Theoretical and Experimental ^{31}P NMR and ESI-MS Study of Hg^{2+} Binding to Fenitrothion

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We present the theoretical and experimental results of ^{31}P NMR and low energy CID MS/MS study of Hg^{2+} binding to fenitrothion (FN). The calculated ^{31}P NMR chemical shifts order for FN with Hg^{2+} complex is in good agreement with experimental ^{31}P NMR chemical shifts order. The experimental and theoretical ^{31}P NMR study of organophosphorus pesticide with Hg^{2+} gives to important information for organophosphorus pesticide metal complexes. ESI-MS and low energy CID MS/MS experiments of Hg^{2+}-FN complexes combined with accurate mass measurements give insight into the metal localization and allow unambiguous identification of fragments and hydrolysis products.

Key Words: Fenitrothion (FN). ^{31}P NMR chemical shift. Electrospray ionization mass spectrometry (ESI-MS). Density functional theory (DFT). Hg^{2+}-FN complex

Introduction

In modern agriculture. forest industries and fields, different pesticides have been widely used. The most of pesticides are harmful to human and animals, and also cause serious environmental damage each year. The organophosphorus (OP) pesticides are widely used today in agriculture for field crop protection and fruit tree treatment. The OP pesticides have been known as a fatal chemicals and they have proven strong dependence on the chemistry of their aquatic environmental.[1] Another problem arises from contamination of pesticides in food through plants affected by pesticide residues that survive through preservation.[1] Due to their toxicity to humans and long-term environmental effects. numerous investigations have been carried out to evaluate the degradation of OP pesticides.[1-3] Fenitrothion (FN) is remarkably less toxic to mammals than other organophosphate insecticides such as methyl parathion and parathion. For this reason. it is used extensively for the control of agricultural and forest pests and also used as a public health insecticide for control of household insects. flies in animal houses. stored product insect pests. mosquito larvae and locusts.[4] Generally. FN degrades in three ways: through hydrolysis. photolysis.[1d] and microbial[1b,1c,2] degradation.[1f] Hydrolysis is one of the most thoroughly studied pathways for the degradation of FN and other OP pesticides. The OH$^-$ (pH > 9.0)$^{2-5}$ nucleophilic attack on FN results in expulsion of the aryloxide[3] in an S$_N$2(P) displacement reaction. However, at low pH (pH ≤ 7.0) and presence of soft nucleophiles such as I$^-$, Br$^-$, H$_2$O. and also in a ctyltrimethylammonium (CTA) micellar system attack on organophosphorus esters such as FN results in expulsion of demethylfenitrothion in an S$_N$2(C) displacement reaction.$^{2-6}$ In OH$^-$/H$_2$O. the S$_N$Ar pathway is indistinguishable from S$_N$2(P) since both produce identical products: however. on changing the base/solvent to EtO$^-$/EtOH. the S$_N$Ar route is observed as a minor
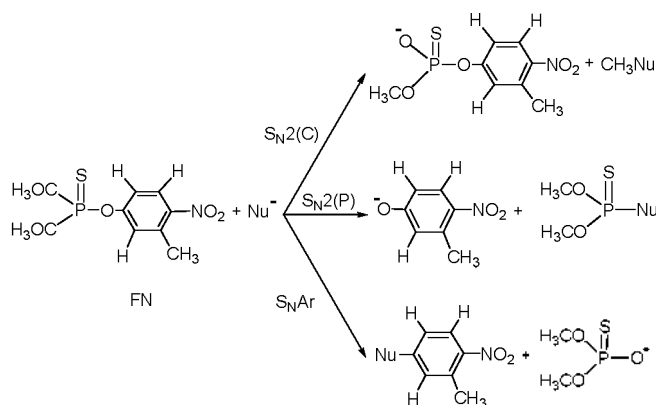
Scheme 1. Competitive pathways observed in the degradation of FN.

(≤ 10 percent) pathway (Scheme 1).^{2,6,7}

Recent research has highlighted the role of metal ions in a biotic degradation processes for the organophosphorus ester and thioester pesticides.$^{7-9}$ The rates of hydrolysis in metal ion solutions. such as Cu^{2+}, Hg^{2+}and Ag$^+$. were 20-3000 times faster than those of the uncatalyzed reactions.^{7,10} However. the former studies do not explain the role of metal ions in hydrolysis of organophosphorus pesticide. There is no direct experimental evidence provided to show organophosphorus pesticide with metal ion interaction. It is postulated that the interaction of metal ions with atoms within O=P or S=P pesticides may enhance their hydrolytic degradation. In order to find direct experimental evidence for organophosphorus pesticides interaction with metal ion. we carried out theoretical studies on ^{31}P NMR chemical shift and low energy CID MS/MS studies for FN and FN with Hg^{2+} complex. From the study of Hg^{2+} complex and their fragmentation products, it may be possible to draw conclusion about the Hg^{2+} metal binding site and that may give insight into the hydrolysis mechanism.

Experimental Section

Reagents. Fenitrothion (*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl phosphorothioate: FN; 96.7%) was gifted from Sumitomo Chemicals, Japan, and it was purified as previously described.[9(b)]

NMR study. The stock solutions of FN were prepared in acetone-d_6 and different concentrations of Hg^{2+} metal ion stock solutions were prepared in D_2O. For NMR analysis, 0.15 mL of metal ion stock solution was added to 0.45 mL of FN stock solution in NMR tube. The pH for stock solution mixtures was ~5.5 for Hg^{2+} metal ion. All ^{31}P NMR experiments on the FN were carried out in the absence and presence of Hg^{2+} metal ion using Bruker 400.302 MHz NMR spectrometer. In the presence of metal ion, ^{31}P NMR spectra were recorded after 5 minute of preparation. ^{31}P NMR chemical shifts (δ) are reported in parts per million (ppm) with respect to 85% H_3PO_4 used as an external chemical shift reference. The spectra were acquired using 90° pulses with 1000-1500 scans for ^{31}P nucleus.

Electrospray Ionization Mass Spectrometry (ESI-MS). 0.10 mL of FN stock solution (0.01 M) prepared in dry acetone was diluted with dry methanol to prepare 1.0×10^{-4} M solution. $HgCl_2$ was dissolved in deuterium depleted water (DDW) for preparation of 5.0×10^{-5} M of Hg^{2+} metal ion solutions. Low micro molar FN solution in methanol and aqueous Hg^{2+} solutions were acidified to a pH = 4 using 0.01 M nitric acid solutions. Measurements were done immediately after the acidified solutions were mixed with varying concentrations of aqueous Hg^{2+}. The mixture was sprayed with a flow rate of 6.0 μL/min into a QSTAR XL QqTOF mass spectrometer (Applied Biosystems/MDS Sciex) employing a regular ESI source setup. Fragmentation of the transition metal-pesticide complex is achieved in two different methods: first, in-source fragmentation by elevating the cone voltage and second, low energy CID in the Q2 collision cell using nitrogen as collision gas. Combination of both methods allows for pseudo-MS^3 analysis of the complexes. Accurate mass measurements enable the correct assignment of molecular formulas for fragments in case of isobaric overlap of possible fragmentation products.

Results and Discussion

NMR study on FN in the absence and presence of Hg^{2+} ion. NMR experiments were performed on FN with various concentrations of the catalytic Hg^{2+} ion. Change in chemical shifts ($\Delta\delta$) was calculated by subtracting the chemical shift values of ^{31}P in the presence of each metal ion from those of the same shifts of ^{31}P in metal-free solution. The upfield change in chemical shift could be attributed to interaction between each metal ion and FN. Preliminary NMR experiments with FN in the presence of the set of metal ions demonstrated that hydrolysis reaction occurred. ^{31}P NMR spectra in the presence of Hg^{2+} ion were recorded after 5 minute of preparation. In order to characterize whether the FN-Hg^{2+} or Hg^{2+}-FN-Hg^{2+} complex are formed during the hydrolysis or not, four samples were prepared; one contained only an aqueous solution of FN, while the other three contained FN and Hg^{2+} in various ratios.

Table 1. ^{31}P NMR chemical shift of FN peaks in the absence and presence of various concentration of Hg^{2+} ion in 25% D_2O-75% Acetone-d_6

$[Hg^{2+}] \times 10^3$ M	$[Hg^{2+}]/[FN]$	pH	δ of $^{31}P^b$	$\Delta\delta$
0.00	-	-	64.67	0.00
10.5	2.50	6.09	64.51	0.15
21.0	5.00	5.60	64.47	0.19
42.0	10.0	5.38	64.42	0.24

$^a[FN] = 4.20 \ 10^{-3} M;$ ^{b31}P NMR chemical shifts (δ) are referenced against 85% H_3PO_4.

The chemical shift of the phosphorus atom bonded to the potential sulfur (S) atom is expected to be sensitive to the coordination of Hg^{2+} to the sulfur atom.[1f] It was found that δ of ^{31}P shifted upfield as the concentration of Hg^{2-} increased.[1f]

As mentioned at the reported works,[1,11] ^{31}P NMR chemical shifts for Demeton S show up-field shifts whenever the electron density of central P atom reduced. The ^{31}P NMR chemical shifts for OPs compounds show upfield shifts when the more electronegative atoms bind to the central P atom. The systematic studies regarding the relative chemical shifts in the ^{31}P NMR spectra of OPs or related compounds are available in the literature.[12-15] Table 1 shows the change in chemical shift ($\Delta\delta$) of ^{31}P as a function of concentration of Hg^{2+} ion. Results in Table 1 clearly show the ^{31}P peak shifted upfield with increasing the concentration of Hg^{2+}, implying that Hg^{2-} bind to


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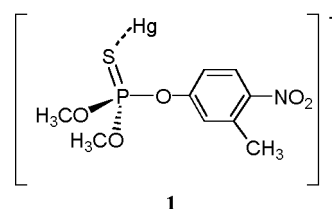
the S atom (1 and 4).

Theoretical ^{31}P NMR chemical shifts studies of the FN-Hg^{2+} complex. All the calculations have been carried out with the Gaussian 98 package.[16] All structures have been fully optimized at the density functional theory (DFT) of Becke's 3-parameter hybrid method using the correlation functional of Lee, Yang, and Parr (B3LYP)[17] at 6-31G(d) level. Relativistic effective core potential of Los Alamos and Double-ζ basis sets (LANL2DZ)[18] were employed for the metal cation. The calculations of absolute shielding constants have been performed using the Gauge-Independent Atomic Orbital (GIAO) perturbation method[19] with HF/6-311+G(2d,p) level. The geometries of FN-Hg^{2-} and FN-Hg^{2+}-FN complexes in the gas phase are shown in Figure 1. Absolute nuclear shielding constants and chemical shifts of the phosphorus and atomic net charges are shown in Table 2. These calculated results are in good agreement with experimental ^{31}P NMR chemical shifts order. In consequence of the above comparison with experimental result, ^{31}P NMR shift of FN-Hg^{2+} complex shows higher upfield change.[20]

Table 2. GIAO calculation of absolute nuclear shieldings (in ppm) and atomic net charges of phosphorus and sulfur atoms

FN and Complexes	σ (δ)	Charge[a](P, S)	Exp(δ)([M[n−]]/[FN])[c]
FN	261.9 (84.4)	0.394, -0.170	64.67
FN-Hg	282.1 (64.2)	0.196, 0.480	64.51(1: 2.5)
FN-Hg-FN[b]	281.3 (65.0)	0.368, -0.107	
H$_3$PO$_4$	346.3 (0)		0.0 (Standard)

[a]HF/6-311+G(d). [b]C$_2$ symmetry point group, [c]From these experiments.
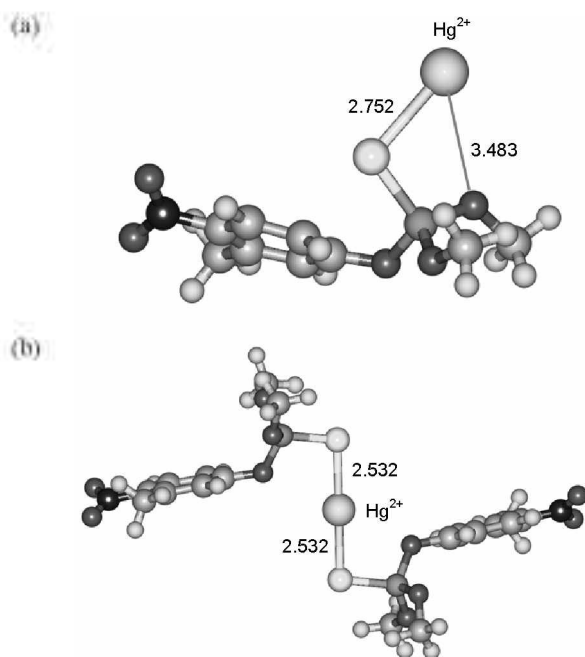
Figure 1. B3LYP/6-31G(d) structures of FN-Hg complex. All values are bond lengths (Å); (a) FN-Hg complex (b) FN-Hg-FN complex.

ESI-MS study in the FN and FN-Hg^{2+} complex. The FN pesticide was chosen to study possible interaction with transition metals. Possible interaction sites on FN are the free electron pairs on the S atom. The transition Hg[2−] metal was chosen due to their known interaction possibilities with sulfur atom containing ligand in solution. The Hg^{2+} ion has strong affinity for S atom[21] in the compounds on the basis of Pearson's hard and soft acid-base theory.[22] MS/MS (CID) spectrum for the FN in methanol is shown in Figure 2. and ESI-MS spectrum for the FN with Hg[2−] ion in aqueous methanol is shown in Figure 3. Mercury ions can be recognized readily by the isotopic pattern of Hg : natural abundance of [196]Hg, [198]Hg, [199]Hg, [200]Hg, [201]Hg, [202]Hg, and [204]Hg. are 0.14, 10.02, 16.84, 23.13, 13.22, 29.80, and 6.85, respectively.[23] All isotopes would be detectable in ESI-MS spectrum except [196]Hg because of the low natural abundance. Since mercury ion cause FN to hydrolyze rapidly, and peaks form FN-mercury complex and FN original compounds (1: m/z = 475, 476, 477, 478, 479, and 481) were too small to be detected. CID spectrum could not be measured for the [FN+Hg][−] peak. This may be interpreted as being a consequence of mercury having greater affinity toward sulfur ligands compared to silver or copper ion. The major peaks in the spectrum of **2**, **3** and **4** are observed at m/z = 262, m/z =481, 482, 483, 484, 485, and 487, and m/z = 659, 660, 661, 662, 663, and 665, respectively (See Table 3 and Figure 3). In this case mercury forms comparable products with the sulfur-phosphorus containing portion of original compound ([2{S − P (=O) (OCH$_3$)$_2$}+Hg+H][−], **3**).

In this case of FN, another interesting product **2** is rapidly formed after exposing mercury ions to FN. The sulfur-oxygen exchange catalyzed by mercury ion leads to this stable compound, which is not subject to hydrolyze immediately compared to the FN. These results regarding Hg[2−]-FN-Hg[2−] complex from the ESI-MS studies support our conclusion made with NMR study.
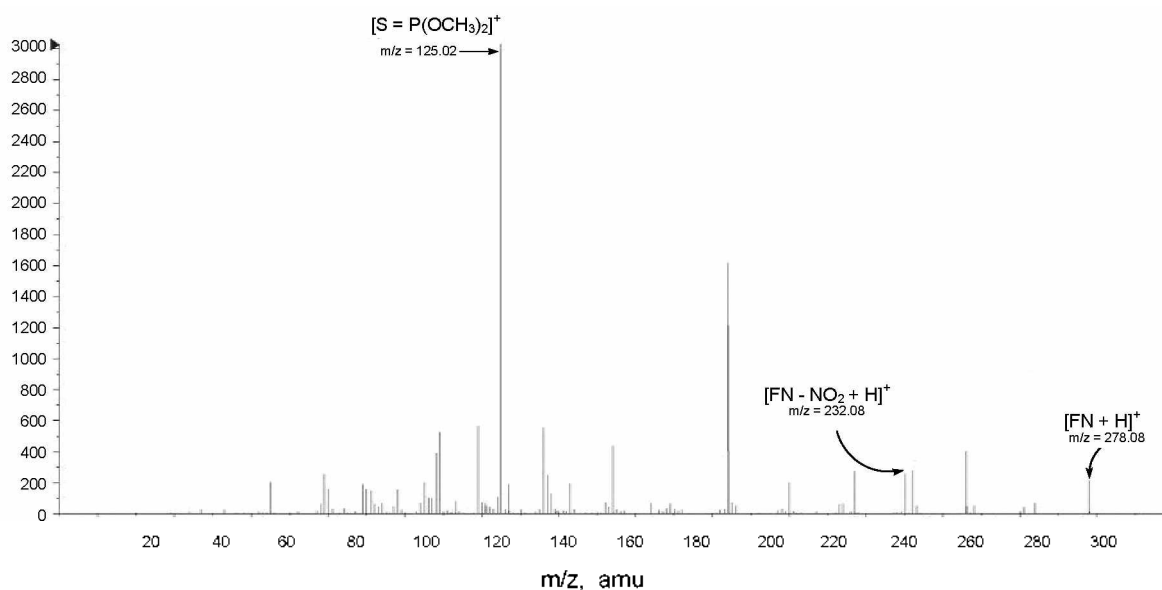
Figure 2. MS/MS (CID) spectrum of [FN+H][+] (m/z = 278.08), which fragments into various ions, collision energy and collision gas pressure were 65 eV and 3.0×10^{−3} Pa, respectively.
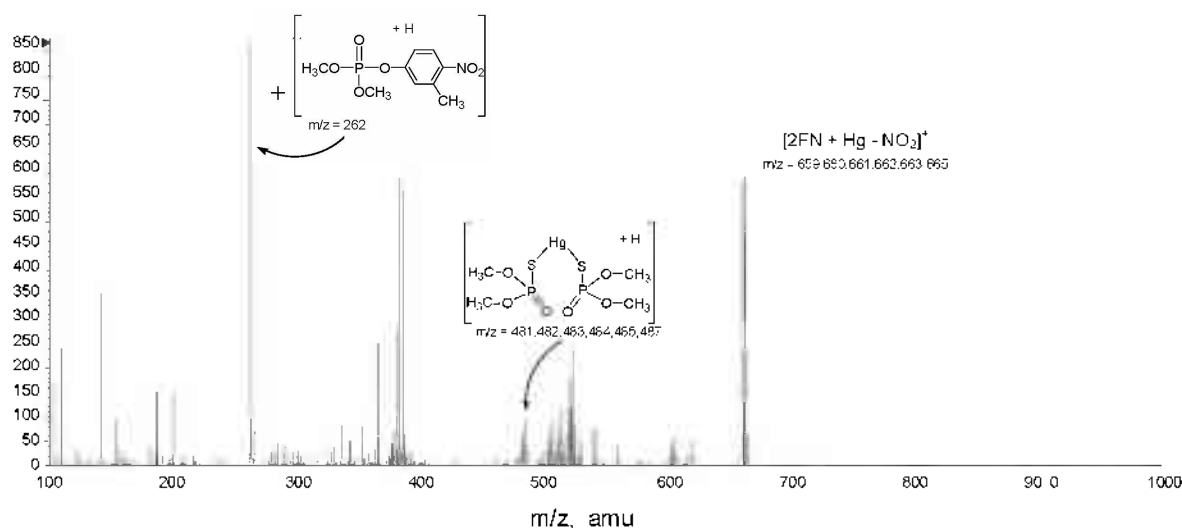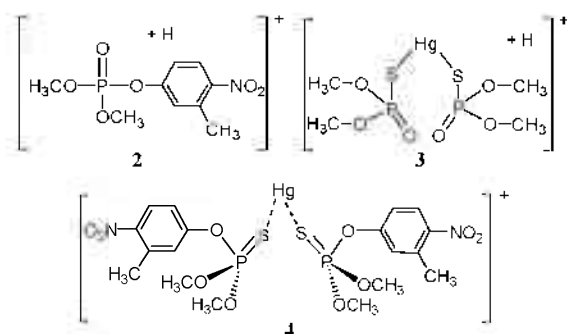
Figure 3. Positive ion ESI-MS spectrum of 50% aqueous methanol solution containing $5.0 \cdot 10^{-5}$ M FN and 2.5×10^{-4} M Hg^{2+} ion.

Comparison of experimental versus theoretical MS study. Verification of assignments of complex: Figure 4 shows the isotopes of mercury, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, and ^{204}Hg. In order to confirm structural assignments, MS spectra for experimental (a) and the theoretical (b) isotopic peaks corresponding to [2{S P(=O)(OCH$_3$)$_2$}+Hg+H]$^+$, **3** are compared. As can be seen from the example given in Figure 4; there is excellent agreement between the theoretical and experimental spectrum. This type of comparison was done for all the metal complexes.

Table 3. ESI-MS and MS/MS data for the FN in methanol and FN with mecury ion in 50%DDW-50%Methanol[a]

Metal salt	Mode	Ions at m/z	Major species
No metal, H	MS	278	[FN+H]
	MS/MS	232	[FN–NO$_2$ – H]
		125	[S P(OCH$_3$)$_2$]
Hg(NO$_3$)$_2$	MS	659, 660, 661, 662, 663,665	[2M – Hg– NO$_2$]
		481, 482, 483, 484, 485, 487	[2{S–P(=O)(OCH$_3$)$_2$}+Hg·H]
		262	[(CH$_3$)(NO$_2$)C$_6$H$_3$(O)P O(OCH$_3$)$_2$+H]

[a] In this table the MS and MS/MS of ion containing mercury is calculated in terms of ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, and ^{204}Hg
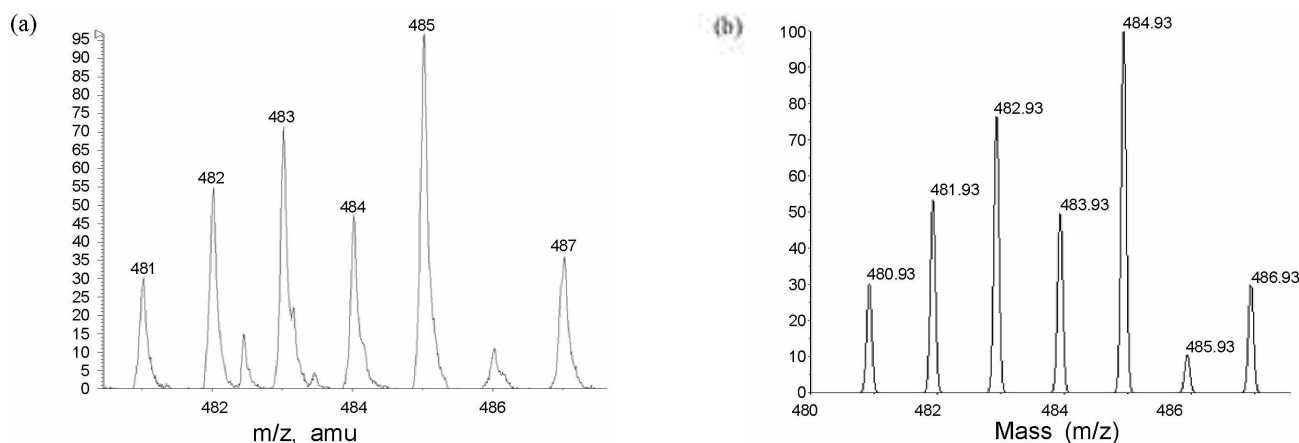

Figure 4. A representative figure showing the agreement between the experimental (a) and the theoretical (b) peaks corresponding to [2{S–P(O)(OCH$_3$)$_2$}+Hg +H] ion from ESI-MS of FN and Hg^{2+}.

Conclusions

³¹P NMR experiments and theoretical studies of FN and FN with Hg²⁺ ion give important information for organophosphorus pesticide metal complexes. In addition, ESI-MS and low energy CID MS/MS experiments of FN with metal complexes combined with accurate mass measurement provided insight into the metal binding site and allow unambiguous identification of fragments and hydrolysis products. Our results provided direct experimental and theoretical evidences for enhanced hydrolysis rates of organophosphorus pesticides in the presence of metal ions because of the feasible nucleophilic attacking to the electron deficient phosphorus atom as mercury ion bind to the adjacent sulfur atom.

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