from the surrounding atmosphere (diphenylamine has an appreciable vapor pressure) in which treated apples were stored. Contamination by contact with used wooden crates and other equipment was also possible.

An amino group on an aromatic ring activates the ortho and para carbons for bromine substitution. Iodine may be used as a catalyst to incorporate one atom of the bromine molecule into an anion, which can act as acceptor for the hydrogen atom to be displaced. The other atom is left as a positively charged ion—i.e., $Br^+(IBr_4)^-$. This may explain the course of bromination of diphenylamine when iodine is the catalyst. The bromination step must be conducted as described. Shorter reaction time leads to a mixture of partially brominated derivatives which will appear earlier in the chromatogram.

Solvents must be distilled before use to avoid an appreciable blank value. Diphenylamine is volatilized by an air stream but the brominated product is not. The bromination is therefore conducted in hexane to obviate evaporation.

Acknowledgment

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INSECTICIDE RESIDUES

Gas Chromatographic Determination of Organophosphorus Insecticides Using the Zeisel Alkoxyl Reaction

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A rapid gas chromatographic method is described for determining organophosphorus insecticides by electron affinity detection. A solution of the insecticide is evaporated in a small vial. Hydriodic acid is introduced, and the vial is sealed and heated. Methyl or ethyl iodides are evolved upon cleavage of the alkoxyl groups. The alkyl iodides are then syringed from the headspace and determined by electron affinity spectroscopy. Ethion and malathion have been determined by the method.

With a few notable exceptions, the organophosphorus insecticides are not exquisitely sensitive to the electron affinity detector as are many of the chlorinated compounds. Iodine imparts much greater sensitivity when introduced into an organic compound than any of the other halogens (*3*). The organophosphorus insecticides usually contain methoxyl or ethoxyl groups and yield the corresponding alkyl iodides upon reaction with hydriodic acid [Zeisel alkoxyl reaction (*4*)]. In this paper, this reaction is the basis for the gas chromatographic determination of ethion, $[(C_2H_5O)_2PS_2]CH_2$, and malathion, $(CH_3O)_2PS_2CH(COOC_2H_5)$-$(CH_2COOC_2H_5)$.

Equipment

A Barber-Colman Model 10 gas chromatograph was used with a battery-operated (*1*, *2*), No. A-4071, 6-cc. detector containing 56 μc. of radium-226. An optimum voltage of 11 volts was applied to the detector, and an electrometer gain of 3000 was used. The recorder was a Wheelco, 0 to 50 mv. equipped with 10-inch chart paper, running 10 inches per hour.

The column was U-shaped made of borosilicate glass, 9-mm. o.d. and 6 feet long. The column packing was 5% ethyl acetate-fractionated Dow Corning high vacuum silicone grease on 80- to 100-mesh, acid-washed Chromosorb W. Connections between the column and detector were made with metal hypodermic tubing, glass elbows, and silicone rubber through-septums. The operating temperatures for the column, flash heater, and detector were 35°, 40°, and 90° C., respectively, and nitrogen (10 cc. per minute) was the carrier gas. The column was conditioned for 16 hours at 230° C. before use.

Procedure

Prepare a series of standard solutions (5 to 20 μg. per ml.) of the insecticide in a suitable solvent. Pipet 1 ml. of the solution into a No. 2 screw-cap vial. Evaporate the solution just to dryness with a gentle air stream. Add 1 ml.

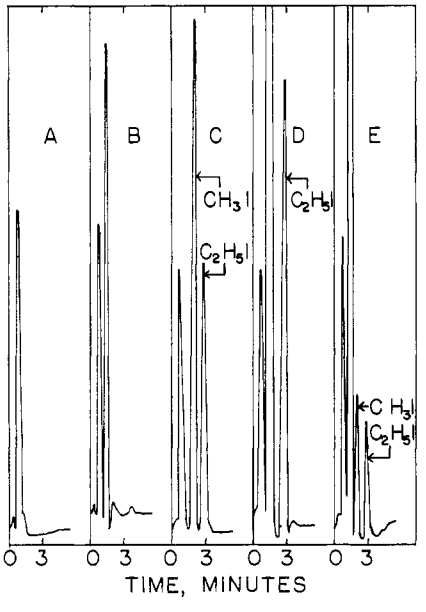

Figure 1. Chromatograms of (*A*) air, (*B*) reagent blank, (*C*) methyl and ethyl iodides in air, (*D*) ethion (13.6 μg.), and (*E*) malathion (9.8 μg.)

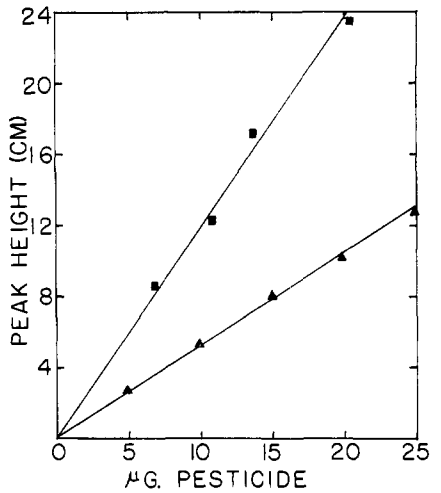

Figure 2. Standard curves of ethion (■——■) based on ethyl iodide and malathion (▲——▲) based on methyl iodide

of 48% hydriodic acid. Immediately place a piece (about 1.5 inches square) of 3-mil polyethylene on top of the vial and screw the cap on tight. The cap used is modified by drilling a $^3/_{16}$-inch hole through it and the paper liner. A silicone rubber half-septum is then fitted tightly in this hole. Immerse the sealed vial about 1 inch deep in a boiling water bath for 10 minutes. Remove and immediately transfer 10 μl. of the headspace gases in the vial into the gas chromatographic column. Plot peak height in centimeters against micrograms of insecticide in the vial.

The syringe was rinsed thoroughly with hexane between injections. Hexane was removed from the syringe by repeatedly drawing air into the syringe.

Results and Discussion

Figure 1 shows the chromatograms obtained by the method. The peak in *A* results from injection of 10 μl. of air. The peak presumably represents capture of electrons by oxygen. In *B* is shown the chromatogram of 10 μl. of the headspace from a vial containing 1 ml. of hydriodic acid which was heated (100° C.) for 10 minutes. One milliliter of acetone had been evaporated to dryness in the vial before introduction of the acid. The first peak represents oxygen. The second peak may be due to an impurity in the hydriodic acid or acetone residue. More likely it is due to the production of iodine in traces by disproportionation of hydriodic acid. Although the caps were placed on the vials as tightly as possible before the incubation step, leakage of the resulting alkyl iodides may have occurred.

Chromatogram *C* represents 10 μl. of the headspace from a vial containing pure methyl and ethyl iodides in air. The retention times for ethyl iodide evolved from ethion (containing only ethoxyl groups) and methyl and ethyl iodides from malathion (containing both methoxyl and ethoxyl groups) are identical with those in *C*.

Figure 2 shows the standard curves for ethion based on the peak height for ethyl iodide and malathion based on methyl iodide. The choice of the methyl iodide peak for developing the standard curve for malathion and of the ethyl iodide peak for ethion was to illustrate that the determination of the organophosphorus compounds could be based on cleavage of either methoxyl or ethoxyl groups. Since ethion contains only ethoxyl groups, malathion was chosen for the study as a common insecticide containing methoxyl (as well as ethoxyl) groups.

The Zeisel reaction yields alkyl iodides when applied to alcohols, ethers, and esters containing alkoxyl groups with not more than three to four carbon atoms. The method may be applicable therefore to analysis of organophosphorus insecticide residues after separation of naturally occurring interferences. It should be directly applicable to analysis of atmospheric samples.

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INSECTICIDE-ACARICIDE RESIDUES

Residue Analysis of Ethion by Cholinesterase Inhibition after Oxidation

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ETHION (*O,O,O',O'*-tetraethyl S,S'-methylene bisphosphorodithioate) is an effective insecticide and acaricide. It can be used for the control of olive scale in olive orchards. For this reason it was necessary to develop a sensitive method for residue analyses of this compound on olives.

The presently available methods for residue analysis of ethion are based on a colorimetric procedure (*4*) and cholinesterase inhibition (*5*). For the latter method, the extract or pure standard is first oxidized to an active form by dilute bromine water. This proved unsuccessful for olives since enough olefinic compounds were present in extracts which reacted preferentially with bromine water and resulted in incomplete activation of the ethion.

For this reason another procedure for the determination of trace amounts of ethion in olives was developed. Essentially, this method is based on the activation of the compound by peracetic acid, similar to the methods for Trithion and phorate (*1, 6*).

Experimental

Reagents. Barbital buffer, pH 8.0. Dissolve 7.42 grams of sodium barbital, 89.46 grams of potassium chloride, and 1.09 grams of monobasic potassium phosphate in 1 liter of distilled water. The pH is adjusted, if necessary, to 8.00 ± 0.10 with acid or base just prior to adjusting the volume to 1 liter.

Acetylcholine bromide, 0.22*M*. Dissolve 4.97 grams in 100 ml. of distilled water.

Saline solution, 0.9%. Dissolve 9 grams of sodium chloride in 1 liter of distilled water.

Glycerol solution, 10% v./v. Add 10 ml. of glycerol to absolute methanol and adjust the volume to 100 ml. with absolute methanol.

Cholinesterase solution. Mix 3 ml. of fresh horse plasma or 4 ml. of outdated human plasma with 2 ml. or 1 ml. of 0.9% saline solution, and 10 ml. of barbital buffer to result in a final volume of 15 ml. Larger amounts of enzyme