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Hg^{2+}-Quinalphos Complexes: Characterized by ^{1}H and ^{31}P NMR

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ABSTRACT

Since Hg^{2+} has catalyzed the hydrolysis of quinalphos and ESI-MS detects species in the gas phase [1], ^{1}H and ^{31}P NMR studies were carried out in this work in order to verify whether the metal ions bind to the quinalphos (Q) and its products in solution. The NMR results show clearly that binding between Hg^{2+} and quinalphos occurs primarily through the S atom. However, small ^{1}H shifts suggest weaker interaction through N. This support strongly that Hg^{2+} may form a six membered ring with quinalphos and that this complex enhancing the hydrolysis rates.

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INTRODUCTION

A number of studies of organic compounds hydrolysis have been reported by different researchers [1-6]. In our previous recent publication [1], kinetics data have shown that Hg^{2+} facilitated the degradation of quinalphos. In another work, we have also reported that copper catalyzed the hydrolysis of quinalphos [6]. NMR is a suitable technique for various studies (e.g., identification, kinetics, etc). As an example, ^{31}P NMR has been reported to be a very useful technique for the analysis of OP compounds [7-9]. Hydrolytic pathways of phorate were studied by following ^{31}P NMR of the substrate and that of the product at pH 8.5. It was found that the reaction followed a single pathway, as the disappearance of ^{31}P peak of phorate was tracked by the simultaneous appearance of ^{31}P of the product. The P-containing product is a diethyl dithiophosphate [7].

NMR has been used also in studying the interaction between dissolved metal ions and various organic compounds. Interaction of Hg^{2+} with ligands containing sulfur by using NMR (400 MHz) [10]. The ligands were 2,3-dimercaptopropanesulfonic acid, di-thioerythritol, dimercaptosuccinic acid, mercaptosuccinic acid, mercaptoacetic acid, cysteine, and penicillamine. ^{1}H NMR spectra have shown that Hg^{2+} binds to S, as the ^{1}H signals of the complexed species shifted downfield. Complexation of Hg^{2+} with the ligands caused also peak broadening [10]. Lomozik et al. have used ^{13}C NMR for studying the interaction of Hg^{2+} with organic compounds containing N atoms in their structures [11]. They compare shifts of ^{13}C NMR signals in the presence of Hg^{2+} to those in metal free solution. Lomozik et al. found that 13C NMR signals for carbons that are neighbours to the donor atom (N) shifted downfield by presence of Hg^{2+}, indicating that Hg^{2+} binds to the N [11]. Other researchers have also used chemical shifts as an indicator for metal ion-organic compounds complexes [12]. They have reported that ^{1}H and ^{13}C NMR shows Ag^{+} has the ability to coordinate compounds (Isoxazole, thiazole, and 1-methylimidazole) containing N or S [12]. More recently and by using NMR technique, we have shown also that coordination between Ag^{+} and quinalphos occurs through both positions S as well as N [13].

In our previous work, electrospray ionization mass spectrometry (ESI-MS) was the used technique to detect the interaction between Hg^{2+} and Q [1]. However, the limitation of ESI-MS is that ions are observed only in the gas phase and that may or may not correlate to species that are present in solution. For this reason, in this paper we use Nuclear Magnetic Resonance (NMR) as an alternative technique for studying the interaction of Hg^{2+} with Q. Also, to check whether NMR will confirm coordination of Hg^{2+} with 2-hydroxyquinoxaline (HQ), hydrolysis product of Q that contains N (not observed by ESI-MS) or not.

METHODS

Quinalphos and its hydrolysis product, 2-hydroxyquinoxaline were obtained from Crescent Chemicals, U.S.A. Both were of the highest purity available and were used as received. A sample of the second product, O, O-diethyl phosphorothioic acid (PA) was prepared at Queen's University as described by Pieda [14]. Q, HQ, and PA were characterized by ESI-MS as well as nuclear magnetic resonance (NMR, 500 MHz). NMR can be employed in studying the interaction between metal ions and organic compounds in solution. In the present study, NMR experiments were carried out on the Q and its degradation products in the absence and presence of Hg^{2+} by using NMR technique (Bruker 500 MHz). Both ^{31}P and ^{1}H NMR of Q, ^{31}P of PA, and ^{1}H NMR of HQ were recorded in the absence and presence of different concentrations of Hg^{2+}. Solutions of mercury (Hg^{2+}) were prepared by dissolving Hg metal (Engelhard, Canada) in conc HNO_3 and then diluted with D_2O. In this work, NMR experiments were performed at pH ~ 1 and temperature 25°C. In fact, pH was not adjusted and that is due to the high concentrations of Hg.

For the NMR analysis, stock solutions of Q, HQ, and PA were prepared in dioxane with a concentration of 3.38×10^{-2}, 1.37×10^{-2}, and 3.38×10^{-2} mol L^{-1}, respectively. In a 3 mL glass vial, 0.1 mL of the metal ion solution (Hg^{2+}) was added individually to 0.5 mL of Q, HQ, or PA stock solutions. In the absence of Hg^{2+}, however, 0.1 mL of D_2O was added to 0.5 mL of each stock solution to lock the samples as well as keep the experiments under the same conditions. Each mixture was then transferred immediately to an NMR tube. Table 1 show the concentrations of Hg^{2+}, Q, or HQ in each NMR tube. Whereas, concentrations of Hg^{2+} and PA are presented in Table 2.

Preliminary NMR experiments of Q in the presence of Hg^{2+} indicate that some hydrolysis had occurred, as two species appeared on the spectra. Therefore, with Q, ^{1}H and ^{31}P NMR was recorded one hour after sample preparation. In the measurements of ^{31}P, a capillary tube containing H_3PO_4 (70 %) was placed into the NMR sample tube to use as reference. Thus, ^{31}P chemical shifts were referenced to H_3PO_4 peak at 0.0 ppm, whereas, ^{1}H chemical shifts were referenced to the dioxane peak at 4.37 ppm. Changes in the chemical shifts (δ) of the substrate induced by the presence of various concentrations of Hg^{2+} were investigated.

Table 1. Concentration of quinalphos, 2-hydroxyquinoxaline, and Hg^{2+} in each NMR tube

NMR tube Number	$[Hg^{2+}] \times 10^3$ mol L^{-1}	$[Q] \times 10^2$ Mol L^{-1}	NMR tube Number	$[Hg^{2+}] \times 10^3$ mol L^{-1}	$[HQ] \times 10^2$ mol L^{-1}
0	0.00	2.82	0	0.00	1.14
1	0.39	2.82	1	0.16	1.14
2	0.88	2.82	2	0.36	1.14
3	1.98	2.82	3	0.81	1.14
4	4.46	2.82	4	1.82	1.14
5	10.0	2.82	5	4.09	1.14
6	22.6	2.82	6	9.20	1.14
7	45.2	2.82	7	18.4	1.14
8	90.4	2.82	8	36.8	1.14

[Q]= concentration of quinalphos, [HQ]= concentration of 2-hydroxyquinoxaline

Note that concentrations higher than 90.4×10^{-3} mol L^{-1} of $[Hg^{2+}]$ were also used, but when such concentrations added to the Q stock solution, a yellow precipitate formed. This prevented NMR studies at higher concentrations.

Table 2. Concentration of diethyl phosphorothioic acid and Hg^{2+} in each NMR tube

NMR tube Number		[Hg^{2+}] x 10^3 Mol L^{-1}	[PA] x 10^3 Mol L^{-1}
0		0.00	9.87
1		0.38	9.87
2		0.75	9.87
3		1.50	9.87
4		3.00	9.87
5		6.00	9.87
6		12.0	9.87
7		23.0	9.87
8		30.0	9.87
9		38.0	9.87

[PA]= concentration of diethyl phosphorothioic acid

RESULTS AND DISCUSSION

Hg^{2+}-Catalyzed the hydrolysis of quinalphos

In our previous paper [1], kinetic data has shown clearly that Hg^{2+} catalyzed the hydrolysis of quinalphos at 25°C and pH 4.0, 7.0, and 10.0 as illustrated in Table 3 below. Hydrolysis rates in the absence of Hg^{2+} were determined to be 6.75 ± 0.11 x 10-8, 9.50 ± 0.17 x 10-8, 17.6 ± 0.0 x 10-8 s-1 at pH 4.0, 7.0, and 10.0, respectively [1].

Table 3. Kinetic data for the hydrolysis of quinalphos in the presence of Hg^{2+} at 25oC

(Reproduced from reference 1)

pH	K_{obs} (dis) x 10^3, s^{-1}	k_{obs} (app.) x 10^3, s^{-1}	k_{obs} (avg) x 10^3, s^{-1}
4.0	3.63 ± 0.02	3.67 ± 0.02	3.65 ± 0.02
7.0	0.207 ± 0.002	0.182 ± 0.002	0.195 ± 0.013
10.0	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000

k_{obs} (dis) = First order rate constant (disappearance of quinalphos)
k_{obs} (app.) = First order rate constant (appearance of the product)
k_{obs} (avg) = Average first order rate constant

NMR in the absence and presence of Hg^{2+}

Although previous studies have shown that ESI-MS is able to reflect gas phase ions that are correlated to the solution structure [15-17], NMR experiments were carried out to demonstrate whether the interactions observed through ESI-MS [1] could also be seen in solution.

To this end, NMR experiments were performed on quinalphos (Q) and its hydrolysis products (HQ and PA) in the presence of various concentrations of the catalytic ion (Hg^{2+}). Various concentrations of Hg^{2+} were added to compare changes in chemical shift associated with various atoms in quinalphos or its product molecules. The chemical shifts (δ) are reported in parts per million (ppm). Changes in chemical shifts (Δδ) Q were calculated by subtracting the chemical shift values of ^{1}H and ^{31}P in the presence of Hg^{2+} from those of the same shifts of ^{1}H and ^{31}P in metal-free solution. Change in chemical shift could then be attributed to interaction between the metal ion and Q, HQ, or PA. Preliminary NMR experiments with quinalphos in the presence of Hg^{2+} demonstrated that hydrolysis occurred. Therefore, for comparison purposes in the case of quinalphos, in the presence of Hg2+, ^{1}H and ^{31}P NMR spectra were recorded 1 hour after preparation.

In order to characterize whether or not the Q - Hg^{2+} complex is formed during the kinetic studies, the interaction of Q with Hg^{2+} was studied in aqueous solutions. Nine samples were prepared of which one contained only an aqueous solution of Q, while the other eight contained Q and Hg^{2+} in various ratios. ^{1}H and ^{31}P NMR spectra were recorded on each of these samples. Overlay ^{1}H NMR spectra of Q in the absence and presence of various concentrations of Hg^{2+} are shown in Figure 1. Table 4 (A and B) lists the chemical shifts (^{1}H) for Q; changes in chemical shifts were also included. However, due to the interference of CH_2 peak with the dioxane signal, the change in chemical shift of the methylene could not be followed.

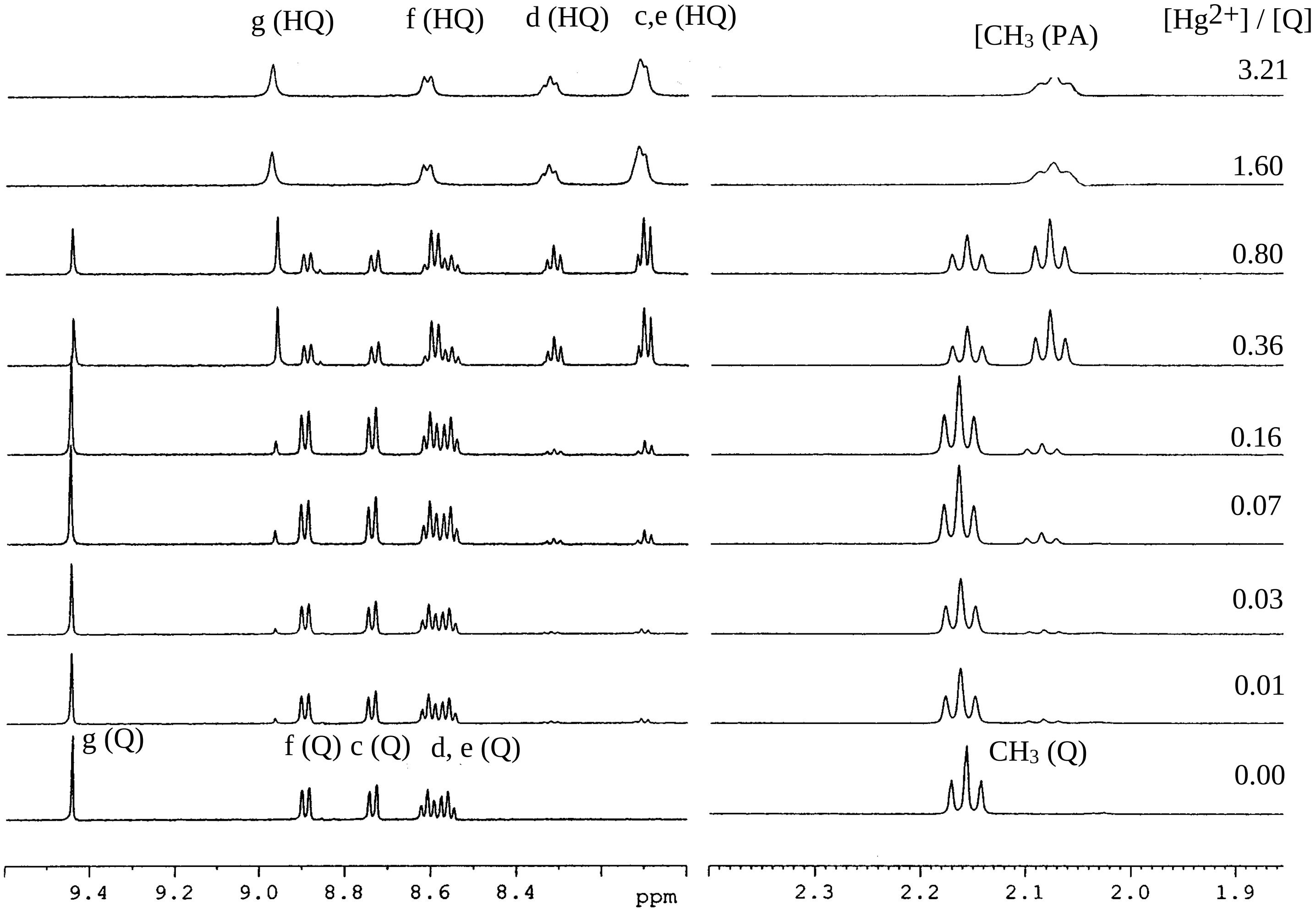


Figure 1. ***^{1}H NMR spectra showing the chemical shift of quinalphos peaks in the presence of various concentrations of Hg^{2+} (after 1 h)***

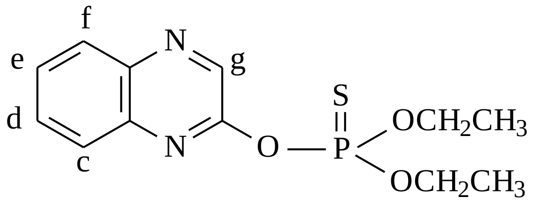


Figure 2. Chemical structure of quinalphos

Table 4 (A). ^{1}H NMR chemical shifts of quinalphos peaks in the absence and presence of various concentrations of Hg^{2+}. [Q] = 2.82 x 10^{-2} mol L^{-1}

Experimental Number	[Hg^{2+}] x 10^3 M	[Hg^{2+}] / [Q]	δ of CH$_3$	Δδ	δ of ^{1}H^g	Δδ	δ of ^{1}H^f	Δδ
0	0.00	-	2.16	-	9.44	-	8.89	-
1	0.39	0.01	2.16	0.00	9.44	0.00	8.89	0.00
2	0.88	0.03	2.16	0.00	9.44	0.00	8.89	0.00
3	1.98	0.07	2.17	-0.01	9.45	-0.01	8.89	0.00
4	4.46	0.16	2.17	-0.01	9.45	-0.01	8.89	0.00
5	10.0	0.36	2.16	0.00	9.44	0.00	8.89	0.00
6	22.6	0.80	2.16	0.00	9.44	0.00	8.89	0.00

Table 4 (B). ^{1}H NMR chemical shifts of quinalphos peaks in the absence and presence of various concentrations of Hg^{2+}.

Experimental Number	[Hg^{2+}] x 10^3 M	[Hg^{2+}]/ [Q]	(δ) of ^{1}H^c	Δδ	(δ) of 1H^d	Δδ	(δ) of 1H^e	Δδ
0	0.00	-	8.73	-	8.61	-	8.56	-
1	0.39	0.01	8.73	0.00	8.61	0.00	8.56	0.00
2	0.88	0.03	8.73	0.00	8.61	0.00	8.56	0.00
3	1.98	0.07	8.73	0.00	8.61	0.00	8.56	0.00
4	4.46	0.16	8.73	0.00	8.61	0.00	8.56	0.00
5	10.0	0.36	8.73	0.00	8.61	0.00	8.56	0.00
6	22.6	0.80	8.73	0.00	8.61	0.00	8.56	0.00

As stated above, any change in the chemical shift in the presence of a metal ion can be attributed to an interaction between the metal ion and the substrate; if there is an interaction between Hg^{2+} and Q, change in 1H chemical shifts, especially ^{1}H close to the interaction site would be expected. In these experiments, in the presence of increasing amounts of Hg^{2+} (experiments 1 to 6, Table 4), the ^{1}H peaks of Q did not shift. The new resonances found at δ 8.9 (s, H^g; HQ), 8.6 (^{d}, H^f, HQ), 8.3 (t, H^d; HQ), 8.1 (overlapping t and d, H^e + H^c; HQ) and δ 2.08 (t, CH$_3$; PA) are ascribed to the hydrolysis products HQ and PA rather than to complexed Q. The assignment of HQ and PA is partly confirmed by the high concentrations of Hg^{2+} (experiments 7 and 8) (Figure 1, two top traces), where only the hydrolysis products (HQ and PA) are present.

In order to test whether Hg^{2+} interacts with the substrate through the S atom as indicated by the MS experiments [1], ^{31}P NMR spectra were recorded in the absence and presence of different concentrations of Hg^{2+} (Figure 3). The chemical shift of the phosphorus bonded to the potential S site of complexation would be expected to be sensitive to any coordination of Hg^{2+} to sulfur. In contrast to the situation with the ^{1}H resonances (Figure 1 and Table 4, A and B), it is found that as the concentration of Hg^{2+} increased, δ of ^{31}P shifted upfield (Table 5 and Figure 4).

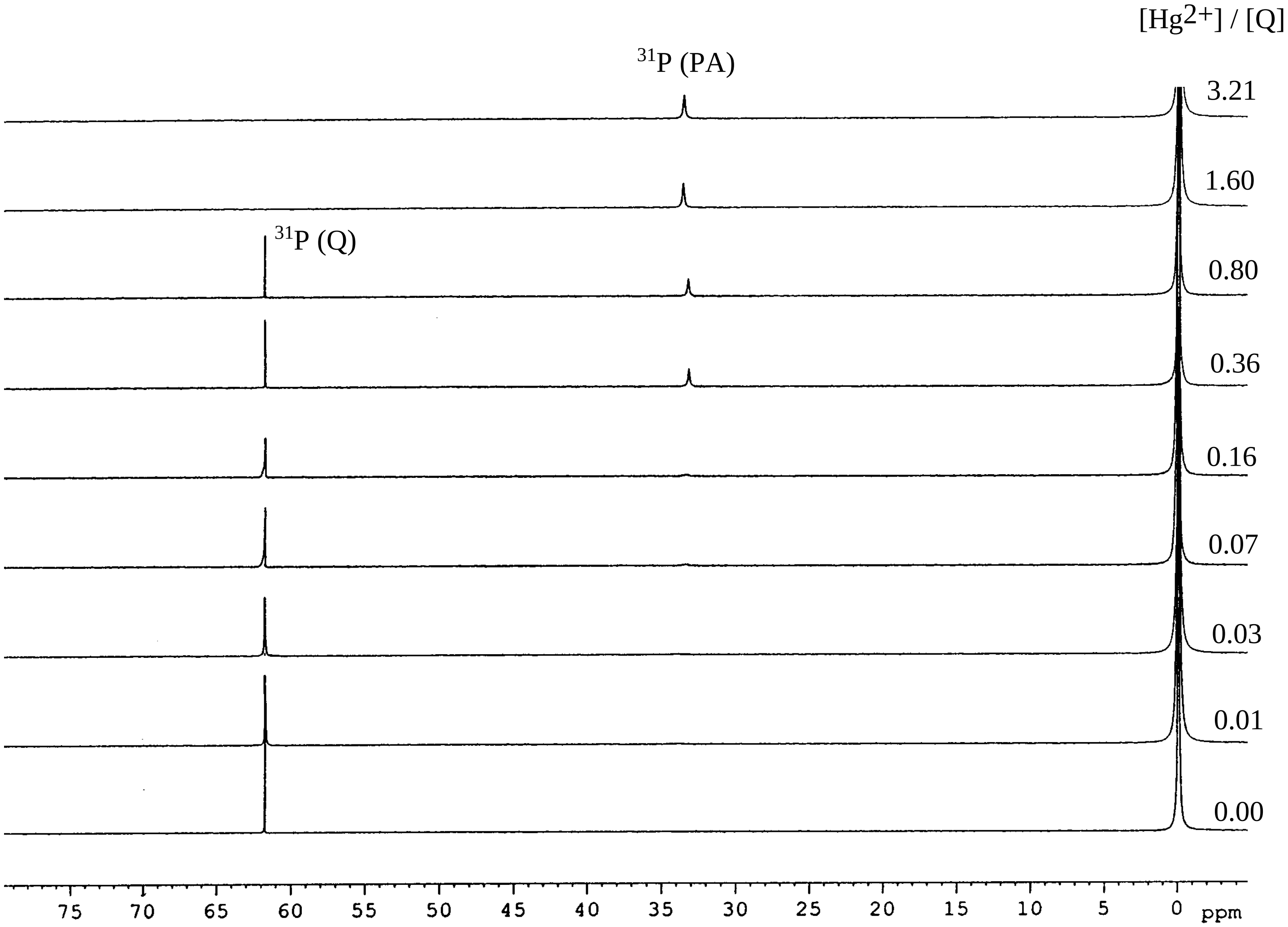


Figure 3. ^{31}P **NMR spectra showing the chemical shift of quinalphos peak in the presence of various concentrations of** Hg^{2+} **(after 1 h)**

Table 5. ^{31}P **NMR chemical shifts of quinalphos peak in the absence and presence of various concentrations of** Hg^{2+}. **[Q] = 2.82 x** 10^{-2} **M**

Experimental Number	$[Hg^{2+}] \times 10^3$ M	[Hg] / [Q]	(δ) of ^{31}P	Δδ
0	0.00	-	61.89	-
1	0.39	0.01	61.81	0.08
2	0.88	0.03	61.79	0.10
3	1.98	0.07	61.76	0.13
4	4.46	0.16	61.73	0.16
5	10.0	0.36	61.69	0.20
6	22.6	0.80	61.66	0.23

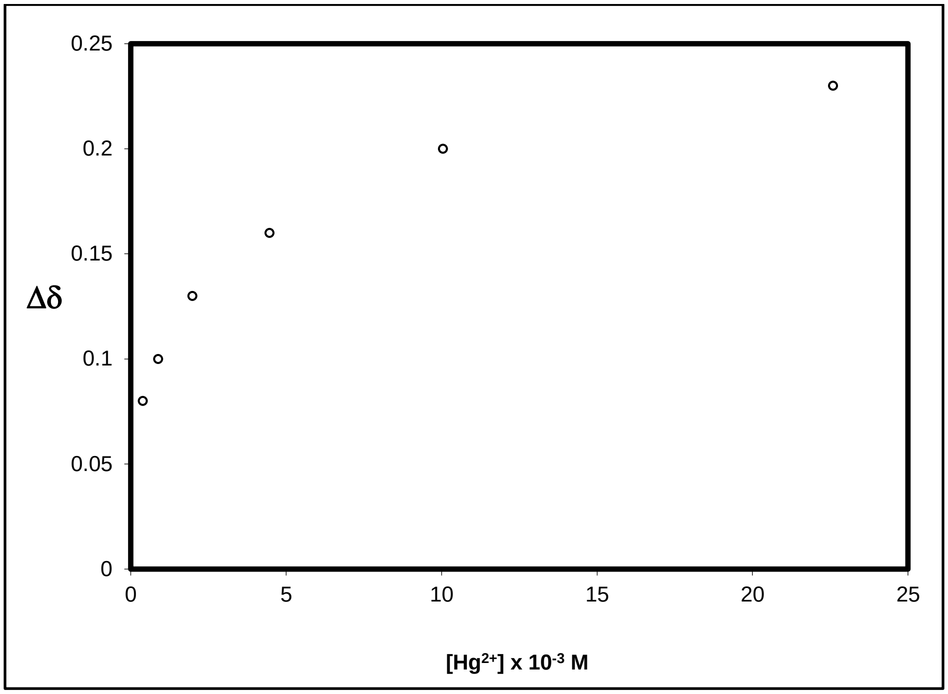


Figure 4. Effect of various concentrations of Hg^{2+} on the chemical shifts of the ^{31}P of quinalphos.

Figure 4 and Table 5 illustrate the change in chemical shift (Δδ) of ^{31}P as a function of increasing concentration of mercury ion. Figure 3 appears to describe a binding isotherm. However, when the data were treated according to a standard double reciprocal plot [18] the data fit the line poorly. Regardless, Figure 4 clearly support that Hg^{2+} binds to the S centre, as the ^{31}P peak shifted up field with increasing the concentration of Hg^{2+}.

The potential for binding of Hg^{2+} to a N atom in Q was studied in more detail by using the HQ product to which Hg^{2+} was added. For this purpose, ^1H NMR spectra of HQ product were recorded in the presence of Hg^{2+} and compared with the chemical shifts of HQ in solution free of metal ion. The ^1H NMR spectra, Figure 6, show that there was a small shift in some protons and the maximum change in the chemical shift occurred in the presence of the two highest concentrations of mercury (data points of experiments 7 and 8; see Table 6 (A and B) and Figure 6.

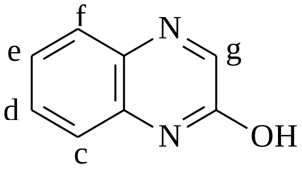


Figure 5. Chemical structure of 2-hydroxyquinoxaline

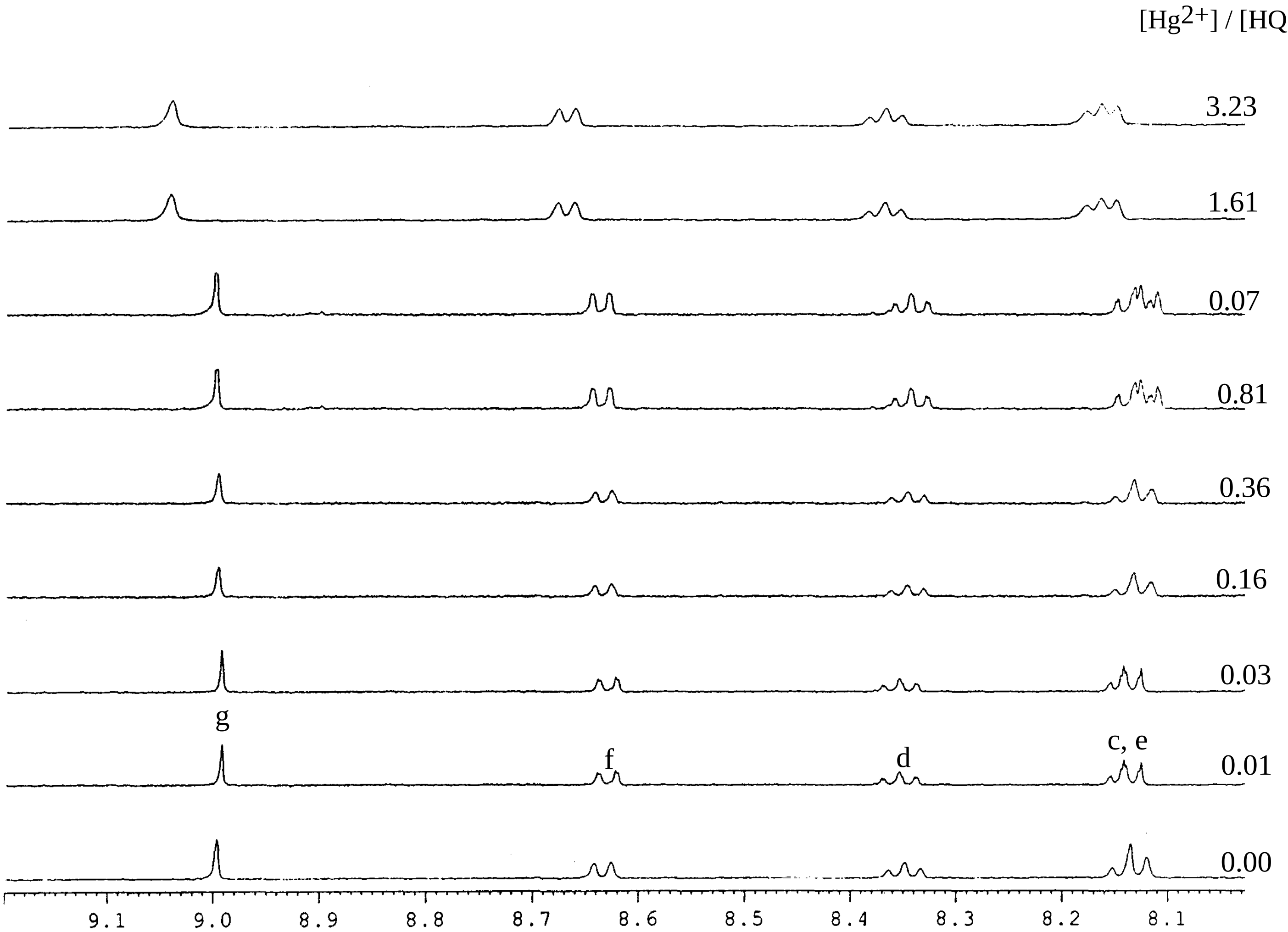


Figure 6. 1H NMR spectra showing the chemical shift of 2-hydroxyquinoxaline peaks in the presence of various concentrations of Hg2+

Table 6 (A). ^{1}H NMR chemical shifts of 2-hydroxyquinoxaline peaks in the absence and presence of various concentrations of Hg^{2+}. [HQ] = 1.14 x 10^{-2} M

Experimental Number	$[Hg^{2+}]$ x 10^3 M	$[Hg^{2+}]$ / [HQ]	δ of H^g	Δδ	δ of H^f	Δδ
0	0.00	-	8.99	-	8.63	-
1	0.16	0.01	8.99	0.00	8.62	0.01
2	0.36	0.03	8.99	0.00	8.62	0.01
3	0.81	0.07	8.99	0.00	8.63	0.00
4	1.82	0.16	8.99	0.00	8.63	0.00
5	4.09	0.36	9.00	-0.01	8.63	0.00
6	9.20	0.81	9.00	-0.01	8.63	0.00
7	18.4	1.61	9.04	-0.05	8.66	-0.03
8	36.8	3.23	9.04	-0.05	8.66	-0.03

Table 6 (B). ^{1}H NMR chemical shifts of 2-hydroxyquinoxaline peaks in the absence and presence of various concentrations of Hg^{2+}.

Experimental Number	$[Hg^{2+}]$ x 10^3 M	$[Hg^{2+}]$ / [HQ]	δ of H^d	Δδ	δ of $H^{e,c}$	Δδ
0	0.00	-	8.35	-	8.14	-
1	0.16	0.01	8.36	-0.01	8.14	0.00
2	0.36	0.03	8.36	-0.01	8.14	0.00
3	0.81	0.07	8.35	0.00	8.13	0.01
4	1.82	0.16	8.35	0.00	8.13	0.01
5	4.09	0.36	8.35	0.00	8.13	0.01
6	9.20	0.81	8.35	0.00	8.13	0.01
7	18.4	1.61	8.37	-0.02	8.17	-0.03
8	36.8	3.23	8.37	-0.02	8.17	-0.03

The feature to be noted from data in table 6 and figure 6 is that as the concentration of Hg^{2+} increases, the resonance assigned to H^g undergoes the largest downfield shift, although it amounts to a maximum change of only 0.05 ppm. The peaks assigned to H^f, H^d and $H^{e,c}$ are less sensitive and undergo smaller downfield shifts (0.02 – 0.03 ppm at most) and then only at the highest concentrations of added Hg^{2+} examined ($\geq 1.84 \times 10^{-2}$ M; ≥ 1.61:1 ratio). Although the change in the chemical shift is small, this may indicate that Hg^{2+} has a limited tendency to bind to an N atom. Coordination to N would be expected to reduce the electron-density in the quinoxaline ring where protons closest to the binding site would be expected to exhibit the greatest loss of electron-density and, hence, exhibit the largest downfield shift. Accordingly, because H^g is closest to the N binding site, it undergoes the greatest downfield shift and the onset of a detectable shift to lower field occurs at a lower added Hg^{2+} concentration (i.e. 4.09×10^{-3} M; 0.36:1 ratio).

However, the relatively small changes in chemical shift, even for H^g preclude any analysis of the magnitude of the binding constant of Hg^{2+} to HQ through N. ^{31}P measurements of PA were also performed in the absence and presence of different concentrations of Hg^{2+}. Sequential ^{31}P NMR spectra, Figure 7, demonstrate an interaction between Hg^{2+} and the PA molecule. The ^{31}P chemical shift of PA in the absence of Hg^{2+} is 64.52 and undergoes progressive upfield shift of ca. 33 ppm as the ratio $[Hg^{2+}]$ / [PA] increases from 0.04 to 3.85. Table 7 and Figure 7 show the correlation in chemical shifts with increasing concentration of Hg^{2+}.

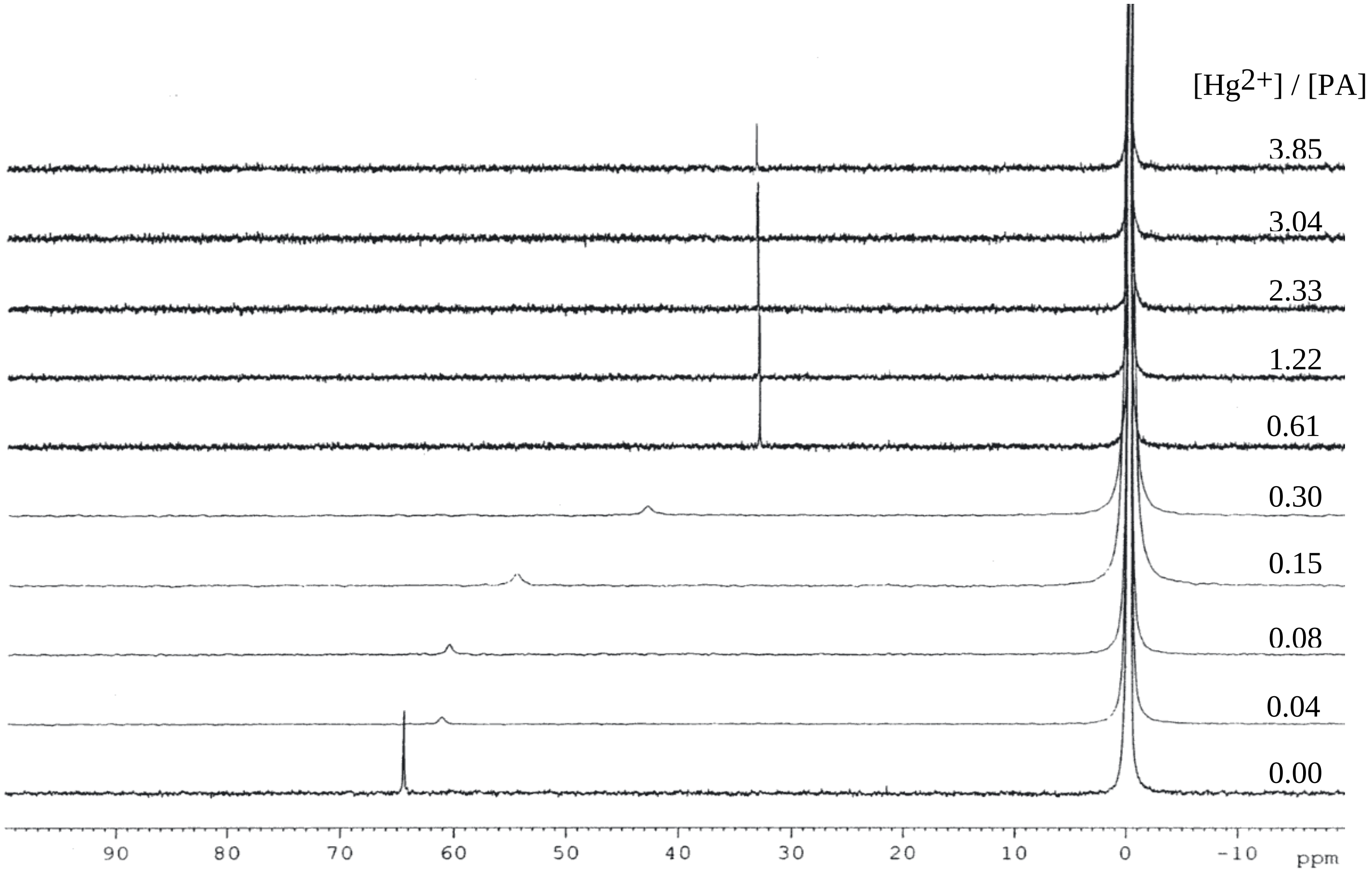


Figure 7. ^{31}P NMR spectra showing the chemical shift of O,O-diethyl phosphorothioc acid peak in the presence of various concentrations of Hg^{2+}.

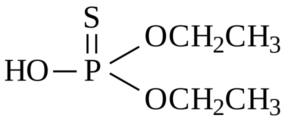


Figure 8. Chemical structure of O, O-diethyl phosphorothioc acid

Table 7. ^{31}P NMR chemical shifts of O, O-diethyl phosphorothioc acid peak in the absence and presence of various concentrations of Hg^{2+}. [PA] = 9.87 x 10^{-3} M

Experimental Number	$[Hg^{2+}] \times 10^3$ M	$[Hg^{2+}] / [PA]$	(δ) of ^{31}P	Δδ
0	0.00	-	64.52	-
1	0.38	0.04	61.19	3.33
2	0.75	0.08	60.59	3.93
3	1.50	0.15	54.60	9.92
4	3.00	0.30	42.97	21.55
5	6.00	0.61	33.08	31.44
6	12.0	1.22	33.18	31.34
7	23.0	2.33	33.24	31.28
8	30.0	3.04	33.31	31.21
9	38.0	3.85	33.37	31.15

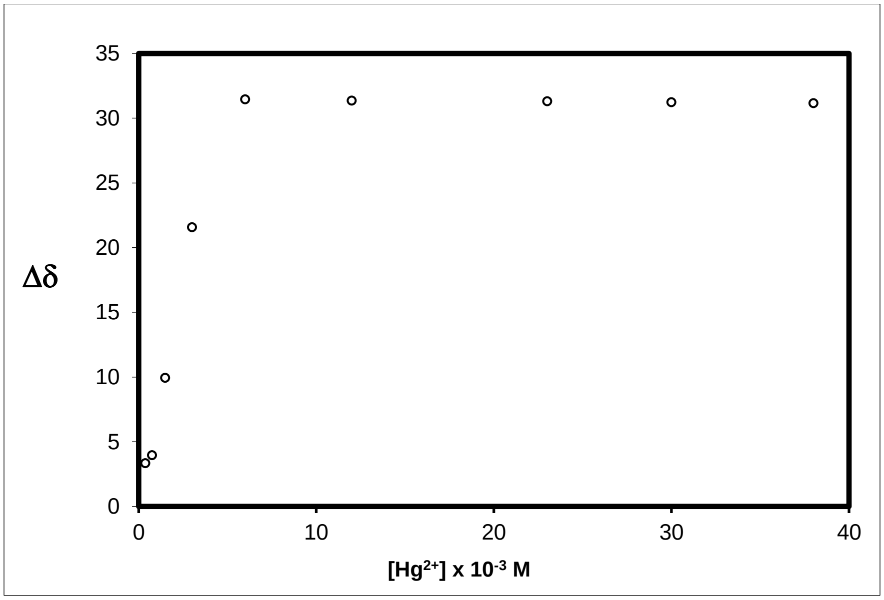


Figure 9. Effect of various concentrations of Hg^{2+} on the chemical shifts of the ^{31}P of O,O-diethyl phosphorothioic acid

The large change in the chemical shift (~31 ppm; Table 7 and Figures 7 and 9) in consistent with Hg^{2+} coordination to the S atom. This suggests that Hg^{2+} can also bind to the parent compound *via* the S atom as well as to N, as outlined above.

In general, the solution NMR results support the ESI-MS studies [1]. The ^1H NMR data presented in Figure 1 show that hydrolysis was enhanced by the presence of Hg^{2+}; this enhancement alone supports that Hg^{2+} coordinates in some manner the substrate. Note, however, that the solutions used for NMR measurements were required to be highly acidic in order to keep the large concentrations of mercury in solution. By analogy to other OP compounds [4] the catalytic effect of Hg^{2+} on hydrolysis is much reduced in such solutions, presumably due to protonation of the coordinated sites.

Figure 1 and Table 4 show no changes in the ^1H chemical shifts were observed when Hg^{2+} is added to Q ($[Hg^{2+}]$ / [Q] from 0.01 to 0.8). We interpret this as due to the low affinity of mercury (II) toward N atoms. Recall that no $[Q + Hg]^{2+}$ ion could be detected by ESI-MS, either [1].

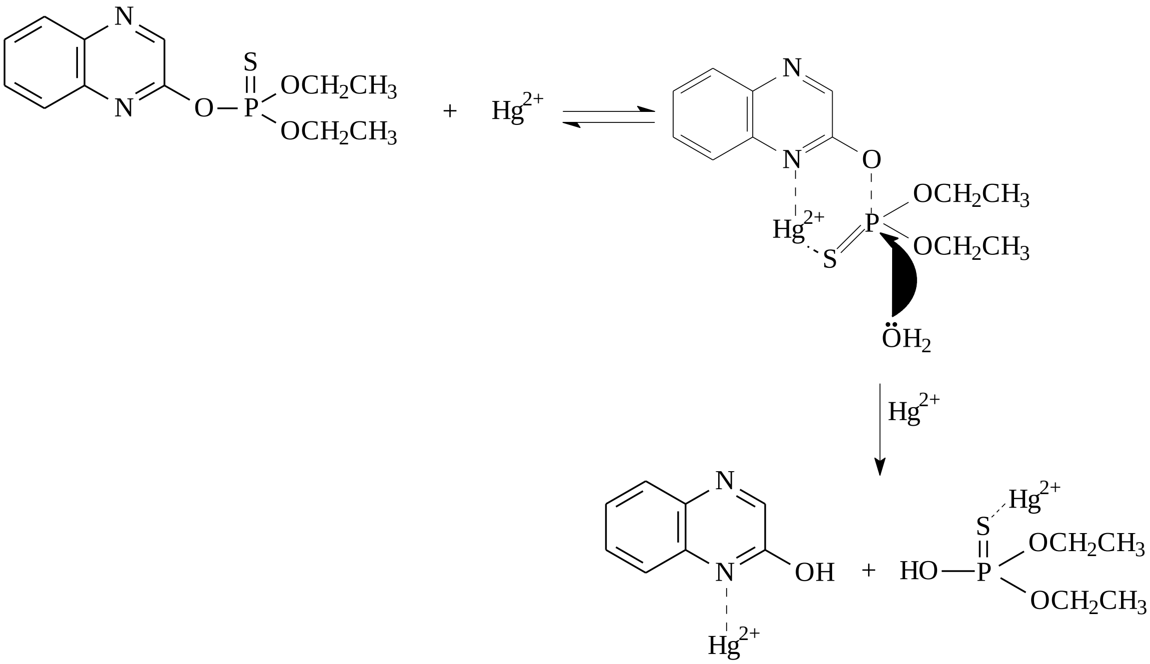
On the other hand, in the case of ^{31}P, Figure 4 and Table 5 show that as the concentration of Hg^{2+} increases so does the $\Delta\delta$, indicating that most likely Q- Hg^{2+} complex is formed, although this complex was not seen in the gas phase ESI-MS studies [1]. As we believe that mercury (II) binds the S atom, this kind of binding can be expected to affect the chemical shift of ^{31}P more than that of ^1H CH_3, as the CH_3 is removed from S; this, as well as the smaller "window" for ^1H chemical shifts as compared to ^{31}P shifts, accounts for the difference in behaviour of these two probes. At high concentrations of Hg^{2+} ($[Hg^{2+}]$ / [Q] > 0.80), the ^{31}P peak belongs to the PA product, as Q underwent hydrolysis. Hg^{2+} ion is known to have a strong affinity for compounds containing sulfur [19-22].

^1H spectra of HQ were also recorded (Figure 6 and Table 6) and show no change in chemical shift up to a ratio $[Hg^{2+}]$ / [HQ] of 0.81. This is consistent with the behavior observed for ^1H of Q, which indicates that the interaction of Hg^{2+} with N is weak. This supports the ESI-MS data (HQ - Hg^{2+} complex was not observed) [1]. However, when the ratio of $[Hg^{2+}]$ / [HQ] increased by 2 and 4-fold (1.61 and 3.22, in turn), ^1H NMR spectra exhibited small downfield shifts (0.05 ppm), indicating that mercury (II) may have a limited ability to bind to HQ through N. Another feature from these spectra is the similarity in ^1H chemical shifts of HQ and Q in experiments 7 and 8 (compare the Figures 1 and 6). This indicates that, as expected in the case of quinalphos, when the ratio of $[Hg^{2+}]$ / [Q] is 1.60 or higher, all quinalphos underwent hydrolysis (Figure 1) and the aromatic proton signals belong to the HQ product. Other researchers [11,23] have also shown that Hg^{2+} has the ability to form complexes with N-containing species. Moder et al. have shown that Hg^{2+} formed complex with bis-tren(tris-pyridine) amine (contains N atoms in its structure) by using ESI-MS technique [23].

In the situation where Hg^{2+} was added to PA, the magnitude of the ^{31}P chemical shift changes upon complexation with mercury ion (see Figures 7 and 9 and Table 7). This significant change in the chemical shift adds evidence that Hg^{2+} binds to PA most likely through S, which was also suggested by the MS results [1]. The upfield shifts in the ^{31}P resonances by presence of Hg^{2+} may be due to changes in the torsion angle of the thiophosphate group; an increase in bond angle results in an upfield chemical shift and vice versa. Gorenstein has reported that "a decrease in the smallest O – P – O bond angle in the molecule results in downfield shift of the phosphorus nucleus" [24]. Similar conclusion was drawn with S – P – S bond angle [25].

Figure 9 and Table 7 show also that the interaction of Hg^{2+} with PA reached equilibrium when the ratio of $[Hg^{2+}]$ / [PA] fell between 0.6 and 1.2; this is probably due to the high affinity of Hg^{2+} toward S, and may be indicative of the formation of a 1:1 complex. The question may arise why the change in the chemical shift ($\Delta\delta$) of ^{31}P in the case of Q is not similar to that of PA. As an example, when the ratio of $[Hg^{2+}]$ / [Q] = 0.8, $\Delta\delta$ was 0.23 ppm, whereas when $[Hg^{2+}]$ / [PA] = 0.6, $\Delta\delta$ was ~ 31 ppm. This large difference between the magnitude of $\Delta\delta$ values may be associated with the aromatic component of the Q structure. Small shifts in ^{31}P resonance in the presence of metal ions were also reported by other researchers on other organophosphorus compounds that have aromatic parts in their structure. Reaction with Mg^{2+} shifted the ^{31}P peak of 5′-monophosphate, AMP ($[Mg^{2+}]$ / [AMP], 10) upfield by 0.4 ppm [26]. The same group also studied the effect of the same metal ion (Mg^{2+}) on the ^{31}P chemical shift of another OP compound (1,4,5 trisphosphate, IP3). They reported that when $[Mg^{2+}]$ / [IP3] is 1.25, the ^{31}P resonances of P1, P4, and P5 shifted upfield by 0.03, 0.5, and 0.6 ppm, respectively. Increasing the concentration of Mg^{2+} shifted the ^{31}P peaks further upfield. When the ratio of $[Mg^{2+}]$ / [IP3] was increased to 2.5, the ^{31}P peaks of P1, P4, and P5 shifted upfield in turn by 0.2, 0.7, and 0.9 ppm [26]. From another study of chemical shifts as an indicator for binding metal ions with organic compounds, the binding of metal ions to lysozyme was investigated by using ^{13}C chemical shifts. The mercury-lysozyme complex formed and ^{13}C resonance shifted downfield by ~ 4.0 ppm [27].

Binding of Hg^{2+} to S and possibly to N in quinalphos (Scheme 1) is probably the main reason for quinalphos being hydrolyzed in the presence of Hg^{2+} [1]. This indicate that a six membered ring may formed and that this complex facilitates nucleophilic attack, enhancing hydrolysis. In Scheme 1, Hg^{2+} the internuclei distance to S is likely shorter, reflecting its stronger affinity for S.



Scheme 1. A transition states for the metal ions-catalyzed hydrolysis of quinalphos

The position of Hg^{2+} in relation to the N and S atom is depicted based on the extent of interaction with parts of the quinalphos molecule as estimated by NMR.

CONCLUSION

In order to demonstrate that the complexes observed *via* ESI-MS (in our previous publication [1]) are correlated with ones in solution, ^{1}H and ^{31}P NMR of Q, ^{1}H of HQ, and ^{31}P of PA were used to provide evidence of metal ion binding to the substrate (Q). In fact, particular attention was paid to the ^{1}H NMR of HQ and ^{31}P of PA to distinguish between the binding of Hg^{2+} to either S, or N, or to both in the substrate. In fact, a small upfield change in ^{31}P chemical shift of Q was observed with increasing concentration of Hg^{2+}. Additions of Hg^{2+} caused larger change in the ^{31}P chemical shift of PA as would be expected if Hg^{2+} binds to Q through S. Small shifts in ^{1}H peaks of HQ with increasing concentrations of Hg^{2+} indicate weak binding of Hg^{2+} to Q through N as well.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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