Application of Nuclear Magnetic Resonance Spectroscopy to the Identification and Quantitation of Pesticide Residues in Soil

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The application of high-resolution Fourier-transform nuclear magnetic resonance (NMR) spectroscopy to the identification of metabolites of the phosphorodithioate ester pesticide sulprofos (Bolstar) was investigated. While differentiation between the various metabolites using either ^{1}H or ^{13}C NMR was unsuccessful, ^{31}P NMR proved to be an exquisitely sensitive probe for these compounds. Treatment of soil samples with sulprofos at a concentration of 7.4 ppm, followed by incubation for up to 90 days, led to the formation of two major metabolites. The residues were extracted, characterized, and quantitated using ^{31}P NMR. Use of ^{14}C radiolabeled material and analysis of extracts by high-performance liquid chromatography and liquid scintillation counting confirmed the results obtained by ^{31}P NMR.

INTRODUCTION

The application of ^{31}P NMR techniques to the identification and characterization of organic compounds has grown extensively since the initial experiments of Dickenson (1951) and Gutowsky and McCall (1951). Monographs by Gorenstein (1984) and Verkade and Quin (1987) illustrate the diversity of the application of this technique to chemical and biochemical research.

The use of ^{31}P NMR to characterize pesticides has seen limited application. Ross and Biros (1970) and Miyata et al. (1988) have characterized phosphorus-containing pesticides using NMR. Lu and co-workers (Lu et al., 1985) correlated ^{31}P NMR data to toxicity values for a number of pesticides. Wayne, et al. (1983) used ^{31}P NMR as a probe to monitor the manufacture and stability of pesticides. The application of ^{31}P NMR to the quantitation of pesticides was carried out by Gurly and Ritchey (1976), and determination of enantiomeric ratios of chiral phosphate ester pesticides was done by Lu and co-workers (Lu et al., 1986). An interesting application of ^{31}P NMR to the analysis of pesticide residues in human body fluids has recently been reported by Nihira and co-workers (Nihara et al., 1990). There appears to be no application to date, however, of the ^{31}P NMR experiment to the analysis of pesticide metabolites in environmental matrices. We report our initial findings on the application of ^{31}P NMR spectroscopy to the analysis of pesticides in soil.

EXPERIMENTAL PROCEDURES

Chemicals. The [*phenyl*-UL-^{14}C]sulprofos [Bolstar, *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate], along with all of the nonradioactive reference standards used in this study, was synthesized in the laboratories of Mobay Corp. Deuterated solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI). Acetonitrile and methanol were of HPLC grade (Burdick and Jackson, Muskegon, MI).

NMR Spectroscopy. NMR data were recorded at ambient temperature for solutions of compounds in CDCl$_3$, CD$_3$OD, perdeuteroacetone, or perdeuterodimethyl sulfoxide. All spectra were recorded using a Varian Model XL-300 (Palo Alto, CA) NMR spectrometer. Observation frequencies were 300.0 MHz for ^{1}H, 75.43 MHz for ^{13}C, and 121.42 MHz for ^{31}P. Chemical shifts (δ) are reported as parts per million downfield from internal tetramethylsilane (TMS) for ^{1}H and ^{13}C spectra and as parts per million downfield from external phosphoric acid (H$_3$PO$_4$) for $^{31}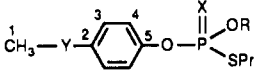$P spectra. ^{31}P NMR spectra of soil extract concentrates were obtained on approximately 500-μg samples using a total spectral acquisition time of 20 h.

High-Performance Liquid Chromatography (HPLC). HPLC was performed on representative samples of soil extract concentrates. Samples (100 μL) were amended with metabolite standards and chromatographed on a YMC AQ-303 S5 120A ODS column (4.6 mm i.d. × 25 cm) using varying proportions of 0.1% aqueous acetic acid (solvent A) and acetonitrile (solvent B) as the mobile phase at a constant flow rate of 1.0 mL/min. The solvent program consisted of eluting for 10 min with a solution of 20% solvent B in solvent A, a linear ramp to 75% solvent B in 5 min, a ramp to 80% solvent B in 30 min, a ramp to 100% solvent B in 10 min, and holding at 100% solvent B for 5 min. The column was equilibrated with 15 mL of 20% solvent B in solvent A immediately prior to each run. The chromatographic system used for all analyses was a Beckman Model 345 consisting of a 112 solvent delivery module, a 340 organizer, a 341 controller, and a 165 variable-wavelength detector. The HPLC system was connected to an IN/US radioactivity monitor (Model Raytest Ramona). All data were compiled and processed using Raytest Ramona data reduction software. Quantitation of radioactive residues was accomplished by integration of the peak areas within each individual HPLC run and confirmed by liquid scintillation counting (LSC) data for recovered HPLC eluates. The reported results are the average of duplicate determinations.

Radioassay. Liquid samples (0.1–1.0 mL) were radioassayed using a Beckman LS 9000 liquid scintillation counter and its associated data reduction software. Aliquots of solid samples were oxidized using a Packard Tri-Carb Model 306 oxidizer, and the total radioactivity was determined by LSC of the evolved ^{14}CO$_2$. Quench curves for both liquid and oxidizer samples were determined and used as prescribed by the instrument manufacturer.
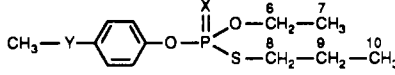
Soil Metabolism Study. The soil used in this study was obtained from Mobay's Howe Research Farm located near Howe, IN. Textural analysis showed it to be sandy loam (66% sand, 22% silt, 12% clay, 2.2% organic matter, pH 6.3, cation-exchange capacity 20 mequiv/g, particle density 2.6 g/mL). The soil was shown to be biologically active by incubating the soil with a known concentration of [^{14}C]glucose and measuring the amount of ^{14}CO$_2$ evolved (Anderson and Domsch, 1978) using the apparatus described by Anderson (1975). Immediately prior to sulprofos treatment, the soil was sieved to a maximum particle size of 2 mm and divided into 100-g (dry weight) aliquots. Aliquots were placed in 250-mL Erlenmeyer flasks with ground glass joints and amended with deionized water sufficient to bring each sample to a moisture level of 75% of 0.33 bar (8.5 g/flask).

Each soil sample was treated with a 100-μL aliquot of a solution containing 0.740 mg of [*phenyl*-UL-^{14}C]sulprofos (specific activity 25.35 mCi/mmol, 174 600 dpm/μg). The soil was thoroughly mixed by tumbling each flask for 2–3 min. The sample flasks

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Table I. ^{13}C Chemical Shifts for the Aryl Portion of Sulprofos Standards

				$\delta\ ^{13}C\ (J_{13C\text{-}31P})^{a,b}$				
compd	X	Y	R	1	2	3	4	5
1	S	S	Et	24.0	136.5	128.5	123.0	149.0
2	S	SO	Et	44.34	144.85 (3.1)	125.87 (1.7)	123.12 (5.0)	153.09 (8.8)
3	S	SO$_2$	Et	44.375	139.302 (2.0)	130.162	123.087 (5.0)	153.09 (8.5)
4	O	S	Et	24.739	136.0 (5.6)	128.773	122.016 (5.0)	144.5 (5.0)
5	O	SO	Et	44.338	144.612 (1.6)	126.076 (1.2)	122.274	153.555 (7.5)
6	O	SO$_2$	Et	44.396	139.016 (0.9)	130.401	122.162 (5.4)	155.198 (12.0)
7	S	S	H	36.202	c	128.624	123.242 (0.8)	132.401 (4.8)
8	O	S	H	33.650	128.85	128.771	122.216	131.45

^{a} Chemical shifts (δ) are expressed as parts per million downfield from TMS. ^{b} Coupling constants (J) are expressed in hertz. ^{c} The indicated resonance was not observed due to extremely slow relaxation.

Table II. ^{13}C Chemical Shifts for the Phosphate Ester Portion of Sulprofos Standards

			$\delta\ ^{13}C\ (J_{13C\text{-}31P})^{a,b}$				
compd	X	S	6	7	8	9	10
1	S	S	65.0	36.5	16.0	16.1	13.5
2	S	SO	65.41 (6.5)	36.60 (4.3)	24.33 (5.7)	16.11 (7.9)	13.34
3	S	SO$_2$	65.596 (3.0)	36.367 (4.0)	24.312 (6.0)	16.073 (8.0)	13.289
4	O	S	64.50	33.735 (4.0)	16.320 (7.2)	16.130 (1.5)	13.195
5	O	SO	64.990 (6.2)	33.815 (4.3)	24.760 (5.6)	16.324 (6.9)	13.173
6	O	SO$_2$	65.234 (6.6)	33.888 (4.5)	24.766 (6.0)	16.313 (7.3)	13.154
7	S	S			24.566 (5.2)	16.853	13.820
8	O	S			24.709 (6.0)	16.230	13.292

^{a} Chemical shifts (δ) are expressed as parts per million downfield from TMS. ^{b} Coupling constants (J) are expressed in hertz.

were fitted with air-permeable sponge plugs and placed in a dark cabinet. At intervals of 1, 30, and 90 days posttreatment, duplicate samples were chosen for analysis. Each sample was covered with 150 mL of methanol and heated, with stirring, at reflux for 2 h. The resulting suspension was vacuum filtered through a Whatman No. 1 paper filter, and the filtered solids were washed with an additional 50 mL of fresh methanol. The filtered solids were air-dried, weighed, and radioassayed. The combined methanol filtrate was evaporated to dryness in vacuo, and the resulting residue was taken up in methanol (10–20 mL). This methanol extract concentrate was radioassayed (triplicate, 100-μL aliquots) by liquid scintillation counting and analyzed by HPLC. The methanol extract concentrate was then evaporated to dryness in vacuo, and the residue was dissolved in perdeuterodimethyl sulfoxide and analyzed by ^{31}P NMR.
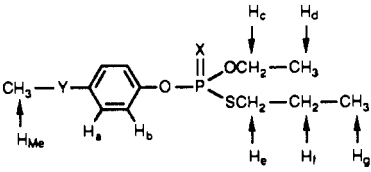
RESULTS AND DISCUSSION

^{13}C NMR. The ^{13}C NMR spectra for the aryl portion of sulprofos (1) and related standards are summarized in Table I. While there is some variation in the chemical shifts of the methyl resonance as a function of the oxidation state of the adjacent sulfur atom, there is insufficient difference to allow the differentiation and quantitation of the various compounds. The ^{13}C NMR spectra for the phosphorothioate or phosphorodithioate ester portion of the standards are summarized in Table II. As before, there was no significant difference in the positions of the ^{13}C resonances to allow the differentiation and quantitation of the possible metabolites. Coupling between ^{13}C and ^{31}P was observed through as many as five bonds, however.

^{1}H NMR. The ^{1}H NMR spectra for sulprofos and possible metabolites are summarized in Table III. As with the ^{13}C spectra, there was very little differentiation among the various compounds. There was significant ^{1}H–^{31}P coupling observed in all of the compounds. The ^{1}H–^{31}P coupling constants are shown in Table IV. In compounds 2 and 6 the coupling is resolved even when the proton is

removed from the phosphorus by five bonds. The ^{1}H–^{31}P coupling is readily seen in the ^{1}H spectrum of sulprofos (Figure 1). The methylene adjacent to the sulfur in the S-propyl side chain is split into a fully resolved doublet of triplets. The magnitude of the ^{1}H–^{31}P coupling suggested the possibility of direct observation of metabolites by ^{31}P NMR.
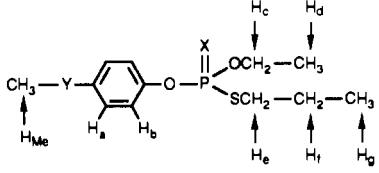
^{31}P NMR. Chemical shift values for ^{31}P resonances in sulprofos and related standards are given in Table V. Due to broad-band proton decoupling during data acquisition, all signals appeared as singlets. Comparisons of compounds containing a phosphorus–sulfur double bond to the corresponding compounds containing a phosphorus–oxygen double bond (compounds 1 and 4, for example) show that the presence of oxygen shifts the phosphorus resonance signal significantly upfield. While the magnitude of this upfield shift can vary from 50 to 70 ppm, this shift readily distinguishes PO and PS compounds. All observed chemical shifts were consistent with previously published data for similar compounds (Gorenstein, 1984) and were found to be insensitive to either solvent or concentration effects (Lerner and Kearns, 1980).

Within each set (PO and PS) there are variations in the chemical shift of the phosphorus resonance due to changes in the electronic character of the molecule. It is worth noting that the phosphorus chemical shift appears to be exquisitely sensitive to remote changes in molecular structure. Changing the oxidation state of a sulfur atom six bonds removed from the phosphorus results in a change in the chemical shift ($\Delta\delta$) of up to 7.3 ppm. The fact that either a remote sulfide or sulfone moiety shifts the resonance downfield compared to the resonance of the corresponding sulfoxide implies that the $\Delta\delta$ is probably not simply a function of the electron-withdrawing ability of the substituent. The sensitivity of ^{31}P NMR as a tool

Table III. ¹H Chemical Shifts and Multiplicities for Sulprofos Standards

compd	X	Y	Hₐ	H_b	H_c	H_d	H_e	H_f	H_g	H_Me
1	S	S	7.66 (d)	7.55 (dd)	4.62 (m)	1.72 (td)	3.33 (dt)	2.07 (tq)	1.32 (t)	2.86 (s)
2	S	SO	7.73 (dd)	7.48 (dd)	4.31 (m)	1.40 (td)	3.01 (dt)	1.72 (tq)	0.98 (t)	2.72 (s)
3	S	SO₂	8.00 (d)	7.54 (dd)	4.33 (m)	1.40 (td)	3.02 (dt)	1.73 (tq)	0.98 (t)	3.14 (s)
4	O	S	7.43 (d)	7.32 (dd)	4.37 (dq)	1.48 (td)	2.99 (dt)	1.79 (tq)	1.07 (t)	2.61 (s)
5	O	SO	7.76 (d)	7.50 (dd)	4.31 (dq)	1.39 (td)	2.92 (dt)	1.68 (tq)	0.96 (t)	2.71 (s)
6	O	SO₂	8.15 (d)	7.68 (dd)	4.45 (dq)	1.52 (td)	3.05 (dt)	1.82 (tq)	1.09 (t)	3.27 (s)
7	S	S	7.41 (d)	7.30 (dd)			2.90 (m)	1.72 (tq)	1.02 (t)	2.56 (s)
8	O	S	7.40 (d)	7.36 (dd)			2.99 (m)	1.78 (tq)	1.06 (t)	2.59 (s)

ᵃ Chemical shifts (δ) are expressed as parts per million downfield from TMS. *ᵇ* Multiplicities are described as follows: s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; td, triplet of doublets; tq, triplet of quartets.

Table IV. ¹H–³¹P Coupling Constants for Sulprofos Standards

			J, Hz				
compd	X	Y	Hₐ	H_b	H_c	H_d	H_e
1	S	S		1.7	1	0.8	13.9
2	S	SO	0.5	1.5	1	0.9	17.2
3	S	SO₂		1.7	1	0.6	16.3
4	O	S		3.2	9.7	1.6	15.1
5	O	SO		1.5	9.0	0.7	15.4
6	O	SO₂	0.7	1.2	8.8	1.5	15.6
7	S	S		2.2			ᵃ
8	O	S		1.8			ᵃ

ᵃ The coupling constant could not be determined.
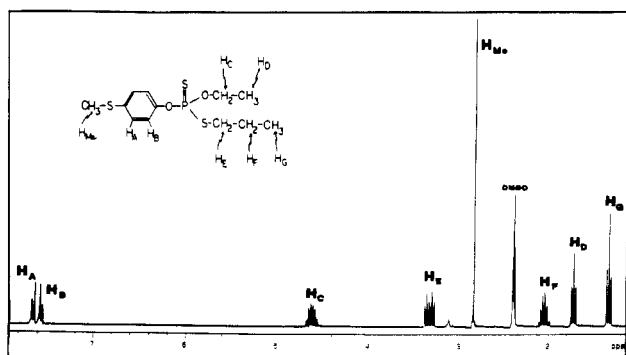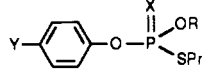
Figure 1. ¹H NMR spectrum of sulprofos (1, Bolstar) showing the effects of ¹H–³¹P coupling.

for the identification and quantitation of sulprofos metabolites in soil was then examined.

Soil Metabolism. While the metabolism of sulprofos in soil has been previously studied (Bull et al., 1976), this study was conducted to determine the metabolic half-life of sulprofos in sandy loam. A total of 98% of the radioactive residue was extracted in the 1-day sample, while 93% and 88% of the total residue was extractable 30 and 90 days posttreatment, respectively. HPLC analysis of soil extract concentrates indicated that sulprofos (1) was rapidly oxidized to the corresponding sulfoxide (2), which was further oxidized to the sulfone (3). Compounds 2 and 3 were additionally slowly hydro-

Table V. ³¹P Chemical Shifts for Sulprofos Standards

compd	X	Y	R	δ ³¹Pᵃ
1	S	CH₃S	Et	100.396
2	S	CH₃SO	Et	93.086
3	S	CH₃SO₂	Et	99.907
4	O	CH₃S	Et	31.398
5	O	CH₃SO	Et	25.889
6	O	CH₃SO₂	Et	27.652
7	S	CH₃S	H	72.545
8	O	CH₃S	H	24.887

*ᵃ* Chemical shifts (δ) are expressed as parts per million downfield from external phosphoric acid.

Table VI. Comparison of Analytical Results from Soil Metabolism Extracts Assayed by both ³¹P NMR and HPLC with Radiodetection

days posttreatment	analysis method	% sulprofos (1)	% sulfoxide (2)	% sulfone (3)
1	³¹P NMR	88	11	0
	HPLC	86	10	0
30	³¹P NMR	61	28	0
	HPLC	58	24	5
90	³¹P NMR	0	64	36
	HPLC	0	52	39

lyzed to yield small amounts (<5%) of the corresponding phenols. The relative amounts of compounds 1–3 in soil extract concentrates at 1, 30, and 90 days after treatment with sulprofos (1) are shown in Table VI. Also shown are the values obtained using ³¹P NMR as the probe for identification and quantitation of metabolites. The values assigned using ³¹P NMR were determined by multiplying the relative NMR peak integrations by the total phosphorus-containing extractable residues as determined by HPLC with radiodetection. Untreated soil extract concentrates showed no observable ³¹P resonances. There is very close agreement for all samples. The inability of the NMR technique to detect the presence of 5% of compound 3 in the 30-day sample indicates a limit of detection of approximately 25 μg of material, corresponding to a 0.25 ppm residue in a 100.0-g sample. The actual ³¹P NMR spectra for the soil extract concentrates are shown in Figure 2.

Conclusion. The use of ³¹P NMR in the characterization and quantitation of pesticide residues in soil is

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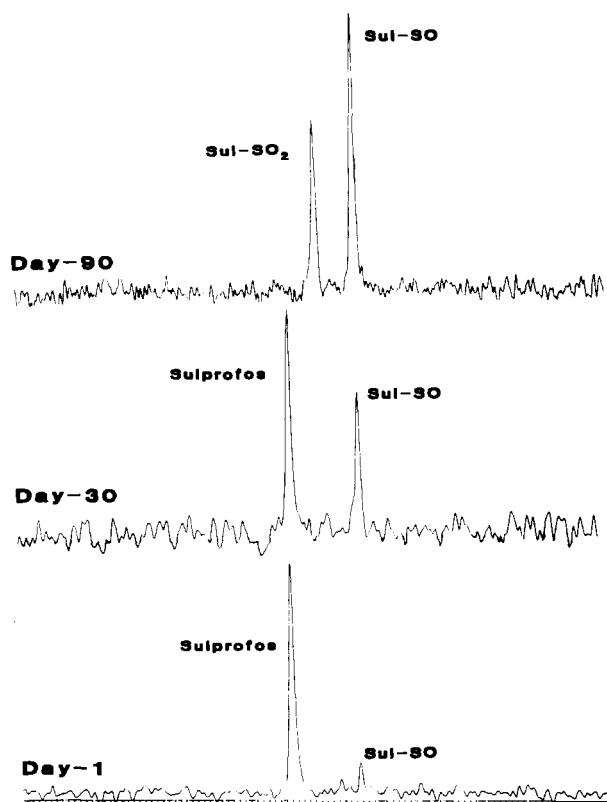
Figure 2. ^{31}P NMR spectrum of soil extracts containing sulprofos (1) and the sulfoxide (2, Sul-SO) and sulfone (3, Sul-SO$_2$) metabolites.

rapid, easy, and accurate. The main advantages of the NMR technique are that it is nondestructive and it simultaneously analyzes for all components of a mixture. The technique described gives only relative amounts of each component. However, the addition of an internal standard would allow the determination of absolute residue levels.

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