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Received for review October 7, 1974. Accepted November 1, 1974.

COMMUNICATIONS

Improved Determination of Residues of Phorate and Its Principal Metabolites

Improvements in methodology for the determination of residues of phorate and its three principal metabolites are described. These refinements include chilling the sample during extraction to prevent emulsion formation, using a microcolumn cleanup procedure resulting in economy of time and materials, and evaporating under nitrogen to preclude oxidation of phorate.

Problems were encountered in applying the method of Suett (1971) to the analysis of field-treated carrots for determining residues of phorate (*O,O*-diethyl *S*-[(ethylthio)methyl] phosphorodithioate) and three of its metabolites. The modifications described here considerably reduce the time needed for analysis, save materials, and prevent oxidation of the labile phorate.

EXPERIMENTAL SECTION

Apparatus. A Sorval Omni-Mixer with a 400-ml sample cup was used for sample extraction. Two gc columns were required: a 6 ft × 0.25 in. o.d. glass column packed with 3% OV-17 on 60–80 mesh Gas-Chrom Q for analysis of phorate and phorate oxygen analog (POA), and a 3 ft × 2 mm i.d. column containing 2.5% XE-60 on 60–80 mesh Gas-Chrom Q for analysis of phorate sulfoxide (PSO) and phorate sulfone (PSO_2) (Saunders and Getzin, 1973). The detector temperature was 200°; column oven temperatures were 195° for the OV-17 and 190° for the XE-60 column. The flow of nitrogen carrier gas was 90 ml/min; hydrogen, 200 ml/min; and air and oxygen, each 20 ml/min.

Reagents. Analytical standards of phorate, POA, PSO, and PSO_2 were supplied by American Cyanamid Co., Princeton, N.J. Nuchar C (Fisher Scientific) was acid washed by refluxing 100 g of charcoal with 500 ml of concentrated HCl for 1 hr, adding 500 ml of distilled water, and again refluxing 1 hr, cooling, and washing with distilled water until neutral to indicator tape. The charcoal was then transferred to a glass jar and heated overnight at 130°. Silane-treated glass wool (Applied Science Lab., Inc.) and Whatman CF11 cellulose powder were used in the microcolumn cleanup.

Procedure. A 50-g sample of shredded whole carrot, with 40 g of anhydrous Na_2SO_4 and 100 ml of chloroform–methanol (9:1, v/v), was blended for 3 min in the Omni-Mixer with the sample cup immersed in an ice water bath. The extract was filtered through a 150-ml Büchner funnel lined with glass fiber paper and fitted to a 250-ml graduated cylinder (Williams and Brown, 1973). The extraction was repeated twice with additional 50-ml portions of chloroform–methanol, and the extracts were pooled.

Ten per cent of the combined extracts, representing 5 g of carrot, was transferred to a 50-ml beaker and evaporated under a stream of nitrogen to about 0.5 ml. A cleanup column was prepared by plugging a Pasteur pipet with a small wad of silane-treated glass wool and adding a 4–5 cm layer of Nuchar C–Whatman CF11 cellulose powder (4:10, w/w). The packing was topped with a smaller plug of glass wool and prewashed with approximately 1 ml of ethyl acetate. The concentrated extract was dissolved in 1 ml of ethyl acetate and transferred to the cleanup column using a Pasteur pipet. As the last of the sample entered the column packing, a total of 5 ml of ethyl acetate was added to strip the column of pesticide. All solvent which eluted after the prewash was collected in a concentrating tube and again evaporated to 0.5 ml under a stream of nitrogen. Each sample was chromatographed twice using a flame photometric detector in the phosphorus mode, first on the OV-17 column to determine phorate and POA and later on the XE-60 column for PSO and PSO_2.

RESULTS AND DISCUSSION

Recoveries from carrots untreated but fortified are shown in Table I. One of the earliest problems was to find a suitable gc column. Grant *et al.* (1969) reported direct determination of phorate and its metabolites including the oxygen analog sulfoxide and sulfone using a 5% DEGS column. However, we were unable to chromatograph these two oxygen analog metabolites even at the 1-μg level. Nor did we achieve satisfactory separation of phorate from POA or PSO from PSO_2, despite variations of column length and support loading. Such disparity of results may be partially explained by the work of Kruppa and Henly (1974). They found that, with conditioning, the DEGS in each column formed a distinct superpolyester and that conditioning was accompanied by severe bleeding of the liquid phase. Thus, each DEGS column was unique and its performance unpredictable. No one column was found which satisfactorily separated all four compounds. The

Table I. Recovery of Phorate and Three Metabolites from Untreated, Fortified Carrots[a]

Fortification, ppm	Percentage recovery			
	Phorate	POA	PSO	PSO_2
0.1	90.5	85.3	107.5	81.5
	88.9	91.3	80.0	88.6
	95.1	79.7	87.2	109.6
	(91.5)	(85.4)	(91.6)	(93.3)
0.5	86.0	86.5	77.6	85.6
1.0	92.9	90.1	83.1	92.6
	99.1	103.7	97.0	80.0
	(96.0)	(96.9)	(90.1)	(86.3)

[a] Average values are in parentheses.

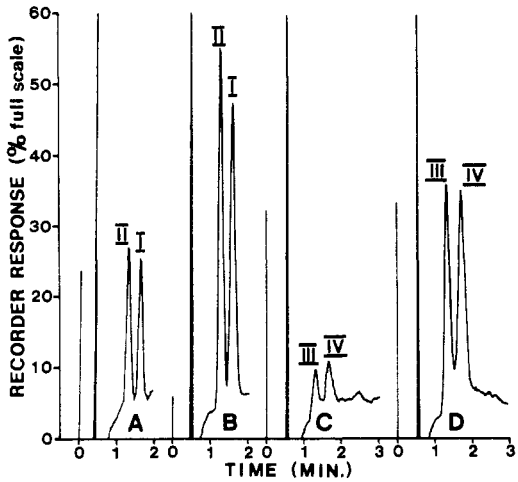

Figure 1. Gas chromatograms showing separation of: (A) 5 ng and (B) 50 ng of phorate (I) and POA (II) on OV-17; (C) 5 ng and (D) 20 ng of PSO (III) and PSO_2 (IV) on XE-60. Sensitivity: 3.2×10^{-8} AFS for (A), (C), and (D); 1.28×10^{-7} AFS for (B).

final choice was made on the basis of separation, sensitivity, and elution time (Figure 1). Conditioning of the XE-60 column at 10° above operating temperature for at least 72 hr was necessary for acceptable separation.

The use of an ice water bath during extraction is essential to prevent emulsion formation. A bath of flaked ice alone does not provide sufficient cooling.

The microcleanup column is used in the interest of speed and economy of materials. The silane-treated glass wool used in the column is necessary because untreated glass wool results in loss of significant amounts of phorate. The make of cellulose powder is also important because some products may reduce recovery of phorate.

All evaporations are done under nitrogen; exposure to an air stream, even in vacuum evaporation, results in large-scale oxidation of phorate, primarily to PSO.

Although these refinements have not yet been applied to other crops, they should provide a basis for analysis of phorate and its principal metabolites. With these few modifications, analyses were easily completed in 1 day.

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Received for review July 1, 1974. Accepted October 1, 1974.

Terpenoid Ethers as Juvenile Hormone Analogs

Several simple terpenoid ethers, derived from various combinations of geraniol, nerol, γ,γ-dimethylallyl alcohol, 3-methyl-2-pentenol, and farnesol show juvenile hormone activity with *Oneopeltus fasciatus* and *Autographa californica*.

Numerous juvenile hormone (JH) analogs have appeared in the literature which simulate the effect of endogenous juvenile hormone when applied to various insect species (Slama, 1971). In order to utilize such compounds as insecticides it would seem desirable that such materials be both readily available and of minimum toxicity to higher animal species. Accordingly we have explored the use of simple terpenoid ethers, devoid of heteroatoms other than oxygen, in order to determine if additional useful compounds could be prepared. In addition, we have examined the general effect of replacing chemically reactive double bonds with less reactive ether oxygen.

Ethers, both terpenoid and nonterpenoid, have occasionally been reported as JH active, the simplest being farnesyl methyl ether (Bowers and Thompson, 1963; Schmialek, 1963) and dodecyl methyl ether (Schneiderman *et al.*, 1965). More complex ethers derived from sesamole or the methylenedioxyphenyl moiety have been reported as particularly effective, especially in the form of mixed ethers with terpenoid side chains (Bowers, 1969). Another series of ethers which has been reported is that derived from α-hydroxy acids (Slama *et al.*, 1973). Ethers derived from the addition of ethanol to terpenoid double bonds are also effective (Wakabayashi, 1969; Brieger, 1971).

The JH molecule itself can be considered as an isoprenoid chain, with functional groupings at each end. It appears that the epoxide group at one terminal is not essential, but certainly enhances the activity. An ester grouping or a related electronegative grouping such as an amide at the other terminus is important for activity (Slama, 1971).

We wanted to determine whether functionality at the ends is absolutely necessary, or whether in fact a simple functional group could be located in other portions of the chain.

We chose to concentrate on ethers derived from the readily available geraniol and citronellol. As indicated in Chart I, various possible combinations of ethers which can be derived from geraniol, citronellol, γ,γ-dimethylallyl alcohol, and 3-methyl-2-pentenol were prepared. In all cases the terminal epoxides were also prepared.

MATERIALS AND METHODS

Infrared spectra were determined on a Beckman I.R-5 spectrophotometer. Nmr spectra were determined using a Varian T-60 spectrometer, using TMS as an internal standard. Gas–liquid chromatographic analyses were performed with a Varian 1200 flame ionization instrument. Generally, a 10-ft, 15% DEGS, Chromosorb W column was used. Elemental analyses (performed by Spang Microanalytical Laboratories, Ann Arbor, Mich.) and nmr spectral data used to characterize the compounds appear