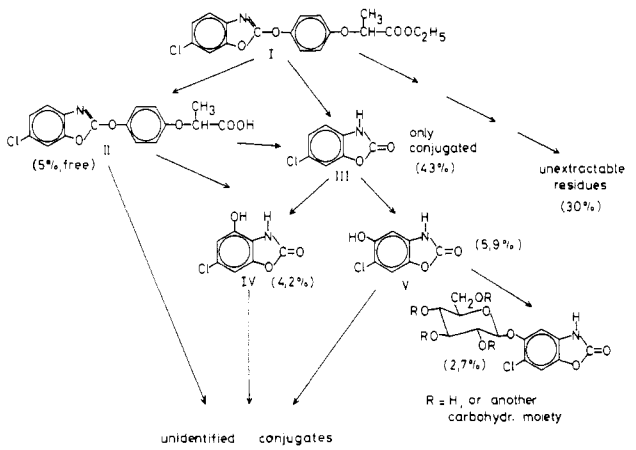

Figure 10. Proposed degradation scheme of Hoe 33171 in soybean with structural formula and chemical names of the compounds: I = Hoe 33171; II = 2-[4-[(6-chloro-2-benzoxazolyl)-oxy]phenoxy]propionic acid; III = 6-chloro-2,3-dihydrobenzoxazol-2-one; IV = 4-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one; V = 5-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one.

or degradation products bound to polymer cell constituents.

Fraction R1B (1.3%) was identified to be the 5-hydroxy isomer (V) by use of GC–MS analysis of the methylated derivatives (Figures 6 and 7). This structural element was bound in the solid residues even after exhaustive extraction and was released from the solid residues after acid treatment (c_{HCl} = 3 mol/L, 6 h, refluxing).

Fraction R1C (5.1%) was identified to be nonhydroxylated product III as revealed by analysis of the methylated derivatives and comparison with the reference compound. This metabolite was also released from the solid residues after acid cleavage.

CONCLUSION

This report provides information on the quality of the residues in soybean plants that originated from Hoe 33171 application (see degradation scheme, Figure 10). The very low residue level in the beans at the normal day of harvest does not enable identification; thus, we characterized the metabolites at that growth stage (day 15) when the ratio of metabolite amount to plant material was the most favorable from an analytical point of view.

In a parallel experiment, Dorn et al. (1983), soybean plants were cultivated post day 15 until maturity. In the fully developed beans no radioactive residues were detected above the limit of quantitation (LOQ = 0.005 μg/g, calculated as μg of active ingredient equiv/g).

Registry No. I, 66441-23-4; II, 73519-55-8; III, 19932-84-4; IV, 88412-28-6; V, 88412-29-7; 1-chloro-3,5-dimethoxybenzene, 7051-16-3; 1-chloro-3,5-dihydroxybenzene, 52780-23-1; 1-chloro-4-nitro-3,5-dihydroxybenzene, 88412-30-0.

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A Simple Single-Step Derivatization Method for the Gas Chromatographic Analysis of the Herbicide Glyphosate and Its Metabolite

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A single-step derivatization method for the herbicide glyphosate [*N*-(phosphonomethyl)glycine] and its major metabolite (aminomethyl)phosphonic acid is reported that allows for their analysis at the μg/mL level by gas chromatography with flame photometric detection. Derivatization is achieved with the reagent *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide, which introduces the dimethyl-*tert*-butylsilyl group at active hydrogens.

Glyphosate [*N*-(phosphonomethyl)glycine] (GLYPH) is a nonselective postemergence herbicide with a growing list of international uses. When used in weed control, it does not injure crops planted immediately after treatment (Sprankle et al., 1975). (Aminomethyl)phosphonic acid (AMPA) has been shown to be the major metabolite in plants, water, and soil (Sprankle et al., 1978).

Various approaches to the analysis of GLYPH and AMPA have been taken. These include gas chromatography (GC) after chemical derivatization ("Pesticide Analytical Manual", 1980; Guinivan et al., 1982), high-performance liquid chromatography (HPLC) utilizing postcolumn fluorogenic labeling (Moye and St. John, 1980; Moye et al., 1983), and thin-layer chromatography (TLC; Sprankle et al., 1978; Young et al., 1977). While the postcolumn fluorogenic labeling procedure has performed well in our hands, some laboratories do not have the instrumentation required and would prefer a GC procedure. However, both published GC procedures require double derivatizations, and one ("Pesticide Analytical Manual", 1980) requires the preparation and use of diazomethane, a highly toxic and explosive reagent.

This report describes the preparation and analysis of derivatives of GLYPH and AMPA using a single reaction, which is rapid, clean, and accomplished with a relatively

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safe, easily obtainable commercial reagent. The derivatives are stable upon storage and easily gas chromatographed under moderate conditions without the need for the removal or destruction of the reagent, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA). This reagent has been used for the derivatization of sulfate, phosphate, and other oxyanions (Mawhinney, 1983), and introduces *tert*-butyldimethylsilyl (TBDMS) groups at sites having active hydrogens.

MATERIALS AND METHODS

Instrumentation. A Hewlett-Packard Model 5840A gas chromatograph equipped with a Model 18805B flame photometric detector operated in the phosphorus mode was used for all measurements. A 1.8 m × 2 mm i.d. silanized glass column was packed with Ultra-Bond 20SE on 80–100-mesh support (Ultra Scientific, Hope, RI). An oxygen trap was installed in front of the column to prevent its deterioration. Column temperature was held at 200 °C for the analysis of GLYPH and 170 °C for the analysis of AMPA. Carrier flow (N_2) was 30 mL/min. Other detector gases were H_2, 220 mL/min, air, 50 mL/min, and O_2, 20 mL/min.

Mass spectra were collected on a Finnigan Model 4021 mass spectrometer. Sample introduction was via the LC belt, operated without heat.

Glassware and Reagents. Derivatization was accomplished with MTBSTFA, supplied by Regis Chemical (Morton Grove, IL). It was stored at room temperature in 25-mL amber bottles equipped with Model SC-24 Mininert valves (Pierce Chemical Co., Rockford, IL). A silylation catalyst, 4-(4-methyl-1-piperidinyl)pyridine (MPP), was obtained from Reilly Chemical Co. (Indianapolis, IN). Nanograde acetonitrile and reagent-grade ethanol and phosphoric acid were used.

Derivatization tubes made from three types of material were examined for reaction efficiency and reproducibility at the μg/mL glyphosate concentration level: borosilicate glass, polypropylene, and stainless steel (316). Culture tubes measuring 15 mm × 125 mm and having Teflon-lined screw caps were used to examine borosilicate glass while 10 mm × 90 mm tubes with polypropylene screw caps were used to examine polypropylene. A 22 mm × 50 mm piece of 316 stainless steel rod was bored out to form a reaction vessel 10 mm × 45 mm (3.5 mL), which was threaded at the top and capped with a stainless steel bolt.

Derivatization Optimization. The relative derivatization efficiencies for both AMPA and GLYPH were determined as a function of catalyst, derivatization tube material, reaction temperature, reaction time, and tube coating material. Tubes other than the conventional borosilicate glass were examined in an effort to correct initial difficulties experienced with the lack of reproducibility of the derivatization efficiency. For the same reason, various coatings, including mineral oil, amino acids, plant extractives, and phosphoric acid were examined for their effectiveness in preventing or reducing apparent adsorption of AMPA and GLYPH onto the derivatization tube surfaces. Borosilicate glass tubes were also silanized with dimethyldichlorosilane.

Since both GLYPH and AMPA have limited solubility in all organic solvents, including MTBSTFA, the possibility of increasing the solubility by forming ion pairs with tetrabutylammonium ion and pyridine was investigated.

The adsorption prevention experiments were conducted in unsilanized borosilicate glass Teflon capped tubes that had been soaked in methanolic KOH and thoroughly rinsed with distilled water. The tubes were coated with either mineral oil (5 μL of oil in CH_2Cl_2), phosphoric acid (100 μg in ethanol), ethyl acetate extracts of endive (equivalent to 25 mg of endive), or trace amounts of glycine or lysine/aspartic acid by rinsing the tubes in a saturated ethanolic solution of the amino acid(s). The solvents were evaporated to dryness, 25.0 μg of GLYPH in water was added, and the water was evaporated at 100 °C under a stream of nitrogen. To this were added 10 μL of a 0.01 mL of MPP in 10 mL of CH_3CN solution, 100 μL of CH_3CN, and 100 μL of MTBSTFA. The tubes were capped and sonicated for 10 min and then incubated at 100 °C for 1 h after which the solutions were analyzed directly by gas chromatography.

Both pyridine and tetrabutylammonium chloride were examined for their ability to reduce adsorption via ion pairing with GLYPH, thereby increasing its solubility in the derivatization solvent. In these experiments, 5 μL of pyridine or 50 μg of tetrabutylammonium chloride in water along with 5 μL of pyridine were added to the tube containing 25 μg of GLYPH in water. The mixture was evaporated to dryness and derivatized as above. In both sets of experiments only the bottom of the tube, where the 200 μL of reagent would come in contact was coated.

Silylations of both glass and polypropylene tubes were achieved by reacting them at 25 °C for 15 min with a 5% (v/v) solution of dimethyldichlorosilane. Glyphosate derivatization was then carried out as above.

Kinetics curves for both GLYPH and AMPA in phosphoric acid treated tubes were determined at 25, 52 and 100 °C (GLYPH, 108 °C) by derivatizing 25 μg of each in 100 μL of CH_3CN and 100 μL of MTBSTFA.

Analytical curves for both GLYPH and AMPA were plotted for concentrations of 1–100 μg/mL.

RESULTS AND DISCUSSION

In an effort to discover a relatively innocuous reagent, which could be used to produce a volatile derivative of GLYPH, $HOC(O)CH_2NHCH_2P(O)(OH)_2$, and AMPA, $NH_2CH_2P(O)(OH)_2$, via a one-step reaction, we investigated a long list of chemicals that have been reported to methylate carboxylic acids, including dimethyl sulfate, methyl iodide, the boron trihalide alcohols, [*m*-(trifluoromethyl)phenyl]trimethylammonium hydroxide, *N*,*N*-dimethylformamide dimethyl acetal, anhydrous HCl alcohols, and the more hazardous diazomethane. Derivatives volatile enough for gas chromatographic analysis were observed for diazomethane only. However, multiple products were verified by GC–MS corresponding to di-, tri-, tetra-, and pentamethylated derivatives that varied in relative intensity depending upon which solvent system was employed. A single major product never could be observed chromatographically with diazomethane as the methylating agent.

Our attention then turned to MTBSTFA, a silylating reagent that has been reported by others to form derivatives that are relatively stable to hydrolysis (Sommer, 1965) and the high temperatures required for gas chromatography (Mawhinney, 1983). The manufacturer also claims that silylations can be achieved even in the presence of small amounts of water (Regis Chemical Co., 1983).

Preparation of separate derivatives of AMPA and GLYPH in MTBSTFA-CH_3CN at the 1 mg/mL level allowed for mass spectra to be taken. Samples were introduced via the LC belt with only partial drying of the MTBSTFA. Ionization in the EI mode (70 eV) produced for both AMPA and GLYPH the strong [M – 57; loss of $-C(CH_3)_3$] and less intense (M – 15; loss of $-CH_3$) ions as was observed by Mawhinney (1983) for the derivatives of the oxyanions. No molecular ions were observed for either derivative. The 438 (M – 15) and 396 (M – 57) ions for

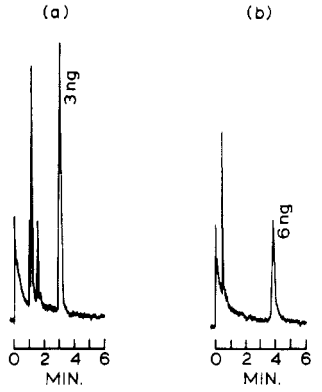

Figure 1. (a) (Aminomethyl)phosphonic acid derivative, 3 ng, 170 °C column temperature; (b) glyphosate derivative, 6 ng, 200 °C column temperature.

AMPA are indicative that three TBDMS groups were introduced into the molecule, ostensibly at the two acid portions of the phosphoric acid and at the amino group. Similarly, the 496 (M - 15) and 454 (M - 57) ions for GLYPH indicate that three TBDMS groups were introduced into this molecule, and a strong 202 ion, due to $(CH_3)_3CSi(CH_3)_2OC(O)CH_2NHCH_2^+$, and a 352 ion, due to $CH_2NHCH_2P(O)(OSi(CH_3)_2C(CH_3)_3)_2^+$, also suggest that the amino group is not silylated.

Initial attempts at derivatization of GLYPH and AMPA with MTBSTFA in glass tubes in the 10–50 ng/μL concentration range met with little success in that chromatographic responses were observed on the order of one-tenth to half of what was expected when compared to those of the insecticide methyl parathion. Additionally, relative standard deviations on the order of 10–50% were observed for replicate samples with $n = 5$. Some improvement was observed in reproducibility when polypropylene tubes were used or when the glass tubes were coated with endive extract. Stainless steel tubes gave very poor yields and very poor reproducibility. Coating polypropylene tubes with endive extract improved their performance somewhat over uncoated tubes. Amino acids coated on glass tubes had little effect. A pronounced improvement was observed, however, when glass tubes were coated with phosphoric acid in that chromatographic responses more than doubled and reproducibility in the analysis of replicates also improved. For example, for replicate analyses of GLYPH, $n = 5$, the relative standard deviation ($s/\mu = 100\%$) was only 3.4%, which compares well with nonderivatization GC methods. Consequently, all further experimentation was conducted in phosphoric acid treated borosilicate glass tubes, prepared by evaporating to dryness 100 μL of a stock solution of phosphoric acid in 95% ethanol (10 mg/10 mL). Similar results were achieved for AMPA. Omitting the MPP catalyst had no effect on derivative response; consequently, it was not used in further experiments. Solubilization was not improved with the ion pairing reagents, pyridine and tetrabutylammonium chloride.

Typical chromatograms of GLYPH and AMPA are shown in Figure 1. The 1.30-min peak in the AMPA chromatogram and the 0.65-min peak in the GLYPH chromatogram are due to a derivative of phosphoric acid; the 1.73-min in the AMPA chromatogram is due to an unknown impurity in the standard. No tailing is observed for either peak on this column, which exhibits 2400 theoretical plates. Before each day's operation a 1 μg/μL solution of the GLYPH derivative is injected 3–4 times (1-μL injections), which is normally sufficient to condition the column for a full day's chromatography. AMPA can be chromatographed at 200 °C just as GLYPH; however, it is not fully resolved from the impurity peaks.

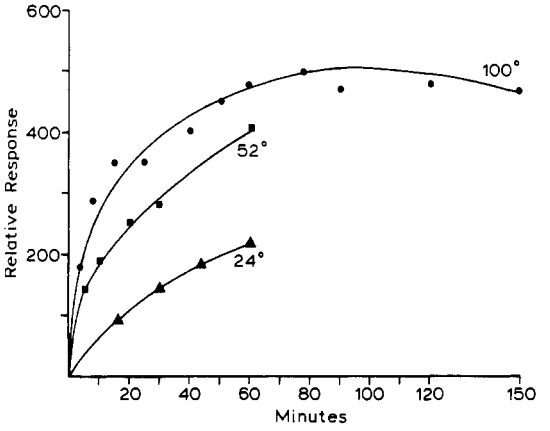

Figure 2. Kinetics curves, (aminomethyl)phosphonic acid derivative.

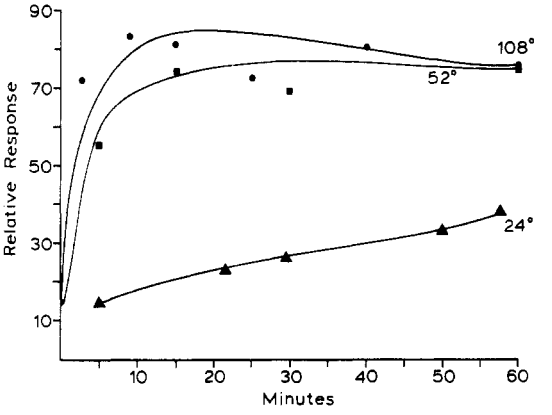

Figure 3. Kinetics curves, glyphosate derivative.

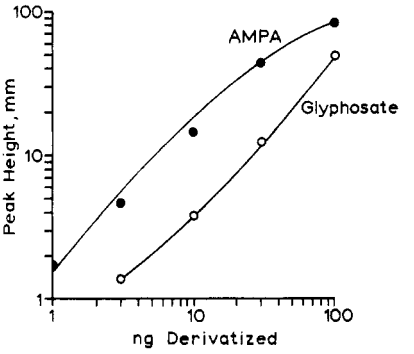

Figure 4. Extended range analytical curves, 100 °C reaction for 1 h.

The time courses for the derivatization of GLYPH and AMPA are shown in Figures 2 and 3. The difference in reaction rates for the two is undoubtedly due to the functionalization of the amino group on AMPA, which does not occur for GLYPH. It is evident that at these concentrations (125 μg/mL) and at 100 °C GLYPH is completely derivatized in 10 min whereas AMPA requires 60 min for completion. Sonication of the tubes for 10 min before incubation had an enhancing effect on GLYPH response and reaction rate as compared to tubes that were not sonicated.

Analytical curves for both AMPA and GLYPH were linear over a concentration range differing by a hundredfold (Figure 4). The lower response for GLYPH remains undetermined and could be due to either lower derivatization efficiency or greater decomposition on the GC column.

The pronounced effect of phosphoric acid treatment of the borosilicate glass derivatization tubes is interesting in light of the observation by Sprankle et al. (1975) that increasing soil phosphate concentrations decreased the amount of GLYPH that adsorbed to the soil. This is consistent with recent observations in this laboratory that indicate that acidic phosphate buffers are reasonably efficient as extraction solvents for removing GLYPH from soil (Moye, 1983).

Work is continuing in this laboratory to employ the presently described derivatization method for the residue analysis of GLYPH on fruits, vegetables, and soil.

Registry No. Glyphosate, 1071-83-6; (aminomethyl)-phosphonic acid, 1066-51-9; *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide, 77377-52-7.

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High-Performance Liquid Chromatographic Determination of Bromoxynil Octanoate and Metribuzin in Runoff Water from Wheat Fields

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A method for the direct determination of the herbicides metribuzin and bromoxynil octanoate and their metabolites DADK and bromoxynil by high-performance liquid chromatography (HPLC) was developed. Aqueous samples were frozen to simulate storage of field samples, thawed in a microwave oven to minimize time of exposure to room temperatures, acidified to pH 3.3, and extracted with dichloromethane/acetonitrile. Compounds were separated by HPLC on an octadecyl reverse-phase column with a water/methanol gradient and determined with a variable-wavelength ultraviolet detector. High-density polyethylene storage containers adsorbed 50–80% of the bromoxynil octanoate from solution, but satisfactory recoveries were obtained by extracting the freshly emptied containers with dichloromethane. In the range of 20–200 ppb, recoveries averaged 83–96%. At 25 ppb standard deviations were 4.8% for metribuzin, 4.0% for DADK, 6.2% for bromoxynil octanoate, and 1.4% for bromoxynil. Almost complete loss of bromoxynil octanoate occurred at room temperatures (20–25 °C) within 72 h, although no losses of the other three compounds were observed. Samples could be stored safely at −15 °C up to 300 days.

Wheatlands in the Pacific Northwest suffer severe runoff and soil erosion during the winter, resulting from a combination of rain or snow melt on saturated soils, steep slopes, and management that leaves little surface cover for soil protection. Adoption of conservation (reduced) tillage systems can cut soil losses from 25 tons acre^{-1} (56 Mg ha^{-1}) to 5 tons acre^{-1} (11 Mg ha^{-1}) or less on the steeply hilled Palouse winter wheat area of Washington, Idaho, and Oregon (Oldenstadt et al., 1982). In this region, herbicides are used extensively for weed control in wheat grown under conventional or reduced tillage. Although most of these chemicals are applied to winter wheat fields in the spring, there is a potential use for, and at times a need for, fall application prior to the winter runoff season.

Two herbicides that have potential use for fall application in wheat are metribuzin (Sencor, Lexan) and bromoxynil octanoate (Brominal). Both chemicals have relatively short half-lives under spring and summer conditions (Stewart et al., 1975). However, little is known about the persistence and runoff of metribuzin and bromoxynil octanoate after fall or winter application to winter wheat. It is known that cold conditions can increase herbicide persistence dramatically (Hörmann et al., 1979). Furthermore, prolonged frozen field conditions occurring shortly after an herbicide treatment followed by a heavy runoff event potentially could lead to high herbicide content in runoff water weeks after the application.

Recently several methods for the determination of bromoxnynil octanoate in wheat plants (Cessna, 1980), wheat products (Lawrence et al., 1980), and soils (Smith, 1980) have been published as have methods for the determination of metribuzin in soils (Vickery et al., 1980),

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