SH1} and K_{S1} can be obtained by nonlinear adjustment of the data to this equation. The value obtained for K_{S1} is the same within experimental error as that reported in the literature which was determined spectrophotometrically (Table III), whereas the value of k_1/k_0 is somewhat lower than the value reported in the literature for the same substrate at pH 6 and 30 °C, namely 16.[7]

For the mechanism described in Scheme III, the dependence of the observed rate constant at pH 10 with β-CD (Figure 3) indicates that in eq 10 $K_{S1}[CD] < 1$ in the range of concentration studied; however, the spectrophotometrically determined value is 100 M^{-1}. Besides the

(16) Huffman, R. W. *J. Org. Chem.* **1980**, *45*, 2675.
(17) Barra, M.; de Rossi, R. H.; de Vargas, E. B. *J. Org. Chem.* **1987**, *52*, 5004.
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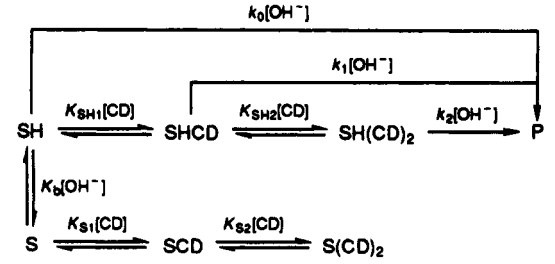
(19) Buvari, A.; Barcza, L. *J. Chem. Soc., Perkin Trans. 2* **1988**, 543.
(20) Kitagawa, M.; Hoshi, H.; Sakurai, M.; Inoue, Y.; Chujo, R. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4225.

Scheme IV



spectrophotometric data indicates that a 1:2 complex is also formed so we have to expand Scheme III to Scheme IV which takes into account the reaction of the 1:2 complex $SH(CD)_2$.

The rate constant k_{corr} for this scheme is given by eq 11 which simplifies to eq 12 when $F \ll 1$. Using the values

$$k_{corr} = ((k_0 + K_{SH1}[CD](k_1 + k_2K_{SH2}[CD]))[HO^-])/(1 + F(K_{SH1}[CD](1 + K_{SH2}[CD])) + (1 - F)(K_{S1}[CD](1 + K_{S2}[CD]))) \quad (11)$$

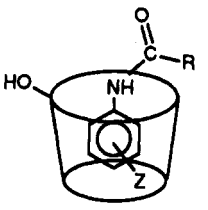
$$k_{corr} = \frac{(k_0 + K_{SH1}[CD](k_1 + k_2K_{SH2}[CD]))[HO^-]}{1 + K_{S1}[CD](1 + K_{S2}[CD])} \quad (12)$$

of the equilibrium constants determined spectrophotometrically, the values of k_1 and k_2 were estimated (Table III).

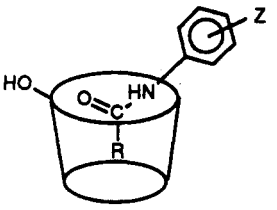
The k_1/k_0 ratio should not be pH dependent if both rate constants have the same dependence on the HO^- concentration. As it can be seen in Table III, the k_1/k_0 ratio for the reactions of 1 increases from pH 12 to pH 8.5 and then decreases at pH 7 where the reactivities of the complexed and uncomplexed substrate are about the same. On the other hand, the k_2/k_0 ratio is about the same between pH 8.5 and 12 but at pH 7 the reaction of the 1:2 complex becomes the only catalyzed pathway. The behavior of HPCD is similar to that of β-CD at the same pH, although only one type of complex is formed and the catalyzed pathway is less efficient than that for the 1:1 complex of β-CD. This is reasonable considering that HPCD have half the number of OH groups available for catalysis (see below). The k_1/k_0 ratio is perhaps coincidentally half the value for β-CD. The fact that the 1:2 complex does not seem to be formed with HPCD may be attributed to the steric restriction imposed for the hydroxypropyl groups attached to the secondary hydroxy groups of the cyclodextrin rim.

Comparing the k_1/k_0 ratio for 1 and 4 we can see that the catalyzed pathway is more favorable for substrate 1 than for substrate 4. Although the difference might be due to the fact that the reactions have been studied at different temperatures, this is not of great important considering reported data.[7] The ratio of the observed rate constant for the hydrolysis of 4 in the presence of 0.03 M of α-CD to the reaction without CD is the same at 30 and 70 °C.[7] On the other hand, for substrate 4 there is strong catalysis through the pathway which involves a 1:2 complex. This result may be due to the structure of the complexes formed. It is known that the nature of the head group of the alkyl-bearing guest has strong influence on the complexation with α- and β-CD.[21] The CF_3 and CH_3 are very different in polarity and hydrophobicity[22] so it may be that compound 1 resides deeper in the cavity of CD than 4 and in a position more appropriate for reaction. Consistent with this explanation is the fact that the 1:2 complex is more effective for reaction in the case of substrate 4.

The reaction of 3 is inhibited by HPCD and by β-CD. The results observed for 1 and 3 contrast with those found for esters of *p*-nitro- and *m*-nitrophenol. The reactions of *m*-nitro-substituted alkanoates are more strongly catalyzed than those of the *p*-nitro-substituted compounds although these differences decrease when the alkyl chain becomes longer. These results have been attributed to a change in the structure of the transition state for the catalyzed pathway from aryl to alkyl inclusion.[5b] The amides 1–4 could also form two types of complexes as shown in 5 and 6.



5



6

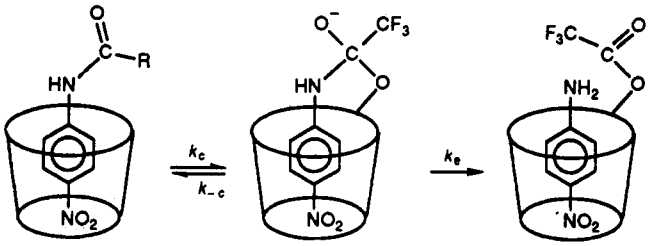
Considering that *p*-nitroaniline[23] and *m*-nitroaniline form inclusion complexes with β-CD, then complexes of the type 5 should also be formed. It is unlikely that the presence of the CF_3CO group decreased so much the affinity of the aromatic ring for the cavity so that 5 would not be formed at all. Then complex 5 is probably formed although it is not necessarily the reacting complex.[24] On the other hand, the possibility of formation of complex 6 cannot be discarded mainly considering the hydrophobicity of the CF_3 group and data regarding reactions with other amides of longer alkyl chain.[25] However, it does not appear reasonable to explain the different behavior of amides when compared with esters just on the basis of the formation of complexes 5 or 6. Besides, the differences in the behavior of 1 and 3 cannot be attributed to changes in rate-determining steps with each substrate, because under the conditions where the reactions with HPCD were studied, the formation of the tetrahedral intermediate is rate determining for both substrates (see Results).

The mechanism for the catalysis of the hydrolysis of 1 by α-CD was explained by the mechanism described in Scheme V. If the reactions of amides 1–4 in the presence of cyclodextrins also occur by a mechanism similar to that described in Scheme V, the value of k_1 is given by eq 14 where K_h is the ionization constant of CD divided by K_w.

$$k_1 = f\frac{k_c k_e}{k_{-c} + k_e} \qquad f = \frac{K_h[HO^-]}{K_h[HO^-] + 1} \quad (14)$$

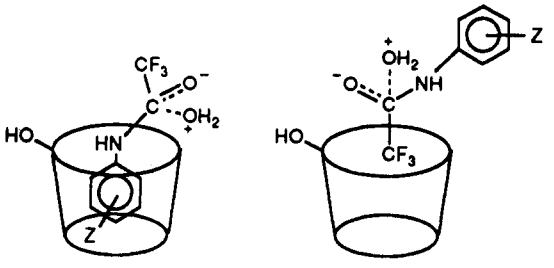
(21) Tee, O. S.; Xian-Xian, Du. *J. Am. Chem. Soc.* **1992**, *114*, 620.
(22) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525.
(23) The X-ray analysis of the β-CD inclusion complex of *p*-nitroaniline shows that the guest is wholy included in the cavity. Harding, M. M.; McLennan, J. M.; Paton, R. M. *Nature* **1978**, *274*, 621.
(24) Tee, O. S. *Carbohydr. Res.* **1989**, *192*, 181.
(25) de Rossi, R. H.; Granados, A. 1ª Conferencia Latinoamericana.8ªConferencia de Físico química Orgánica, Florianopolis, Brasil, 1991, 69.

Scheme V



If k_c and k_e are pH independent,[26] k_1 should increase linearly with the hydroxide ion at low hydroxide concentration ($K_h \ll 1$) but then level off when $K_h[HO^-] > 1$; consequently the k_1/k_0 ratio should decrease as the pH increases as observed. The inhibition in the reactions of **2** and **3** is probably due to a strong contribution of k_e (eq 14) to the value of k_1, since the leaving groups aniline and *m*-nitroaniline are poorer than *p*-nitroaniline. The fact that there is stronger catalysis in the reaction of esters than that of amides may also be explained along the same lines since *m*- and *p*-nitrophenol should be better leaving groups than *p*- and *m*-nitroaniline.[28]

For the neutral solution, the main reaction may not be that shown in eq 5 but just the reaction of water as nucleophile[10,11] for the reaction of the free and included substrate. In this case the CD does not react as nucleophile as it does in Scheme V, but it serves as a reaction vessel just like it does in other reactions.[29] The lack of catalysis indicates that there is no extra stabilization in the transition states **7a** or **7b**.

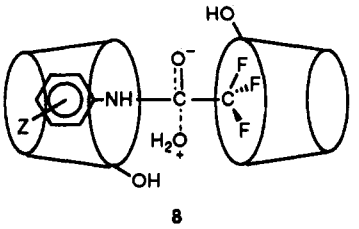


7a **7b**

On the other hand, the catalysis by CD through the formation of the 1:2 complex may be explained considering that the transition state requires additional solvation of the transition state over the ground state of four water molecules.[30] This additional solvation may not be required for the transition state for the substrate in the 1:2 complex **8** because the stabilization is provided by the hydroxyl groups at the rim of CD.

Conclusions

The reactivity for the hydrolysis of *p*-nitrotrifluoroacetanilide is higher than that of *p*-nitrophenylacetate, but the catalysis by cyclodextrins is weaker for the former than for the latter. Besides, there is inhibition by cyclodextrins in the reactions of amides where the leaving group is aniline or *m*-nitroaniline. Since cyclodextrins are acylated by amides, the catalysis or inhibition reflect the unfavorable partition of the tetrahedral intermediate toward products as the leaving amine became a poorer leaving group. In the case of substrates **1** and **4** a second reaction pathway, which involves the formation of 1:2 (substrate:cyclodextrin) complex, was identified. Although the binding of the second cyclodextrin is quite weak, particularly for **4**, it provides a very efficient pathway and for the reactions of **1** at pH 7 it is the only one that leads to acceleration of the reaction. These results are new examples of complexes involving two cyclodextrin units which are responsible for catalysis of reactions.



8

Experimental Section

Aqueous solutions were made up from water purified in a Millipore apparatus. Acetonitrile Merck HPLC grade was used as received.

α-Cyclodextrin (Aldrich), β-cyclodextrin, γ-cyclodextrin (FDS publications, Hungary), HPCD (Roquette), and soluble starch (Sigma) were used as received, but the purity was periodically checked by UV spectroscopy. HPCD has an average of one hydroxypropyl group per glucose unit.

The substrates **1–3** were prepared by the reaction of the appropriate aniline with trifluoroacetic anhydride in acetonitrile at room temperature. The mixture was stirred for 2 h in the dark, and the product was precipitated by addition of cold water. The solid was filtered out, washed with aqueous bicarbonate and water, and dried under vacuum. **1**, mp 141–142 °C (lit.[9] mp 147 °C); **2**, mp 79–80 °C (lit.[9] mp 86.5 °C); and **3**, mp 82–83 °C (lit.[9] mp 88 °C). **4** was prepared from *p*-nitroaniline and acetic anhydride, mp 213–214 °C (lit. mp 216 °C).[31] The purity of all the substrates was checked by thin-layer chromatography, and the spectrum of a completely hydrolyzed solution was compared with a mock solution of the corresponding aniline.

Kinetic Procedures. The reactions were initiated by adding the substrate dissolved in acetonitrile to a solution containing all the other constituents. The total acetonitrile concentration was less than 1%.

All reactions were run at 25 °C and at constant ionic strength (0.5 M) using NaCl as compensating electrolyte. The observed rate constants were determined by following the appearance of the *p*-nitroaniline, for **1** and **4**, at 380 nm. The reactions of **2** and **3** were followed by measuring the disappearance of the substrates at 253 and 263 nm, respectively. The change in absorbance during a kinetic run was recorded on a Shimadzu 260 recording spectrophotometer with a thermostated cell compartment. The reactions were followed up to 80–90% conversion. We did not detect any deviation from pseudo-first-order behavior in these conditions.

The pH of the solutions containing varying concentrations of cyclodextrin was adjusted by adding a drop of dilute acid or base. At pH 7 the buffer was $PO_4H_2^-$, and at pH 8 and 10 the buffer was CO_3^{2-}.

The p*K* values of **2** and **3** were determined by measuring the change in absorbance with pH at 240 nm. The values are 9.5 and 8.2, respectively.

(26) The aminolysis of esters in general base and general acid catalyzed (see ref 27); therefore it might be that k_e and/or k_c are pH or buffer dependent, but from our data we cannot determine this.

(27) (a) Kovack, I. M.; Belz, M.; Larson, M.; Rousy, S.; Schowen, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 7360. (b) Cox, M.; Jencks, W. P. *J. Am. Chem. Soc.* **1981**, *103*, 580.

(28) (a) DeTar, D. F. *J. Am. Chem. Soc.* **1982**, *105*, 7205. (b) Gravitz, N.; Jenks, W. P. *J. Am. Chem. Soc.* **1982**, *96*, 507.

(29) de Rossi, R. H.; Barra, M.; de Vargas, E. B. *J. Org. Chem.* **1986**, *51*, 2157.

(30) Shaskus, J.; Haake, J. *J. Org. Chem.* **1983**, *48*, 2036.

(31) *Handbook of Chemistry and Physics*, 72th ed.; CRC Press: Boca Raton, FL, 1991–1992; pp 3–17.

The association equilibrium constant for 1 with β-CD, for 3 with HPCD, and for *m*-nitroaniline with β-CD and HPCD were determined from the difference spectrum of solutions containing the appropriate concentration of CD and the substrate. The values for 1 and 3 are reported in Table III and for *m*-nitroaniline are 72 ● 4 M^{-1} (HPCD) and 60 ± 4 M^{-1} (β-CD).

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Supplementary Material Available: Table I containing the observed rate constants for the reactions of 1–3 as a function of pH and Table II containing the observed rate constants for the hydrolysis of 1–4 in the presence of cyclodextrins (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.