A Convenient Method Using Methanol/Benzyltrialkylammonium Reagents for Simultaneous Extraction and Methylation of 2,4-Dichlorophenoxyacetic Acid in Soil, with Subsequent Analysis via Gas Chromatography

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A novel analytical procedure for the determination of 2,4-dichlorophenoxyacetic acid in soil samples has been developed. The acid is extracted from the soil and simultaneously esterified in situ to its methyl ester by means of a benzyltrialkylammonium-type organic solubilizer. Quantitation was carried out by capillary gas chromatography with an electron capture detector. Recovery was over 96%. The procedure offers possible extension to other chlorophenoxy acids.

Since World War II, chlorophenoxy acids have been used extensively for the control of weeds in agriculture and forestry (Bovey and Young, 1980). Of the chlorophenoxy acids, the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is widely distributed in the natural environment, causing public concern (Ware, 1983). Monitoring of residual levels of 2,4-D is essential to determine the extent of environmental pollution.

A number of methods for the determination of 2,4-D are reported in the literature. Mainly these are by gas chromatography with electron capture or nitrogen–phosphorus detection (Kan et al., 1981; Rivers et al., 1970; Zhang et al., 1982; Cotterill, 1982; Lee et al., 1986; Smith, 1984; Steinwandter, 1989; Horner et al., 1974). However, it also has been determined via an electroanalytical method (Chiang et al., 1990) and by enzyme immunoassay (Fleeker, 1987). In common with many acidic compounds, chlorophenoxy acids have not been analyzed directly by GC because of their low vapor pressures and high polarities (Haque and Sexton, 1968; Darbre, 1978). Such problems can be eliminated by conversion of the acid into a suitable derivative that is nonpolar and which has a moderately low boiling point. A number of derivatives have been proposed to facilitate the GC analysis of such acids. These are principally esters, especially the methyl esters which frequently are the most convenient.

Diazomethane is the most commonly used methylating agent (Rivers et al., 1970). Other procedures for the esterification of the acids include ion-pair alkylation (IPA) (Cotterill, 1982; Zhang et al., 1982), the production of esters containing three or five fluorine atoms to increase the response sensitivity of the electron capture detector (Smith, 1984), and mineral acid-catalyzed esterifications (Steinwandter, 1989) and silylation (Horner et al., 1974).

While these methods are effective, they suffer a number of disadvantages. First, they tend to be time-consuming; for example, Gurka et al. (1987) found that as long as 3 h may be required to prepare pentafluorobenzyl esters. Also, many side products may be formed during the esterification reactions, which are not separated from the ester in the GC analysis, thus making quantitation difficult or even impossible. Often, only moderate recovery rates are possible. Finally, in some cases, especially diazomethane, the starting material is carcinogenic (Sax, 1984).

In the present work, a novel approach has been undertaken. By means of a benzyltrialkylammonium salt organic solubilizer, 2,4-D is extracted from a soil sample and esterified in situ. Quantitation is then carried out by GC with electron capture detection.

EXPERIMENTAL PROCEDURES

Reagents. 2,4-Dichlorophenoxyacetic acid (2,4-D) was obtained from Aldrich Chemical Co. and recrystallized from methanol. Its purity was confirmed by melting point measurement [140 °C, cf lit. 140–141 °C (Zimmerman, 1943)] and from ^1H and ^{13}C NMR spectra. All the organic solubilizers employed were also obtained from Aldrich and used as 40% solutions in methanol.

HPLC grade methanol and "Nanograde" hexane (Mallinckrodt) were used without further treatment.

Deionized water obtained from a Millipore filtration system was employed throughout the work, and the sulfuric acid used was A.R. grade (M and B Chemicals).

Florisil (Aldrich, 60–100 mesh) was activated at 650 °C for 24 h and then held in a vacuum desiccator until it reached room temperature. It was partially deactivated by spiking into it ca. 1% water and mixing for 1 h. The prepared material was stored in a tightly stoppered flask until ready for use.

An authentic sample of 2,4-dichlorophenoxyacetic acid methyl ester was prepared by mineral acid (H_2SO_4) catalyzed esterification in refluxing methanol (3 h). The solid was recrystallized from hexane and confirmed by melting point measurement [38 °C, cf. lit. 39 °C (van Peteghem and Heyndrickx, 1975)] and from ^1H and ^{13}C NMR spectra. A stock solution containing 1000 ppm was prepared and stored for use.

Soil Samples. Lunette soil (sandy loam originally from Willaura, Western District of Victoria, Australia), obtained from the School of Agriculture, La Trobe University, was passed through a 100-mesh sieve, dried at 105 °C over 24 h, and stored in a vacuum desiccator at room temperature. Composition of this soil is presented in Table I.

Apparatus. (*i*) *Gas Chromatography.* A Hewlett-Packard Model 5880A instrument, equipped with a ^{63}Ni electron capture detector, a GC-411V autosampler, and a splitless capillary column injection port with a carrier gas flow rate of 10 mL min^{-1}, was employed in this work. The column employed was an HP-5 cross-linked 5% phenylmethylsilicone: 25 m × 0.2 mm i.d. × 0.33-μm film thickness. Injection volumes were 0.5 μL. The column head pressure was 140 kPa.

The operating temperatures were as follows: injection port, 250 °C; detector, 320 °C.

An argon–methane (95:5) mixture was employed as the detector makeup gas: flow rate, 30 mL min^{-1}.

Two temperature programs were employed after initial values of 50 °C and initial time 1 min: rate 1, 15 °C min^{-1}, final value

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Table I. Characteristics of Lunette Soil

organics, %	4.07	sand, %	56.14
clay, %	10.60	pH	6.15
silt, %	33.26		

220 °C, final time 0 min; rate 2, 20 °C min^{-1}, final value 270 °C, final time 5 min.

The GC autosampler was set up with a cycle time of 32 min, flush time of 20 s, and dwell time of 6 s.

(*ii*) *GC–MS.* A Hewlett-Packard Model 5890 GC instrument coupled to a 5970 series mass selective detector was employed, along with a 9000 series data processing system. The GC column was an HP-5, maintained as above.

An IB-7RDJ microsyringe was used; injection volumes were 0.5 μL.

Preliminary Investigations. The initial aims of the experiments were to extract 2,4-D from soil samples with methanol solutions of the organic solubilizers benzyltrimethylammonium methoxide (BTMAM) or the corresponding hydroxide (BTMAH) since both had been used successfully by us in other work (Chiang et al., 1989, 1990). The extracted 2,4-D was then to be esterified with a traditional esterification reagent. However, it was found, before any formal esterification had been employed, that both BTMAM and BTMAH, in addition to extracting 2,4-D, had simultaneously formed the 2,4-D methyl ester (2,4-DME). Therefore, it was decided to develop this simultaneous extraction/esterification procedure. Further investigations revealed that the reagents benzyltrimethylammonium chloride (BTMAC) and benzyltriethylammonium chloride (BTEAC) were even better.

Extensive investigations were carried out to ascertain the minimum amount of organic solubilizer needed to obtain quantitative recoveries of the analyte. Although 2,4-D was present in the soil only at parts per million levels, quite large amounts of solubilizer were required to achieve the extraction. Unsatisfactory results were achieved when 5 mL of 10%, 20%, and 30% solutions of BTMAM was used, but 7 mL of a 40% solution was sufficient to generate the ester in high yield. Obvious precipitation was noted when the analyte and solubilizer solutions were concentrated in the absence of soil. However, in the presence of the soil, the concentration process was carried out successfully. It is clear that the majority of the solubilizer was consumed by the organic components present in the soil, as well as by the added sulfuric acid. Consequently, the minimum amount of reagent required should be found experimentally when different types of soil with different organic content are involved.

Since the methanol solvent causes rapid deterioration of the coating of the GC column (Zweig, 1986), it was not employed as an eluent and the 2,4-DME was extracted into hexane instead.

For a "cleanup" procedure, both alumina and florisil columns were examined. Since 2,4-DME tended to saponify on alumina, florisil was employed throughout. In addition, it was found desirable to cover the top of the column with a 0.5 cm depth layer of anhydrous sodium sulfate to protect it from contact with moisture (which deactivates the florisil).

Procedure. Dried soil (5.0 g) and 0.5 g of a 1% H_2SO_4 solution were equilibrated for 0.5 h in a sample jar. The acid prevents loss by adsorption of 2,4-D on the glass container wall (Kan et al., 1982). Aliquots of fresh 2,4-D/methanol stock solution (1 mL containing 10 ppm or 1 mL containing 5 ppm of 2,4-D) diluted daily from a stock solution were spiked into the soil sample which was equilibrated for a further 3 h. A 40% solution (7 mL) of BTMAC organic solubilizer in methanol was added to the soil sample and the mixture shaken for 10 min before filtering. The filtrate was collected and the solid in the funnel washed with 20 mL of methanol.

The extracted solution was concentrated to 1 mL on a water bath set at 35 °C, evaporation being assisted by a gentle stream of N_2 gas (2,4-DME is volatile and easily lost with any harsher treatment). The 2,4-DME in the resulting solution was extracted by a 25-mL aliquot of hexane by intermittent shaking over 10 min in a separatory funnel. The methanol layer was removed and the hexane layer transferred to a 50-mL beaker, where it was reduced to a final volume between 1 and 3 mL by a gentle flow of N_2 gas through the solution.

This concentrate was passed through a florisil column and eluted with 40 mL of hexane. The eluate was again concentrated by a N_2 stream at room temperature to a volume less than 10 mL and then transferred to a 10-mL volumetric flask and made up to volume with hexane. Aliquots of this solution were ready for injection into the GC instrument.

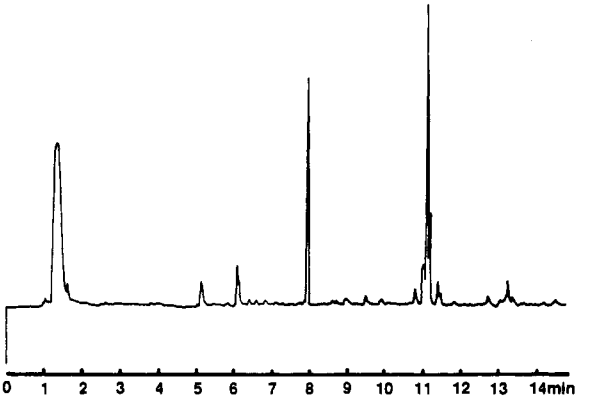

Figure 1. Gas chromatogram for the fortified soil extract (2 ppm level).

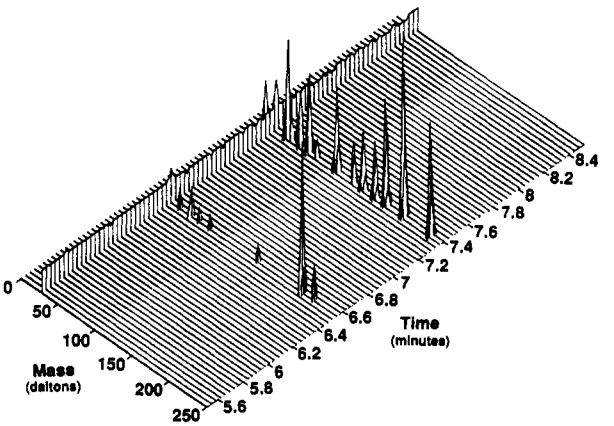

Figure 2. GC–MS plot for the soil extract.

RESULTS AND DISCUSSION

The chromatogram shows the presence of several minor components (Figure 1), all of which occur in the soil "blank" (i.e., when the soil does not contain any added 2,4-D), and the nature of these was not generally pursued further. However, those with retention times of ca. 5–6 min appear to be relatively nonpolar materials, while the component eluting at 7.99 min was positively identified as 2,6-bis-(1,1-dimethylethyl)-4-methylphenol from its mass spectrum. The 2,4-DME has a retention time of 11.19 min under the conditions employed and is readily identified as the major component.

In the GC–MS experiment, the two major components were identified (Figure 2). The different retention times compared with the GC experiment reflect only differences in the programming rates employed on the two instruments.

The efficiency of the procedure was measured by determining recovery rates for a range of samples by means of the calibration curve (Figure 3) prepared from 2,4-DME standard samples.

Recovery was tested by using five different organic solubilizers. The results obtained as the mean of five determinations for each solubilizer at two 2,4-D concentration levels are shown in Table II. Very high recovery rates were obtained under these conditions for BTMAC and BTEAC, while tetramethylammonium hydroxide (TMAH) was the least satisfactory presumably because it did not esterify 2,4-D or extract it from the soil. The fact that BTEAC also allowed a considerably high recovery of 2,4-DME indicates that the transferred methyl group does not originate from the solubilizer but from the solvent.

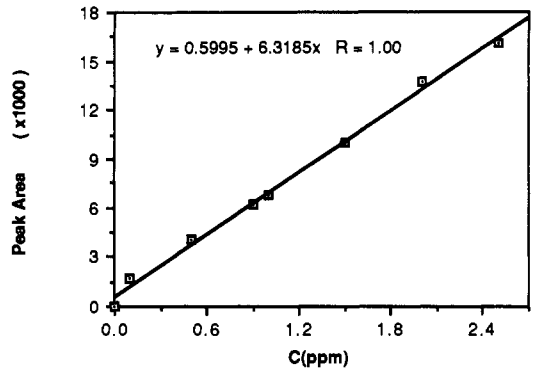

Figure 3. Calibration graph for the 2,4-D methyl ester.

Table II. Recoveries of 2,4-D with Different Organic Solubilizers

solubilizer	% recovery from standards	
	2.0 ppm	0.4 ppm
BTMAC	96.4	95.5
BTEAC	91.7	91.1
BTMAH	50.9	50.1
BTMAM	50.7	50.1
TMAH	31.8	30.0

Since 2,4-D is not esterified to any appreciable extent by methanol alone under the same conditions, the solubilizer would appear to catalyze the esterification reaction.

Although a number of liquid–solid extraction methods are known (Rosenfeld et al., 1986; Zhu and Xu, 1984; Di Corcia et al., 1989; Wang and Huang, 1989), they are either inefficient when applied to trace analysis (Cotterill, 1982) or inapplicable to molecular compounds such as 2,4-DME. Therefore, the classic liquid–liquid extraction and column cleanup procedure were employed in this case.

The extraction and concentration procedure reported here has not been optimized and so does not preclude the use of other techniques. A major objective, however, was to avoid the addition of more reagents which could have introduced other impurities into the system.

The solubilizers BTMAH and BTMAM were not as efficient as originally anticipated. This was due to their high apparent pH values (each ca. 10.8), which affected the esterification or the stability of any 2,4-DME formed. A high pH accelerates the hydrolysis of 2,4-DME. In contrast, the pH of the BTMAC solution is 4.8, which appears to stabilize the 2,4-DME formed by this reagent. Consequently, BTMAC was adopted for use in the recommended procedure. It is evident that the elimination of a separate esterification step leads to a more efficient procedure and one which appears to lend itself to the determination of other chlorophenoxy acids in soils. Studies on such systems are continuing.

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