Cyclodextrins as Enzyme Models in Nitrosation and in Acid−Base-Catalyzed Reactions of Alkyl Nitrites

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Abstract: The widely studied cyclodextrin-mediated reactions of esters but not those of alkyl nitrites, together with the marked differences between the chemistry of esters and alkyl nitrites, prompted us to investigate the influence of β-cyclodextrin (β-CD) on the reactions of alkyl nitrites. Due to the particular characteristics of alkyl nitrite reactions, the system β-cyclodextrin−alkyl nitrites allows us to explore cyclodextrin's behavior under several experimental conditions, contrary to the case of esters. Therefore, general acid−base-catalyzed hydrolysis and nitrosation of amines by alkyl nitrites are studied. Alkyl nitrites of a particular structure have been chosen to clearly evidence the mimicry of enzyme catalysis by β-CD. Addition of β-CD strongly inhibits the acid hydrolysis of alkyl nitrites (a very fast reaction in water), except in the case of ethoxyethyl nitrite, where no effect is detected. The retardation of the reaction is attributed to a separation of the reagents: β-CD and alkyl nitrites form host−guest 1:1 inclusion complexes, but simple cations, such as H_3O^+ in the present case, did not prove to include into the β-CD cavity. In fact, at constant β-CD concentrations, addition of dodecyltrimethylammonium bromide monomers (DTABr), which strongly compete with alkyl nitrites for the hydrophobic β-CD cavity and, thus, expel the alkyl nitrites, catalyzes the reaction. On the contrary, in alkaline medium, when a secondary hydroxy group of β-CD is ionized, addition of β-CD to the reaction medium strongly catalyzes the basic hydrolysis of alkyl nitrites (an extremely slow reaction in water). The degree of catalysis depends on the alkyl nitrite structure, varying from a factor higher than 100 in the case of 3-phenyl-1-propyl nitrite, to 0 (no reaction is observed) in the case of 2-phenyl-2-propyl nitrite. The effective molarities calculated for the catalysis evidence a base-catalyzed mechanism for the reaction. The strong catalysis observed with 1-phenyl-1-propyl nitrite upon the addition of DTABr is indicative of an example of allosteric activation. Finally, the nitrosation of pyrrolidine, piperidine, and cyclohexylamine by ethoxyethyl nitrite is slightly catalyzed by the presence of β-cyclodextrin. The degree of the observed catalysis depends on both the amine concentration and the structure.

Introduction

Enzymes promote very fast reactions by bringing the reacting groups together under the special conditions of the enzyme−substrate complex, but it is clear that a major part of the very large rate enhancements observed are simply due to the way the functional groups involved are brought together.[1] Enzyme mimics catalyze reactions by mechanisms that are demonstrably enzyme-like. General conclusions from work on models are that efficient catalysis involve an initial binding interaction between the substrate and the catalyst.[2,3] Consequently, the easiest way to improve binding, and for that the catalytic effect, is to modify the substrate.

Cyclodextrins (CDs) have proved to be the most enduringly popular enzyme mimics, catalyzing various reactions.[4] The cleavage of esters in basic medium, mediated by CDs, is the system most studied.[5,6] In some cases, such as that with *meta*-substituted phenyl acetates, cleavage is strongly accelerated; on the contrary, the reaction of *para*-substituted isomers is accelerated only modestly. The observed effects depend on the chain length of the ester, the CD, and the position of the substituent on the phenyl group. The mechanism typically involves nucleophilic attack by the ionized secondary hydroxy groups of CD, but in other cases, general base catalysis competes with nucleophile catalysis.

The present work reports the results obtained in the kinetic study of the acidic and basic hydrolyses of alkyl nitrites (R−O−N=O) (the case of general acid−base-catalyzed reactions) and of the nitrosation reaction of amines by RONO (nucleophilic reactions) carried out in the presence of β-cyclodextrin, with three main objectives in mind: first, to see the β-CD effects under conditions of neutral and ionized β-CD in two typical mechanistic reactions; second, to understand how the substrate structure conditions the results; and third, to examine the RONO−CD interactions. Therefore, we studied the acid hydrolysis of ethoxyethyl (EEN), 1-phenyl-1-propyl (1PhPN), 2-phenyl-2-propyl (2PhPN), and 3-phenyl-1-propyl (3PhPN) nitrites in acetic acid−acetate buffer; the basic hydrolyses of those alkyl nitrites and also of *n*-butyl (BuN) and *n*-pentyl (PeN) nitrites in alkaline medium; and the nitrosation of pyrrolidine, piperidine, and *N*-methylcyclohexylamine in alkaline conditions.

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Scheme 1

Acid catalyzed hydrolysis   Basic catalyzed hydrolysis
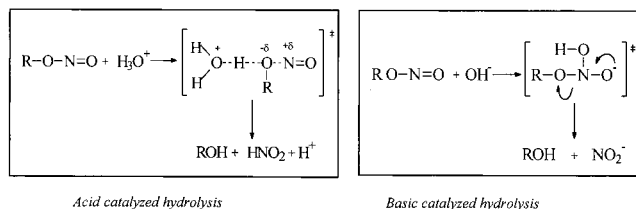
The chemistry of esters and alkyl nitrites shows marked differences. The acid-catalyzed hydrolysis of alkyl nitrites is quite fast,[7] 500-fold faster than the reaction of esters;[8] by contrast, the basic hydrolysis of esters is a very fast reaction,[9] whereas the basic hydrolysis of alkyl nitrites is an extremely slow process;[9] finally, the reaction of alkyl nitrites with amines is relatively fast.[10–12] The fact that nitrogen is more electronegative than carbon and has a lone pair probably explains the significant differences between the chemistry of alkyl nitrites and that of carboxylic esters: carboxyl chemistry is dominated by the formation of tetrahedral intermediates, whereas it is assumed that alkyl nitrites transfer the N=O group intact. That is, if the acid- and base-catalyzed hydrolyses of alkyl nitrites take place through a concerted mechanism (Scheme 1), in the case of esters the reactions proceed via an addition–elimination pathway, either A-1 or A-2 in the acid-catalyzed hydrolysis, or through the well-known $B_{AC}2$ mechanism in the nucleophilic attack by OH^-.[13]

Finally, it is convenient to indicate that, apart from the mere mechanistic interest of the present study, a biochemical importance must be indubitably attributed to the present results. On one hand, CDs are human foods, either in the form of orally administered pharmaceuticals or as food additives, in both cases being present as free cyclodextrins or as their inclusion complexes, containing a drug, flavorer, or other guest substance.[14] On the other hand, alkyl nitrites have pharmaceutical applications in the control of blood pressure due to their well-known properties as vasodilators. Alkyl nitrites cause muscle relaxation by releasing NO, through their reaction with thiols to afford unstable nitrosothiols that release the vasodilatory NO.[15–17] Alkyl nitrites typically lose their NO group by transferring it to amines, carbanions, thiols, etc., and as we describe in this work, alkyl nitrites may generate NO in

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quantitative yield in basic media by reaction with the anion of β-cyclodextrin.

Experimental Section

Alkyl nitrites were synthesized by treating the corresponding alcohol with sodium nitrite in aqueous sulfuric acid,[18] purified by fractional distillation, and stored at low temperature over 3-Å molecular sieves to prevent their hydrolysis. β-CD was purchased from Aldrich Co. and was used without further purification. All other reagents were supplied by Merck and were used as received. Sodium hydroxide was standardized against primary standard potassium acid phthalate. All solutions were prepared with doubly distilled water obtained from a permanganate solution.

Kinetic experiments were monitored by using a Kontron-Uvikon (model 941) UV–vis double-beam spectrophotometer, provided with a multiple-cell-carrier thermostated by circulating water. In either the acid or basic hydrolysis, the consumption of alkyl nitrites was followed by recording the decreasing absorbance in the 240–250-nm region. The kinetics of the nitrosation reaction of amines were studied by recording the increase in absorbance due to the formation of *N*-nitrosoamine. All the experiments were performed at 25 °C, unless otherwise indicated.

Stock solutions of the alkyl nitrites were prepared in dioxane. Reactions were initiated with the addition of 20 μL of a solution of alkyl nitrite in dioxane to the rest of the reaction mixture. The percentage of dioxane in the final reaction mixture was less than 1 vol %. The concentration of alkyl nitrite used was $(1–4) \times 10^{-4}$ M. Kinetic experiments were carried out under pseudo-first-order conditions, with the acid (or base) concentration at least 50 times greater than of the alkyl nitrite. In each case, the integrated method was followed, fitting the experimental absorbance–time data to the first-order integrated equation and obtaining satisfactory correlation coefficients (>0.999) and residuals. In what follows, k_o denotes the observed pseudo-first-order rate constant, whose value was usually reproducible to within 2%.

Results and Discussion

Acid Hydrolysis. Alkyl nitrites (RONO) hydrolyze in acid medium to yield the corresponding alcohol and nitrous acid. The reaction is general acid catalyzed; the protonation of the alcoholic-O atom, being simultaneous with the breaking of the O–N bond in a concerted mechanism, is the rate-limiting step of the reaction. The catalytic constant for the hydrogen ion ($k_H \approx 500–1000$ M^{-1} s^{-1}) catalysis is much more important than the catalytic constant for the undissociated form of a weak acid (HA),[7] e.g., acetic acid, $k_{HA} < 0.20$ M^{-1} s^{-1}.

The acid hydrolyses of EEN, 1PhPN, 2PhPN, and 3PhPN were studied in aqueous acetic acid–acetate buffer of pH 4.89 in the absence and presence of β-cyclodextrin. Figure 1 shows typical results of the variation of k_o as a function of total buffer concentration for the cases of 2PhP and 3PhP nitrites. As expected, the rate constant increases moderately with [buffer], describing a straight line, in accordance with eq 1, where K_a represents the acidity constant of acetic acid (pK_a 4.76).[19] The

$$k_o = k_H[H^+] + \frac{k_{HA}[H^+]}{K_a + [H^+]}[\text{buffer}] \qquad (1)$$

experimental data also show a strong inhibition of the reaction by the presence of β-CD. Least-squares fitting of the data to eq 1 gave the results reported in Table 1; we can note that the degree of catalysis depends on the structure of RONO.

Subsequently, we studied the influence of [β-CD] at a constant buffer concentration (0.02–0.04 M), i.e., in conditions

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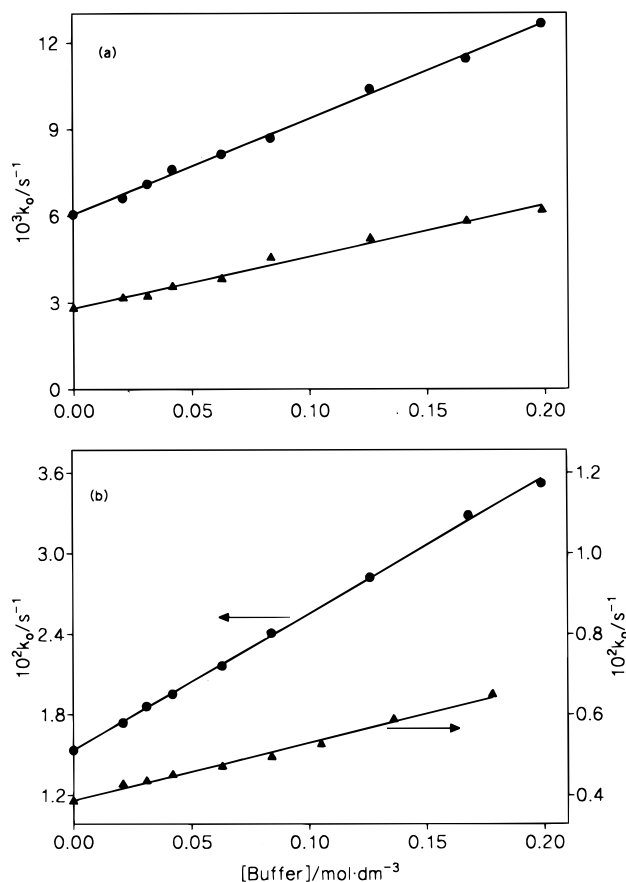
Figure 1. Influence of the concentration of acetic acid−acetate buffer of pH 4.89 in the acid hydrolysis of (a) 3-phenyl-1-propyl nitrite (●) in the absence of β-CD and (▲) in the presence of 4.4 mM of β-CD, and of (b) 2-phenyl-2-propyl nitrite (●) in the absence of β-CD and (▲) in the presence of 5.5 mM of β-CD.

Table 1. Intercept and Slope Values of the Linear Plots of k_o vs [buffer]$_t$ Obtained in the Acid Hydrolysis of 3-Phenyl-1-propyl and 2-Phenyl-2-propyl Nitrites Performed at pH 4.89 (Acetic Acid−Acetate Buffer) in the Absence and Presence of β-Cyclodextrin at 25 °C

RONO	[β-CD]/M	Intercept/s^{-1}	slope/M^{-1}s^{-1}	k_H/M^{-1}s^{-1}	k_{HA}/M^{-1}s^{-1}
⬡−(CH₂)₃−ONO	0.0	$(6.05\pm0.07)\times10^{-3}$	$(3.30\pm0.06)\times10^{-2}$	469	0.078
⬡−(CH₂)₃−ONO	4.4×10^{-3}	$(2.80\pm0.08)\times10^{-3}$	$(1.79\pm0.08)\times10^{-2}$	217[a]	0.042[b]
⬡−C(CH₃)₂−ONO	0.0	$(15.3\pm0.1)\times10^{-3}$	$(10.2\pm0.1)\times10^{-2}$	1163	0.243
⬡−C(CH₃)₂−ONO	5.5×10^{-3}	$(3.87\pm0.05)\times10^{-3}$	$(1.43\pm0.05)\times10^{-2}$	300[c]	0.034[d]

[a] Value of $(k_H + k_H{}^c K_c{}^N[\beta\text{-CD}])/(1 + K_c{}^N[\beta\text{-CD}])$. [b] Value of $(k_{HA} + k_{HA}{}^c K_c{}^N[\beta\text{-CD}])/(1 + K_c{}^N[\beta\text{-CD}])$. [c] Value of $k_H/(1 + K_c{}^N[\beta\text{-CD}])$. [d] Value of $k_{HA}/(1 + K_c{}^N[\beta\text{-CD}])$.

where the major part of the hydrolysis reaction proceeds via specific catalysis (or H$^+$ ion). In the case of 3PhPN, the kinetic study was performed at six temperatures ranging between 10 and 37 °C. Representative results are displayed in Figure 2. As can be seen, the increase in [β-CD] has nearly no effect on the reaction of EEN, whereas the observed rate constant for the cases of 1PhP and 2PhP nitrites strongly decreases as [β-CD] increases, dropping to saturation kinetics, and the reciprocal plot of k_o (see Figure 2b) varies proportionally with [β-CD]; finally, the observed rate constant for the hydrolysis of 3PhPN also decreases with [β-CD] increase, but $1/k_o$ values do not vary linearly with β-CD concentration.

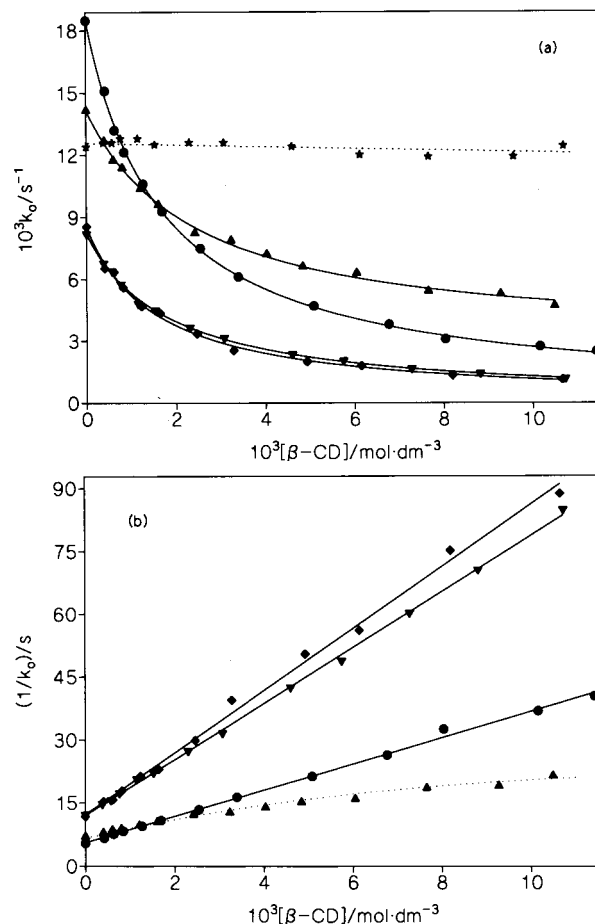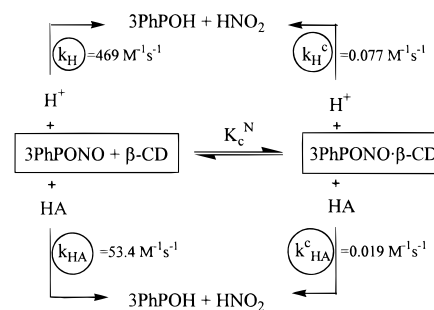**Figure 2.** Influence of [β-CD] in the acid hydrolysis (acetic acid−acetate buffer of pH 4.89) of (a) 2-phenyl-2-propyl nitrite (●); 3-phenyl-1-propyl nitrite at 31 °C (▲); ethoxyethyl nitrite (★), and 1-phenyl-1-propyl nitrite at (▼) [buffer]$_t$ = 20 mM and (◆) [buffer]$_t$ = 40 mM; solid lines fit to eq 2. (b) Reciprocal plot of k_o ($1/k_o$) against [β-CD]; solid lines fit the linear regression.

Scheme 2

The kinetic results obtained in the presence of CDs are often interpreted on the basis of the similarity between the special CD behavior and enzyme catalysis. Assuming the formation of a 1:1 inclusion complex between CD and RONO, from Scheme 2, put forward for the case of 3PhP nitrite, one easily arrives at eq 2, which relates the variation of k_o with the host concentration ([β-CD]).

$$k_o = \frac{(k_H[H^+] + k_{HA}[HA]) + (k_H{}^c[H^+] + k_{HA}{}^c[HA])\, K_c{}^N[\beta\text{-CD}]}{1 + K_c{}^N[\beta\text{-CD}]}$$

(2)

Table 2. Experimental Conditions, Rate Constants (k_o^w, k_o^c), and Stability Constants (K_s^N) of the Complex RONO·CD, Obtained in the Kinetic Study of the Influence of β-Cyclodextrin Concentration in the Acid Hydrolysis of Alkyl Nitrites in Acetic Acid−Acetate Buffer of pH 4.89 (Experimental Kinetic Results Were Fitted to Eq 2)

RONO	t/°C	[buffer]/M	effect	$10^3 k_o^w$/s^{-1}	$10^3 k_o^c$/s	K_c^N/M^{-1}	
EEN	25	0.020	no change	12.5			
1PhPN	25	0.020	inhibition	8.21	ncr	543 ± 13	556[a]
1PhPN	25	0.040	inhibition	8.53	ncr	643 ± 21	606[a]
2PhPN	25	0.030	inhibition	18.5	ncr	589 ± 11	562[a]
3PhPN	10.4	0.040	inhibition	1.96	0.135 ± 0.03	401 ± 20	
3PhPN	15.0	0.040	inhibition	2.80	0.36 ± 0.03	358 ± 74	
3PhPN	20.0	0.040	inhibition	4.91	0.59 ± 0.08	343 ± 17	
3PhPN	25.0	0.040	inhibition	7.58	1.15 ± 0.09	354.5 ± 14	
3PhPN	31.0	0.040	inhibition	14.2	2.75 ± 0.20	407 ± 19	
3PhPN	37.1	0.040	inhibition	23.4	5.25 ± 0.18	400 ± 13	

$$\ln k_o^w = A - B(1/T), B = -(8.1 \pm 0.2) \times 10^3 \text{ K}^{-1}; \ln k_o^c = A - B(1/T), B = -(11.9 \pm 0.2) \times 10^3 \text{ K}^{-1}$$

[a] From the linear regression analysis of $1/k_o$ vs [β-CD]. EEN, ethoxyethyl nitrite; 1PhPN, 1-phenyl-1-propylnitrite; 2PhPN, 2-phenyl-2-propyl nitrite; 3PhPN, 3-phenyl-1-propyl nitrite. ncr means "no complex reaction".

The shape of k_o vs [β-CD] profiles depends on the structure of the alkyl nitrite and can be explained by considering the formation of a nonreactive or reactive complex, which is a consequence of the possible conformation adopted by the host−guest complex. Nonlinear regression analysis of the experimental data k_o − [β-CD] to eq 2 yielded the results collected in Table 2. The following observations can be drawn: either EEN does not form an inclusion complex with β-CD, or the reaction rate of the complex is the same as that with the uncomplexed EEN, since no effect of β-CD is observed; the branched alkyl nitrites, 2PhP and 1PhP, form a nonreactive complex with β-CD, and the complex shows the same stability in both cases; and finally, 3PhPN forms a reactive 1:1 complex with β-CD, but the complex is less stable than the previous alkyl nitrites (compare values of K_c^N).

The noninfluence of β-CD on the acid hydrolysis of EEN might be understood on the basis of a possible interaction of EEN with β-CD, forming hydrogen−bonds between the ether-O atom of EEN and the secondary hydroxy groups of β-CD. This geometry of the complex will leave the −ONO group quite outside the β-CD cavity; i.e. the nucleophilic center toward H$^+$ (alcoholic-O atom) is immersed in aqueous medium, and therefore no changes in the reactivity are expected.

The results obtained from the study of the influence of temperature on the acid-catalyzed hydrolysis of 3-phenyl-1-propyl nitrite at an acetic acid−acetate buffer concentration of 0.040 M and pH 4.89 indicate that the inclusion equilibrium constant K_c^N remains practically unchanged with varying temperature. This fact means that the position of equilibrium is determined mainly by the entropy of the inclusion process. Bearing in mind the structures of both 2PhP and 1PhP nitrites, only hydrophobic interactions with the β-CD interior could arise; thus, steric effects hinder the approach of both the hydroxy β-CD groups and the −O−NO group of the alkyl nitrite in forming hydrogen bonds.

The Arrhenius plot corresponding to the rate constants k_o^w ($=k_H[H^+]$) and k_o^c ($=k_H^c[H^+]$), for the case of the acid hydrolysis of 3PhP nitrite, describes good straight lines in both cases whose slopes yield activation energy values of 68.9 and 98.5 kJ/mol for the reaction through the uncomplexed RONO and for the reaction of the complex RONO·β-CD, respectively. Both figures are in agreement with the characteristics of the reaction and also point to the formation of a complex that is less reactive. This lower reactivity, or nonreactivity, of the complex could be attributed to the restricted access of the reagent H$_3$O$^+$ to the CD cavity. In fact, whereas the binding of anions of many types by CDs has been observed, and it can be quite strong,[20−22] the binding of cations has rarely been

observed, and only for large organic dyes,[23] long-chain surfactants,[24] and metal ions with organic ligands.[25] In contrast, the binding of simple cations appears to be relatively unfavorable; for example, piperidinium ion binds only weakly and much less than neutral piperidine.[26] Therefore, we might conclude that the main effect of the presence of β-CD in the acid hydrolysis of alkyl nitrites is a separation of the reagents.

Further arguments corroborating this point can be acquired from the analysis of the results obtained in the study of the influence of dodecyltrimethylammonium bromide (DTABr). Figure 3 shows the variation of k_o as a function of [DTABr] for the representative cases of 3PhP and 1PhP nitrites studied at acetic acid−acetate buffer concentration of 0.040 M and pH 4.89 and in the presence of a fixed amount of β-CD.

Addition of DTABr to the reaction medium at concentrations below the *critical micelle concentration* (cmc^o = 1.2 × 10^{-3} M determined in water at [HCl] = 0.014 M by following the method of benzoylacetone solubilization[27]) causes an increase of the reaction rate constant. The k_o vs [DTABr] profile describes a sigmoidal curve, typical of an effect modifying an equilibrium process. In this sense, in the absence of β-CD, the addition of DTABr at concentrations below the cmc^o has no effect on the reaction,[28] but in the presence of β-CD, due to the fact that the binding constant of DTABr to β-CD (K_s = 3000 M^{-1})[29] is higher than that of alkyl nitrites (see Table 2), an efficient competition for the CD cavity between both substrates emerges. Then, an increase in [DTABr] increases the [RONO] in water, i.e., not forming inclusion complexes, and the reaction rate consequently increases until reaching again the value of k_o

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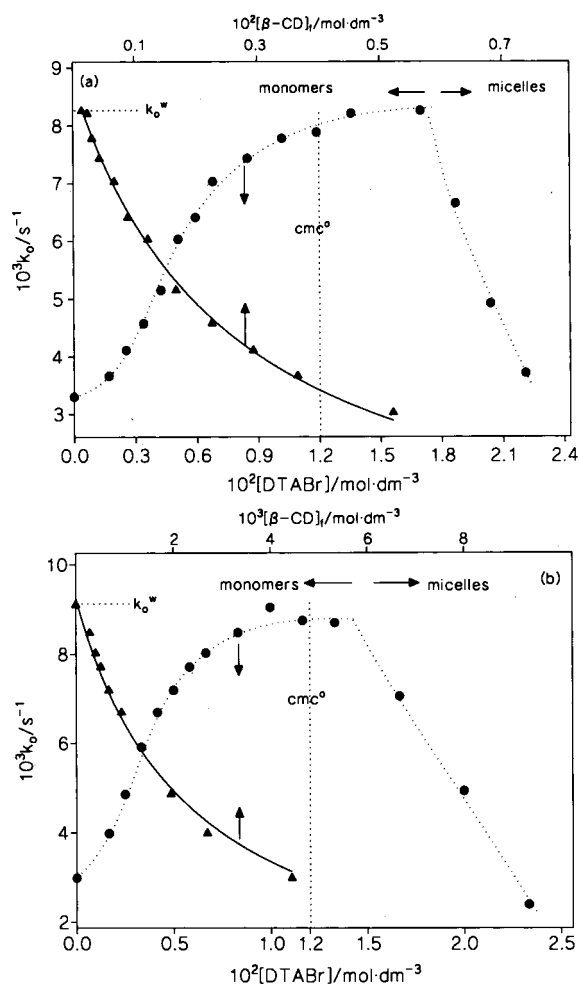
Figure 3. (●) Influence of DTABr concentration in the acid hydrolysis of (a) 3-phenyl-1-propyl nitrite at [β-CD] = 5.2 mM and (b) 1-phenyl-1-propyl nitrite at [β-CD] = 3.7 mM; acetic acid–acetate buffer of pH 4.89 and at 0.040 M. (▲) Plot of the data against free β-CD concentration, calculated according to eq 3 by assuming $K_s = 3000$ M^{-1}; solid lines fit eq 2; for parameters, see text.

measured in the absence of cyclodextrin, i.e., when all the complexed RONO was pushed out of the CD cavity by the surfactant monomers.

In the same figure, a plot of k_o against free CD concentration is also displayed. To calculate [β-CD]$_f$ in line with treatments previously described,[21,30] free β-CD concentration was determined at each surfactant concentration by solving eq 3. Fitting

$$[\beta\text{-CD}]^2 + [\beta\text{-CD}]\left(\frac{1}{K_s} + [\text{surfactant}]_t - [\beta\text{-CD}]_t\right) - \frac{[\beta\text{-CD}]_t}{K_s} = 0 \quad (3)$$

the data thus obtained to eq 2, one determines $k_o^w = (8.78 \pm 0.08) \times 10^{-3}$ s^{-1} and $K_c^N = 392 \pm 13$ M^{-1} for the case of 3-phenyl,1-propyl nitrite, and $k_o^w = (9.1 \pm 0.3) \times 10^{-3}$ s^{-1} and $K_c^N = 515 \pm 22$ M^{-1} in the acid hydrolysis of 1-phenyl-1-propyl nitrite. These values compare quite well with the data in Table 2. (At the low β-CD concentration used in these experiments, we did not consider the reaction through the complex in the fitting process in any case; i.e., we assumed that $k_o^c = k_H^c[H^+] + k_{HA}^c[HA]$ was negligible.)

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At [DTABr] > cmc°, a value which increases slightly in the presence of β-CD, as one can realize from the results in Figure 3, micelles of DTABr are also formed. The presence of cationic micelles strongly inhibits the acid hydrolysis of alkyl nitrites: alkyl nitrites solubilize into micelles, but H$^+$ is excluded from the micellar interface (where the reaction takes place) due to electrostatic repulsion, and consequently a separation of the reagents occurs.[28]

Basic Hydrolysis. The basic hydrolysis of alkyl nitrites is a very slow process, contrary to what occurs with the corresponding esters. The nucleophilic catalysis of OH$^-$ in the hydrolysis of carboxylic esters is remarkably greater than that for alkyl nitrites; but other marked differences also appear, such as the absence of concurrent oxygen exchange between the nitroso oxygen of the alkyl nitrite and OH$^-$ during the reaction, or the importance of steric requirements in the ester hydrolysis, whereas polar effects of substituents on the leaving alkoxide group are greater for alkyl nitrites than for esters. These differences are often rationalized by saying that carboxyl chemistry is dominated by the formation of tetrahedral intermediates,[31] whereas in alkyl nitrite reactions the N=O group is transferred intact.[11,12] The relatively high electronegativity of the N-atom and the presence of a lone pair on the nitrogen ensure that alkyl nitrites are soft electrophiles and their nucleophilic reactions are orbital-controlled processes.[32] Consequently, as OH$^-$ is the hardest nucleophile, in accordance with Pearson's principle of hard and soft acids and bases,[33] the reaction of RONO with OH$^-$ is a slow process, in which polar effects of substituents on the leaving alkoxide group accelerate the reaction; e.g., bromoethylnitrite hydrolyzes faster than ethylnitrite.[28]

The basic hydrolysis of RONO in water is catalyzed by OH$^-$; the observed rate constant increases proportional to [OH$^-$], i.e., $k_o = k_{OH}[OH^-]$.[34] The pK_a of trifluoroethanol (TFE) is 12.4,[35] close to that of β-CD (pK_a = 12.3).[14,36] For comparative purposes, the hydrolysis of some alkyl nitrites catalyzed by TFE, reacting as its anion, was studied in aqueous solution of 0.20 M sodium hydroxide. We found a linear increase in k_o with TFE concentration, i.e., $k_o = k_{OH}[OH^-] + k_{TFE}[TFE]$; then the appropriate second-order rate constant k_{TFE} was determined from the slope of the straight lines (see Figure 4). Values of k_{TFE} and k_{OH} (obtained from the intercept) are collected in Table 3, along with the values of the derived constant k_2 (vide infra) for comparison purposes. The second-order rate constant for the reaction with TFE anion is more than 2-fold that with OH$^-$, and the hydrolyses of EEN and 1PhPN are faster than those of the other studied alkyl nitrites, in accordance with the accelerating effects of electron-withdrawing substituents. Electron-withdrawing substituents, or substituents with resonance effects, make easy the stability of the leaving group, the alkoxide, R–O$^-$.

The influence of [OH$^-$] at constant β-CD concentration (fixed between 3.5 and 5.2 mM) was also analyzed. Figure 5 shows the results corresponding to EEN, 3PhP, 1PhP, and *n*-pentyl nitrites. A strong catalysis of the reaction can be observed in

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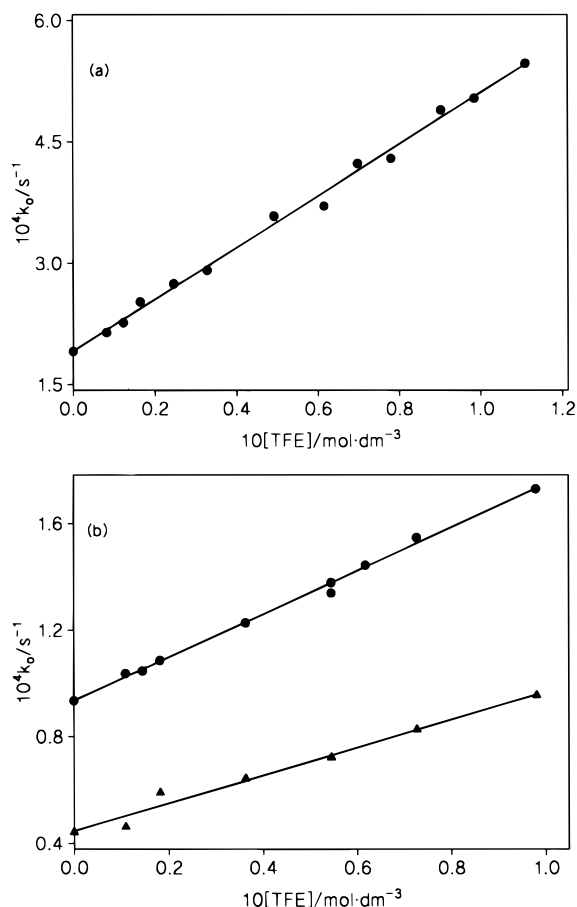(36) Li, S.; Purdy, W. C. *Chem. Rev.* **1992**, *92*, 1457.

Figure 4. Catalysis of TFE concentration in the basic hydrolysis of (a) ethoxyethyl nitrite and (b) 1-phenyl-1-propyl (●) and 3-phenyl-1-propyl (▲) nitrites at [OH⁻] = 0.20 M.

Table 3. Bimolecular Rate Constants for the Reaction of RONO with OH⁻ (k_{OH}) and with Trifluoroethoxide Ion (k_{TFE}) Obtained from the Study of the Influence of [TFE] at [OH⁻] = 0.20 M

RONO	$CH_3CH_2O(CH_2)_2ONO$	⬡—CH—CH₂CH₃ (ONO)	⬡—(CH₂)₃—ONO	$CH_3(CH_2)_4ONO$	$CH_3(CH_2)_3ONO$
k_{OH} /M⁻¹s⁻¹	9.5×10⁻⁴	4.7×10⁻⁴	2.2×10⁻⁴	0.99×10⁻⁴	0.75×10⁻⁴
k_{TFE} /M⁻¹s⁻¹	(31.9±0.7)×10⁻⁴	(8.1±0.2)×10⁻⁴	(5.3±0.1)×10⁻⁴	(2.57±0.06)×10⁻⁴	(2.3±0.4)×10⁻⁴
k_2/M⁻¹s⁻¹	0.29	0.18	0.76	0.10	0.11

[a] $k_2 = k_c K_c$, see Table 4 for values of k_c and K_c.

every case. With 3PhPN, the variation of k_o with [OH⁻] displays an ascending curve up to saturation level reached at approximately [OH⁻] > 0.15 M; the reaction of *n*-pentylnitrite does not depend on hydroxide ion concentration (between 0.03 and 1.1 M, the range investigated), but a strong catalysis by the presence of 3.5 mM β-CD can be detected; thus, the observed rate constant at [OH⁻] = 0.10 M in the absence of β-CD is 0.99 × 10⁻⁵ s⁻¹ (see Table 3). Finally, the plot of k_o vs [OH⁻] in the hydrolysis of EEN and 1PhPN becomes a straight line at [OH⁻] higher than approximately 0.15 M.

Subsequently, we studied the influence of β-CD concentration at fixed [OH⁻] (0.10 or 0.20 M). In the case of 3PhPN, the set of kinetic experiments varying the [β-CD] at [OH⁻] = 0.20 M was performed at six different temperatures (in the range 10−37 °C). Representative results are shown in Figure 6; some of the results obtained in the case of 3PhPN are shown in Figure 6b, and it can be seen that the experimental data sets obtained at 25 °C and 0.10 or 0.20 M of [OH⁻] do not differ. Pseudo-first-order rate constant, obtained over a range of (0.5−10) × 10⁻³ M β-CD, gave rise to saturation kinetics in every case,
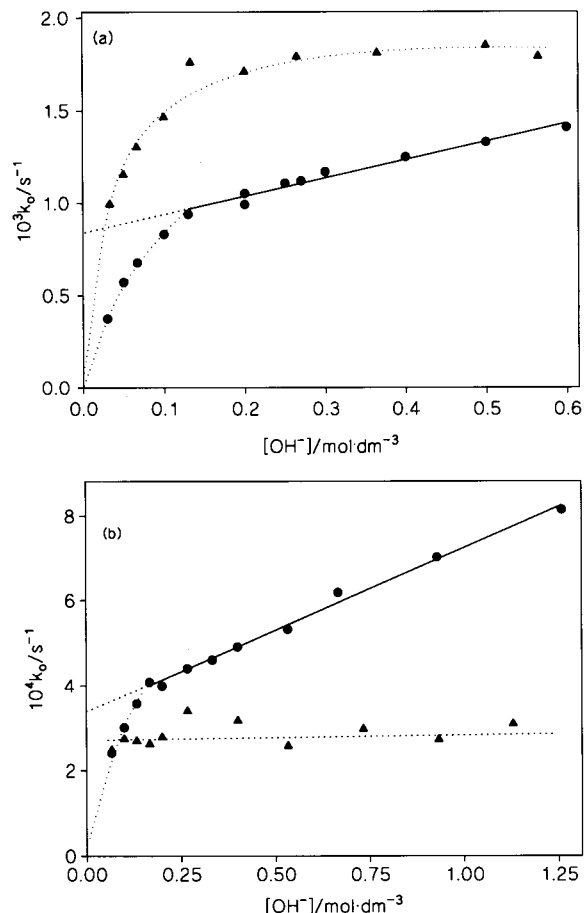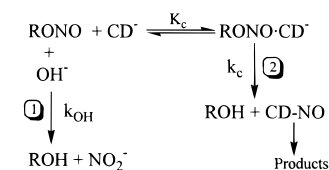

Figure 5. Variation of the observed rate constant, k_o, as a function of [OH⁻] in the basic hydrolysis of (a) 3-phenyl-1-propyl nitrite at [β-CD] = 5.2 mM (▲), ethoxyethylnitrite at [β-CD] = 3.8 mM (●); (b) 1-phenyl-1-propyl nitrite at [β-CD] = 4.5 mM (●), and *n*-pentylnitrite at [β-CD] = 3.5 mM (▲).

Scheme 3

meaning that 1:1 inclusion complexes between RONO and CD are formed. The experimental behavior observed conforms very well to reaction between RONO and OH⁻ in the medium, along with reaction through a complex RONO·CD. Scheme 3 can be proposed, from which eq 4 may be obtained to relate the variation of k_o with the concentration of β-CD.

$$k_o = \frac{k_{OH}[OH^-] + k_c K_c[\beta\text{-CD}]}{1 + K_c[\beta\text{-CD}]} \qquad (4)$$

The catalysis observed can be qualitatively explained if CD participates directly in the reaction; in other words, the complex RONO·CD is more reactive than the reaction of RONO not complexed. As the pK_a of β-CD is 12.3, at the above experimental conditions, a secondary hydroxy group in the wider rim of β-CD is ionized. Then, by comparison with TFE anion, the reaction of the complex involves the N═O transfer to an ionized secondary hydroxy group of β-CD, resulting in the direct formation of the nitrosocyclodextrin, but a big difference emerges: *the nucleophilic catalysis of the bimolecular process*

Table 4. Experimental Conditions and Parameters Obtained in the Study of the Influence of β-CD Concentration on the Basic Hydrolysis of Alkyl Nitrites by Fitting the Experimental Data to Eq 4, Where $k_o^w = k_{OH}[OH^-]$

RONO	$t/°C$	$[OH^-]/M$	k_o^w/s^{-1}	k_c/s^{-1}	K_c/M^{-1}	EM^a
EEN	25.0	0.10	9.5×10^{-5}	$(3.8 \pm 0.1) \times 10^{-3}$	64.5 ± 2.4	
EEN	25.0	0.20	1.8×10^{-4}	$(4.52 \pm 0.04) \times 10^{-3}$	65.4 ± 0.7	1.42
n-BuN	25.0	0.20	$\sim 2.0 \times 10^{-5}$	$(2.0 \pm 0.2) \times 10^{-3}$	51.5 ± 6.7	8.7
n-PeN	25.0	0.20	$\sim 1.5 \times 10^{-5}$	$(2.1 \pm 0.2) \times 10^{-3}$	50.5 ± 8.0	8.2
1PhPN	25.0	0.10	3.8×10^{-5}	$(5.6 \pm 0.1) \times 10^{-4}$	279 ± 13	
1PhPN	25.0	0.20	7.5×10^{-5}	$(7.1 \pm 0.2) \times 10^{-4}$	253 ± 20	$0.88/2.45^b$
2PhPN	25.0	0.20		reaction too slow; not good first-order reactions		
3PhPN	25.0	0.10	2.0×10^{-5}	$(2.34 \pm 0.02) \times 10^{-3\,c}$	360 ± 10	
3PhPN	25.0	0.20	3.5×10^{-5}	$(2.53 \pm 0.07) \times 10^{-3\,c}$	301 ± 19	4.8
3PhPN	10.4	0.20	~ 0	$(0.76 \pm 0.02) \times 10^{-3\,c}$	336 ± 22	
3PhPN	15.0	0.20	~ 0	$(1.13 \pm 0.03) \times 10^{-3\,c}$	324 ± 23	
3PhPN	20.1	0.20	$\sim 0.5 \times 10^{-5}$	$(1.79 \pm 0.05) \times 10^{-3\,c}$	278 ± 18	
3PhPN	31.0	0.20	5.2×10^{-5}	$(4.5 \pm 0.1) \times 10^{-3\,c}$	287 ± 21	
3PhPN	37.1	0.20	1.0×10^{-4}	$(7.34 \pm 0.09) \times 10^{-3\,c}$	281 ± 10	

^{a} Effective molarity ($=k_c/k_{TFE}$). ^{b} By taking the maximum value of k_c on the analysis of the influence of [DTABr]. ^{c} $\ln k_c = A - B(1/T)$, $B = -(7.53 \pm 0.18) \times 10^3$ K^{-1}, $A = 19.4 \pm 0.6$.
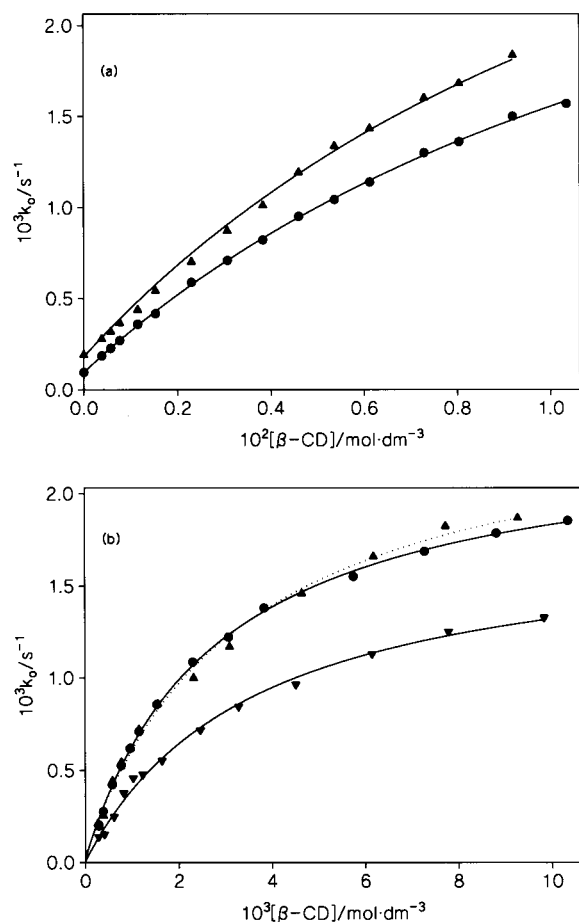

Figure 6. Catalysis of β-CD in the basic hydrolysis of (a) ethoxyethyl nitrite at $[OH^-] =$ (●) 0.10 and (▲) 0.20 M, and of (b) 3-phenyl-1-propylnitrite at $[OH^-] =$ (●) 0.10 M and 25 °C, (▲) 0.20 M and 25 °C, and (▼) 0.20 M and 20 °C. Solid lines fit to eq 4; for parameters, see Table 4. In plot (b), both data sets (●,▲) obtained at equal temperature are fitted together.

(or the intermolecular catalysis) in the case of TFE ion becomes an intramolecular catalysis in the reaction of the complex RONO·CD$^-$.[4,37]

As a productive complex requires ionized β-CD, increasing $[OH^-]$ also increases the ionized β-CD concentration; i.e. β-CD $+ OH^- \rightleftharpoons \beta$-CD$^- + H_2O$, whose equilibrium constant is equal to $K = K_a/K_w \approx 40$ M^{-1} at the experimental conditions of this

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work. When all β-CD molecules are ionized (which occurs at approximately $[OH^-] > 0.15$ M), the excess of OH^- catalyzes the reaction via step 1 (see Scheme 3). In those alkyl nitrites where $v_1 = k_{OH}[OH^-]$ is much slower than $v_2 = k_c$ (the cases of 3PhPN and *n*-PeN), the excess of OH^- does not influence the reaction; by contrast, when $v_1 \sim v_2$, the excess of $[OH^-]$ increases the k_o values proportionally to the degree of k_{OH} (the cases of EEN and 1PhPN), see Figure 5.

In the same way, the experiments carried out at $[OH^-] = 0.20$ M displayed in Figure 6 correspond to conditions where all β-CD molecules are ionized, and then an increase in [β-CD] also increases the concentration of productive complexes. Solid lines fit to eq 4, and the experimental conditions, along with the values determined for the constants k_c and K_c, are reported in Table 4. A detailed inspection of the results suggests the following.

Values of k_2, the second-order rate constant for the reaction of RONO + CD → *products*, determined as $k_2 = k_c K_c$ in M^{-1} s^{-1}, are reported in Table 3. This derived constant measures the reactivity of CD toward the alkyl nitrite and indicates the ability of CD to select between different RONO under nonsaturating conditions. As we can see, the value of k_2 corresponding to 3PhPN is the highest, also observing the highest degree of catalysis. On the other hand, k_2 values for the different alkyl nitrites exhibit a range of catalytic ability of CD anion, showing in all cases to be a better catalyst than TFE anion. This fact rules out the possible action of β-CD anion as simple general base catalyst; on the contrary, a significant inclusion of the alkyl nitrite in the CD cavity in the transition state must take place.

In comparison with the results in Table 2, the stability constants of RONO formed with neutral β-CD are shown to be higher than those formed with ionized β-CD$^-$. In some cases, such as that with 1PhPN, the difference is very important: $K_s^N \approx 2K_c$. In total contrast, EEN forms inclusion complexes with ionized β-CD, but it appears to not form inclusion complexes with neutral β-CD. Finally, in the case of 3PhPN, the stability constants formed with either neutral or ionized β-CD no longer differ from each other (compare values of K_s^N in Table 2 and K_c in Table 4); in addition, as for K_s^N, there is no influence of temperature on K_c.

Another important fact is the difference in reactivity of both processes, steps 1 and 2. The reaction rate of the complex, v_2, is more than 30 times faster than the rate of the bimolecular process, v_1, which in some cases cannot be detected. From the analysis of the influence of temperature for the case of 3PhPN, we can estimate a value of 89 kJ/mol for the activation energy

Chart 1

Chart 2
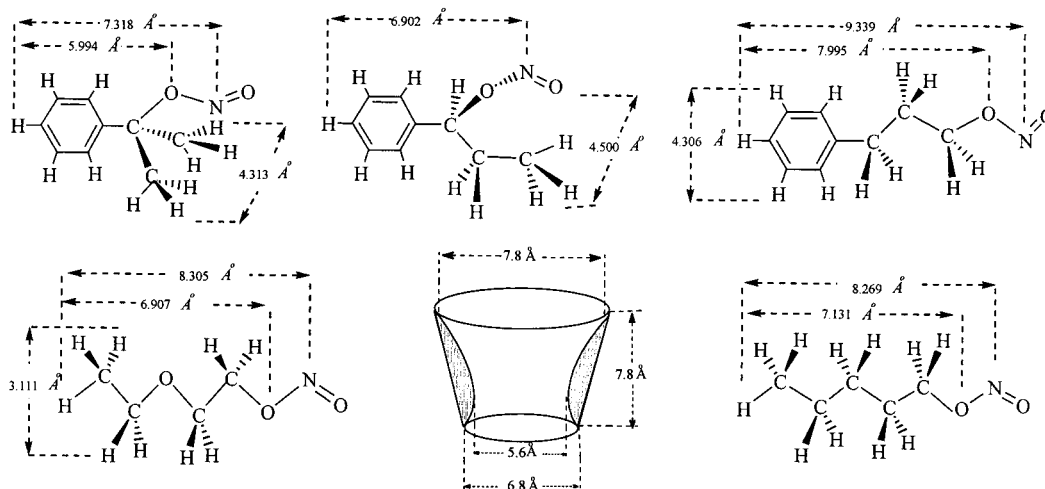
corresponding to the k_{OH} process, i.e., for the bimolecular reaction, whereas the Arrhenius plot corresponding to the rate constant k_c (Table 4) gives a value of 62.8 kJ/mol for the activation energy of the hydrolysis mediated by the ionized β-cyclodextrin. These results indicate an important stabilization of the transition state of the reaction through the complexed 3PhPN with respect to the reaction via OH⁻. In this sense, making use of the approach developed by Kurz[5,38] for estimating the stabilization of a transition state by a catalyst, we define $K_{TS} = [TS\cdot CD]/\{[TS][CD]\} = k_c K_c/k_o{}^w$, i.e., the apparent stability constant of formation of the transition state of the CD-mediated reaction, symbolized by TS·CD, from the transition state of the normal reaction (TS) and the CD. This quantity takes values of 1642, 2395, 5200, 7000, and 21 758 M⁻¹, corresponding to EEN, 1PhPN, n-BuN, n-PeN, and 3PhPN, respectively. This variation of K_{TS} with alkyl nitrite structure is an additional proof of the transition-state binding of CD.

Along the same line, the efficiency of *intramolecular catalysis*, like the complex reaction, is conveniently measured in terms of the *effective molarity* (EM) of the catalytic group.[37] The EM is the ratio of the first-order rate constant for the intramolecular reaction, k_c in our case, and the second-order rate constant for the *intermolecular* reaction that proceeds by the same mechanism under the same conditions, i.e., k_{TFE} since the pK_a of TFE is quite similar to that of β-CD. The EM is formally the concentration of the catalytic group (R−O⁻ in this study) required to make the intermolecular reaction proceed at the observed rate of the intramolecular process.

The values of EM ($= k_c/k_{TFE}$) appear in Table 4 (entry 7). Since these values are less than 80 M, the mechanism of β-CD-mediated base hydrolysis of alkyl nitrites is considered a *general base catalysis*.[37] Surprisingly, the highest EM is observed with those alkyl nitrites forming the least stable complexes. We must find the reason for this in the structure of RONO, which obviously determines the way of including into the β-CD cavity, and then the efficiency of CD⁻ catalysis. The simple systems' tighter binding to CD increases the EM, and thus makes the desired net increase in transition-state binding possible. Molecular mechanics calculations were carried out to obtain the dimensions and optimal geometries of guest molecules from MOPAC calculations (PM3) for comparison purposes with the host molecule cavity.[3,39] The energy-minimized structures are shown in Chart 1.
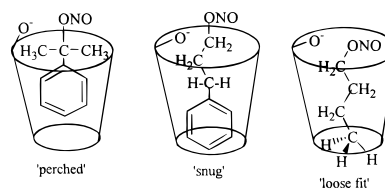
In line with this, 2-phenyl-2-propyl nitrite binds "perched" to β-CD⁻, and thus the NO group cannot come near the ionized −O⁻ group of β-CD, as a consequence making the reaction impossible; the same applies to 1-phenyl-1-propyl nitrite, but to a lesser extent, as the branching effects do not completely surround the reaction center (−NO) (perching the RONO molecule in the CD cavity in only one side). Contrarily, n-pentyl- and n-butyl nitrites can sit "loosely" in the β-CD cavity, so both molecules can adjust the deepness in order to closely approach the reacting groups; consequently, the EM is the greatest observed. In an intermediate position is 3-phenyl-1-propyl nitrite, which has no steric hindrance, but it sits inside the β-CD snugly and, as the length of the molecule slightly exceeds the deepness of the CD cavity, the reaction between the functional groups is forced (see Chart 2).

Potential Inhibitors (PI). To shed light on the host−guest interaction process, we studied finally the effect of the addition of DTABr to the reaction medium. Typical results obtained at [OH⁻] = 0.20 M in the presence of a constant [β-CD] (fixed in the range of 2.5−3.7 mM) are plotted in Figure 7. Normally, addition to the reaction mixture of an inert species, i.e., a potential inhibitor here (PI), that forms a complex with CD reduces the concentration of free CD, so that less CD·RONO complex is formed and k_o is decreased.

Surprisingly, the addition of DTABr at concentrations lower than the cmc° causes a slight increase of k_o in the hydrolysis of 3PhPN but a 3-fold increase in the case of 1PhPN. We must also note that, contrary to the case of acid hydrolysis, k_o vs [DTABr] profiles do not describe a sigmoidal course typical of a modification of an equilibrium step; instead, k_o increases with [DTABr]. As expected, at [DTABr] >cmc°, an inhibition is observed. This is because once micelles are formed, alkyl nitrites also solubilize in the micellar pseudophase with the concomitant decrease in the productive complex concentration. Although, contrary to the case of the reaction by H⁺, cationic micelles increase the [OH⁻] at the micellar interface (also due to electrostatic effects); but, as the intermolecular catalysis by OH⁻

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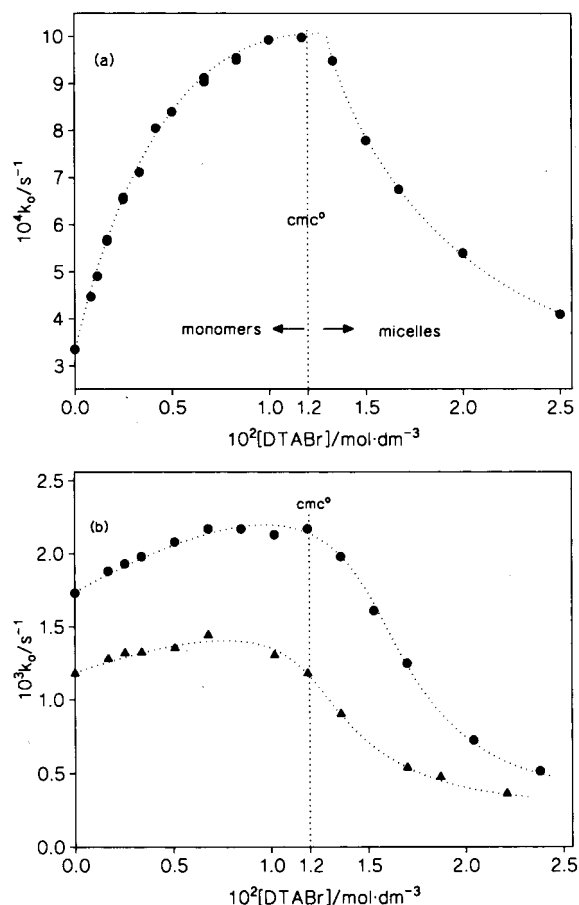
Figure 7. Influence of [DTABr] in the basic hydrolysis of (a) 1-phenyl-1-propyl nitrite at [OH$^-$] = 0.20 M and [β-CD] = 3.7 mM, and of (b) 3-phenyl-1-propyl nitrite at [OH$^-$] = 0.20 M and [β-CD] = (●) 5.2 mM and (▲) 2.5 mM.

Table 5. Maximum Degree of Catalysis, Measured as k_o^{max}/k_o^w, with k_o^{max} Being the Pseudo-First-Order Rate Constant at the Maxium [β-CD] Used (∼0.01 M) and k_o^w the Pseudo-First-Order Rate Constant Observed for Each Amine at the Concentration Given in the Absence of β-CD

	[amine]			
	1.7×10^{-3} M	3.2×10^{-3} M	6.4×10^{-3} M	7.8×10^{-3} M
PyR	1.8	1.4	1.2	
PIP	4.3	2.6		
MCH		4.1		2.8

surrounding of the host−guest complex by the DTABr monomers, displacing the hydration water molecules of the reacting groups. (Note that the hydration shell decreases the natural nucleophilicity of a nucleophile.) Similar catalysis by potential inhibitors has been found in the cleavage of *p*-nitrophenyl alkanoates by the addition of alcohols and alkanoate or sulfonate anions.[30,40] In any event, there is no doubt that the present results provide an example of allosteric activation;[41] that is, substances which are not substrate analogues attach to some site of the β-CD·RONO complex (in this case) with a resulting effect on the catalytic reactivity.

Nitrosation of Amines. The nitrosation reaction of pyrrolidine (PyR), piperidine (PIP), and *N*-methylcyclohexylamine (MCH) by ethoxyethyl nitrite (EEN) has also been studied in the presence of β-CD. Reactions between alkyl nitrites and secondary amines produce N-nitrosamines in quantitative yield. The reaction in water is of first order with respect to the amine, i.e. $k_o^w = k_2^w$[amine], with k_o^w being the observed rate constant. It has been postulated that alkyl nitrites transfer their nitroso group to amines through a concerted mechanism with a four-center transition state, although the possibility of a six-membered ring, which includes a water molecule, was also considered.[10a,11c,12]

The inclusion equilibrium constants (K_c^A) of PyR, PIP, and MCH with β-CD are reported in the literature as 6,[34b] 50,[26] and 550 M^{-1},[42] respectively. These host−guest inclusion constants are for the neutral form of the amine, since the corresponding ammonium ions have proved to not include into the β-CD cavity.[26]

In this study, we worked with [OH$^-$] = 0.10 M, where all the amine is in its unprotonated form. The nitrosation reaction by EEN of the aforementioned amines is catalyzed by the presence of β-CD. The degree of catalysis, measured as the ratio of k_o^{max}/k_o^w (k_o^{max} being the maximum value of the observed rate constant measured at the higher [β-CD] used (∼0.01 M), and k_o^w the observed rate constant determined in the absence of β-CD at the same experimental conditions), first decreases as the amine concentration increases, and second depends strongly on the amine, giving the highest catalysis with MCH and the lowest catalysis with PyR, see Table 5. To perform a clear exposition of the results, we will comment on the results of PyR separately for the other two cases.

Nitrosation of Pyrrolidine. The influence of β-CD on the nitrosation of PyR by EEN has been studied at two pH values: (i) in pyrrolidine−pyrrolidinium chloride buffer of pH 11.15, i.e., under conditions where the major part of β-CD molecules are not ionized (pK_a 12.30), and (ii) in the presence of [OH$^-$]

is much lower than the intramolecular catalysis by CD$^-$ (see Table 4), an inhibition of the reaction is also observed.

It should be emphasized here that the normal expected behavior through the entire [DTABr] range was an inhibition of the reaction. As in the previous section, due to the competition between DTABr monomers and RONO, addition of DTABr should expel the included RONO, with the consequent retardation of the reaction due to the lower reactivity of the noncomplexed RONO. The key difference, which it is important to note here, is that, in alkaline medium, the complex between RONO and ionized β-CD is the *transition state* of the hydrolysis reaction, i.e., a true compound in which the functional groups are covalently bound.

The high catalysis degree observed with 1PhPN by DTABr addition is better viewed as being the reaction between the PI·CD complex and 1PhPN; or, more exactly, due to the fact that saturation kinetics is obtained at [DTABr] < cmc°, this implies the formation of ternary complexes (PI·CD·RONO) that are more reactive than the binary complex CD·RONO. Quantitative analysis of the results is complicated because it contains two concentration variables, [PI] and [CD]; however, as the most plausible cause for a qualitative reason for the observed facts, we believe that DTABr monomers could protrude through the narrow rim of β-CD, pushing out 1PhPN molecules, thus acquiring a close proximity between the reacting groups. The EM calculated using the maximum value observed here (k_o^{max} = 9.98 × 10^{-4} s^{-1} at [β-CD] = 3.69 mM; using eq 4 and data in Table 4 gives k_c^{max} = 2.0 × 10^{-3} s^{-1}) increases to 2.45 M. Another possibility could be found in a particular

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(42) Tee, O. S.; Gadosy, T. A.; Giorgi, J. B. *Can. J. Chem.* **1996**, *74*, 736.

Table 6. Pseudo-First-Order Rate Constants Obtained in the Nitrosation of Pyrrolidine by Ethoxyethyl Nitrite in Pyrrolidine–Pyrrolidinium Chloride Buffer of pH 11.15 and Total Buffer Concentration of 0.020 M as a Function of β-CD Concentration

$10^3[\beta\text{-CD}]/M$	0.0	0.383	0.575	0.767	1.15	1.53	2.30	3.07	4.60	6.13	7.28	9.20	11.1
$10^2 k_o/s^{-1}$	1.30	1.27	1.25	1.26	1.27	1.30	1.21	1.19	1.15	1.13	1.10	1.14	1.09

Table 7. Experimental Conditions Used in the Nitrosation of Pyrrolidine, Piperidine, and *N*-Methylcyclohexylamine in Basic Medium ($[OH^-] = 0.10$ M) by Ethoxyethyl Nitrite Performed in the Presence of β-CD[e]

[amine]/M	k_o^w/s^{-1}	$10^3\alpha/s^{-1}$ [a]	$10^3\alpha/s^{-1}$ [b]	K_c^A/M^{-1}	$k_2^w/M^{-1}\,s^{-1}$	k_c/s^{-1}	$k_2^c/M^{-1}\,s^{-1}$
			Pyrrolidine				
1.7×10^{-3}	2.45×10^{-3}	7.77 ± 0.08	7.9 ± 0.1	6	1.45	4.52×10^{-3}	1.93
3.2×10^{-3}	4.67×10^{-3}	9.51 ± 0.06	9.7 ± 0.1	6	1.46	4.52×10^{-3}	1.59
6.4×10^{-3}	9.45×10^{-3}	13.7 ± 0.2	13.5 ± 0.3	6	1.48	4.52×10^{-3}	1.29

[amine]/M	k_o^w/s^{-1}	$\gamma/s^{-1}\,M^{-1}$	$\delta/s^{-1}\,M^{-2}$	K_c^A/M^{-1}	$k_2^w/M^{-1}\,s^{-1}$	$k_2^c K_c + k_2^{c\prime} K_c^A/s^{-1}\,M^{-2}$	$k_2^{cc}/M^{-1}\,s^{-1}$
			Piperidine				
1.7×10^{-3c}	0.57×10^{-3}	0.332 ± 0.005	23.5 ± 0.8	50	0.34	50.9	2.0
1.7×10^{-3d}	0.57×10^{-3}	0.328 ± 0.009	24.0 ± 1.2	50	0.34	47.3	2.1
3.2×10^{-3c}	1.05×10^{-3}	0.387 ± 0.005	25.9 ± 0.8	50	0.32	42.4	1.3
3.2×10^{-3d}	1.05×10^{-3}	0.388 ± 0.004	25.7 ± 0.6	50	0.32	43.9	1.28
			Methylcyclohexylamine				
3.8×10^{-3c}	0.67×10^{-3}	1.22 ± 0.02	205 ± 4	550	0.176	251	0.51
3.8×10^{-3d}	0.67×10^{-3}	1.18 ± 0.02	214 ± 3.5	550	0.176	245	0.57
7.7×10^{-3c}	1.25×10^{-3}	2.10 ± 0.02	263 ± 9	550	0.164	252	0.46
7.7×10^{-3d}	1.25×10^{-3}	2.20 ± 0.02	279 ± 8	550	0.164	254	0.52

[a] Experimental data fitted to eq 5. [b] Least-squares fit of $k_o(1 + K_c[CD])$ vs $[\beta\text{-CD}]$. [c] Experimental data fitted to eq 6 [d] Analysis of k_o^{corr} as a function of free $[\beta\text{-CD}]$. [e] Observed rate constants, k_o^w, measured in the absence of β-CD, and determined values for the rate constant of the nitrosation processes. To obtain k_2^c, $k_2^{c\prime}$, and k_2^{cc}, we have used the values of $k_c = 3.8 \times 10^{-3}\,s^{-1}$ and $K_c = 65\,M^{-1}$, previously determined.

$= 0.10$ M, in which case a great part of secondary hydroxy groups of β-CD are ionized.

Kinetic results obtained at pH 11.15 and total pyrrolidine buffer concentration ($[\text{PyR}]_t$) of 0.020 M as a function of $[\beta\text{-CD}]$ are collected in Table 6. As one can see, there is no change in rate except for a very slight decrease. The average value of k_o is $1.13 \times 10^{-2}\,s^{-1}$. At this pH value, the concentration of neutral pyrrolidine is determined as $[\text{PyR}] = [\text{PyR}]_t K_a/(K_a + [H^+])$, with K_a being the acidity constant of pyrrolidinium ion ($pK_a = 11.30$).[43] Then, the bimolecular rate constant for the reaction between neutral PyR and EEN is calculated as $k_2 = 1.36\,M^{-1}\,s^{-1}$. This value is in perfect accordance with those reported in Table 7 (vide infra) and with that obtained from the study in aqueous alkaline medium of the influence of [PyR] (see Figure 8b): k_o varies linearly with [PyR], with the straight line passing through the origin and whose slope value is equal to $1.48 \pm 0.01\,M^{-1}\,s^{-1}$, i.e., the k_2 value for the reaction in water.

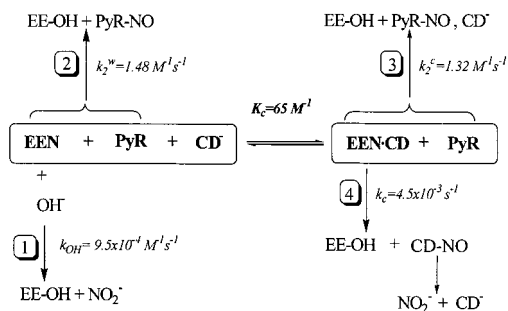Kinetic results obtained at $[OH^-] = 0.10$ M and $[\text{PyR}] = 3.2 \times 10^{-3}$ M are displayed in Figure 8a. Pseudo-first-order rate constants increase with $[\beta\text{-CD}]$, giving rise to simple saturation kinetics. Remembering that the stability constant of the inclusion complex formed between PyR and β-CD has been estimated as $K_c^A = 6\,M^{-1}$, working at $[\text{PyR}] = 1.6 \times 10^{-3}$, 3.2×10^{-3}, or 6.4×10^{-3} M, the amount of amine forming inclusion complexes will be insignificant. On the contrary, EEN binds to ionized β-CD, with $K_c = 65\,M^{-1}$ being the equilibrium constant of complex formation, and in the presence of OH^-, the basic hydrolysis reaction via both free and complexed EEN occurs (see previous section). But, on the other hand, the reaction rate of the nitrosation process is much higher than that of the basic hydrolysis; in fact, we found $k_{OH} = 9.5 \times 10^{-4}\,M^{-1}\,s^{-1}$, whereas from the study of the nitrosation reaction in the absence of β-CD, we found $k_2^w = 1.48\,M^{-1}\,s^{-1}$. Therefore, as a starting hypothesis, we can consider that the nitrosation of PyR could take place through both free EEN and the EEN·CD⁻ complex. On the basis of these considerations, Scheme 4 can be proposed.

(43) Andraos, J.; Kresge, A. J. *J. Am. Chem. Soc.* **1992**, *114*, 5643.
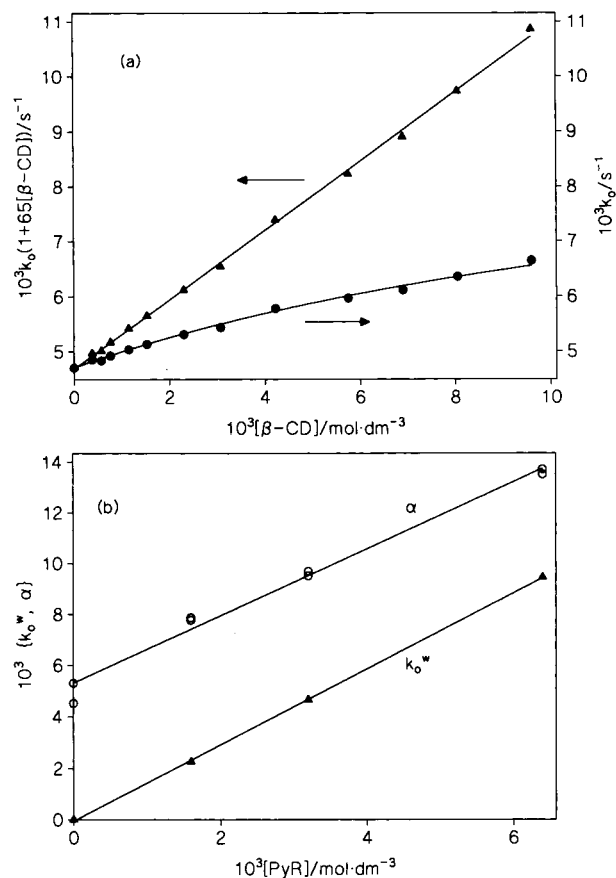
Figure 8. (a) (●) Influence of β-CD concentration in the nitrosation of pyrrolidine by ethoxyethyl nitrite at $[OH^-] = 0.10$ M and $[\text{PyR}] = 3.2 \times 10^{-3}$ M; (▲) linearization of the data according to eq 5. (b) Plot of k_o^w and α (= $k_c + k_2^c[\text{PyR}]$) against [PyR].

The rate of disappearance of EEN is the sum of the rate of the four reactive steps; nevertheless, in the experimental conditions of this work, $v_1 \ll v_2$. In fact, plotting k_o^w against [PyR], the straight line goes through the origin (Figure 8b).

Scheme 4

Considering, then, the following mass-balance equations, $[EEN]_t = [EEN] + [EEN \cdot CD]$, $[PyR]_t = [PyR]$, and $[CD]_t = [CD]$, one easily arrives at eq 5.

$$k_o = \frac{k_o{}^w + \alpha K_c[\beta\text{-CD}]}{1 + K_c[\beta\text{-CD}]}, \quad \text{with } \alpha = k_c + k_2{}^c[PyR] \quad (5)$$

Experimental values of $k_o - [\beta\text{-CD}]$ were fitted to this equation by means of nonlinear regression analysis, with $k_o{}^w$ and K_c being input parameters. Table 7 contains the obtained parameters along with the experimental conditions. Values of α have been plotted against [PyR]; as expected, a good straight line is described (Figure 8b) with intercept ($\equiv k_c = (5.3 \pm 0.2) \times 10^{-3}$ s^{-1}) and slope ($\equiv k_2{}^c = 1.32 \pm 0.06$ M^{-1} s^{-1}) statistically significant values; in other words, the complexed EEN reacts via transferring the −NO group both to the ionized hydroxy group of β-CD and to pyrrolidine, and also k_c compares quite well with the value obtained in the absence of PyR (see Table 4).

Further support for eq 5 is obtained from the linear plot of the data in the form of $k_o(1 + K_c[\beta\text{-CD}])$ against $[\beta\text{-CD}]$ with $K_c = 65$ M^{-1}. The plot corresponding to $[PyR] = 3.2 \times 10^{-3}$ M is also shown in Figure 8a, and, as expected, a good straight line was described; this was also the case with the other PyR concentrations (plots not shown). Values of $k_2{}^w$, k_c, and $k_2{}^c$ are collected in Table 7. The comparison of $k_2{}^w$ and $k_2{}^c$ values indicates that both free and complexed EEN reacts practically at the same rate with PyR. This means that the observed catalysis is due only to the comparable rates of steps 3 and 4; but, increasing [PyR], the rate of step 3 is being increased, while that of step 4 remains unchanged. Consequently, the degree of catalysis is reduced on increasing [PyR].

Nitrosation of Piperidine and Methylcyclohexylamine. Typical results obtained in the nitrosation of PIP and MCH by EEN at $[OH^-] = 0.10$ M in the presence of β-CD are shown in Figure 9a. These amines are less reactive than PyR and more hydrophobic, in the sense that the stability constants of the corresponding inclusion complexes with β-CD are much higher than those of PyR. Therefore, with PIP and MCH, the amount of complexed amine is not negligible. Thus, we suggest that our kinetic scheme of reaction may be represented as in Scheme 5, put forward for the case of MCH, where, besides the basic hydrolysis reaction via either OH$^-$ (step 1) or ionized β-CD (step 2), the possibility of nitrosation reactions of free EEN with free amine (step 3), the complexed amine (MCH·CD) with the free EEN (step 4), or its kinetically equivalent reaction of the free amine with the complexed EEN (step 6), and the complexed EEN and complexed amine (step 5), are represented. The rate of the reaction is the sum of the six reaction steps, but as with PyR, the rate of step 1 may be ignored. Then, taking into account that the concentrations of the species are given by $[EEN]_t = [EEN] + [EEN \cdot CD]$, $[MCH]_t = [MCH] + [MCH \cdot CD]$, and
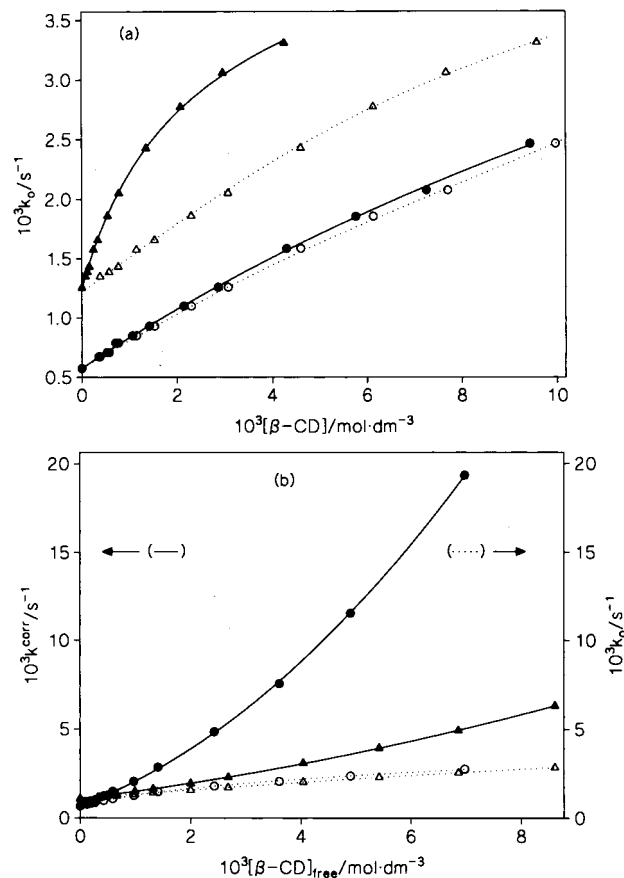
Figure 9. (a) Influence of β-CD concentration in the nitrosation reaction by ethoxyethyl nitrite at $[OH^-] = 0.10$ M of piperidine (●,○) at $[PIP] = 1.67 \times 10^{-3}$ M and of *N*-methylcyclohexylamine (▲,△) at $[MCH] = 7.7 \times 10^{-3}$M. Open points refer to total $[\beta$-CD], and solid points refer to free $[\beta$-CD]; solid lines fit to eq 6, for parameters, see Table 7. (b) Plots of k^{corr} $\{= k_o(1 + K_c[\beta\text{-CD}])(1 + K_c{}^A[\beta\text{-CD}])\}$ (●,▲) and of k_o (△,○) against free $[\beta$-CD] obtained in the nitrosation by ethoxyethylnitrite, of piperidine (triangles) at $[PIP] = 3.3 \times 10^{-3}$ M ($K_c = 65$ M^{-1} and $K_c{}^A = 50$ M^{-1}), and of *N*-methylcyclohexylamine (circles) at $[MCH] = 3.8 \times 10^{-3}$ M ($K_c = 65$ M^{-1} and $K_c{}^A = 550$ M^{-1}). Solid lines fit to $k^{corr} = k_o{}^w + \gamma[\beta\text{-CD}] + \delta[\beta\text{-CD}]^2$, according to eq 6; for parameters, see Table 7.

Scheme 5
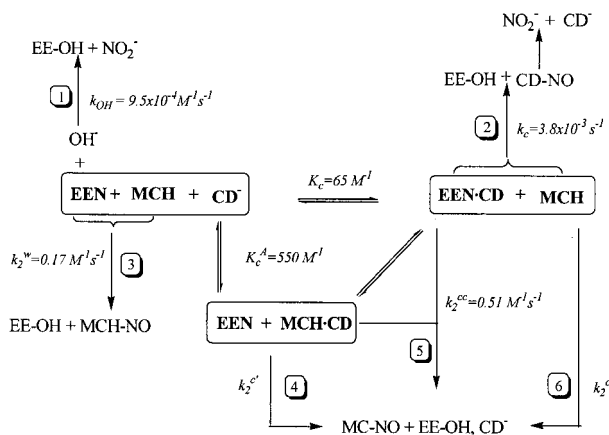
$[CD]_t = [CD] + [MCH \cdot CD]$, from the expressions of K_c and $K_c{}^A$ given in Scheme 5, one arrives easily to eq 6, where $\gamma = \{k_c K_c + (k_2{}^c K_c + k_2{}^{c'} K_c{}^A)[MCH]\}$ and $\delta = \{k_c K_c K_c{}^A + k_2{}^{cc} K_c K_c{}^A[MCH]\}$ (or [PIP]) and $[\beta\text{-CD}]$ represents free cyclodextrin concentration, which has been determined by solving the equation $[CD]^2 + [CD]\{[\text{amine}]_t - [CD]_t + 1/K_c{}^A\} - [CD]_t/$

Chart 3
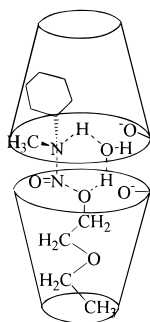
$K_c^A = 0$, with $K_c^A = 550$ M^{-1} for the case of MCH, and $K_c^A = 50$ M^{-1} for the association of PIP to CD.

$$k_o = \frac{k_o^w + \gamma[\beta\text{-CD}] + \delta[\beta\text{-CD}]^2}{(1 + K_c[\beta\text{-CD}])(1 + K_c^A[\beta\text{-CD}])} \quad (6)$$

Since k_o^w and the equilibrium constants are known, we could calculate γ and δ values by nonlinear regression analysis of the experimental data $k_o - [\beta\text{-CD}]$ fitted either to eq 6 or to its modification in the form of $k_o^{corr} = k_o(1 + K_c[\beta\text{-CD}])(1 + K_c^A[\beta\text{-CD}])$ vs $[\beta\text{-CD}]$. Solid lines in Figure 9a and b correspond to the fit of the experimental data in the two alternatives, respectively. Values of γ and δ determined from both procedures are collected in Table 7, along with the experimental conditions and the input parameters used in the fitting process.

Values of γ, together with k_c and K_c values reported in Table 7, afford the sum $k_2^c K_c + k_2^{c'} K_c^A$. In the case of piperidine, values of K_c and K_c^A are similar, and they are also similar to the observed value of the aforementioned sum. If we assume that $k_2^c = k_2^{c'} = \gamma/(K_c + K_c^A)$, a value of 0.40 M^{-1} s^{-1} can be obtained for these rate constants. Obviously, other combinations are also possible, but there is no reason for a big difference in the values of the rate constants for the reaction between the complex EEN·CD (or PIP·CD) and free PIP (or free EEN). In the same manner, a value of 0.41 M^{-1} s^{-1} can be obtained for $k_2^c = k_2^{c'}$ in the case of *N*-methylcyclohexylamine; or since with this amine $K_c^A \gg K_c$, considering the sum must be equated to $k_2^{c'} K_c^A$ unless $k_2^c \gg k_2^{c'}$, then we could estimate $k_2^{c'} = 0.46$ M^{-1} s^{-1}. That is, both possibilities could work.

On the other hand, from δ values, one determines k_2^{cc}, that is the rate constant for the reaction between the complexes EEN·CD and amine·CD. As we can see, this rate constant is more than 3-fold the rate constant between free substrates. In other words, there is an important decrease in free energy when the complexed substrates react together. This may be due to the possibility of formation of channel-like structures which, besides serving to fix the reacting species in close proximity, might intervene in the formation of the transition state, possibly in the way shown in Chart 3.

In fact, the nitrosation of amines by alkyl nitrites goes through highly ordered transition states, based chiefly on the solvent isotope effects observed that are greater than unity that evidence a rate-controlling step involving a proton transfer, and on the large negative entropies of activation determined for these reactions.[11a,b] Similar effects of CDs have been found in the nuclophilic aromatic substitution reactions of 1-chloro-2,4-dinitrobenzene and 1-fluoro-2,4-dinitrobenzene with amines.[26]

Mechanism Characteristics. The measured reactivities of the substrates studied here, as well as that with ClO$^-$ ion[44] and
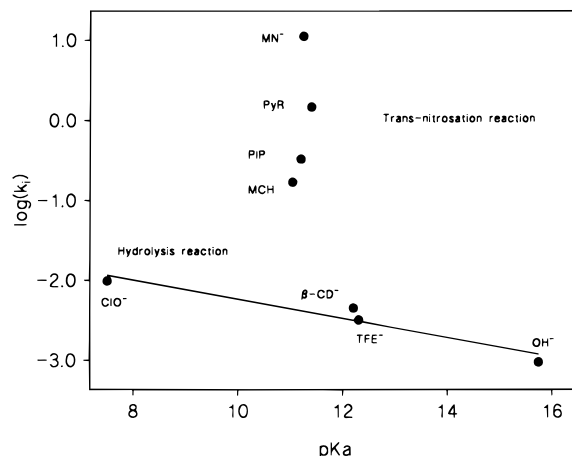

Figure 10. Brønsted plot of the reactivities of EEN toward oxygen anions, β-CD anion, carbanion of malonitrile (MN), and amines.

malononitrile anion (MN$^-$),[45] with EEN (log k_i) are plotted against their pK_a in Figure 10. Bimolecular reactions of EEN with oxygen nucleophiles OH$^-$, TFE$^-$, ClO$^-$, and CD$^-$ display a good Brønsted plot with a slope $\alpha = -0.12 \pm 0.03$; meanwhile, the reactivities with amines or the carbanion of MN do not show any clear trend. In accordance with the values of EM, the mechanism of the reaction of EEN with oxygen nucleophiles is a general base-catalyzed reaction, while amines and MN$^-$ behave as intrinsic nucleophiles; then, their reactivities with alkyl nitrites are better correlated with Ritchie's N$_+$ parameter,[46] as our group has demostrated previously.[12] It seems that N$_+$ measures some property of the nucleophiles that strongly influences reactivity of reactions and, in particular, those of NO-transfer. Since N$_+$ is determined kinetically, it seems that it must contain information on transformations that take place in the nucleophile during the course of the chemical reaction.

Conclusions

Acid hydrolysis of 1PhP, 2PhP, and 3PhP nitrites is inhibited by the presence of β-CD. These RONO form 1:1 inclusion complexes with β-CD, but H$_3$O$^+$, a simple cation, does not bind to CD. The energy of activation of CD-mediated acid hydrolysis is much higher than that of the reaction between free substrates. The acid hydrolysis of EEN is not influenced by the presence of β-CD, which has been attributed to the special way EEN interacts with neutral CD.

On the contrary, the basic hydrolysis of ethoxyethyl, *n*-butyl, *n*-pentyl, 1-phenyl-1-propyl, and 3-phenyl-1-propyl nitrites is strongly catalyzed by the presence of ionized β-CD. The catalysis is due to the formation of productive 1:1 inclusion complexes between RONO and ionized β-CD; that is, the complex is the transition state of the reaction in which the reactive groups, $-$NO and the ionized hydroxy group of CD, are brought together. The stability constant of the transition state for the CD-mediated reaction depends on the structure of RONO, being higher for the alkyl nitrites with linear hydrocarbon chains. This characteristic explains the nonobserved catalysis in the case of 2PhPN, in which steric hindrance of methyl groups at both sides of the NO-group prevent reactant groups from approaching each other. Addition of potential inhibitors, such as DTABr, catalyzes the reaction, contrary to what is expected. The effect is quite strong in the case of 1PhPN

(44) Leis, J. R.; Peña, M. E.; Rios, A. M. *J. Chem. Soc., Perkin Trans. 2* **1995**, 587.

(45) Iglesias, E. *J. Chem. Res. (S)* **1995**, 98.

(46) Ritchie, C. D. *Can. J. Chem.* **1986**, *64*, 2239. Ritchie, C. D. *Acc. Chem. Res.* **1972**, *5*, 348.

and can be viewed as an example of allosteric activation; that is, DTABr monomers induce conformational changes in the transition state.

Nitrosation of amines by ethoxyethyl nitrite in alkaline medium is enhanced by the presence of β-CD. PyR does not form inclusion complex with β-CD, and free and complexed EEN react with PyR at practically the same reaction rates. PIP and MCH form inclusion complexes with β-CD. The nitrosation can occur via either free substrates or complexed substrates, or between amine (or EEN) complex and free EEN (or amine), with the reaction rate through both complexes being nearly 3-fold that of free EEN and MCH.

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