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Effect of cyclodextrin on the hydrolysis of the pesticide fenitrothion [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl)-phosphorothioate]†

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ABSTRACT: The hydrolysis of fenitrothion was studied at HO^- concentrations between 0.099 and 0.999 M. The second-order rate constant is 2.0×10^{-3} M^{-1} s^{-1}. The reaction is inhibited by β-cyclodextrin at HO^- concentrations of 0.5, 0.1 and 0.05 M. Saturation kinetics are observed in all cases. From the kinetic data, it is concluded that the main reaction pathway for the cyclodextrin-mediated reaction is the reaction of HO^- with the substrate complexed with the anion of β-cyclodextrin. The rate constant for this reaction, k_3, is about four times smaller than that for the reaction of the substrate with HO^- in the solvent. The inhibition is attributed to the inclusion of the substrate with the phosphate group in an orientation that is difficult to reach by the ionized secondary OH groups of the cyclodextrin and protected for the attack of an external OH. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: fenitrothion; hydrolysis; cyclodextrin; kinetics

INTRODUCTION

The vital role of organophosphorus and other classes of pesticides in increasing the world's food supplies is widely recognized.[1] However, many of them are highly toxic to humans; therefore, it is important to learn about their chemistry in order to determine their persistence in the environment and to find ways to destroy them rapidly and safely.

Fenitrothion [*O, O*-dimethyl *O*-(3-methyl-4-nitrophenyl)phosphorothioate], **1**, is used as a broad spectrum insecticide and acaricide,[2] but it is also classified as an anticholinesterase neurotoxic.[3] Hence the chemistry of **1** is important to a wide range of interests.

Cyclodextrins are cyclic oligomers of α-D-glucose which have a well defined cavity surrounded by the secondary OH in the wider rim and by the primary OH in the smaller rim.[4] They are soluble in water and the cavity is relatively non-polar compared with the solvent, so it provides a microenvironment for organic reactions. Cyclodextrins have attracted interest in several different areas, e.g. they have been used as models for enzyme-catalyzed reactions[5] and also for many industrial applications.[6]

We are particularly interested in the effect of cyclodextrins on the mechanism of hydrolysis of esters[7,8] and amides,[9] and therefore we undertook a study of the reaction of fenitrothion in basic aqueous solution in the presence of cyclodextrin.

Some reactions of organophosphorus compounds have been shown to be catalyzed by cyclodextrins[10,11] and some others, including that of fenitrothion, inhibited by this host.[12] We report here that the basic hydrolysis of this pesticide is inhibited by complexation with β-cyclodextrin (β-CD) mainly because the phosphate group is protected from external attack by HO^- groups. We also show that the usual pathway responsible for catalysis of ester hydrolysis does not take place in the case of this phosphate ester.

RESULTS

Rate constants, k_{obs}, for the hydrolysis reaction of **1** were measured spectrophotometrically under pseudo-first-order conditions at 25 °C at several NaOH concentrations and at constant ionic strength. The plot of k_{obs} vs HO^- is linear (Fig. 1) and from the slope the value of the second-

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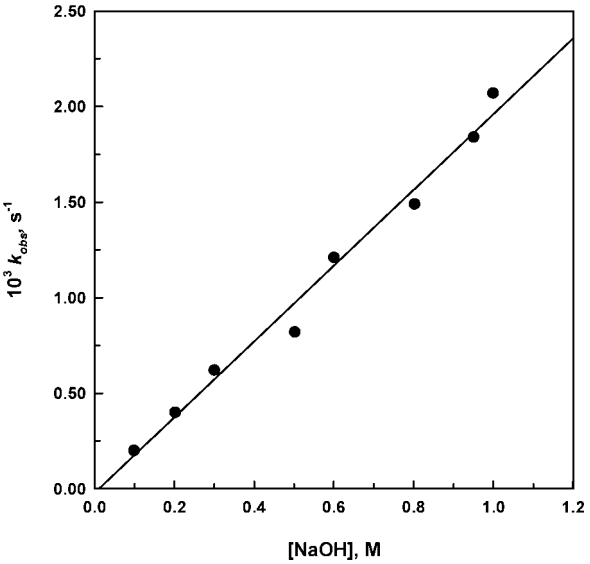


Figure 1. Plot of k_{obs} vs [NaOH] for the hydrolysis of **1** at 25 °C. Solvent contains 2% ACN; ionic strength $\mu = 1$ M (NaCl); $[\mathbf{1}]_0 = 5.37 \times 10^{-5}$ M

order rate constant $k_0 = (2.0 \pm 0.1) \times 10^{-3}\ M^{-1}\ s^{-1}$ was calculated. This value is in good agreement with one value reported [the second-order rate constant reported by Omakor *et al.*[13] is $2.79 \times 10^{-3}\ M^{-1}\ s^{-1}$ determined at pH between 10.9 and 12.1; we think that they obtained this value from a plot of the concentration of HO^- calculated as $10^{-(14-pH)}$, which is equivalent to $\log a_{OH}$, whereas we use HO^- concentration; hence in order to compare the two values, our rate constant must be divided by the activity coefficient of HO^-, which is 0.709[14]], but is somewhat lower than another value taken from the literature, $3.32 \times 10^{-3}\ M^{-1}\ s^{-1}$,[12] but the authors of the latter work reported that under their reaction conditions they found some deviation from strictly first-order behavior. As shown in Fig. 1, we do not observe any deviation up to 1 M NaOH concentration. We did, however, have problems in finding the right concentration of the substrate to study the kinetics because its solubility in water is very low. Kamiya and Nakamura[12] did not report the use of any organic co-solvent in their reactions, but we were unable to obtain stable solutions in pure water.

The effect of β-CD was determined at three HO^- concentrations and the data are summarized in Table 1. The addition of β-CD produces a decrease in the observed rate constants and shows a saturation effect (Fig. 2). In order to compare the results, the effects of 0.02 M α- and γ-CD at an HO^- concentration of 0.50 M were determined. The observed inhibition was 15, 67 and 40% for α-, β- and γ-CD, respectively (Table 2). The effect of a disaccharide, sucrose, in a weight amount equal to a solution 0.02 M in β-CD was also determined and the effect observed was about the same as that of γ-CD.

Under all our reaction conditions in solutions with or without cyclodextrin, the spectrum of the product matches that of 3-methyl-4-nitrophenoxide at the expected concentration; therefore, the only reaction taking place is P—O bond fission, Eqn. (1). It was previously demonstrated that above pH 8, the only reaction taking place is elimination of the phenol.[2]

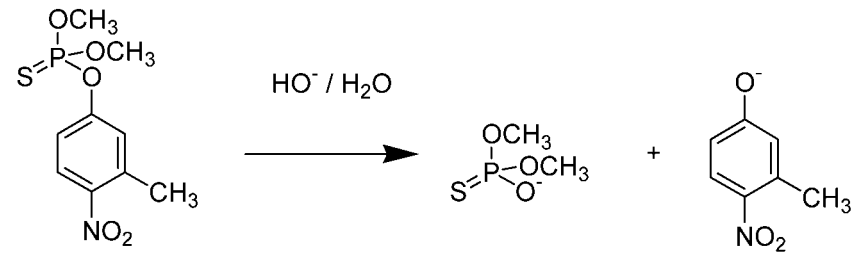
Table 1. Dependence on [β-CD] of observed rate constants for the hydrolysis of fenitrothion at 25 °C[a]

$[NaOH]_{eff}$ (M)[b]	10^2 [β-CD] (M)	$10^4\ k_{obs}$ (s^{-1})[c]
0.500	0.0506	9.4 ± 0.3
	0.100	8.6 ± 0.2
	0.100	5.0 ± 0.3
	0.151	5.2 ± 0.2
	0.200	7.2 ± 0.1
	0.250	6.5 ± 0.1
	0.300	6.6 ± 0.1
	0.381	5.2 ± 0.1
	0.438	5.5 ± 0.2
	0.560	3.8 ± 0.2
	0.740	3.6 ± 0.2
	0.920	3.76 ± 0.06
	0.920	3.4 ± 0.2
	1.00	4.5 ± 0.1
	1.28	3.56 ± 0.09
	1.64	3.45 ± 0.09
	2.00	3.24 ± 0.05
	2.50	3.23 ± 0.04
	3.00	3.20 ± 0.04
0.100	0.100	1.51 ± 0.02
	0.152	1.340 ± 0.006
	0.199	1.31 ± 0.01
	0.401	1.07 ± 0.02
	0.560	0.886 ± 0.007
	0.701	0.80 ± 0.01
	0.920	0.759 ± 0.007
	1.28	0.68 ± 0.01
	1.64	0.607 ± 0.007
	2.00	0.591 ± 0.002
0.050	0.101	0.72 ± 0.02
	0.150	0.65 ± 0.03
	0.300	0.526 ± 0.008
	0.701	0.335 ± 0.005
	1.00	0.277 ± 0.003
	1.50	0.236 ± 0.006

[a] Solvent contains 2% ACN; ionic strength $\mu = 1$ M (NaCl); $[\mathbf{1}]_0 = (5.34–7.07) \times 10^{-5}$ M.
[b] Calculated from the stoichiometric concentration of NaOH and β-CD and the pK_a of CDOH (12.2) taken from Ref. 15
[c] Errors are the standard deviation of the absorbance vs time plot from a single exponential.

DISCUSSION

The reaction studied was affected by the addition of β-CD at the three HO^- concentrations used. In order to determine the importance of the size of the cavity in the observed effect, the rate constants were determined in the presence of sucrose in similar weight concentration to that of β-CD. In Table 2 the data are collected and it can be seen that sucrose and γ-CD produce approximately the same inhibition and α-CD is the less effective, but in all



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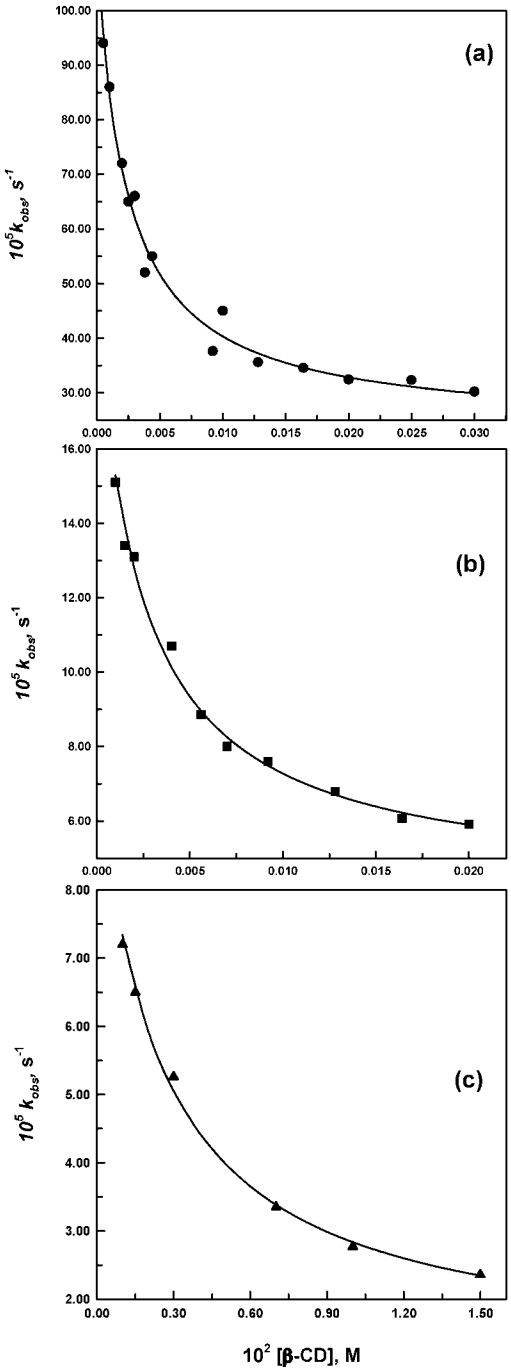


Figure 2. Plots of k_{obs} vs [β-CD] for the hydrolysis of **1** at 25 °C at constant [NaOH]. Solvent contains 2% ACN; ionic strength μ = 1 M (NaCl); $[\mathbf{1}]_0 = (5.34–7.07) \times 10^{-5}$ M. $[NaOH]_{eff}$ = (a) 0.500, (b) 0.100 and (c) 0.050 M

Table 2. Effect of α-, β- and γ-CD and sucrose on the hydrolysis of fenitrothion at 25 °C[a]

CD	10^2[CD] (M)	$10^4 k_{obs}$ (s^{-1})	Inhibition (%)
	0	9.9	
α-	2.00	8.4	15
β-	2.00	3.2	67
γ-	0.250–2.60	6.0[b]	40
Sucrose	—[c]	6.3	36

[a] Solvent contains 2% ACN; ionic strength μ = 1 M (NaCl); $[\mathbf{1}]_0 = (5.34–7.07) \times 10^{-5}$ M; $[NaOH]_{eff}$ = 0.500 M.
[b] Represents the average value of the rate constants obtained at six different HO^- concentrations.
[c] Weight amount equal to a solution 0.02 M in β-CD.

cases the effect is smaller than that for β-CD. The inhibition of the reaction by sucrose can be attributed to a medium effect or to some type of unspecific association of the substrate with the sugar. We do not think that the effect is due to changes in pH because at the high OH^- concentration used, the consumption of hydroxide ion due to reaction with the HO of the sugar is very small since the pK_a of sucrose is not expected to be lower than that of β-CD (12.2).[15] On the other hand, the effect of β-CD should be attributed to specific interactions. The addition of β-CD to a solution of **1** gives an induced circular dichroism spectrum (not shown) similar to that reported in the literature[12] (a positive peak at the same wavelength as reported, i.e. 269 nm,[12] is obtained but we could not measure the negative peak, which should be obtained at about 218 nm, because the instrument was too noisy at this short wavelength), indicating that the substrate forms an inclusion complex.[16] From the circular dichroism spectrum Kamiya *et al.*[12] concluded that the compound is included in the cavity of cyclodextrin as shown in Fig. 3 with the nitro group inside the cavity and with an angle of 19° between the longitudinal axis of 4-nitrophenoxy moiety (arrow A in Fig. 3) relative to the symmetry axis of the cyclodextrin cavity (arrow B in Fig. 3). The structure of compound **1** shown in Figure 3 is the optimized geometry obtained by first executing MM2 minimization and then minimizing the conformational energy using the quantum chemistry program MOPAC [these calculations were done with the software included in Chem3D Pro, version 5.0 (CambridgeSoft)].

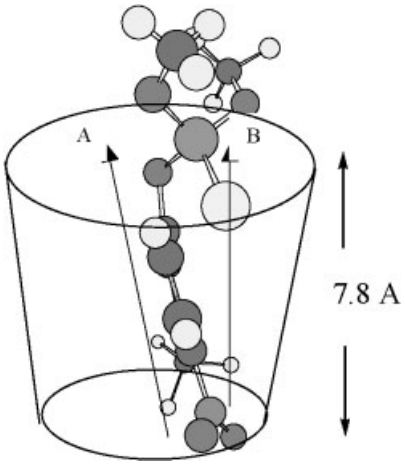


Figure 3. Three-dimensional representation of **1** as calculated by MOPAC with cyclodextrin represented schematically. Arrow A represents the longitudinal axis of the *p*-nitrophenyl moiety of the molecule and arrow B represents the axis in the center of the cavity pointing from the smaller to the larger rim of β-CD

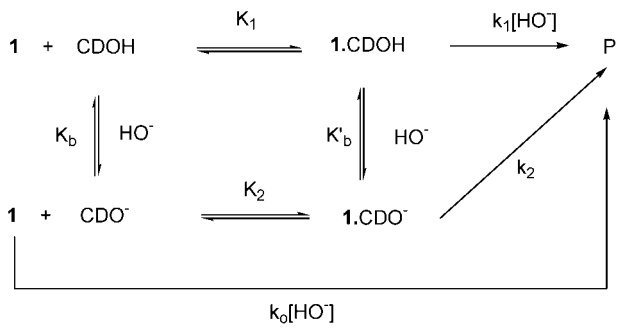
The pK_a of β-CD is 12.2[15] so, as demonstrated in previous work with other substrates,[17] the reaction may take place as indicated in Scheme 1, where k_0, k_1 and k_2 represent the reactions of the free substrate, the substrate complexed with neutral β-CD (CDOH) and the reaction with ionized β-CD (CDO^-), respectively.

The observed rate constant for Scheme 1 is given by Eqn. (2), where *f* represents the fraction of ionized β-CD as defined in Eqn. (3) with $K_b = K_w/K_a$ and CD is the stoichiometric concentration of β-CD. Under the condition of a constant HO^- concentration of 0.50 M, f $\approx$ 1; then Eqn. (2) simplifies to Eqn. (4).

$$k_{obs} = \frac{k_0[HO^-] + k_1K_1[HO^-](1-f)[CD] + k_2K_2f[CD]}{1 + K_1(1-f)[CD] + K_2f[CD]} \quad (2)$$

$$f = \frac{[HO^-]}{[HO^-] + K_b} \quad (3)$$

$$k_{obs} = \frac{k_0[HO^-] + k_2K_2[CD]}{1 + K_2[CD]} \quad (4)$$



Scheme 1

The reactions in the presence of β-CD were studied at three HO^- concentrations, i.e. 0.050, 0.100 and 0.500 M. For each HO^- concentration, the observed rate constant was fitted to an equation of the form of Eqn. (5) with $a = k_0[HO^-]$, *b* and *c* being adjustable parameters given in Eqns (6) and (7):

$$k_{obs} = \frac{a + b[\beta - CD]}{1 + c[\beta - CD]} \quad (5)$$

$$b = (k_1K_1[HO^-](1-f) + k_2K_2f) = (k_1K_1K_b + k_2K_2)f \quad (6)$$

$$c = K_1(1-f) + K_2f \quad (7)$$

The values of *a, b* and *c* obtained using Eqn. (5) to fit the data (non-linear fitting was carried out using the software included in SigmaPlot, version 3.02) are given in Table 3, where the experimentally determined value of $k_1[HO^-]$ is also shown.

Within experimental error, the value of *c* is about the same at the three HO^- concentrations, indicating that K_2 is not significantly different from K_1 and it is in good agreement with the value reported in the literature, namely 417 M^{-1} s^{-1}.[12]

The parameter *b* divided by *f* should be constant [see Eqn. (6)]. It can be seen in the last column of Table 3 that *b/f* increases with increase in HO^- concentration, indicating that there must be a pathway involving another HO^-. Hence we should add to the mechanism of Scheme 1 the reaction of the substrate complexed with CDO^- reacting with HO^- to give the products P with rate constant k_3 [Eqn. (8)]:

$$\mathbf{1}.CDO^- + HO^- \xrightarrow{k_3} P \quad (8)$$

If reaction (8) takes place, a term $k_3K_2[HO^-]$ should be included in parameter *b* [Eqn. (9)]:

$$b = (k_1K_1K_b + k_2K_2 + k_3K_2\lfloor HO^- \rfloor)f \quad (9)$$

A plot of *b/f* vs HO^- concentration (not shown) is linear with slope 0.22 M^{-2} s^{-1}. The intercept of this line is indistinguishable from zero within experimental error, indicating that Eqn. (8) is the main reaction pathway. Taking the value K_2 as the value of *c* at 0.5 M HO^-, k_3 is calculated as 5.30×10^{-4} M^{-1} s^{-1}, which is about four times smaller than the value for the rate constant for the reaction of the free substrate, k_0. This may be attributed in part to electrostatic repulsion of the negative hydroxide ion and in part to steric hindrance to nucleophilic attack imposed by the cyclodextrin rim. The fact that the main reaction pathway for the cyclodextrin-mediated reaction is the reaction of HO^- with the complexed substrate and that there appears to be no significant nucleophilic reaction of the secondary OH of the cyclodextrin may

Table 3. Parameters of Eqn. (5) for the hydrolysis of fenitrothion in the presence of β-CD at 25 °C

$[NaOH]_{eff}$ (M)	$10^4 a$	b	c	f	b/f
0.500	11.0 ± 0.5 (10)[a]	0.10 ± 0.02	415 ± 72	1.00	0.10
0.100	1.87 ± 0.08 (2.0)[a]	0.012 ± 0.002	326 ± 20	0.86	0.014
0.050	0.92 ± 0.03 (1.0)[a]	0.002 ± 0.001	311 ± 52	0.76	0.0026

[a] Values in parenthesis are the second-order rate constants for the hydrolysis of **1** in the absence of β-CD times HO^- concentration.

indicate that in the structure of the complex the phosphate group is not at an appropriate distance from the ionized secondary OH of the cyclodextrin rim.

We conclude that fenitrothion forms a complex with β-CD which is less reactive towards hydrolysis than the substrate itself because the phosphate group is buried in the cavity of the cyclodextrin so that it is protected from nucleophilic attack.

EXPERIMENTAL

Materials. Fenitrothion was isolated from a commercial sample of Sumithion (Sumitomo Chemical) by column chromatography over silica gel as a yellow oil. It was identified by ^{1}H NMR spectroscopy and GC–MS.

β-Cyclodextrin (Roquette) (a gift from Ferromet, Buenos Aires, Argentina), α-cyclodextrin (Aldrich) and γ-cyclodextrin (a gift from Cerestar) were used as received, but the purity was periodically checked by UV spectroscopy.

Aqueous solutions were prepared using water purified with a Millipore Milli-Q apparatus. Acetonitrile (Sintorgan, HPLC grade) was used as received.

All of the inorganic reagents were of analytical-reagent grade and were used without further purification.

UV spectra and kinetic measurements were recorded on a Shimadzu UV-2101 spectrophotometer and the change in absorbance during a kinetic run was measured on the same instrument. Circular dichroism spectra were recorded on a JASCO Model J-810 spectropolarimeter which was calibrated with 10-camphorsulfonic acid. The circular dichroism measurements were always made using the free β-CD solution as a reference system. NMR spectra were recorded on a Bruker ACE 200 instrument and GC–MS was performed on a Shimadzu Model CQ5050 instrument.

Kinetic procedures. Reactions were initiated by adding the substrate dissolved in ACN to a solution containing all the other constituents. The reaction temperature was 25.0 ± 0.1 °C, the ionic strength was 1 M and NaCl was used throughout as compensating electrolyte. The solvent contained 2% ACN.

All kinetic runs were carried out under pseudo-first-order conditions, with substrate concentrations of about $(5–7) \times 10^{-5}$ M.

The reactions were followed by measuring the increase in absorbance of the reaction mixture at 397 nm, the λ_{max} of the phenoxide formed. The solutions were maintained in the dark during the kinetic run in order to diminish photolytic effects.

The $[HO^-]_{eff}$ values reported in Tables 1 and 2 and Fig. 2 were calculated from the stoichiometric concentration of NaOH and β-CD and the pK_a of CDOH (12.2).[15]

Acknowledgements

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