Differences in the activity of neutral and ionized β-cyclodextrin on the nitrosation of amines by phenylpropyl nitrites

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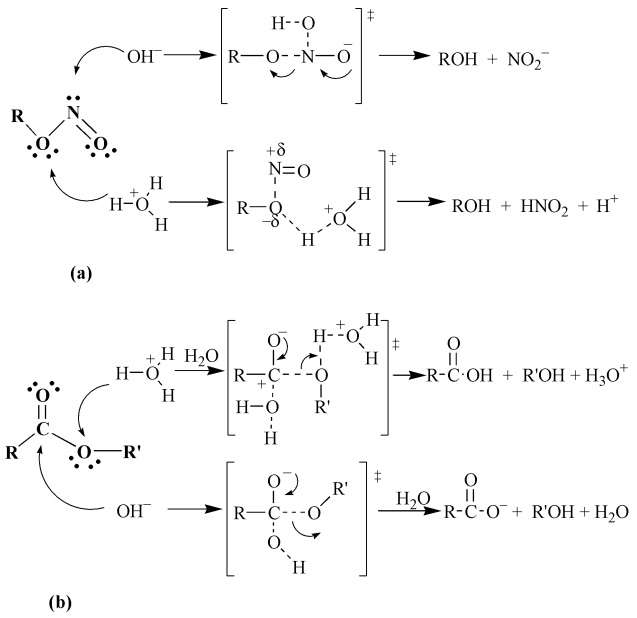
The influence of β-cyclodextrin (β-CD) on the base-catalyzed hydrolysis reaction of 1-phenyl-1-propyl, 2-phenyl-1-propyl and 3-phenyl-1-propyl nitrites and on the nitrosation of pyrrolidine, piperidine and *N*-methylcyclohexylamine by the aforementioned alkyl nitrites (RONO) is studied in aqueous buffers of the amines and in an alkaline medium with $[OH^-] = 0.20$ M. The hydrolysis reaction is catalyzed by the presence of β-CD owing to the formation of reactive 1 : 1 inclusion complexes between the alkyl nitrite and the ionized β-CD; the addition of potential inhibitors, such as dodecyltrimethylammonium bromide monomers, accelerates the reaction even more. The effect is quite significant in the case of 1-phenyl-1-propyl nitrite and is viewed as a case of *allostery*. In the presence of neutral β-CD, the nitrosation by 1-phenyl-1-propyl nitrite, either of pyrrolidine or piperidine, is inhibited by β-CD addition; however, the nitrosation reaction of piperidine by 2-phenyl-1-propyl nitrite is catalyzed (passing through a maximum) by β-CD, whereas the nitrosation of pyrrolidine promoted by 3-phenyl-1-propyl nitrite exhibits practically no change upon β-CD addition. In alkaline media (containing ionized β-CD) the nitrosation of pyrrolidine by 1-phenyl-1-propyl nitrite is inhibited by the presence of β-CD; in contrast, the nitrosation of both piperidine and *N*-methylcyclohexylamine is catalyzed in all cases, but the degree of catalysis depends not only on the alkyl nitrite structure, but also on the type and concentration of the amine. Kinetic results are quantitatively interpreted on the basis of the proposed reaction mechanism in each case, and the kinetic rate constants of the different steps are determined. Comparison of the results obtained in aqueous alkaline media and in aqueous buffers of the amines themselves allows us to establish important characteristics of the *transition state* of the reaction.

Cyclodextrins (CDs) constitute a very popular family of molecular hosts, capable of forming inclusion complexes, even in aqueous solutions, with a wide range of low molecular weight compounds, which are included into the CD cavities.[1–3] Regardless of the stabilizing force involved, geometric factors (size selectivity) and the hydrophobicity of the guest molecules are decisive in determining the complex formation in a considered medium. The process of forming inclusion complexes is that of a dynamic equilibrium and in general can be detected directly because both physical (absorption of light,[4] fluorescence,[5] NMR spectra,[6] *etc.*) and/or chemical[7] properties of the guest are modified. The stability of the inclusion complex can be described in terms of the formation equilibrium constant.

Over the last few years, numerous studies have been centered on the influence of cyclodextrins on the basic hydrolysis of carboxylic esters. The cleavage of aryl esters in basic aqueous solutions is accelerated by the presence of cyclodextrins.[8–10] The chemistry of carboxylic esters is often compared with the chemistry of alkyl nitrites (RONO). Specifically, while the acid catalyzed hydrolysis of alkyl nitrites is a very fast process,[11] the base catalyzed hydrolysis is a very slow one;[12] however, the contrary applies in the case of carboxylic esters, which hydrolyze faster in alkaline conditions than in acidic media.[13] This different behavior can be understood in light of the Lewis molecular structure for both families of compounds as shown in Scheme 1. The lone pair on the N atom, along with the higher electronegativity of N *vs.* C, explains not only why RONO have higher reactivity than carboxylic esters towards electrophiles, but also the difference in the chemistry of carboxylic esters, dominated by the formation of tetrahedral intermediates, and in the chemistry of RONO, which transfer the nitroso group (–N=O) intact. Both the acid and base catalyzed hydrolyses of alkyl nitrites take place through concerted mechanisms;[11,12] whereas the hydrolysis of esters proceeds *via* an addition-elimination pathway[13] in which the direct participation of a water molecule is



Scheme 1 (a) Mechanism of hydrolysis of alkyl nitrates. (b) Mechanism of hydrolysis of carboxylic esters.

required: in acid hydrolysis, the water molecule provides the OH^- ion and thus acts as a base, while in basic hydrolysis, the water molecule provides a H^+ and acts as an acid.

We recently reported the formation of 1 : 1 inclusion complexes between β-cyclodextrin (β-CD) and alkyl nitrites in aqueous media.[14,15] As a consequence, the acid hydrolysis of alkyl nitrites is strongly inhibited by the presence of β-CD. The lower reactivity, or nonreactivity, of the complexed alkyl nitrite was attributed to the restricted access of the H_3O^+ to the β-CD cavity since the inclusion of simple cations appears to be relatively unfavorable, except for large organic dyes,[16] long-chain surfactants[17] or metal ions with organic ligands.[18] On the other hand, the basic hydrolysis of alkyl nitrites is strongly catalyzed by ionized β-CD. As the pK_a of β-CD is 12.3,[19] working in an alkaline medium with $[OH^-] = 0.20$ M leads to all the β-CD molecules having an ionized secondary –OH group. The stability of the inclusion complex formed between ionized β-CD (CD^-) and an alkyl nitrite proves to be lower than the complex formed with the neutral β-CD and in some cases the differences are quite important.[15] Catalysis in alkaline conditions occurs because the complex is the transition state of the hydrolysis reaction; that is the nucleophilic catalysis of the *bimolecular* process RONO + OH^- becomes an *intramolecular catalysis* of the reaction through the complex $RONO \cdot CD^-$. In the present article, we report the results of kinetic studies of the β-CD mediated reactions of 1-phenyl-1-propyl (1P1P), 2-phenyl-1-propyl (2P1P) and 3-phenyl-1-propyl (3P1P) nitrites with OH^- and with pyrrolidine (PyR), piperidine (PiP) and *N*-methylcyclohexylamine (MCH) in basic media. The work was conceived as a comparative study of the results obtained with three guests of closely related structures in order to gain a better understanding of the inclusion process and the catalytic effect.

Experimental

1P1P, 2P1P and 3P1P nitrites were synthesized by treating the corresponding alcohols with sodium nitrite in aqueous sulfuric acid;[20] they were 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. All solutions were prepared with doubly distilled water obtained from a permanganate solution.

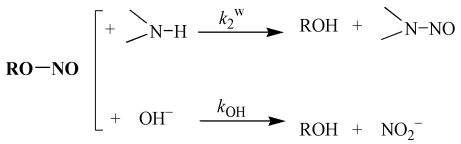
Kinetic experiments were monitored by using a Kontron–Uvikon (model 941) UV-VIS double beam spectrophotometer, provided with a multiple cell carrier thermostatted by circulating water. The consumption of alkyl nitrites was observed by recording decreasing absorbance in the 245–250 nm region. The kinetics of the nitrosation reaction of amines were studied by recording the increase in absorbance due to the formation of *N*-nitrosoamine (also in the 245–250 nm region). All experiments were performed at 25 °C.

Stock solutions of the alkyl nitrites were prepared in dioxane. Reactions were initiated by 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% by volume. 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 OH^- (or amine) concentration at least 50 times greater than that of the alkyl nitrite. For every case, the integrated method was followed, fitting the experimental absorbance-time data with 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, the values which were usually reproducible to within 2%.

1 Reaction in the absence of β-cyclodextrin

The NO transfer from alkyl nitrites in basic media is a much slower process than that which occurs in acid media. Nevertheless, NO transfer to an N-nucleophile, such as a secondary amine, can be a fast process, depending on the amine nucleophilicity.[21] The reaction between an alkyl nitrite and secondary amines in basic media yields stable *N*-nitrosamines; some of which are potential carcinogens. The reaction takes place through a concerted mechanism in which the NO group is transferred directly to an unprotonated amine molecule.

Fig. 1(a) shows the results obtained for the nitrosation of PyR, PiP and MCH by 3P1P nitrite, in a basic medium ($[OH^-] = 0.20$ M). For comparative purposes, Fig. 1(b) shows the plot of the observed rate constants obtained in the nitrosation of piperidine by the three alkyl nitrites considered in this study. The observed rate constant k_o ($=k_{OH}[OH^-] + k_2^w$ [amine]) increases linearly with the amine concentration, and, in the case of PyR, the intercept at the origin appears to be negligible; that is the reaction proceeds only up to the formation of *N*-nitrosopyrrolidine where the competitive reaction of the base catalyzed hydrolysis of RONO is insignificant (Scheme 2).



Scheme 2

For a given amine [see the case of PiP in Fig. 1(b)], the secondary alkyl nitrite 1P1P proves to be much more reactive

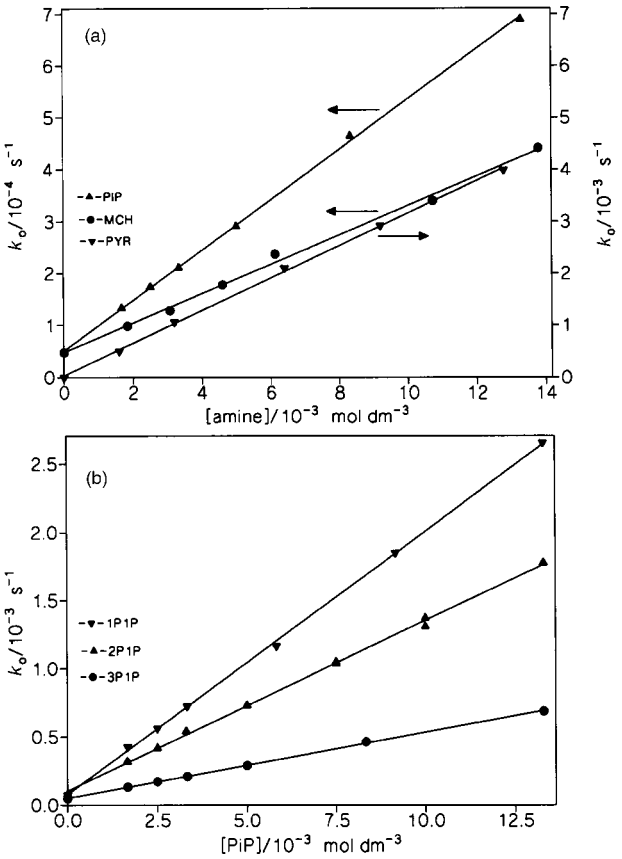


Fig. 1 (a) Influence of the amine concentration on the observed rate constant of the nitrosation by 3-phenyl-1-propylnitrite of pyrrolidine (PyR), piperidine (PiP), and *N*-methylcyclohexylamine (MCH) at $[OH^-] = 0.20$ M. (b) Variation of the observed rate constant as a function of [PiP] in the nitrosation of PiP by 3-phenyl-1-propyl (3P1P), 2-phenyl-1-propyl (2P1P), and 1-phenyl-1-propyl (1P1P) nitrites in aqueous alkaline solutions of $[OH^-] = 0.20$ M.

than the primary alkyl nitrite 3P1P. In general, reactivity is related to the stability of the leaving alkoxide in the rate controlling step. One would expect the leaving alkoxide to be strongly hydrogen-bonded to the solvent, and this strong solvation accounts for the highly negative value of the activation entropy.[21] Therefore, the more electronegative the R substituent (RO–NO), or the greater the stabilization (*e.g.* by the resonance effect) of the negative charge on the O atom, the faster the nitrosation reaction.

Table 1 reports the experimental results along with values of k_2^w/k_{OH}, which gives the ratio [nitrosamine]/[NO_2^-] that one would expect to obtain in a reaction mixture of equal amine and OH^- concentrations. In each trial, this ratio is favorable to nitrosamine formation; nevertheless, as the [OH^-] used here is always higher than the [amine] (from 13- to 120-fold), the hydrolysis process must be taken into account, mainly in those experiments carried out at low amine concentrations.

2 Reactions in the presence of β-CD

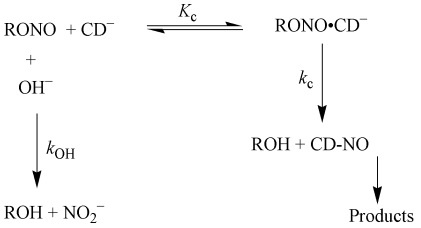
(A) Hydrolysis reaction

Alkaline hydrolysis of alkyl nitrites is an extremely slow process when no harsh conditions are used, such as moderate [OH^-]. The addition of β-CD strongly enhances the rate of the hydrolysis reaction. The degree of catalysis depends on the alkyl nitrite structure. Fig. 2(a) (solid points) shows the variation of the observed rate constant k_o obtained in the cleavage of 2P1P and 1P1P nitrites at [OH^-] = 0.20 M, as a function of β-CD concentration. The primary alkyl nitrite 2P1P experiences the largest catalytic effect (data for 3P1P not shown).

Kinetic experimental profiles of k_o *vs.* [β-CD] are explained quantitatively by considering the formation of reactive inclusion complexes between ionized β-CD molecules (CD^-) and the alkyl nitrite. The presence of saturation kinetics in every case is indicative of a 1 : 1 stoichiometry for the inclusion complex. Therefore, using Scheme 3 and taking into account that [RONO] ≪ [β-CD], we obtain eqn. (1) to relate values of k_o to [β-CD].

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

Solid lines in Fig. 2 (solid points) correspond to the theoretical fit of eqn. (1) to the experimental points. The resulting parameters of k_{OH} ($=k_o^w/[OH^-]$), k_c and K_c are listed in Table 2, along with values of k_2 ($=k_c K_c$), that is, the second-order rate constant for the reaction RONO + CD^- → *products*, which measures the ability of CD to select among different RONO under nonsaturating conditions. The highest k_2 value is observed with 2P1P nitrite and the smallest one with 1P1P nitrite. This finding illustrates the importance of the guest geometry in affecting the host in the reactivity of the complex. 2P1P possesses the most suitable structure for residing in the β-CD cavity in such a way that the reactive centers (–N=O in the alkyl nitrite and $–O^-$ in the β-CD) closely approach each other in a favorable configuration. Branching substituents attached to the C atom attached to the –NO group make the reaction difficult due to steric hindrance.



Scheme 3

The effect of DTABr. The catalysis observed with 1P1P nitrite is strongly magnified if the reaction is carried out in the presence of a fixed amount of dodecyltrimethylammonium

Table 1 Experimental conditions and bimolecular rate constants obtained in the basic hydrolysis (k_{OH}) and in the nitrosation of amines (k_2^w) by phenylpropyl nitrites in an aqueous alkaline medium with [OH^-] = 0.20 M

RONO	Amine	[Amine][a]/10^{-3} M	k_o^w[b]/10^{-4} s^{-1}	k_2^w/dm^3 mol^{-1} s^{-1}	k_2^w/k_{OH}
1P1P	PyR	1.5–13	0.61 ± 0.35	1.073 ± 0.005	3100
1P1P	PiP	1.6–13	0.8 ± 0.1	0.193 ± 0.002	550
1P1P	MCH	1.5–12	0.75 ± 0.06	0.092 ± 0.001	260
2P1P	PyR	1.0–13	0.65 ± 0.20	0.691 ± 0.007	2000
2P1P	PiP	1.5–13	0.80 ± 0.12	0.124 ± 0.002	350
2P1P	MCH	1.5–15	1.15 ± 0.09	0.055 ± 0.001	100
3P1P	PyR	1.5–13	0.83 ± 0.66	0.298 ± 0.006	1200
3P1P	PiP	1.5–13	0.50 ± 0.03	0.0485 ± 0.001	190
3P1P	MCH	1.5–13	0.48 ± 0.06	0.0283 ± 0.0008	110

[a] Range of [amine]. [b] $k_o^w = k_{OH}[OH^-]$.

Table 2 Parameters obtained in the study of the influence of β-CD concentration on the basic hydrolysis of phenylpropyl nitrites in an aqueous alkaline medium ([OH^-] = 0.20 M) at 25 °C by fitting the experimental data to eqn. (1)

RONO	[DTABr][a]/10^{-3} M	k_{OH}/10^{-4} dm^3 mol^{-1} s^{-1}	k_c/10^{-3} s^{-1}	K_c/dm^3 mol^{-1}	k_2/dm^3 mol^{-1} s^{-1}
3P1P	—	2.8	2.45 ± 0.05	315 ± 10	0.77
2P1P	—	3.5	3.43 ± 0.07	265 ± 13	0.91
2P2P[b]	—	Too slow	No effect	—	—
1P1P	0.0	3.25	0.72 ± 0.02	279 ± 22	0.20
1P1P	3.4	3.25	1.37 ± 0.04	295 ± 25[c]	0.40
1P1P	5.1	3.25	1.69 ± 0.04	246 ± 13[c]	0.41
1P1P	6.8	3.25	2.17 ± 0.06	179 ± 11[c]	0.39
1P1P	10.2	3.25	2.78 ± 0.08	137 ± 7[c]	0.38

[a] Concentration of dodecyltrimethylammonium bromide. [b] Ref. 15. [c] K_c^{ap}.

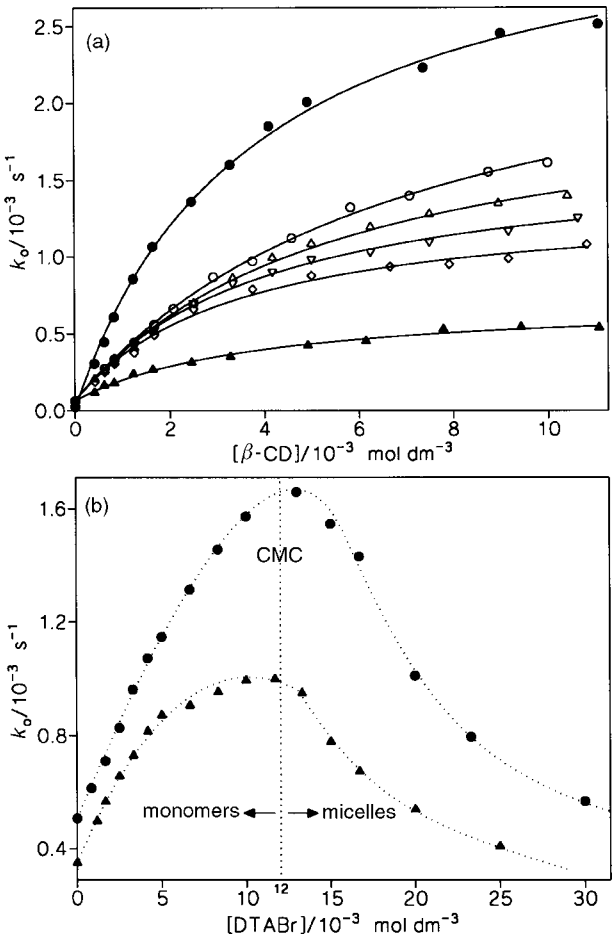


Fig. 2 (a) Variation of k_o as a function of [β-CD] in the basic hydrolysis ([OH^-] = 0.20 M) of 2P1P (●) and 1P1P nitrite (▲) and of 1P1P in the presence of different concentrations of DTABr monomer (◇) 3.4, (▽) 5.1, (△) 6.8 and (○) 10.2 mM. (b) Variation of k_o as a function of [DTABr] in the basic hydrolysis of 1P1P at [β-CD] of (●) 7.38 and (▲) 3.7 mM.

bromide (DTABr). Fig. 2(a) (open points) shows the variation of k_o as a function of [β-CD] in the presence of fixed DTABr concentrations below the CMC (critical micelle concentration: for this surfactant, CMC = 12 mM).[22] As one can see, the effect is greater if one increases both the β-CD and DTABr monomer concentrations. This behavior is better represented in Fig. 2(b), which shows the influence of [DTABr] on k_o at fixed [β-CD], and at surfactant concentrations below and above the CMC. Once micelles of DTABr are formed (above the CMC), the system becomes more complicated; the net observed effect represents an inhibition of the reaction because of the solubilization of the alkyl nitrite into the micelles, a process that favors the uncatalyzed reaction, which is much slower than the reaction mediated by β-CD.

In acidic media, DTABr monomers compete with the alkyl nitrite for the β-CD cavity by expelling the included RONO molecules.[14,15] If the same effect were to occur here, the addition of DTABr monomers would result in inhibition of the reaction because the RONO·CD^- complex is more reactive than free RONO. Nevertheless, the contrary is observed: addition of DTABr strongly catalyzes the basic hydrolysis of 1P1P. By fitting the experimental results obtained under these conditions to eqn. (1), the curves in Fig. 2(a) are drawn, and the obtained values of k_c and K_c^{ap} are also reported in Table 2. K_c^{ap} values decrease slightly on increasing the concentration of DTABr monomers, while k_c increases strongly with [DTABr]. The stability constant for the complex formation between β-CD and dodecyltrimethylammonium monomers is reported in the literature[23,24] as 3000 dm^3 mol^{-1}. Comparing this value with K_c values and taking into account that [RONO] is lower than [DTABr], one can explain the present results only by considering a case of *allostery* by analogy with enzymes.[25,26] In this sense, DTABr monomers may be seen as *modifiers* or as *effectors* that influence the catalytic efficiency of β-CD by inducing conformational changes or a more proper disposition of the guest, thus altering the reactivity of the complex. In fact, k_c increases linearly with DTABr concentration, being 4 times the value corresponding to the unmodified reaction (no added DTABr) at 0.01 M of DTABr monomer (the maximum monomer concentration possible); but k_2, the second-order rate constant for the reaction of complexed 1P1P with the host (ionized β-CD), doubles its value in the presence of a small amount of DTABr. This means a reduction of 1.5 kJ mol^{-1} in the activation energy of the reaction.

Since urea is an important protein denaturing agent, we also analyzed the influence of urea on the basic hydrolysis of 1P1P carried out in the presence of β-CD. We found no effect on reactivity, even at 3.0 M urea. Previous related results indicated that the presence of 7 M urea decreases the binding constant of *p*-toluidinonaphthalene sulphonate[27] to α-CD from 120 to 55 dm^3 mol^{-1}, and to β-CD, from 2200 to 95 dm^3 mol^{-1}. In contrast, the presence of 8 M urea increases the binding constant of 2-naphthoate anion[28] to β-CD from 320 to 3990 dm^3 mol^{-1}; thus, the observed effect seems to depend on the guest.

(B) Reaction with amines

Self-buffered solution of the amine. *Nitrosation of pyrrolidine.* The nitrosation of PyR by 3P1P and 1P1P nitrites under conditions of neutral β-CD, that is in aqueous buffered solutions of pyrrolidine–pyrrolidinium chloride of pH 11.20 and $[PyR]_t = 0.020$ M, has been studied. The observed rate constants k_o are displayed in Fig. 3 as a function of [β-CD]. While k_o appears to be unchanged by the presence of β-CD in the nitrosation by 3P1P nitrite, the reaction with 1P1P proves to be strongly inhibited by increasing amounts of β-CD. The difference in behavior between 3P1P and 1P1P nitrites could be explained if the former did not enter into the β-CD cavity, but this possibility must be ruled out because the acid hydrolysis of both alkyl nitrites is strongly inhibited by β-CD, from which experiments we determined the corresponding stability constants for complex formation: $K_c^N = 350$ dm^3 mol^{-1} for 3P1P and $K_c^N = 590$ dm^3 mol^{-1} for 1P1P.[15] (Notice that these equilibrium constants for complex formation between the alkyl nitrite and neutral β-CD are higher than those corresponding to the inclusion into the ionized β-CD, K_c; see Table 2.)

A reasonable explanation could be due to the possibility of H-bonding between RONO and C-2 or C-3 secondary –OH groups of neutral β-CD: R–O(NO)···H–O–, which is impossible with ionized β-CD.[7c] On the other hand, neutral PyR binds to β-CD very slightly; the stability constant for the complex formation was estimated as 6 dm^3 mol^{-1}.[14] Therefore, we can expect that neutral PyR would locate closely to the –OH groups on the wide rim of the β-CD molecule, interacting by H-bonding with these groups. Protonated hydrophilic amines are not seen to include into the hydrophobic β-CD cavity.[29] Following these considerations, the lack of influence of β-CD in the reaction of PyR + 3P1P could be understood if there were no remarkable difference between the reactivity of free and complexed 3P1P nitrites toward PyR. By considering the concentrations of both PyR (*i.e.* $[PyR]_t = [PyR]$) and β-CD forming complexes (because [RONO] ≪ [β-CD], then $[\beta\text{-CD}]_t = [\beta\text{-CD}]$) to be negligible, one arrives at eqn. (2) from Scheme 4, put forward for the case of 1P1P.

$$k_o = \frac{k_2^w + k_2^c K_c^N[\beta\text{-CD}]}{1 + K_c^N[\beta\text{-CD}]}[PyR]_n \qquad (2)$$

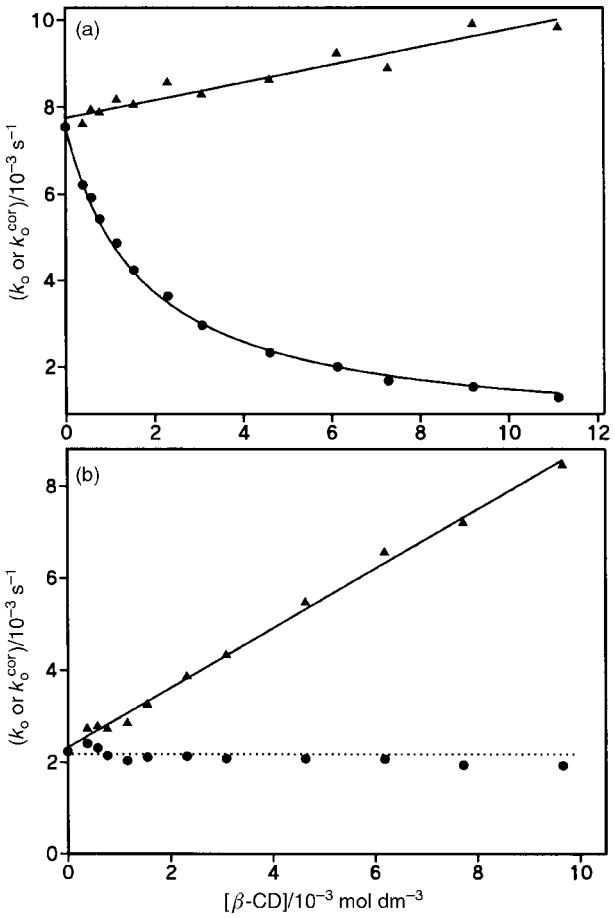
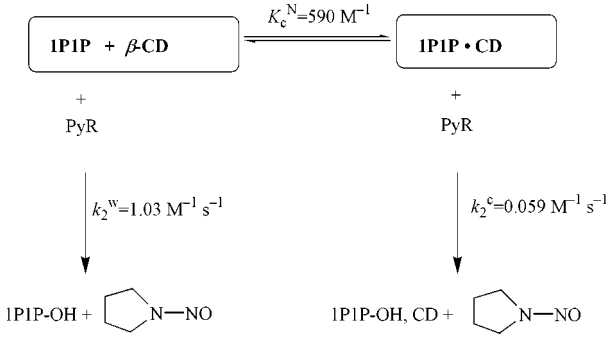


Fig. 3 Values of (●) k_o and (▲) k_o^{cor} $\{=k_o(1 + K_c^N[\beta\text{-CD}])\}$ as a function of [β-CD] obtained in the nitrosation of PyR by (a) 1P1P and (b) 3P1P in a buffer of pyrrolidine–pyrrolidinium chloride of pH 11.20 and $[PyR]_t = 0.020$ M. Solid lines fit eqn. (2); for parameters, see Table 3.



Scheme 4

In eqn. (2), $[PyR]_n$ refers to the unprotonated PyR, which was determined as $K_a[PyR]_t/(K_a + [H^+])$, with K_a being the acidity constant of pyrrolidinium cation ($pK_a = 11.40$)[30,31] and with k_2^w being the rate constant for the bimolecular reaction in water determined in the preceding section. Also shown in Fig. 3 is the plot of k_o^{cor} $\{=k_o(1 + K_c^N[\beta\text{-CD}])\}$ *vs.* [β-CD], resulting in a straight line, in agreement with eqn. (2). The linear regression analysis of the data gives values of (2.29 ± 0.07) and $(7.75 \pm 0.09) \times 10^{-3}$ s^{-1} for the intercepts $(=k_2^w[PyR]_n)$ and values of 0.66 ± 0.02 and 0.27 ± 0.03 dm^3 mol^{-1} s^{-1} for the corresponding slopes $(=k_2^c K_c^N[PyR]_n)$ of the plots obtained in the nitrosation of PyR by 3P1P and 1P1P nitrites, respectively. [In the case of 1P1P, the experimental data were also fitted to eqn. (2) by using a non-linear regression analysis. The values determined for the unknown parameters are independent of the fitting method].

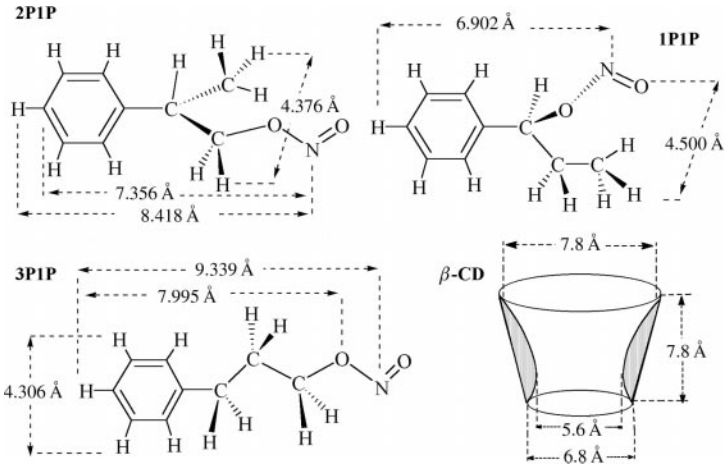
Using these results, along with the values of $[PyR]_n$ and K_c^N, one obtains the k_2^w and k_2^c values displayed in Table 3. The values of k_2^w compare quite well with those in Table 1, which were found by studying the reaction in the absence of β-CD. But the most striking feature of these results are the similar values determined for k_2^w and k_2^c in the case of 3P1P; the reactivity of this alkyl nitrite, free or complexed to β-CD, is practically the same. In contrast, the complexed 1P1P is more than 20-fold less reactive than free 1P1P nitrite. The explanation for this finding can be arrived at by looking at the structure and dimensions of a molecule of both alkyl nitrites in comparison to the size of the β-CD cavity: while the NO group in 3P1P must lie completely outside the β-CD cavity, which results in no difference in its reactivity, the same group in the case of 1P1P lies completely inside the β-CD cavity (see Scheme 5). Thus, the restricted access of PyR molecules to the β-CD interior, together with the low polarity of this microenvironment, makes the reaction slower than it is in water.

Nitrosation of piperidine. We also tested the reaction of 1P1P and 2P1P with PiP in a buffer of piperidine–piperidinium chloride of pH 11.02 and $[PiP]_t = 0.020$ or 0.033 M. The experimental results are shown in Fig. 4. The addition of β-CD strongly inhibits the nitrosation of PiP by 1P1P, but the observed pseudo-first order rate constant k_o goes through a maximum when the nitrosation is effectivated by 2P1P nitrite. Unlike PyR, neutral piperidine forms inclusion complexes with β-CD; the equilibrium constant for the complex stability has been reported[29] as $K_c^A = 50$ dm^3 mol^{-1}. Under the above experimental conditions, the neutral piperidine concentration is $[PiP]_n$ $\{=K_a[PiP]_t/(K_a + [H^+])\} = 7.15 \times 10^{-3}$ or 0.0124 M (with K_a being the acidity constant of piperidinium ion, $pK_a = 11.24$).[30,31] We must consider here the amount of β-CD forming complexes with neutral PiP, since both concentrations are comparable. (It should be noted that we do not consider the total PiP concentration, due to the

Table 3 Experimental conditions and rate constants obtained in the kinetic study of the influence of [β-CD] in the nitrosation of pyrrolidine and piperidine by phenyl-1-propyl nitrites in aqueous self-buffered solutions of the amine at pH 11.20 (for PyR) and pH 11.02 (for PiP)

RONO	Amine	$[Amine]_t$/M	K_c^N/dm^3 mol^{-1}	X^a/dm^3 mol^{-1} s^{-1}	$k_o^w/10^{-3}$ s^{-1}	k_2^w/ dm^3 mol^{-1} s^{-1}	k_2^c or $k_2^{c'}$[b]/ dm^3 mol^{-1} s^{-1}
3P1P	PyR	0.020	355[c]	0.66 ± 0.02	2.29 ± 0.07	0.296	0.243
1P1P	PyR	0.020	590[c]	0.27 ± 0.03	7.75 ± 0.09	1.03	0.045
			528 ± 15[d]				(0.059)
1P1P	PiP	0.020	590[c]	0.145 ± 0.013	1.49 ± 0.02	0.207	0.029
			582 ± 14[d]				(0.033)
2P1P	PiP	0.020	406[c]	0.75 ± 0.05	0.93 ± 0.02	0.129	0.218
			430 ± 34[d]				(0.216)
2P1P	PiP	0.033	406[c]	1.26 ± 0.07	1.82 ± 0.05	0.144	0.195
			435 ± 30[d]				(0.204)

[a] $X = k_2^c K_c^N[PyR]_n$ or $(k_2^c K_c^N + k_2^{c'} K_c^A)[PiP]_n$. [b] Values obtained from the linear plot of k_o^{cor} *vs.* [CD] (or from the nonlinear regression analysis of k_o *vs.* [CD]). [c] Determined in the acid hydrolysis[15] and used to draw the linear plots of k_o^{cor} *vs.* free [β-CD]. [d] Obtained by fitting k_o *vs.* [β-CD], eqn. (2) (PyR) or eqn. (4) (PiP) with $K_c^A = 50$ dm^3 mol^{-1}.



Scheme 5

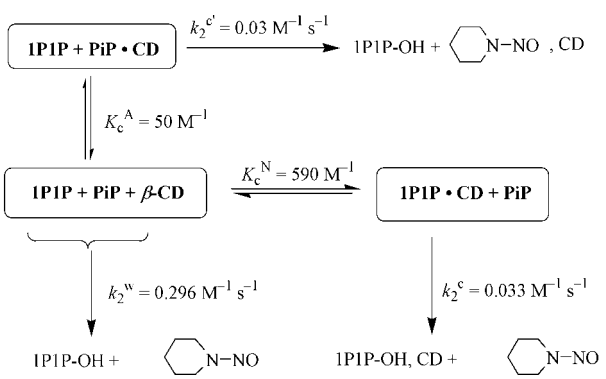
negligible binding of piperidinium cations with β-CD). Taking into account the mass balance equations $[\beta\text{-CD}]_t = [\text{CD}] + \beta\text{-CD}\cdot\text{PiP}]$ and $[\text{PiP}]_n = [\text{PiP}] + [\beta\text{-CD}\cdot\text{PiP}]$ and Scheme 6, one arrives at eqn. (3) to determine the free β-CD concentration ([CD]).

$$[\text{CD}]^2 + [\text{CD}]([\text{PiP}]_n + \frac{1}{K_c^A} - [\beta\text{-CD}]_t) - \frac{[\beta\text{-CD}]_t}{K_c^A} = 0 \quad (3)$$

By solving this second-order equation at each experimental $[\beta$-CD], one may calculate the free CD concentration, [CD]. Fitting the experimental data to eqn. (4) by using $K_c^A = 50$ dm^3 mol^{-1} provides the results given in Table 3.

$$k_o = \frac{k_2^w + (k_2^c K_c^N + k_2^{c'} K_c^A)[\text{CD}]}{(1 + K_c^N[\text{CD}])(1 + K_c^A[\text{CD}])}[\text{PiP}]_n \quad (4)$$

The values of k_2^w obtained from those of k_o^w $(=k_2^w[\text{PiP}]_n)$ agree perfectly with those in Table 1. In addition, the K_c^N values determined in this section, in aqueous buffered solutions of the amines at pH *ca.* 11, compare quite well with those determined in the study of the acid hydrolysis (aqueous solutions of acetic acid–acetate buffer of pH *ca.* 5). In Fig. 4 it is also evident that k_o^{cor} $\{=k_o(1 + K_c^N[\text{CD}])\cdot(1 + K_c^A[\text{CD}])\}$ increases linearly with [CD] when the nitrosation is carried out either by 1P1P or by 2P1P nitrites. This behavior is evidence that there is no reaction between the two complexed reactants (*vide infra*) and that the rate constants corresponding to either the reaction between PiP and complexed RONO (k_2^c) or between free RONO and complexed PiP ($k_2^{c'}$) should be comparable to the rate constant of the reaction between free reagents (k_2^w). Nevertheless, with 2P1P the bimolecular rate constant k_2^c (or its kinetically equivalent $k_2^{c'}$) should be slightly higher than k_2^w to be able to explain the smooth increase of k_o at low $[\beta$-CD], that is when the concentration of PiP·CD is not significant, but at higher $[\beta$-CD], when the complexed [PiP] becomes important, k_o decreases. In contrast, in the system PiP + 1P1P, k_2^c should be much smaller than k_2^w in order to be able to explain the strong inhibition. To determine k_2^c and $k_2^{c'}$, a reasonable assumption may be that both constants are equal; thus, the site of the reaction should not be very different. We then obtain the rate constants k_2^c (or $k_2^{c'}$) reported in Table 3. As expected, the reaction between complexed 1P1P (or PiP) and free PiP (or 1P1P) is nearly 7-fold slower than the reaction between free reagents; in contrast, the complexed 2P1P (or PiP) is nearly 2-fold more reactive towards free PiP (or 2P1P) than the free reagents. Possible reasons for these results are: firstly, the different behavior of the alkyl nitrites is a consequence of their structures, and secondly, the lower polarity and hydration of the reaction site, along with the restricted access of PiP molecules to the N atom of 1P1P included inside the β-CD cavity, explain the lower reactivity that results in the observed inhibition. However, we will present the results of the next section before further discussing this point.



Scheme 6

Nitrosation in alkaline solution. The influence of $[\beta$-CD] on the nitrosation of PyR, PiP, and MCH by phenylpropyl nitrites was also studied in an alkaline aqueous medium with $[\text{OH}^-] = 0.20$ M, that is under conditions where the majority of the β-CD molecules possesses an ionized secondary –OH group.

Reaction with pyrrolidine. The kinetic results obtained for the reaction of PyR with 1P1P and 3P1P nitrites are displayed in Fig. 5. In the case of 1P1P, the pseudo-first order rate constant decreases as $[\beta$-CD] increases, but the opposite occurs with 3P1P, with simple saturation kinetics resulting in both cases. The experimental behavior of k_o *vs.* $[\beta$-CD] obtained with 2P1P resembles that obtained with 3P1P, catalysis is observed. Nevertheless, the degree of catalysis (or inhibition, in the case of 1P1P) depends on the molecular structure of the alkyl nitrite. Table 4 (3rd column) shows the values of the observed rate constants obtained at the maximum $[\beta$-CD] used (*ca.* 0.010 M), k_o^{max}. The ratio k_o^{max}/k_o^w is indicative of the degree of catalysis (or inhibition). One may verify that k_o^{max}/k_o^w decreases as [PyR] increases for the cases of 2P1P and 3P1P, and at constant [amine] the ratio is higher with 3P1P than with 2P1P. The contrary applies to the case of 1P1P: the strongest inhibition effect is observed at $[\text{PyR}] = 0.010$ M and is practically negligible (no effect of β-CD addition) at $[\text{PyR}] = 1.5 \times 10^{-3}$ M.

Remembering that the stability constant of the inclusion complex formed between PyR and neutral β-CD has been estimated as $K_c^A = 6$ dm^3 mol^{-1}, and even working at $[\text{PyR}] = 0.015$ M (the maximum concentration used), one will find that the amount of amine forming inclusion complexes will be insignificant; nevertheless, we have to consider the

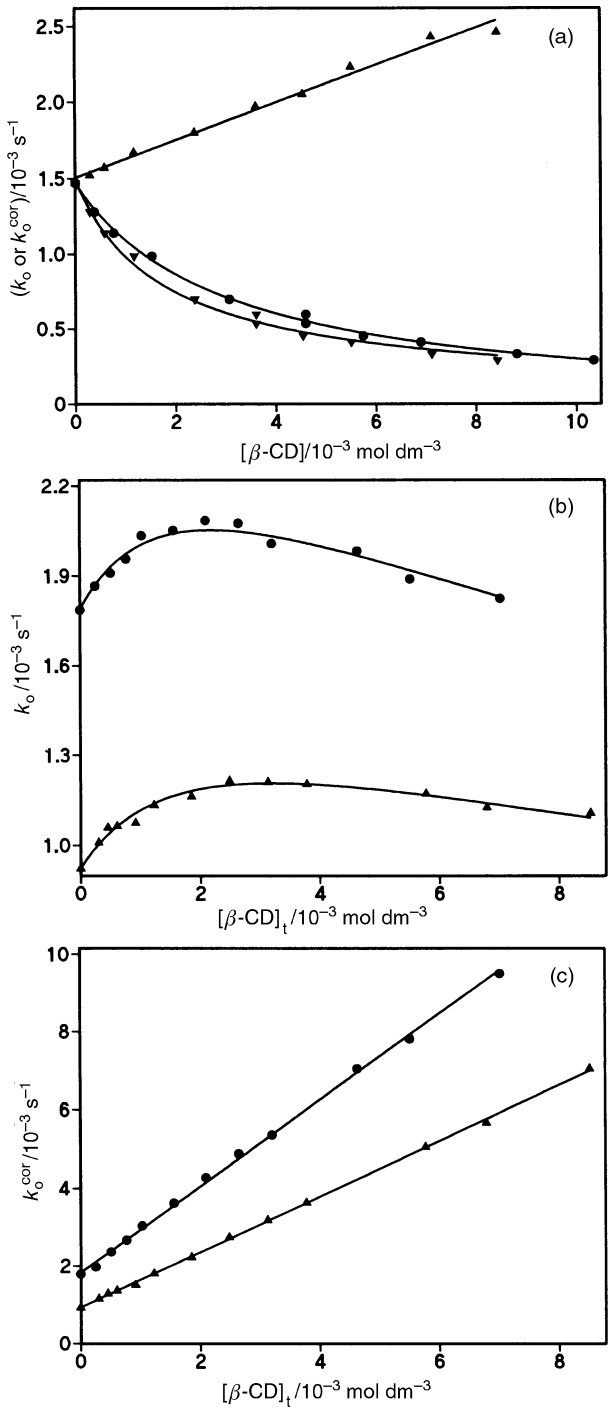


Fig. 4 (a) Values of (●) k_o and (▲) k_o^{cor} $\{=k_o(1 + K_c^N[\beta\text{-CD}])(1 + K_c^A[\beta\text{-CD}])\}$ obtained in the nitrosation of PiP by 1P1P in a buffer of piperidine–piperidinium chloride of pH 11.02 and $[PiP]_t = 0.020$ M as a function of $[\beta$-CD] and (▼) of free $[\beta$-CD]. Variation of (b) k_o, the pseudo-first order rate constant and of (c) k_o^{cor} obtained in the nitrosation of PiP by 2P1P in a buffer of piperidine–piperidinium chloride of pH 11.02 and $[PiP]_5 =$ (▲) 0.020 or (●) 0.033 M as a function of free $[\beta$-CD]. Solid lines are fits from eqn. (4), for parameters, see Table 3.

inclusion into the ionized β-CD of the alkyl nitrites studied here, whose equilibrium constants for complex formation are around 300 dm^3 mol^{-1} (see Table 2). In the presence of OH^- the basic hydrolysis reaction *via* free (k_{OH}) or complexed (k_c) RONO must be taken into account, along with the nitrosation process of PyR through free RONO (k_2^w) and the RONO·CD$^-$ complex (k_2^c). As we have shown in our study of the reaction in water, the reaction rate of the nitrosation process is much higher than that corresponding to the basic hydrolysis, which results in a simplification of the rate equation. On the basis of these considerations, eqn. (5) is deduced when one recalls that the rate of disappearance of RONO is the sum of the rate of the three steps (hydrolysis of complexed RONO, k_c, and nitrosation of PyR by free, k_2^w, and complexed, k_2^c RONO).

$$k_o = \frac{k_o^w + \alpha K_c[\beta\text{-CD}]}{1 + K_c[\beta\text{-CD}]} \quad (5)$$

with $\alpha = k_c + k_2^c[PyR]$ and $k_o^w = k_2^w[PyR]$.

When we fit the experimental data with eqn. (5), by using nonlinear regression analysis, we obtain the values of α and K_c reported in Table 4, by using the known values of k_o^w listed in the table. Solid lines in Fig. 5 correspond to the theoretical fit of the model to the experimental points. On the other hand, as eqn. (5) indicates, the graph of k_o^{cor} $\{=k_o(1 + K_c[\beta\text{-CD}])\}$ against $[\beta$-CD] should result in straight lines. To draw these plots, we used the K_c values determined from the basic hydrolysis, $K_c = 265$ or 300 dm^3 mol^{-1} for 1P1P and 3P1P, respectively. As is shown in Fig. 6, the observed experimental behavior is in accordance with eqn. (5). In addition, the values of α determined at each [PyR] (either from the slope of the linear plots or from the nonlinear regression analysis) should increase proportionally to [PyR]. This is indeed the experimental behavior observed. (Plots are not shown; instead, we report k_2^c values determined at each α–[PyR] pair.)

Viewing the results obtained, we must comment on the following. The rate constant of the reaction of complexed 2P1P (or 3P1P) with PyR, k_2^c, is practically equal to k_2^w, that is complexed alkyl nitrites react at the same rate as free RONO. The

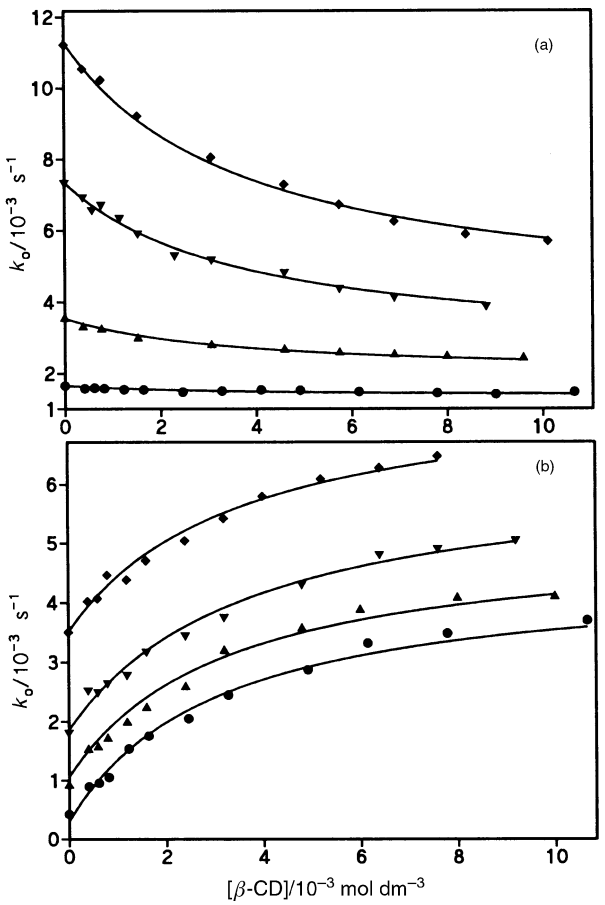


Fig. 5 Variation the observed rate constant k_o as a function of β-CD concentration obtained in the nitrosation in an alkaline medium ($[OH^-] = 0.20$ M) of pyrrolidine by (a) 1-phenyl-1-propyl nitrite at [PyR] equal to (●) 1.6, (▲) 3.2, (▼) 6.4 and (◆) 10 mM; and by (b) 3-phenyl-1-propyl nitrite at [PyR] of (●) 1.6, (▲) 3.2, (▼) 6.4 and (◆) 12.8 mM. Solid lines are fits to eqn. (5); for parameters, see Table 4.

Table 4 Experimental conditions used in the nitrosation of pyrrolidine in an aqueous alkaline medium ([OH^-] = 0.20 M) by 1P1P, 2P1P and 3P1P nitrites performed in the presence of β-CD and parameters obtained by fitting the experimental data to eqn. (5)

[PyR]/10^{-3} M	$k_o^w/10^{-3}$ s^{-1}	$k_o^{max\,a}/10^{-3}$ s^{-1}	$\alpha/10^{-3}$ s^{-1}	K_c/dm^3 mol^{-1}	k_2^w/dm^3 mol^{-1} s^{-1}	$k_c/10^{-3}$ s^{-1}	k_2^c/dm^3 mol^{-1} s^{-1}
1P1P							
1.5	1.51	1.41	1.3 ± 0.2	270 ± 30	1.03	0.72	0.387
3.2	3.54	2.42	1.93 ± 0.03	272 ± 13	1.11	0.72	0.378
6.4	7.35	3.89	2.52 ± 0.05	268 ± 9	1.16	0.72	0.281
10	11.2	5.63	3.81 ± 0.04	264 ± 9	1.12	0.72	0.309
2P1P							
1.6	1.04	3.41	4.39 ± 0.05	254 ± 12	0.65	3.43	0.619
3.2	1.96	4.6	5.69 ± 0.06	263 ± 8	0.61	3.43	0.707
3P1P							
1.6	0.42	4.01	4.65 ± 0.09	315 ± 14	0.237	4.2	0.281
3.2	0.85	4.07	5.14 ± 0.20	303 ± 32	0.267	4.2	0.294
6.4	1.8	5.74	6.18 ± 0.08	295 ± 13	0.281	4.2	0.309
12.8	3.52	6.60	7.68 ± 0.08	288 ± 15	0.275	4.2	0.272

[a] Value of k_o obtained at the highest β-CD concentration used (*ca.* 0.010 M).

same observation may be seen when 3P1P and neutral β-CD are used (see Table 3). In contrast, with 1P1P (a secondary alkyl nitrite) the reaction between the complex 1P1P · CD and PyR is nearly 3-fold slower than the reaction of PyR with free 1P1P, although the inhibition has greater importance when β-CD is not ionized because reaction in the CD anion is 7 times faster that in the neutral form: k_2^c(ionized) ≈ $7k_2^c$(neutral). These results account for the inhibition observed in the reaction of 1P1P + PyR on addition of β-CD. The catalysis observed with the other two alkyl nitrites is due to the hydrolysis of the complex (step k_c in Scheme 3), whose rate constant is comparable to the nitrosation rate constant, but the important association of these alkyl nitrites to β-CD makes the hydrolysis process more noticeable.

Piperidine and N-methylcyclohexylamine. Typical results obtained in the nitrosation of PiP by 2P1P and of MCH by 3P1P at [OH^-] = 0.20 M in the presence of β-CD are shown in Fig. 7(a) and 8 (insert), respectively. In every case a catalytic effect was observed with increasing amounts of β-CD. 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 that with PyR: K_c^A = 50 dm^3 mol^{-1} for PiP[29] and K_c^A = 550 dm^3

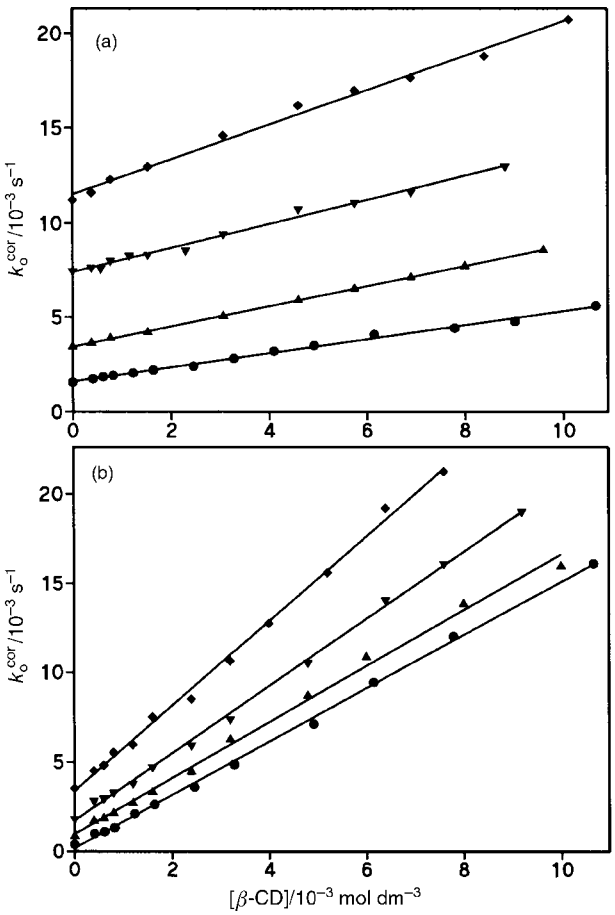


Fig. 6 Plot of k_o^{cor} {$=k_o(1 + K_c[\beta\text{-CD}])$} against [$\beta$-CD] obtained in the nitrosation of PyR at several [PyR] with (a) 1-phenyl-1-propyl nitrite (K_c = 265 dm^3 mol^{-1}) and (b) 3-phenyl-1-propyl nitrite (K_c = 300 dm^3 mol^{-1}). See Fig. 5 caption for the [PyR] values.

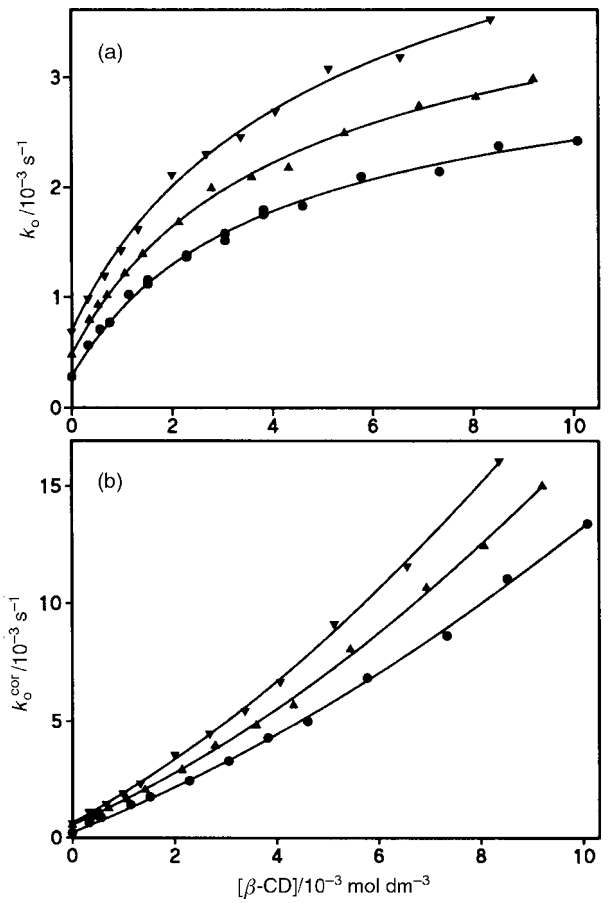
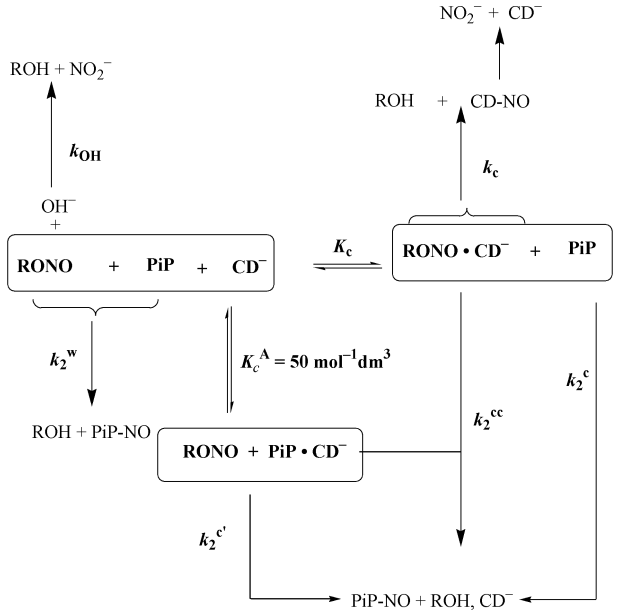


Fig. 7 (a) Variation of k_o as a function of free β-CD concentration obtained in the nitrosation in an aqueous alkaline medium ([OH^-] = 0.20 M) of piperidine by 2-phenyl-1-propylnitrite at [PiP] of (●) 1.67, (▲) 3.3 and (▼) 5.0 mM. (b) Plot of k_o^{cor} {$=k_o(1 + K_c[\beta\text{-CD}]_f)(1 + K_c^A[\beta\text{-CD}]_f)$} with K_c = 265 dm^3 mol^{-1} and K_c^A = 50 dm^3 mol^{-1}. Solid lines are fits to eqn. (6); for parameters see Table 5.

mol^{-1} for MCH.[32] Therefore, the amount of amine forming complexes is not negligible, but we may neglect the β-CD concentration forming complexes with the alkyl nitrite (due to the much lower concentration of the latter). From the corresponding mass balance equations, one may devise an equation of type (3) to ascertain the free [β-CD] at each stoichiometric value; it must be remembered that in the present experimental conditions all the amine is in the neutral form. {For the sake of comparison, we can see in Fig. 8 the values of k_o plotted against total β-CD concentration (insert) and against free [β-CD].}

A reaction mechanism is proposed here in Scheme 7 for the case of PiP, in which the following are represented: the basic hydrolysis reaction *via* either OH^- (k_{OH}) or ionized β-CD (k_c), the possibility of nitrosation reactions of free RONO with free amine (k_2^w), the complexed amine (PiP·CD) with the free RONO ($k_2^{c'}$), or its kinetically equivalent reaction of the free amine with the complexed RONO (k_2^c), and the complexed RONO and complexed amine (k_2^{cc}). The rate of the reaction is the sum of the six reaction steps; but, as with PyR, the reaction *via* k_{OH} may be ignored. Taking into account that the concentrations of the species are given by $[RONO]_t = [RONO] + [RONO \cdot CD]$; $[PiP]_t = [PiP] + [PiP \cdot CD]$, and $[CD]_t = [CD] + [PiP \cdot CD]$, the expressions of K_c and K_c^A given in Scheme 7, one arrives easily at eqn. (6), where $\gamma = k_c K_c + (k_2^c K_c + k_2^{c'} K_c^A)[PiP]_t$ and $\delta = k_c K_c K_c^A + k_2^{cc} K_c K_c^A [PiP]_t$ (or [MCH]) and [CD] represents free β-cyclodextrin concentration.



Scheme 7

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

Since k_o^w and the equilibrium constants are known, we can calculate γ and δ values by a nonlinear regression analysis of the experimental data k_o–[β-CD], fitted either to eqn. (6) or to its modification in the form of k_o^{cor} $\{=k_o(1 + K_c[CD])(1 + K_c^A[CD])\}$ *vs.* [CD]. Solid lines in Fig. 7(a) and 8 correspond to the fit to eqn. (6) of the experimental k_o values and [CD], and those in Fig. 7(b) correspond to the fit of k_o^{cor} $(=k_o^w + \gamma[CD] + \delta[CD]^2)$ against free [β-CD]. Values of γ and δ determined in the nitrosation of PiP are collected in Table 5, and those corresponding to the nitrosation of MCH are listed in Table 6, along with the experimental conditions and the input parameters used in the fitting process.

A comparison of the data plotted in Fig. 4 and 7(b), corresponding to the variation of k_o^{cor} as a function of free [β-CD] obtained in the nitrosation of PiP by 1P1P in a buffer of the amine (neutral CD) and by 2P1P in an alkaline medium, respectively, indicates that the δ parameter, for the reaction *via* both complexed substrates (RONO and PiP), is important only in alkaline medium and can be disregarded (the perfect straight line of k_o^{cor} *vs.* free [β-CD]) when β-CD is neutral. In addition, the plot of γ or δ (obtained in the nitrosation of MCH by 3P1P) against the amine concentration gives a per-

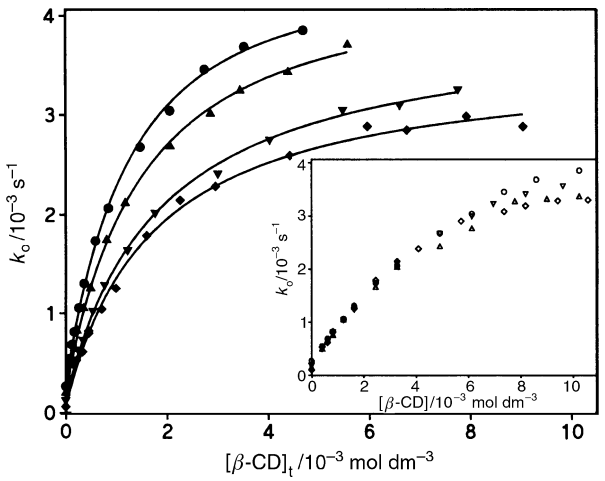


Fig. 8 Plot of k_o against free β-CD concentration obtained in the nitrosation in aqueous alkaline medium ($[OH^-] = 0.20$ M) of *N*-methylcyclohexylamine by 3-phenyl-1-propylnitrite at [MCH] of (●) 7.7, (▲) 5.4, (▼) 3.1 and (◆) 1.8 mM. (insert) Plot of k_o against total β-CD concentration. Solid lines are fits to eqn. (6); for parameters, see Table 6.

Table 5 Parameters obtained in the nitrosation of piperidine in an aqueous alkaline medium ($[OH^-] = 0.20$ M) by 3P1P, 2P1P and 1P1P nitrites performed in the presence of β-CD; rate and equilibrium constants obtained by fitting the experimental data to eqn. (6)

[PiP]/10^{-3} M	$k_o^w/10^{-3}$ s^{-1}	γ/dm^3 mol^{-1} s^{-1}	δ/dm^6 mol^{-2} s^{-1}	K_c/dm^3 mol^{-1}	K_c^A/dm^3 mol^{-1}	$k_c/10^{-3}$ s^{-1}	k_2^w/dm^3 mol^{-1} s^{-1}	k_2^c, $k_2^{c'}$/dm^3 mol^{-1} s^{-1}	k_2^{cc}/dm^3 mol^{-1} s^{-1}
3P1P									
1.67	0.133	1.04 ± 0.02	73 ± 4	294 ± 10	47 ± 3	4.2[a]	0.0485	0.153	0.884
3.30	0.215	1.16 ± 0.01	99 ± 2	314 ± 5	51 ± 2	3.1[b]	0.0485	0.153	0.884
5.80	0.334	1.26 ± 0.02	131 ± 5	318 ± 8	55 ± 3	3.2[c]	0.0485	0.153	0.884
2P1P									
1.67	0.28	0.89 ± 0.02	41 ± 4	308 ± 20	51 ± 9	3.4[a]	0.124	0.279	0.922
3.3	0.52	1.02 ± 0.02	62 ± 4	278 ± 15	51 ± 3	2.5[b]	0.124	0.279	0.922
5.0	0.69	1.21 ± 0.03	84 ± 7	284 ± 12	45.5 ± 5	1.4[c]	0.124	0.279	0.922
1P1P									
1.67	0.36	0.21 ± 0.01	14 ± 1	254 ± 15	54 ± 2	0.72[a]	0.193	0.037	0.20
3.3	0.65	~0.25	~16	265	50	0.72[a]	0.193	~0.057	~0.15

[a] Obtained in the basic hydrolysis, Table 3. [b] Obtained from the plot of γ *vs.* [PiP]: $\gamma = k_c K_c + (k_2^c K_c^A + k_2^{c'} K_c)[PiP]$. [c] Obtained from the intercept of the plot of δ *vs.* [PiP]: $\delta = k_c K_c K_c^A + k_2^{cc} K_c K_c^A [PiP]$.

Table 6 Parameters obtained in the nitrosation of *N*-methylcyclohexylamine in an aqueous alkaline medium ([OH^-] = 0.20 M) by 3P1P, 2P1P and 1P1P nitrites performed in the presence of β-CD; rate and equilibrium constants obtained by fitting the experimental data to eqn. (6)

[MCH]/10^{-3} M	$k_o^w/10^{-3}$ s^{-1}	$k_o^{max}/10^{-3}$ s^{-1}	γ/dm^3 mol^{-1} s^{-1}	δ/dm^6 mol^{-2} s^{-1}	K_c/dm^3 mol^{-1}	K_c^A/dm^3 mol^{-1}	$k_c/10^{-3}$ s^{-1}	k_2^c, $k_2^{c'}$/dm^3 mol^{-1} s^{-1}	k_2^{cc}/dm^3 mol^{-1} s^{-1}
3P1P									
1.84	0.062	2.83	1.79 ± 0.07	593 ± 20	307 ± 8	548 ± 13	2.6[a]	0.36	0.26
3.07	0.128	3.35	2.21 ± 0.05	651 ± 12	299 ± 5	551 ± 9	4.0[b]	0.36	0.26
5.37	0.199	3.70	2.98 ± 0.05	769 ± 17	315 ± 6	552 ± 10	3.0[c]	0.36	0.26
7.70	0.265	3.85	3.62 ± 0.06	854 ± 22	320 ± 15	580 ± 45	—	0.36	0.26
2P1P									
1.53	0.197	1.97	1.29 ± 0.07	349 ± 9	273 ± 12	552 ± 7	3.4[a]	0.53	0.30
3.07	0.291	2.59	1.87 ± 0.09	441 ± 14	250 ± 8	554 ± 16	2.3[b]	0.53	0.30
6.10	0.459	3.62	3.32 ± 0.04	584 ± 11	243 ± 10	512 ± 17	2.1[c]	0.53	0.30
7.67	0.624	3.70	3.86 ± 0.06	612 ± 25	295 ± 13	563 ± 21	—	0.53	0.30
1P1P									
3.07	0.282	0.915	0.62 ± 0.02	155 ± 6	264 ± 10	552 ± 19	0.72[d]	0.163	0.093
6.13	0.653	0.983	0.90 ± 0.03	168 ± 11	298 ± 20	565 ± 32	0.72[d]	0.163	0.061

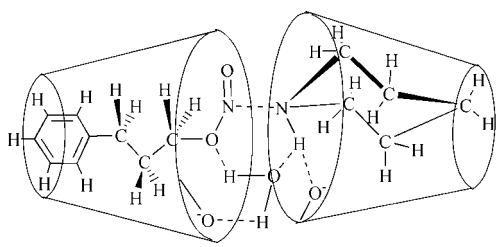
[a] Obtained in the basic hydrolysis, Table 3. [b] Obtained from the intercept of the γ *vs.* [MCH] plot $\{\gamma = k_c K_c + (k_2^c K_c + k_2^{c'} K_c^A)[MCH]\}$. [c] Obtained from the intercept of the δ *vs.* [MCH] plot ($\delta = k_c K_c K_c^A + k_2^{cc} K_c K_c^A[MCH]$). [d] Assumed value to determined k_2^c and k_2^{cc} from γ and δ values, respectively.

fectly straight line, as predicted by eqn. (6); the bimolecular rate constants k_2^c (or $k_2^{c'}$) and k_2^{cc} have been determined respectively from the slopes of the corresponding plots: γ (or δ) *vs.* [MCH] (or [PiP]) and their values are given in Tables 5 (for PiP) and 6 (for MCH).

Discussion

The most striking features of the results in Tables 4 to 6 are as follows. Free 1P1P is the most reactive alkyl nitrite towards all the amines studied here, but the reactivity of complexed 1P1P decreases with respect to that of uncomplexed substrate, and the decrease depends upon whether the β-CD molecule is neutral or ionized; k_2^c for the system PyR + 1P1P is more than 20-fold lower than k_2^w when the reaction occurs at pH 11.20 (neutral β-CD), but a reduction of only 3-fold is observed in alkaline conditions (ionized β-CD). In contrast, the reactivity of complexed 2P1P or 3P1P with PyR (k_2^c) is the same as that of the free substrate, while the reaction between complexed 2P1P or 3P1P with free PiP is faster than the reaction between free substrates. The enhancement is quite strong in the case of the complexed reactants with ionized β-CD, and the effect of increasing reactivity is even higher for the reaction between both complexed substrates (k_2^{cc}); a 10-fold increase is found in the systems 3P1P + MCH and 2P1P + PiP mediated by β-CD.

An increase in the rate constant of the rate determining step means a decrease in the activation energy; therefore, this must occur when the reaction goes through both complexed substrates. As the increasing reactivity of the complexed reagents is especially important when the complexation occurs with ionized β-CD, a particular way of fixing both reagents in the *transition state* of the reaction should take place with ionized β-CD. A possible explanation could be the formation of channel-like structures that, besides serving to fix the reacting species in close proximity, might intervene in the formation of the transition state. In the reaction of secondary amines with alkyl nitrites, a four-center transition state[33] has been postulated. Even the possibility of a six-membered ring, in which the participation of a water molecule is required, has been considered. The strong solvation of the leaving alkoxide undoubtedly plays a crucial role in the transition state of the process. The removal of the alcohol is particularly favored when the NO transfer to an amine is promoted by an alkyl nitrite (of suitable structure) inside the cavity of an ionized β-CD molecule. The ionized $-O^-$ of a CD molecule will form H bonds with either water molecules or with the amine also included in the CD cavity. This special arrangement of the reactants in the transition state, shown in Scheme 8 with a water molecule participating in the fixation of the reagents to the ionized host, should result in a strong decrease in the activation energy, even though the gain in the entropy of activation will not be negligible. Only a transition state like this, however, accounts for the higher k_2^{cc} values over those of k_2^w. Likewise, this picture for the transition state may explain the lower reactivity of 1P1P because, due to the hydrophobicity of the $-CH_2CH_3$ moiety, 1P1P is more deeply enclosed inside the β-CD cavity; thus an arrangement like that of Scheme 8 would force the ethyl moiety to leave the β-CD cavity. The proposed structure for the transition state also explains the much lower k_2^c values compared to k_2^w, and the absence of step k_2^{cc} when the reaction is studied in a buffer of the amine, that is to say in conditions of non-ionized β-CD. Finally, the structure of 3P1P appears the most suitable for producing a transition state like that described in Scheme 8; in fact, this alkyl nitrite, which is the least reactive in the absence of β-CD, shows the greatest catalytic effect in the presence of β-CD and it is higher with PiP than with MCH.



Scheme 8 Postulated transition state for the nitrosation between complexed PiP and 3P1P nitrite.

Conclusions

Ionized β-CD catalyzes the basic hydrolysis of alkyl nitrites. The present study reveals the crucial importance of the substrate structure to the degree of the observed catalysis, which is due to the formation of productive 1 : 1 inclusion complexes between RONO and ionized β-CD. The β-CD behavior in the catalysis of the process mimics the enzyme action, especially in the case of 1-phenyl-1-propyl nitrite: potential inhibitors, such as the monomer of dodecyltrimethylammonium bromide, influence the "*activity*" of β-CD in a clear case of *allostery*. Ionized β-CD also influences the nitrosation of PyR, PiP, and MCH by phenyl-1-propyl nitrites. The nitrosation of PyR by 3P1P and 2P1P is enhanced by the presence of β-CD, but it is inhibited when NO transfer to PyR is promoted by 1P1P. The

nitrosation of PiP and MCH is catalyzed by β-CD, no matter which alkyl nitrite transfers the nitroso group. The rate constant of the reaction of complexed amine with free 3P1P (or 2P1P), or of complexed RONO with free amine, or between both complexed reactants, proved to be much higher than the rate constant corresponding to the reaction between free substrates. Finally, comparison of the data obtained in the study of the nitrosation reaction carried out in buffers of its own amine (conditions of neutral β-CD) and in alkaline media leads us to describe important characteristics of the *transition state* of the reaction, in which the solvation of the alcoholic O atom, along with the participation of the ionized $-O^-$ group of a β-CD molecule that fixes the reactants in the highly ordered transition state, are assumed. All these characteristics are in agreement with the large negative entropies of activation found for the reaction in water and account for the higher values of the rate constants corresponding to the processes mediated by β-CD, as compared to the same processes occurring in water.

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