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Reactivity of the insecticide chlorpyrifos-methyl toward hydroxyl and perhydroxyl ion. Effect of cyclodextrins

Raquel V. Vico[a], Rita H. de Rossi[a] and Elba I. Buján[a]*

The reactivity of Chlorpyrifos-Methyl (1) toward hydroxyl ion and the α-nucleophile, perhydroxyl ion was investigated in aqueous basic media. The hydrolysis of 1 was studied at 25 °C in water containing 10% ACN or 7% 1,4-dioxane at NaOH concentrations between 0.01 and 0.6 M; the second-order rate constant is 1.88×10^{-2} M^{-1} s^{-1} in 10% ACN and 1.70×10^{-2} M^{-1} s^{-1} in 7% 1,4-dioxane. The reaction with H_2O_2 was studied in a pH range from 9.14 to 12.40 in 7% 1,4-dioxane/H_2O; the second-order rate constant for the reaction of HOO^- ion is 7.9 M^{-1} s^{-1} whereas neutral H_2O_2 does not compete as nucleophile. In all cases quantitative formation of 3,5,6-trichloro-2-pyridinol (3) was observed indicating an $S_N2(P)$ pathway. The hydrolysis reaction is inhibited by α-, β-, and γ-cyclodextrin showing saturation kinetics; the greater inhibition is produced by γ-cyclodextrin. The reaction with hydrogen peroxide is weakly inhibited by α- and β-cyclodextrin (β-CD), whereas γ-cyclodextrin produces a greater inhibition and saturation kinetics. The kinetic data obtained in the presence of β- or γ-cyclodextrin for the reaction with hydroxyl or perhydroxyl ion indicate that the main reaction pathway for the cyclodextrin-mediated reaction is the reaction of HO^- or HOO^- ion with the substrate complexed with the anion of the cyclodextrin. The inhibition is attributed to the inclusion of the substrate with the reaction center far from the ionized secondary OH groups of the cyclodextrin and protected from external attack of the nucleophile. Sucrose also inhibits the hydrolysis reaction but the effect is independent of its concentration.

Supporting information may be found in the online version of this article.

Keywords: organophosphorus insecticides; cyclodextrins; host–guest systems; hydrolysis; hydrogen peroxide; reaction mechanisms; inclusion complexes

INTRODUCTION

Organophosphorus compounds are an economically important class of chemical compounds with numerous uses, such as pesticides, industrial fluids, flame retardants, therapeutics, and nerve agents. Many of the synthetic organophosphorus compounds are tailor-made to inhibit acetylcholinesterase (AChE), an enzyme essential for life in humans and other animal species.[1,2] Hence the chemistry of organophosphorous compounds is important to a wide range of interests. Available methods for destroying organophosphorous compounds include thermic, enzymatic, or photochemical degradation as well as reactions with strong oxidants or nucleophiles.[1–6] Detailed knowledge of the kinetics of the alkaline and neutral hydrolysis pathways is critical to several areas of environmental chemistry;[7] they are particularly important to understand their persistence and fate in natural environments and to find ways to destroy them rapidly and safely. Hydrogen peroxide has been used for decontamination of chemical warfare agents,[1,2] and in advanced oxidation processes for environmental remediation, such as Fenton and photo Fenton.[8–11] This oxidant has the advantage over others that is environment friendly leading in some cases to the total mineralization of the organic pollutant.[8]

Chlorpyrifos-Methyl (*O,O*-dimethyl *O*-(3,5,6-trichloro-2-pyridynil)-phosphorothioate) (**1**) is a cholinesterase inhibiting pesticide registered for use in the control of insect pests on certain stored grain, including wheat, barley, oats, rice, and sorghum as well as for empty grain bins.[12] Annual usage of Chlorpyrifos-Methyl is an estimated 80 000 pounds active ingredient for approximately 267 497 000 bushels of grain.[12] 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 primary OH in the smaller rim.[13–15] They are soluble in water and the cavity is relatively non-polar compared with the solvent, so it provides a special microenvironment for organic reactions. Cyclodextrins have attracted interest in several different areas, e.g., they have been used as models for enzyme-catalysed reactions[16–19] and also for many industrial applications.[20]

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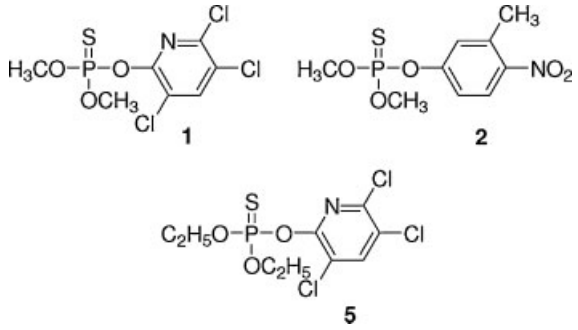
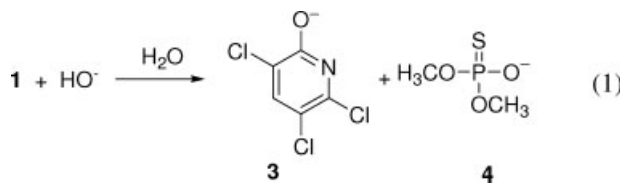


We are particularly interested in the effect of cyclodextrins on the mechanism of hydrolysis of esters[21–23] and amides,[24] and therefore we undertook a study of the reaction of phosphorothioates in basic aqueous solution in the presence of cyclodextrins.[25] We have previously reported that the basic hydrolysis of Fenitrothion (*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl)-phosphorothioate) (**2**) is inhibited by complexation with β-cyclodextrin (β-CD) and that the main reaction pathway for the cyclodextrin-mediated reaction is the reaction of HO^- ion with the substrate complexed with the anion of β-CD.[25] Since **1** is an insecticide of the same family as **2**, we considered of interest to study its hydrolysis, the reaction with hydrogen peroxide and the effect of cyclodextrins on them. We found that both compounds behave in a similar way although **1** is more reactive and HOO^- is more than 400 times more reactive than HO^- toward **1**. The hydrolysis of **1** is inhibited in different magnitude by complexation with β- and γ-CD with saturation kinetics whereas the reaction with hydrogen peroxide is only inhibited with saturation kinetics by γ-CD. The difference in the magnitude of the inhibition observed with the two CDs is due to differences in the binding abilities of the two hosts with the guest.

RESULTS AND DISCUSSION

Hydrolysis reaction

The hydrolysis of **1** was studied under pseudo-first-order conditions in 10% ACN/H_2O at 25 °C at several NaOH concentrations and at constant ionic strength ($I = 0.6$ M). The UV–Vis spectrum of the product matches that of 3,5,6-trichloro-2-pyridinol (**3**) at the expected concentration; therefore, the only reaction taking place is P—O bond fission, (Eqn (1)). Some authors have previously demonstrated for Chlorpyrifos (**5**), that above pH 8, the only reaction that occurs is elimination of the pyridinol.[7,26] Jans and coworkers[27] had reported on the hydrolysis of **1** between pH 5.4 and 9.7 indicating that the observed hydrolysis rate is independent of pH over the range 5.4–8.6, and that at pH 9.0, formation of **3** accounts for 76% of the substrate that reacted, desmethyl chlorpyrifos-methyl being the other product formed by attack of H_2O on the aliphatic α-carbon.



Our results were confirmed by ^{31}P NMR. The degradation of a 1×10^{-3} M solution of **1** in 1,4-dioxane/D_2O/H_2O (20:40:40) in the presence of 0.6 M NaOH was followed by ^{31}P NMR over a period of 6 h. The initial reading showed a single peak at 64.42 ppm, corresponding to **1**. After 3 h of reaction a single signal was observed at 58.23 ppm corresponding to *O,O*-dimethylphosporothioate (**4**),[28,29] this signal remain unchanged over the period of time studied. (Fig. 1).

The kinetics of the hydrolysis of **1** was followed by measuring the increase in absorbance at 320 nm, the λ_{max} of **3**. The observed rate constants for this reaction are shown in Table S1. The plot of k_{obs} *versus* HO^- concentration is linear (Fig. 2) and from the slope the value of the second-order rate constant $k_{OH} = (1.88 \pm 0.05) \times 10^{-2}\ M^{-1}\ s^{-1}$ was calculated. The intercept of this plot is equal to zero, within experimental error, indicating that H_2O does not compete with HO^- ion as nucleophile; this is in agreement with the fact that $t_{1/2}$ for the reaction of **5** in natural waters ranges from 19 to 80 days,[7,26,30] and $t_{1/2}$ reported for the hydrolysis of **1** at neutral pH ranges from 8 to 23 days.[31] The value of k_{OH} calculated for the hydrolysis of **1** is one order of magnitude higher than that for **2**, i.e., $(2.0 \pm 0.1) \times 10^{-3}\ M^{-1}\ s^{-1}$.[25] The difference in reactivity of the two insecticides, **1** and **2**, can be explained by the difference in pK_a of their leaving groups. The pK_a of 3-methyl-4-nitrophenol, the leaving group of **2**, is 7.26,[32] and that of **3**, determined by us, is 4.77 (Lit.[26] 4.55). Similar leaving group effect was observed previously in the reaction of PhO^- with a series of substituted dimethyl posphorothioates.[33] Besides, it is a well documented fact that in many families of esters, their reactivity increases with the decrease in the pK_a of the leaving group.[34–37]

Other factors that may contribute to the difference in reactivity observed between **1** and **2** are the ionic strength and the organic solvent content in the reaction medium. The hydrolysis of **2**[25] was studied at $I = 1$ M and 2% ACN/H_2O whereas for **1** it was necessary to work at $I = 0.6$ M and 10% ACN/H_2O due to its lower solubility in water. It was previously reported that **5** is less soluble in sea waters than in distilled water and the rate of hydrolysis in natural waters is highly dependent on the medium salinity.[26] Williams and coworkers[38] informed that the second-order rate constant for the alkaline hydrolysis of 4-nitrophenyl diphenyl phosphate increases by a factor of 1.6 as the ionic strength of the reaction medium decreases from 0.5 to 0.1 M. On the other hand, the increase in polar aprotic solvent content produces an increase in the reactivity of some phosphate and phosphorothioate esters.[39,40]

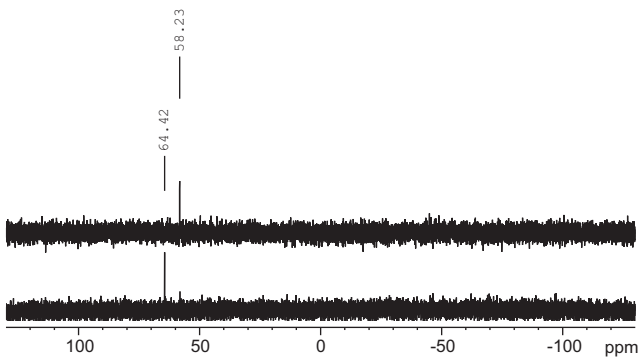


Figure 1. ^{31}P NMR spectrum in 1,4-dioxane/H_2O/D_2O (20:40:40) at room temperature of **1** (0.080 M) (lower) and of **1** (1.1×10^{-3} M) with 0.62 M NaOH after 6 h of reaction (upper)

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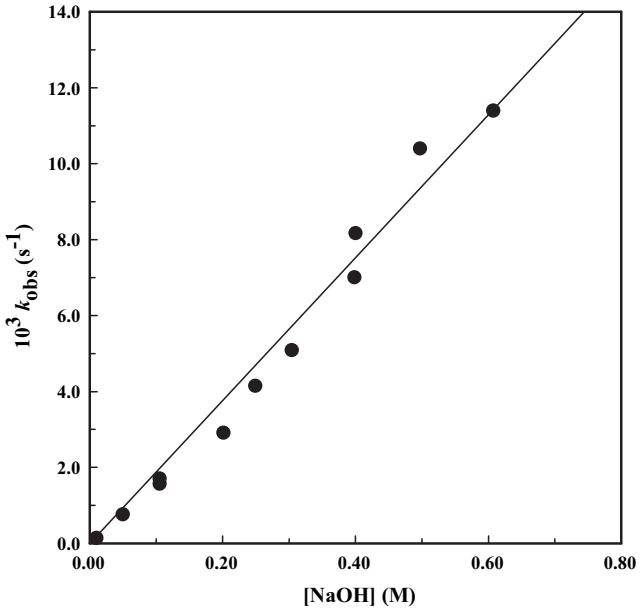


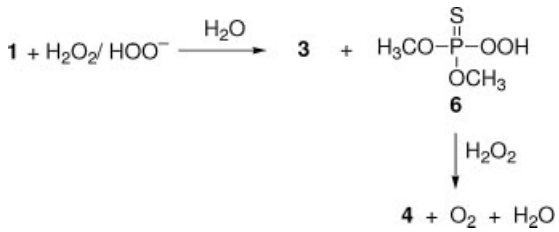
Figure 2. Plot of k_{obs} *versus* [NaOH] for the hydrolysis of **1** at 25 °C. Solvent contains 10% ACN; ionic strength $I = 0.6$ M (NaCl); $[\mathbf{1}]_0 = 3.04 \times 10^{-5}$ M

The hydrolysis of **1** was also studied in 7% 1,4-dioxane/H_2O at $I = 0.6$ M (NaCl) at 25 °C with 0.101 M < [NaOH] < 0.600 M. As it was observed in 10% ACN/H_2O, **3** was formed quantitatively. From the slope of the linear plot of k_{obs} *versus* [NaOH] (Fig. S1), k_{OH} was calculated as $(1.70 \pm 0.03) \times 10^{-2}\ M^{-1}\ s^{-1}$. This value is similar to that obtained in ACN/H_2O indicating that both organic solvents have similar influence on the reaction rate.

Reaction with H_2O_2

The reaction of **1** with H_2O_2 was studied in a pH range from 9.14 to 12.40 in 7% 1,4-dioxane/H_2O, $I = 0.6$ M (NaCl) at 25 °C; buffers of H_2O_2 or 0.05 M Borax were used to control the pH of the solutions. We have used this organic solvent due to the known reaction of ACN with H_2O_2.[41] The reaction was followed by measuring the appearance of **3** at 320 nm. Quantitative formation of **3** indicates that the only reaction taking place is $S_N2(P)$ as was observed in the hydrolysis reaction. Considering the fact that **3** was formed quantitatively, that during the kinetic measurements evolution of bubbles was observed and taking into account reported data,[42,43] we suggest that the reaction taking place is that shown in Scheme 1.

Values of k_{obs} were determined at different pH values varying the analytical concentration of H_2O_2 ($[H_2O_2]_0$) at each pH; the results are shown in Table S2. The nucleophiles present in the reaction solutions are H_2O, HO^-, H_2O_2, and HOO^-, so the expression for k_{obs} is given by Eqn (2). The plots of k_{obs} *versus* $[H_2O_2]_0$ according to Eqn (2) at each pH are linear (Fig. 3) and from the slope of these plots the values of the second-order rate constants ($k_{[H_2O_2]_0}$) at each pH, were calculated (Table S3). The intercepts of the plots of Fig. 3 are indistinguishable from zero, within experimental error, indicating that H_2O and HO^- ion do not compete with H_2O_2. The value of $k_{[H_2O_2]_0}$ is given by Eqn (5) and it can be seen that it increases with pH.



Scheme 1.

$$k_{obs} = k_{H_2O} + k_{HO^-}[HO^-] + k_{H_2O_2}[H_2O_2] + k_{HOO^-}[HOO^-]$$
$$= k_{H_2O} + k_{HO^-}[HO^-] + (k_{H_2O_2}X_{H_2O_2} + k_{HOO^-}X_{HOO^-})[H_2O_2]_0 \quad (2)$$

$$X_{H_2O_2} = \frac{[H_2O_2]}{[H_2O_2]_0} \quad (3)$$

$$X_{HOO^-} = \frac{[HOO^-]}{[H_2O_2]_0} \quad (4)$$

$$k_{[H_2O_2]_0} = k_{H_2O_2}X_{H_2O_2} + k_{HOO^-}X_{HOO^-}$$
$$= k_{H_2O_2} + (k_{HOO^-} - k_{H_2O_2})X_{HOO^-} \quad (5)$$

From a plot of $k_{[H_2O_2]_0}$ *versus* X_{HOO-} according to Eqn (5), the second-order rate constants for H_2O_2 and HOO^- can be calculated from the intercept at $X_{HOO-} = 0$ and 1, respectively (Fig. 4). The intercept at $X_{HOO-} = 0$ is zero, within experimental error; therefore H_2O_2 does not compete as nucleophile with HOO^-. The intercept at $X_{HOO-} = 1$ gives $k_{HOO-} = 7.9 \pm 0.4\ M^{-1}\ s^{-1}$.

The reaction of **1** with H_2O_2 was evaluated also from another set of data. A series of experiments was conducted at constant $[H_2O_2]_0 = 4.71 \times 10^{-3}$ M over a pH range from 9.14 to 12.4 and the results are collected in Table S4. When $k_{obs}/[H_2O_2]_0$ was plotted against X_{HOO-} a straight line was obtained (Fig. S2), and the value of k_{HOO-} was calculated as $7.8 \pm 0.1\ M^{-1}\ s^{-1}$, in good agreement with the value obtained before. By plotting together all the values

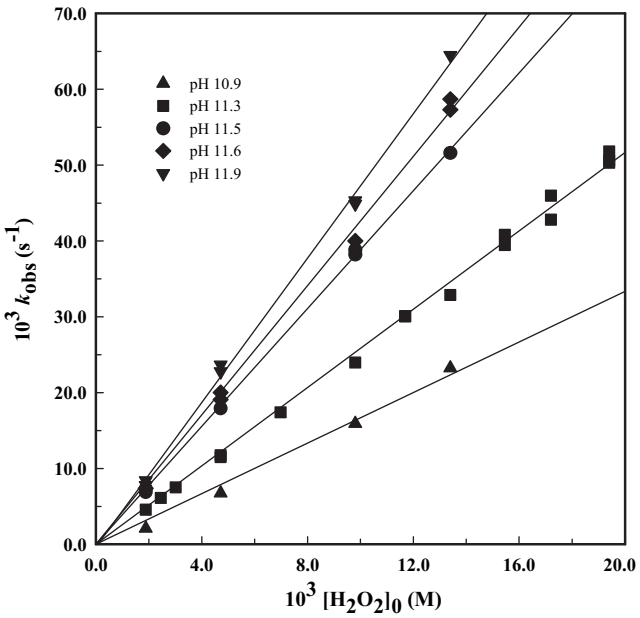


Figure 3. Plot of k_{obs} *versus* $[H_2O_2]_0$ for the reaction of **1** with H_2O_2 at 25 °C at different pHs. Solvent contains 7% 1,4-dioxane; ionic strength $I = 0.6$ M (NaCl); $[\mathbf{1}]_0 = (3.07–3.10) \times 10^{-5}$ M

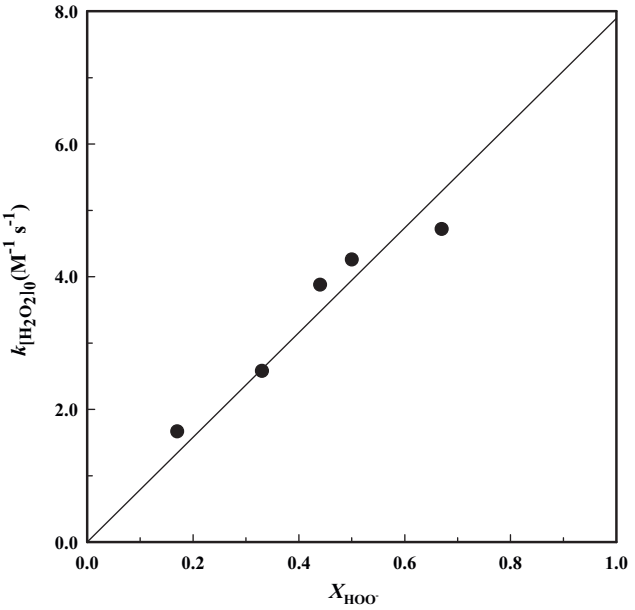


Figure 4. Plot of $k_{[H202]0}$ *versus* $X_{HOO}-$ for the reaction of **1** with H_2O_2 at 25 °C. Solvent contains 7% 1,4-dioxane; ionic strength $I = 0.6$ M (NaCl); $[\mathbf{1}]_0 = (3.07–3.10) \times 10^{-5}$ M

of k_{obs} obtained in the two series of experiments against $[HOO^-]$ (Fig. 5), $k_{HOO}-$ was calculated as $7.9 \pm 0.1\ M^{-1}\ s^{-1}$.

As the pK_a of H_2O_2 is 11.6,[33] in the pH range used for this study, both H_2O_2 and HOO^- are present in the reaction solution. The results indicate that only the HOO^- ion acts as nucleophile toward **1**. Besides, this anion is 465 times more reactive than HO^- in spite of the fact that it is less basic than the latter ($pK_a^{H2O} = 15.74$);[44] this could be attributed to the fact that HOO^- is an α-nucleophile.

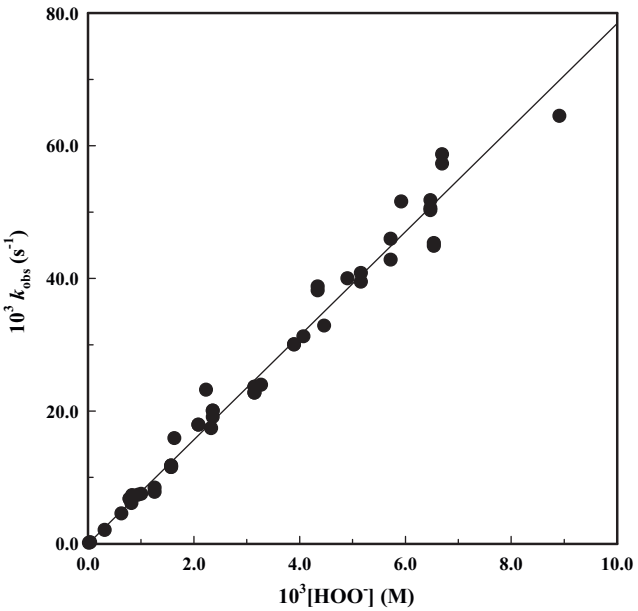


Figure 5. Plot of k_{obs} *versus* $[HOO^-]$ for the reaction of Chlorpyrifos-Methyl (**1**) with H_2O_2 at a pH range from 9.14 to 12.40 at 25 °C. Solvent contains 7% 1,4-dioxane; ionic strength $I = 0.6$ M (NaCl); $[\mathbf{1}]_0 = (3.07–3.10) \times 10^{-5}$ M

A number of studies have demonstrated the α-effect in nucleophilic attack at the P center.[33,38,42,43,45,46] It was reported an α-effect of 210 for the HOO^-/HO^- pair for the reaction with **2**,[33] this is nearly half of the value that we calculated. However, the same authors note that the magnitude of the α-effect is substrate-dependent.[33]

Effect of cyclodextrins

The effect of β-CD on the hydrolysis of **1** was determined in 10% ACN/H_2O at three HO^- ion concentrations and the data are summarized in Table S5. The addition of β-CD produces a nonlinear decrease in the observed rate constants and a saturation effect as shown in Fig. 6.

In order to determine the importance of the size of the cavity of the cyclodextrin in the observed effect, the rate constants were determined in the presence of α- and γ-CD at 0.50 M NaOH. The effect of sucrose, a non-reducing disaccharide, was also investigated. The data are collected in Table 1. It can be seen that the greater inhibition is produced by γ-CD whereas the effect of α- and β-CD are similar. On the other hand, it was previously observed that β-CD produces a larger inhibition of the hydrolysis of **2** than the others CDs.[25] These contrasting results indicate a high specificity of cyclodextrins in the recognition of the nature of the guest.

As the greater inhibition was observed with γ-CD, we investigated the effect of increasing concentrations of γ-CD on the hydrolysis of **1** at 0.50 M HO^- ion concentration (Table 2). As was observed with β-CD, the addition of γ-CD produces a nonlinear decrease in the observed rate constants and saturation kinetics as shown in Fig. S3.

We have also studied the effect of increasing amounts of sucrose on the hydrolysis of **1** in weight amount equal to solutions $(0.05–0.2) \times 10^{-2}$ M in β-CD at 0.50 M NaOH. The addition of sucrose produces an inhibition of the hydrolysis of **1** which is independent of the amount of sugar; the observed rate constants under these conditions have a mean value of $(6.1 \pm 0.6) \times 10^{-3}\ s^{-1}$ (Table S6).

The effect of β- and γ-CD on the reaction of **1** with H_2O_2 was determined at pH 11.30 at constant $[H_2O_2]_0 = 4.71 \times 10^{-3}$ M in 7% 1,4-dioxane/H_2O, $I = 0.6$ M (NaCl) at 25 °C and the data are summarized in Table S7. The presence of increasing amounts of β-CD produces a slight decrease in k_{obs} independent of β-CD concentration with a mean value of $(11.4 \pm 0.3) \times 10^{-3}\ s^{-1}$. On the other hand, γ-CD produces a nonlinear decrease in the observed rate constants and a saturation effect as shown in Fig. 7.

We have also evaluated the effect of α-CD and sucrose on the reaction of **1** with H_2O_2 under the same conditions. In Table 3 are summarized the results for all the sugars. It can be seen that again, γ-CD produces the greater inhibition whereas α- and β-CD show only a little inhibitory effect and sucrose has no effect, within experimental error.

The observation of inhibition of the reaction with saturation kinetics on the hydrolysis upon addition of β- and γ-CD and on the reaction with H_2O_2 upon addition of γ-CD, indicates that these hosts form inclusion complexes with **1**. The fact that the greater inhibition is observed with γ-CD may indicate a better fit of the insecticide in the cavity of this CD.

The addition of cyclodextrins or sucrose does not affect the reaction products; therefore the only reaction taking place, with or without CDs, is P—O bond fission (Eqn (1)).

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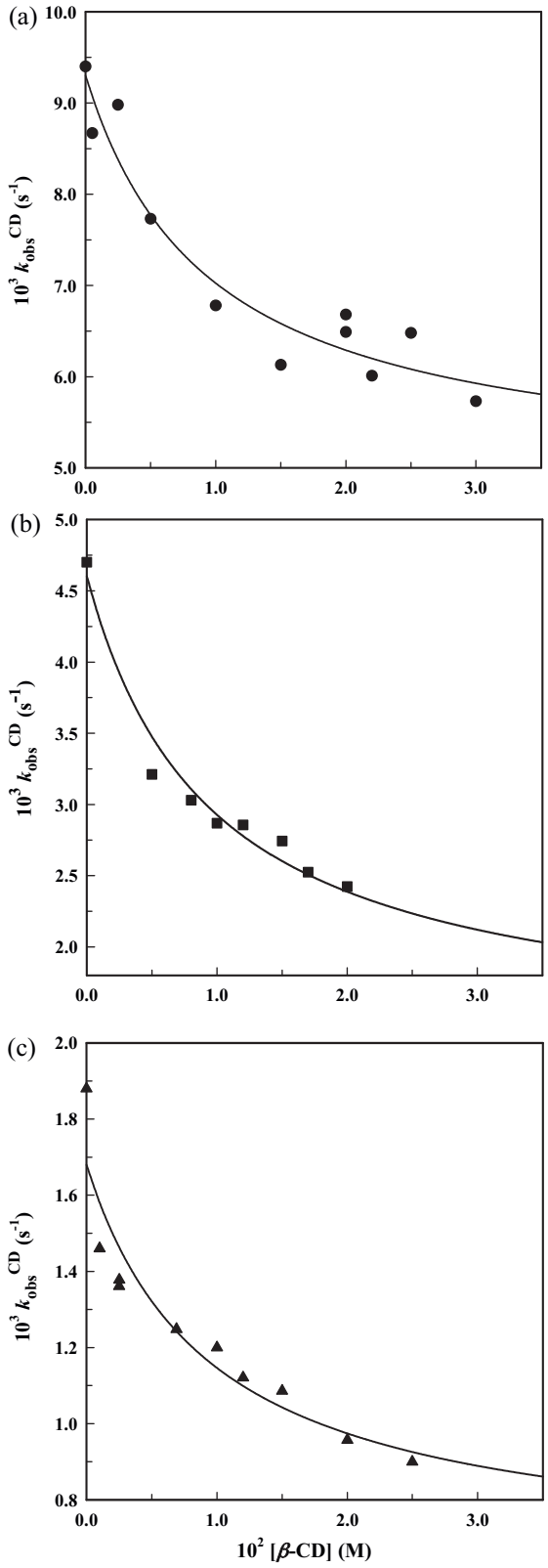

Figure 6. Plots of k_{obs}^{CD} *versus* [β-CD] for the hydrolysis of **1** at 25 °C at constant [NaOH]. Solvent contains 10% ACN; ionic strength $I = 0.6$ M (NaCl); $[\mathbf{1}]_0 = (2.90–3.10) \times 10^{-5}$ M; $[NaOH]_{eff}$ = (a) 0.500, (b) 0.250, and (c) 0.100 M

Table 1. Effect of α-, β-, and γ-CD and sucrose on the hydrolysis of Chlorpyrifos-Methyl (**1**) at 25 °C at 0.5 M NaOH[a]

CD	[CD] (M)	$10^3\,k_{obs}^{CD}$ (s^{-1})	$k_{obs}^{CD}/k_{obs}^{OH}$
	0	9.40	
α	0.020	6.52	0.69
β	0.020	6.49	0.69
γ	0.020	4.68	0.50
Sucrose	[b]	6.48	0.69

[a] Solvent contains 10% ACN; ionic strength $I = 0.6$ M (NaCl); $[1]_0 = (2.90–3.10) \times 10^{-5}$ M; $[NaOH]_{eff} = 0.500$ M.
[b] Weight amount equal to a solution 0.02 M in β-CD.

It was reported before that α-, β-, and γ-CD have promotive inclusion-catalytic effects on the degradation of some organophosphorous pesticides solubilized in neutral aqueous media; pesticide degradations were especially accelerated in the systems α-CD plus Diazinon and β-CD plus **5**.[47] Kamiya *et al.*[48] have also informed that in buffer solution (pH = 8.5) containing humic acids, β-CD inhibits the hydrolysis of Parathion and Methyl-Parathion and catalyses the hydrolysis of Paraoxon while γ-CD inhibits the hydrolysis of the three pesticides. To interpret the medium dependence of CD effects they proposed that as in alkaline media the hydrogen-bond linkages of the peripheral hydroxyl groups in the rim of CD cavity are destructed by their ionizations, it is highly probable that electrostatic repulsions of the ionized hydroxyl groups lead to widening of the CD torus which would allow deep inclusions of the pesticides in alkaline media. This may cause the degradation inhibition of the included pesticides.[47]

As 1,4-dioxane was used instead of ACN in the reaction of **1** with H_2O_2, the absence of saturation kinetics in this reaction in the presence of increasing amounts of β-CD and the lower inhibition observed in the presence of γ-CD, may be a consequence of the fact that 1,4-dioxane has a greater

Table 2. Dependence of observed rate constants on [γ-CD] for the hydrolysis of Chlorpyrifos-Methyl (**1**) at 25 °C[a]

10^2 [γ-CD] (M)	$10^3\,k_{obs}$ (s^{-1})[b]
0.0512	9.0 ± 0.1
0.200	6.3 ± 0.6
0.501	5.874 ± 0.008
0.701	4.6 ± 0.1
1.00	4.57 ± 0.01
1.20	5.303 ± 0.007
1.50	4.7 ± 0.2
1.70	3.9 ± 0.2
2.00	3.8 ± 0.1
2.00	4.679 ± 0.003

[a] Solvent contains 10% ACN; ionic strength $I = 0.6$ M (NaCl); $[1]_0 = 3.10 \times 10^{-5}$ M; $[NaOH]_{eff} = 0.500$ M.
[b] Errors are the standard deviation of the fit of the absorbance *versus* time data to a single exponential equation.

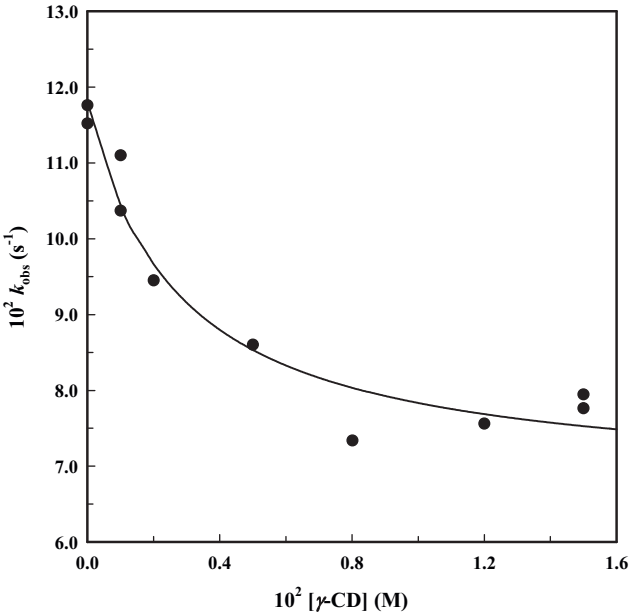

Figure 7. Plot of k_{obs} *versus* [γ-CD] for the reaction of **1** with H_2O_2 at 25 °C. Solvent contains 7% 1,4-dioxane; ionic strength $I = 0.6$ M (NaCl); $[\mathbf{1}]_0 = 3.07 \times 10^{-5}$ M; $[H_2O_2]_0 = 4.71 \times 10^{-5}$ M; pH = 11.30

association constant with β- or γ-CD than ACN. Reported values for these constants are $K_{ass} = (20.0 \pm 2.5)\ M^{-1}$ for a 1:1 association of 1,4-dioxane with β-CD or its anion, $K_{ass} = (0.60 \pm 0.05)\ M^{-1}$ and $(0.50 \pm 0.05)\ M^{-2}$ for a 2:1 association of ACN with β-CD and its anion, respectively;[49] $K_{ass} = 0.54\ M^{-1}$ for a 1:1 association of ACN with β-CD.[50] The organic co-solvent competes with **1** toward the cavity of the CD; as the affinity of the co-solvent with the host increases, a greater amount of the substrate will be displaced from the cavity of the host, increasing the amount of free substrate and decreasing the observed inhibition effect.[51] The kinetically determined association constant for **1** with β-CD in ACN/H_2O (see bellow) 105 M^{-1} is small, so as 1,4-dioxane has a greater affinity with β-CD than ACN, it is reasonable to think that in the presence of dioxane, a lower concentration of **1**:β-CD would be responsible for the absence of saturation kinetics in the reaction with H_2O_2. Assuming a greater K_{ass} for 1,4-dioxane with γ-CD than for ACN, the smaller inhibition produced by γ-CD on the reaction of **1** with H_2O_2 than in its hydrolysis can be explain by the same arguments.

Table 3. Effect of α-, β-, and γ-CD and sucrose on the reaction of Chlorpyrifos-Methyl (**1**) with H_2O_2 at 25 °C and pH = 11.30[a]

CD	[CD] (M)	$10^3\ k_{obs}^{CD}$ (s^{-1})	$k_{obs}^{CD}/k_{obs}^{OOH}$
	0	11.76	
α	0.015	11.08	0.94
β	0.015	10.96	0.93
γ	0.015	7.853	0.67
Sucrose	[b]	11.94	1.02

[a] Solvent contains 7% 1,4-dioxane; ionic strength $I = 0.6$ M (NaCl); $[1]_0 = 3.07 \times 10^{-5}$ M; $[H_2O_2]_0 = 4.71 \times 10^{-3}$ M.
[b] Weight amount equal to a solution 0.015 M in β-CD.

Although the magnitude of the inhibition of the hydrolysis of **1** produced by sucrose is similar to that observed with α- and β-CD, the origin of the inhibition is different as no saturation kinetics was observed. 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. At the high OH^- ion 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- or γ-CD. It is known that starch and some linear oligosaccharides can self-associate and form complexes with various types of compounds.[52] There are examples in the literature of changes in the reaction rates, accelerations, or inhibitions of different substrates produced by the presence of linear saccharides.[34,53,54]

Spectroscopic studies

Inclusion complex formation between CD and different guest molecules can be detected by a variety of spectroscopic methods.[13] UV–Vis spectroscopy is one of the most frequently used technique to determine the association constants between an organic compound and CDs.[55]

We intended to determine the binding constant of **1** with β- and γ-CD by UV–Vis spectroscopy. Thus, we carried out the spectra of **1** in the presence of increasing amounts of β- or γ-CD. The UV–Vis spectrum of **1** in the presence of β-CD is identical to that in the presence of γ-CD. Although the spectrum of **1** in the presence of any of the CDs shows a small hypsochromic shift, the changes in absorbance observed are not great enough to enable the determination of the association constants (Fig. S4).

We have also studied the interaction of **1** with β- and γ-CD by circular dichroism. The addition of β-CD to a solution of **1** gives an induced circular dichroism spectrum (ICD) with a negative peak at 230 nm and a positive one at 289 nm; these peaks coincide with the maxima in the UV–Vis spectrum (Fig. 8). On the other hand, γ-CD produces a more intense ICD spectrum with two negative peaks at 240 and 269 nm (Fig. 8). As is observed in other cases where CD is the host,[56–59] these peaks are slightly displaced from the maxima in the UV–Vis spectrum. The fact that the ICD signal at the higher wavelength changes from negative to positive and the ellipticity of that signal decreases when the host changes from γ- to β-CD indicates an extrusion of the guest from the cavity as was suggested before by others.[60] If we compare the distance between the OCH_3 groups on P (5.573 Å)[61] or between the Cl substituents on C3 and C5 (6133 Å)[61] of insecticide **1** (Fig. 9) and the cavity diameters of β-CD (internal diameter = 5.6 Å)[14] and γ-CD (internal diameter = 6.8 Å),[14] it is evident that **1** can be more deeply included in the cavity of γ-CD than in the cavity of β-CD.

We have also studied the interaction of **3** with β- and γ-CD by circular dichroism. As can be seen in Fig. 8, the spectra obtained are less defined and intense than those observed with **1** suggesting a weaker interaction with the hosts than that of the insecticide.

In order to confirm that the formation of inclusion complexes between **1** and β- or γ-CD is responsible of the ICD spectra observed, we studied the interaction of **1** with sucrose and raffinose, two non-reducing saccharides. Although the UV–Vis spectra of **1** in the presence of sucrose or raffinose are identical to that in the presence of β-CD, these compounds induce weak ICD

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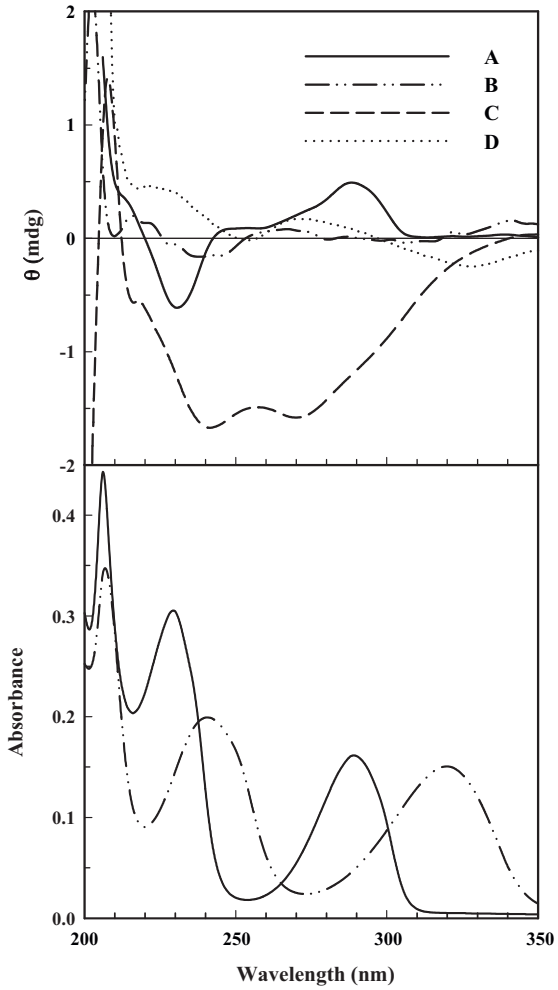


Figure 8. UV–Vis (lower) and ICD (upper) spectra in 10% ACN/H_2O, ionic strength $I = 0.6$ M at 25 °C of (A) **1** (3.10×10^{-5} M) with 0.010 M β-CD; (B) **3** (3.07×10^{-5} M) with 0.010 M β-CD; (C) **1** (3.10×10^{-5} M) with 0.008 M γ-CD; (D) **3** (3.07×10^{-5} M) with 0.008 M γ-CD

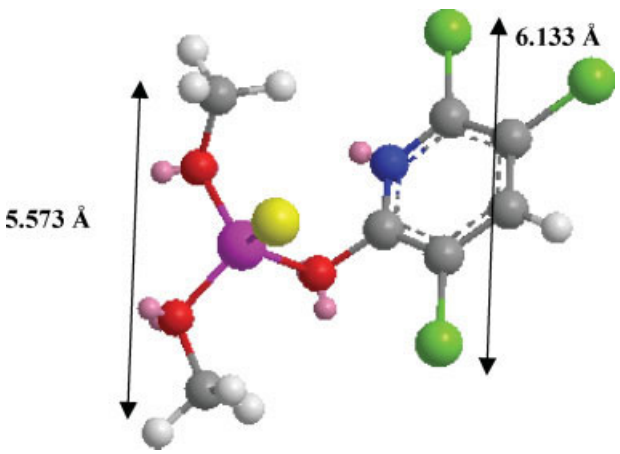
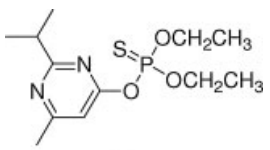


Figure 9. Schematic representation of the structure of **1** as calculated by Hyper Chem 7.5

signals (Fig. S6), different to those observed with the CDs, which can be attributed to some type of unspecific association of the substrate with the sugar.

NMR is the most widely used method for structural elucidation of organic compounds in solution. It is also very useful to provide evidence for the formation of inclusion complexes in solution since this will affect the nuclei environment in both the host and the guest and will hence be reflected by chemical shift variation. It allows the calculation of the binding constant between the host and the guest as well as the binding modes in the complexes.[55,62,63] Complex formation between **1** and β-CD was also investigated by ^{31}P and ^{1}H NMR.

Proton-decoupled ^{31}P NMR spectra were recorded for **1** (8.4×10^{-4} M) in aqueous media in the absence and presence of 8.7×10^{-4} M β-CD. In the presence of β-CD two signals were observed, one at 64.43 ppm corresponding to **1** and the other at 0.38 ppm downfield from that of **1** (Fig. 10) corresponding to **1** included in β-CD. Downfield changes in the ^{31}P NMR chemical shift for the organophosphorus pesticide diazinon (**7**) were reported before in the presence of α-, β-, and γ-CD and interpreted as formation of an inclusion complex.[64]



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The spectra of β-CD in 1,4-dioxane/D_2O (10:90) were recorded in the absence and the presence of **1**. All the signals corresponding to β-CD in the presence of **1** appear at lower fields than in its absence (Table S8). The fact that $\Delta\delta$H3 < $\Delta\delta$H5 indicates that the guest is deeply included inside the cavity of the host.[65] As was observed in ^{31}P NMR, in the ^{1}H NMR of **1** in the presence of β-CD, two signals for each type of protons appears corresponding to free and complexed substrate. In Table S9 are listed the chemical shifts of **1** in the presence and absence of β-CD; it can be observed that all the signals are shifted to higher fields in the presence of the host and the most affected is the signal corresponding to the aromatic proton.

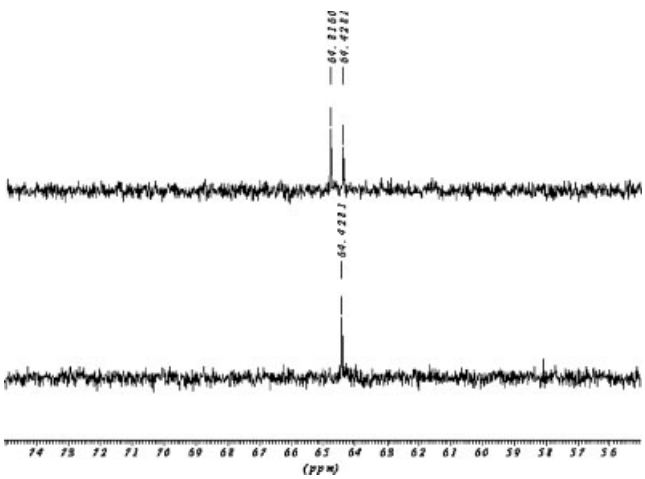


Figure 10. ^{31}P NMR spectrum in 10% [D_8]1,4-dioxane/D_2O at room temperature of **1** (8.4×10^{-4} M) (lower) and of **1** in the presence of β-CD 8.7×10^{-4} M (upper)

A 2D ROESY was conducted for **1** in the presence of β-CD. Due to the low solubility of the pesticide in water, ROESY-2D spectra have low resolution and only interaction between the aromatic proton of the guest with H-3 of β-CD could be clearly detected. The signals corresponding to the OCH_3 groups of the guest in the complex appear close to those of β-CD and no definitive conclusion about their interaction could be established.

The NMR experiments indicate that **1** is deeply included in the cavity of β-CD, there is an important interaction of the aromatic proton with H-3 of β-CD and the rate of exchange of **1** between the cavity and the bulk solution is slower than the time scale of NMR since two signals for the H or P in the guest were observed in the presence of the host.

Reaction mechanism in the presence of CDs

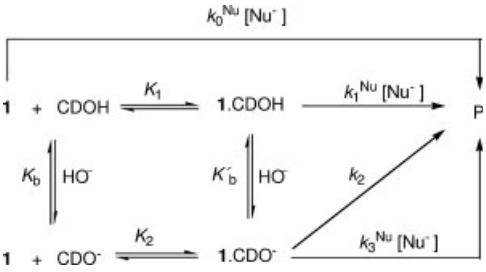
We have found that H_2O does not compete with HO^- ion in the hydrolysis of **1** and that H_2O, HO^-, and H_2O_2 do not compete with HOO^- ion in the reaction with **1**. Considering that the pK_a of β-CD is 12.2 and that of γ-CD is 12.08,[66] as demonstrated in previous work with other substrates,[67] and with **2**,[25] the reaction of **1** with HO^- or HOO^- in the presence of CDs may take place as indicated in Scheme 2. In Scheme 2 Nu^- represents HO^- or HOO^- and k_0^{Nu}, k_1^{Nu}, k_2, and k_3^{Nu} represent the reactions of the free substrate, the substrate complexed with neutral β- or γ-CD (CDOH), the reaction with ionized β- or γ-CD (CDO^-) and the reaction of the substrate complexed with CDO^- reacting with HO^- or HOO^- ion, respectively.

The observed rate constant for the mechanism shown in Scheme 2 is given by Eqn (6), where f represents the fraction of ionized β- or γ-CD as defined in Eqn (7) with $K_b = K_W/K_a$, and CD is the stoichiometric concentration of β- or γ-CD.

$$k_{obs} = \frac{k_0^{Nu}[Nu^-] + k_1^{Nu}K_1[Nu^-](1-f)[CD] + (k_2 + k_3^{Nu}[Nu^-])K_2 f[CD]}{1 + K_1(1-f)[CD] + K_2 f[CD]} \quad (6)$$

$$f = \frac{[HO^-]}{[HO^-] + K_b} \quad (7)$$

In all cases where saturation kinetics was observed, the observed rate constants were fitted to an equation of the form of Eqn (8) where $a = k_0^{Nu}$ $[Nu^-]$ and b and c are adjustable parameters given in Eqn (9) and (10).



Scheme 2.

$$k_{obs} = \frac{a + b[CD]}{1 + c[CD]} \quad (8)$$

$$b = k_1^{Nu}K_1[Nu^-](1-f) + (k_2 + k_3^{Nu}[Nu^-])K_2 f$$

$$= \{k_1^{Nu}K_1K_b + (k_2 + k_3^{Nu}[Nu^-])K_2\}f \quad (9)$$

$$c = K_1(1-f) + K_2 f \quad (10)$$

The hydrolysis reactions in the presence of β-CD were studied at 0.10, 0.25, and 0.50 M NaOH while 0.50 M NaOH was used for the reactions carried out in the presence of γ-CD. Under the condition of a constant HO^- ion concentration of 0.50 M, $f \approx 1$; then Eqn (6) simplifies to Eqn (11).

$$k_{obs} = \frac{k_0^{HO}[HO^-] + (k_2 + k_3^{HO}[HO^-])K_2[CD]}{1 + K_2[CD]} \quad (11)$$

The values of a, b, and c obtained using Eqn (8) to fit the data are given in Table 4, where the experimentally determined value of k_0^{HO} $[HO^-]$ is included.

Taking the value K_2 as the value of c at 0.5 M HO^-, we can calculate the association constants of **1** with β- and γ-CD as 105 and 461 M^{-1}, respectively. A good fit to Eqn (8) was obtained for all the $[HO^-]$ studied in the presence of β-CD using the same value for c. So, according to Eqn (10), K_2 must not be significantly different from K_1, as was previously reported for other guests.[68] A value of $K_{ass} = 90 \pm 28$ M^{-1} was informed for the complexation of **5** with β-CD, in good agreement with that calculated here.

A plot of b/f *versus* HO^- ion concentration (Fig. S6) for the data in the presence of β-CD, is linear with slope 0.9 $M^{-2}s^{-1}$. The intercept of this line is indistinguishable from zero within experimental error, indicating that the main reaction pathway of

Table 4. Parameters of Eqn (8) for the reaction of Chlorpyrifos-Methyl (**1**) with HO^- or HOO^- in the presence of β- and γ-CD at 25 °C[a]

Nu^-	CD	$[Nu^-]_{eff}$ (M)	10^3 a[b]	b	c	f	b/f
HO^-	β	0.50	9.3 ± 0.2 (9.4)	0.51 ± 0.03	105 ± 4	1.00	0.51
HO^-	β	0.25	4.6 ± 0.1 (4.7)	0.14 ± 0.01	105[c]	0.94	0.149
HO^-	β	0.10	1.68 ± 0.06 (1.88)	0.067 ± 0.009	105[c]	0.86	0.078
HO^-	γ	0.50	9.6 ± 0.4 (9.4)	1.7 ± 0.1	461 ± 22	1.00	1.7
HOO^-	γ	0.00157	11.8 ± 0.2 (11.76)	2.47 ± 0.08	366 ± 9	0.11	0.73

[a] Solvent contains 10% ACN for the reaction with HO^- or 7% 1,4-dioxane for the reaction with HOO^-.
[b] Values in parenthesis are k_0^{Nu} $[Nu^-]$.
[c] The fit was made keeping c constant.

Scheme 2 is that including k_3, as was previously observed for **2**.[25] With $K_2 = 105\ \text{M}^{-1}$, k_3^{HO} is calculated as $8.57 \times 10^{-3}\ \text{M}^{-1}\ \text{s}^{-1}$, which is about two times smaller than the value for the rate constant for the reaction of the free substrate, k_0^{Nu}. As we proposed before for the hydrolysis of **2**,[25] 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^- ion with the complexed substrate and that there appears to be no significant nucleophilic reaction of the ionized secondary OH of the cyclodextrin may indicate that in the structure of the complex the thiophosphate group is not at an appropriate distance from the ionized secondary OH of the cyclodextrin rim. The electronegativity of sulfur (2.44) is smaller than that of oxygen (3.50) whereas the atomic radio of sulfur (1.27 Å) is greater than that of oxygen (0.60 Å).[69] Besides, the value of log $P_{O/W}$ for **1** is 4.28 whereas that for the phosphate analog is 3.55.[70] Thus, the thiophosphate group is more hydrophobic than the phosphate and the first one may be more deeply included in the cavity of the CDs. This is in agreement with the fact that monothiophosphates form inclusion complexes with CDs that are more stable than those of the corresponding monophosphates.[69]

In Table 5 are summarized the values of the rate and equilibrium constants calculated for the mechanism depicted in Scheme 2 for the hydrolysis of **1** in the presence of β- and γ-CD along with those previously calculated for **2**.[25]

Comparing the results of **1** with β-CD with those for **2**,[25] we found that for the latter compound K_2 is about four times greater and k_3^{HO} is sixteen times smaller than those for compound **1**. On the other hand, K_2 for **1** with γ-CD is comparable to that for **2** with β-CD.[25] With $K_2 = 461\ \text{M}^{-1}$ for γ-CD, k_3^{HO} is calculated as $7.37 \times 10^{-3}\ \text{M}^{-1}\ \text{s}^{-1}$, which is about 2.5 times smaller than the value for the rate constant for the reaction of the free substrate, k_0^{HO}. With the values of k_3^{HO} with γ- and β-CD shown in Table 5, we can calculate $k_3^{HO(\gamma)}/k_3^{HO(\beta)}$ as 0.86, indicating that the rate constants for the reaction of the substrate included in the cavity of β and γ-CD are very similar. Therefore, the observed higher decrease in rate for the reactions with γ-CD is mainly due to the higher binding constant.

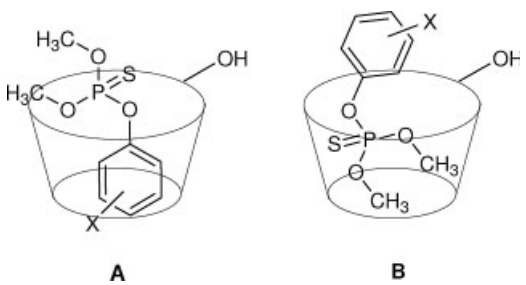
Table 5. Rate and equilibrium constants calculated for the hydrolysis of Chlorpyrifos-Methyl (**1**) and Fenitrothion (**2**) and for the reaction of **1** with H_2O_2

	1	2[a]
$10^2\ k_0^{HO}$ ($\text{M}^{-1}\ \text{s}^{-1}$)[b]	1.88 ± 0.05	0.20 ± 0.01
$10^2\ k_0^{HO}$ ($\text{M}^{-1}\ \text{s}^{-1}$)[c]	1.70 ± 0.03	
$K_2^{\beta-CD}$ (M^{-1})[b]	105 ± 4	415 ± 72
$10^3\ k_3^{HO\beta-CD}$ ($\text{M}^{-1}\ \text{s}^{-1}$)[b]	8.57	0.53
$K_2^{\gamma-CD}$ (M^{-1})[b]	461 ± 22	
$10^3\ k_3^{HO\gamma-CD}$ ($\text{M}^{-1}\ \text{s}^{-1}$)[b]	7.37	
k_0^{HOO} ($\text{M}^{-1}\ \text{s}^{-1}$)[c]	7.9 ± 0.1	
$K_2^{\gamma-CD}$ (M^{-1})[c]	366 ± 9	
$k_3^{HOO\gamma-CD}$ ($\text{M}^{-1}\ \text{s}^{-1}$)[c]	4.30	

[a] Taken from Reference [25].
[b] Solvent contains 10% ACN.
[c] Solvent contains 7% 1,4-dioxane.

The kinetic data of the reaction of **1** with H_2O_2 in the presence of γ-CD were fitted to Eqn (8) and the values of parameters *a*, *b*, and *c* obtained are shown in Table 4, where the experimentally determined value of k_0^{HOO} [HOO^-] is also included. Assuming that $K_1 \approx K_2$, and that the main reaction pathway is that involving k_3^{HOO}, as was observed previously in the hydrolysis reaction,[71] the association constant of **1** with γ-CD in 7% 1,4-dioxane/H_2O could be calculated from parameter *c* and k_3^{HOO} from parameter *b* (Table 5). The value $366 \pm 9\ \text{M}^{-1}$ obtained for K_2 is 20% smaller than that kinetically determined in the hydrolysis reaction in the presence of 10% ACN. In the presence of γ-CD, the ratio $k_3^{HOO}/k_3^{HO} = 583$ indicates a greater α effect for the reaction of the substrate included in the cavity with the nucleophile than for the free substrate with the nucleophile.

There are two possible modes of inclusion of the insecticide **1** in the cavity of the CDs as shown in Scheme 3, with the aromatic moiety or with the thiophosphate moiety inside the cavity. For most of the esters having an aromatic group it is proposed that this region is included in the cavity of the CDs. This was suggested for **2**, Parathion, Pharathion-Methyl, and Paraoxon.[48,72,73] It is known that the observed acceleration in the release of phenol from substituted acetates in the presence of CDs is independent of the electronic nature of the substituents but it strongly depends on the position that they occupied in the aromatic ring. In the substrates having a *meta* substituent in the aromatic ring, the ring is not deeply included in the cavity and the carbonylic carbon of the ester is located near the secondary OH of CD, thus catalysis of the reaction of phenol release is observed.[34] On the other hand, in esters having *para* substituted phenols, the inclusion is deeper placing the carbonylic group far away from the secondary OH with a weaker catalysis of the reaction.[34] For instance, it was suggested that the *t*-butyl group of *m*-*t*-butylphenylacetate is included in the cavity of α-CD whereas the aromatic region remains in the aqueous solution consistent with the fact that the UV–Vis spectrum of the complex shows only a little perturbation compared with that of the substrate.[34] In contrast, *p*-*t*-butylphenylacetate shows an important spectral change in the presence of α-CD suggesting the inclusion of the aromatic region.[34] Moreover, the inhibition of the hydrolysis of the esters $CF_3(CF_2)_nCOOPh$ ($n = 1$ and 2) produced by β-CD was suggested to arise from the fact that the reaction take place with the hydrophobic perfluorinated alkyl chain included in the cavity.[22] The complexation of diazinon (**7**), with α-, β-, and γ-CD was investigated by NMR and computational studies.[64] Binding constants determined by ^1H and ^{31}P NMR follow the order γ-CD > α-CD = β-CD, being that for γ-CD, i.e., $390 \pm 63\ \text{M}^{-1}$,[64] similar to K_2 determined by us for **1**. The computational



Scheme 3.

calculations indicate that while in α- and β-CD the aromatic moiety of **7** is located inside the cavity and the phosphoryl residue is largely outside it, in γ-CD the heterocyclic residue and the phosphoryl residue of the guest are both largely encrypted in the CD cavity with the methyl substituent on C5 emerging from the wider rim of CD.

In this work, we present clear evidence of the formation of inclusion complexes between **1** and α-, β-, or γ-CD, although we cannot assure which of the two possible modes of inclusion depicted in Scheme 3 is taking place with each of the CDs.

CONCLUSIONS

Chlorpyrifos-Methyl (**1**) forms inclusion complexes with α-, β-, and γ-CD in aqueous solutions as is evidenced by kinetic and spectroscopic methods.

Chlorpyrifos-Methyl is decomposed quantitatively to 3,5,6-trichloro-2-pyridinol (**3**) in basic aqueous media by HO^- or HOO^- through a $S_N2(P)$ pathway with an important α-effect. The hydrolysis of **1** is inhibited by α-, β-, and γ-CD and sucrose; the greater inhibition was observed with γ-CD and saturation kinetics was found in the case of β- and γ-CD. The reaction of **1** with H_2O_2 is slightly inhibited by α- and β-CD and inhibited by γ-CD with saturation kinetics. The effect of γ-CD is greater for the hydrolysis reaction than for the reaction with H_2O_2; this may be a consequence of differences in solvation/desolvation of the two nucleophiles, HO^- or HOO^- as was proposed before to explain the α-effect in the reactions of other phosphorous compounds.[46] The kinetic data indicate that the main reaction pathway for the cyclodextrin-mediated reaction is the reaction of HO^- or HOO^- ion with the substrate complexed with the anion of the cyclodextrin, as was previously found for the hydrolysis of Fenitrothion (**2**).[25]

EXPERIMENTAL SECTION

Materials

Chlorpyrifos-Methyl (**1**) was characterized by ^1H and ^{13}C NMR and GC–MS. The hydrolysis product 3,5,6-trichloro-2-pyridinol (**3**), was obtained by hydrolysis of Chlorpyrifos-Ethyl (**5**) with KOH in 20% ACN at room temperature and characterized by ^1H and ^{13}C NMR and GC–MS. α-, β-, γ-cyclodextrin, sucrose and raffinose were used as received, but the purity was periodically checked by UV–Vis spectroscopy.

Aqueous solutions were prepared using water purified with a Millipore Milli-Q apparatus. Acetonitrile (HPLC grade) was used as received and 1,4-dioxane was purified as described previously.[74] All of the inorganic reagents were of analytical-reagent grade and were used without further purification. Hydrogen peroxide solutions were titrated with a $KMnO_4$ solution standardized by sodium oxalate.

UV–Vis spectra were recorded on a Shimadzu UV-2101 or HP Multispect 1501 spectrophotometer and the change in absorbance during a kinetic run was measured on the same instruments. Circular dichroism spectra were recorded on a JASCO Model J-810 spectropolarimeter which was calibrated with D-(+)-amonium camphorsulfonate. ^{31}P and ^1H NMR spectra were recorded on a Bruker Ultra Shield 400. GC–MS were performed on a Shimadzu Model CQ5050 instrument.

pK_a determination

The pK_a of 3,5,6-trichloro-2-pyridinol (**3**) was determined by spectrophotometric titration in 10% ACN/H_2O at 25 °C and ionic strength 0.6 M. Solutions for the various pH ranges were prepared using HCl (2.00–3.04), acetic acid/sodium acetate (4.00–5.50), K_2HPO_4/KH_2PO_4 (5.40–8.00), borax (8.80), $Na_2CO_3/NaHCO_3$ (9.70), and NaOH (12.0).

Kinetic procedures

Reactions were initiated by adding the substrate dissolved in ACN or 1,4-dioxane to a solution containing all the other constituents. The reaction temperature was 25.0 ± 0.1 °C, the ionic strength was 0.6 M and NaCl was used throughout as compensating electrolyte. The solvent contained 10% ACN (hydrolysis) or 7% 1,4-dioxane (hydrolysis and reaction with H_2O_2). In the reactions with H_2O_2, water and 1,4-dioxane were degassed and the stock solution of NaCl was filtered by nylon membranes before preparing the reaction solutions. After the pH was adjusted to the desired value, the solution was sonicated for 5 min and thermostated to 25 °C before the substrate was added.

All kinetic runs were carried out under pseudo-first-order conditions, with substrate concentrations of $(2.9–3.1) \times 10^{-5}$ M.

The reactions were followed by measuring the increase in absorbance of the reaction mixture at 320 nm, the λ_{max} of **3**. The $[HO^-]_{eff}$ values reported in Tables 1–4 were calculated from stoichiometric concentration of NaOH and CD and the pK_a of CDOH (12.2 or 12.08 for β- or γ-CD, respectively).[66]

Spectroscopic procedures

The circular dichroism measurements were recorded at 25 °C in 10% ACN/H_2O at an ionic strength of 0.6 M by the addition of NaCl; $[\mathbf{1}]_0 = 3.1 \times 10^{-5}$ M; the concentration of β- or γ-CD was equal to 0.01 M while sucrose or raffinose were used in a weight amount equal to a solution 0.01 M in β-CD. To remove background contributions, circular dichroism measurements were always made using the free CD, sucrose or raffinose solution as a reference system. Water and aqueous salt solutions were filtered by nylon membranes before the measurements were done.

NMR experiments

The ^{31}P NMR spectra were recorded on a spectrometer operating at 161.97 MHz. Chemical shifts were measured with respect to the external standard of 85% H_3PO_4 in D_2O/H_2O (75:25). Spectra were acquired with a longitudinal relaxation time (D1) of 30 s with 128 accumulations and proton decoupling. In the experiments conducted for products identification (Fig. 1), 1,4-dioxane was used while in those for investigation of complex formation [D_8]1,4-dioxane was used.

Sample preparation: For complex investigation by ^{31}P NMR, 70 µl of a stock solution of **1** 8.06×10^{-3} M in [D_8]1,4-dioxane was added to 0.6 ml of D_2O or to 0.6 ml of β-CD solution 9.9×10^{-4} M in D_2O. The final concentrations were: $[\mathbf{1}]_0 = 8.4 \times 10^{-4}$ M, $[\beta\text{-CD}] = 8.7 \times 10^{-4}$ M, [D_8]1,4-dioxane = 10%.

For complex investigation by ^1H NMR a stock solution of β-CD was prepared by dissolving 0.004 g in 3 ml of D_2O ($[\beta\text{-CD}] = 1.17 \times 10^{-3}$ M). For the spectra of β-CD, 70 µl of [D_8]1,4-dioxane were added to 0.6 ml of the stock solution of β-CD. Final concentrations were: $[\beta\text{-CD}] = 1.04 \times 10^{-3}$ M, [D_8]1,4-dioxane = 10%. For the ^1H NMR and ROESY-2D

experiments of **1** in the presence of β-CD, to 0.6 ml of the stock solution of β-CD, 60 μl of [D_8]1,4-dioxane and 70 μl of a stock solution of **1** 8.06×10^{-3} M in [D_8]1,4-dioxane were added. Final concentrations were: $[\mathbf{1}]_0 = 7.73 \times 10^{-4}$ M, [β-CD] $= 9.56 \times 10^{-4}$ M, [D_8]1,4-dioxane = 18%. All chemical shifts for ^{1}H are relative to TMS using ^{1}H (residual) chemical shifts of [D_8]1,4-dioxane as a secondary standard.

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