properties of the carbon and to determine the recovery of a given pesticide.

Other problems potentially present in the cleanup step are the loss of the pesticide because of multiple handling steps and volatility. Chlorobenzilate was not volatile under the conditions used. Also, because of the development of detectors with increased sensitivity, the organic reagents that are used in the isolation of the pesticide must be free of impurities, such as halogens, which will be detected in microgram or picogram amounts on the gas chromatograph.

Crafts (*4*) has demonstrated that the isopropyl ester of 2,4-D undergoes hydrolysis on the leaf surface of a plant whereby the acid is adsorbed and translocated. The authors also have evidence that esters of 2,4-D revert to the parent acid in plant materials obtained from experimental plots. Therefore, the question arises whether or not chlorobenzilate may partially hydrolyze to the free acid during weathering of the chemical on the plant in the field. No studies were made on this phase of the subject. The free acid would not chromatograph under the conditions of column operation used in this study.

The application of the gas chromatographic procedure to the analysis of grapes and cottonseed for chlorobenzilate residues eliminated several tedious and painstaking fractionation and chemical conversion steps and gave more precise and specific residue data.

Acknowledgment

Special recognition for technical assistance in this work is due Nancy Nash of this laboratory.

Literature Cited

(1) Beckman, H. F., Bevenue, A., *J. Chromatog.* **10,** 231 (1963).
(2) *Ibid.*, **12,** 109 (1963).
(3) Blinn, R. A., Gunther, F. A., Kolbezen, J., J. AGR. FOOD CHEM. **2,** 1080 (1954).
(4) Crafts, A. S., *Weeds* **8,** 19 (1960).
(5) "Dohrmann Operational Manual," Dohrmann Instruments Co., San Carlos, Calif., 1962.
(6) Harris, H. J., J. AGR. FOOD CHEM. **3,** 939 (1955).
(7) Schechter, M. S., Soloway, S. B., Hayes, R. A., Haller, H. L., *Ind. Eng. Chem., Anal. Ed.* **17,** 704 (1945).

Received for review April 1, 1963. Accepted July 31, 1963. Division of Agricultural and Food Chemistry, 144th Meeting, Los Angeles, Calif., April 1963.

HERBICIDE RESIDUES

Determination of Amiben in Tomatoes by Electron Affinity Gas Chromatography

CARL A. BACHE, WALTER H. GUTENMANN, and DONALD J. LISK

Pesticide Residue Laboratory, Department of Entomology, New York State College of Agriculture, Cornell University, Ithaca, N. Y.

A method is described for the determination of 3-amino-2,5-dichlorobenzoic acid (amiben) in tomatoes. It involves extraction of amiben at pH 2 into benzene from an alkali-hydrolyzed tomato extract. The acid is then methylated with BF_3-methanol, and the ester is determined by electron affinity gas chromatography. Recovery of the herbicide from 0.05 to 1.25 p.p.m. ranged from 70 to 123%. About 0.02 p.p.m. of the herbicide may be detected. Residues observed in field-treated samples are reported.

THE HERBICIDE 3-amino-2,5-dichlorobenzoic acid (amiben) may be used for weed control in vegetables and certain other crops. In this paper, a method is described for the determination of residues of this compound in tomatoes based on electron affinity gas chromatography of the methyl ester.

Equipment

A Barber-Colman Model 10 gas chromatograph was used with a battery-operated (*4, 5*) Barber-Colman No. A-4071, 6-cc. detector containing 56 μc. of radium-226. The detector was operated at 3.5 volts, which was found to be its optimum for electron capture by chlorinated compounds. A 90,000-megohm resistor was added to the electrometer to give additional gains of 3,000; 10,000; and 30,000. The 3,000 and 10,000 settings were used in this study. The recorder was a Wheelco, 0 to 50 mv. equipped with 10-inch chart paper, running 10 inches per hour.

The column was borosilicate glass, U-shaped, 9-mm. o.d., and 6 feet long. The packing was 5% Dow Corning high-vacuum silicone grease (ethyl acetate–fractionated) on 80- to 100-mesh acid-washed Chromosorb W. Connections between the column and detector were made with metal hypodermic tubing and glass elbows, using silicone rubber through septums. The operating temperatures for the column, flash heater, and detector were 200°, 265°, and 235° C., respectively, and nitrogen (35 cc. per minute) was the carrier gas. The column was conditioned for 16 hours at 230° C. before use.

Procedure

Weigh 10 grams of well-blended tomatoes in a 100-ml. beaker. Add 10 ml. of 2*N* sodium hydroxide, cover with a watch glass, and boil the mixture for 30 minutes. Transfer the digest quantitatively to a 50-ml., glass-stoppered centrifuge bottle with about 10 ml. of 0.1*N* sodium hydroxide and centrifuge at 2000 r.p.m. Filter the supernatant liquid through No. 41 Whatman paper into a 200-ml. beaker. Shake the residue in the bottle with 25 ml. of 0.1*N* base and centrifuge as above. Decant the supernatant liquid and use this liquid to rinse the filter. Repeat this step using 25 ml. of the 0.1*N* base and again centrifuge and filter the solution. Combine the alkali filtrates.

Adjust the pH of the solution to 7.5 with 5*N* hydrochloric acid using a pH meter and transfer the solution to a 250-ml. separatory funnel. Extract the solution four times with 50 ml. of diethyl ether and once with 50 ml. of benzene. Discard the organic solvents. Adjust the pH of the aqueous solution to 2.0 with 5*N* hydrochloric acid and extract

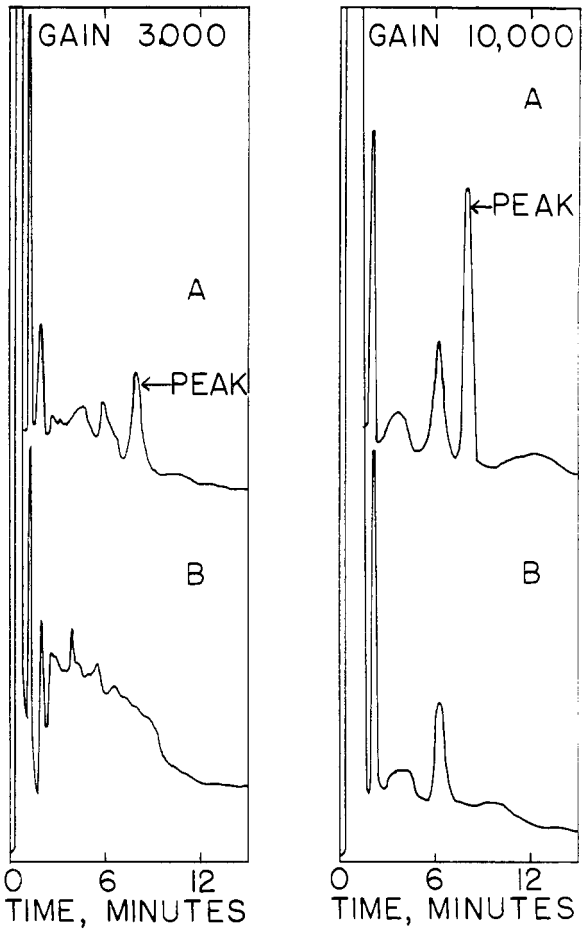

Figure 1. Chromatograms of (A) field-treated tomatoes containing 0.05 p.p.m. of amiben and (B) untreated tomatoes at electrometer gain settings of 3,000 and 10,000

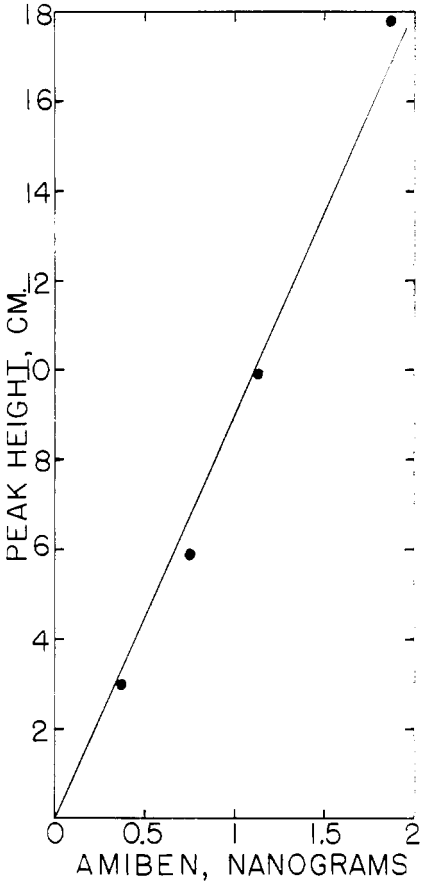

Figure 2. Standard curve of amiben

four times with 50 ml. of benzene. Combine the benzene extracts in a 500-ml., round-bottomed flask and evaporate to dryness, using a rotating evaporator and reduced pressure.

Dissolve the residue in the flask with exactly 5 ml. of acetone. Transfer 1 ml. of the acetone solution to a 25-ml. volumetric flask and evaporate the acetone using an air stream. Add 2 ml. of BF_3-methanol reagent (*6*) to the flask, and hold the flask in a boiling water bath for 2 minutes with frequent swirling. Cool the flask and repeat the esterification twice more with 2-ml. portions of fresh BF_3-methanol reagent. Cool the flask each time before addition of the reagent. Add 1 ml. of hexane to the cooled flask, make to volume with 2% sodium sulfate solution, and shake the contents vigorously for 30 seconds. Inject 10 μl. of the upper hexane layer into the chromatographic column. The retention time for the methyl ester of amiben was approximately 8 minutes.

Develop the standard curve for amiben as follows. Pipet 0, 0.1, 0.2, 0.3, 0.4, and 0.5 ml. of amiben (0.42 μg. per ml.) in acetone into a series of 25-ml. volumetric flasks. Evaporate the acetone and proceed as in the analysis of tomatoes beginning with the addition of 2 ml. of BF_3-methanol.

Results and Discussion

Figure 1 shows chromatograms of untreated tomatoes and field-treated tomatoes calculated to contain 0.05 p.p.m. of amiben at electrometer gain settings of 3000 and 10,000. The first two peaks appearing after the solvent peak at the lower setting were due to constituents in the saponified tomato extract. With the exception of the amiben ester peak and these two peaks, all of the other peaks were present in the reagent blank (zero standard) chromatogram. The first peak after the solvent peak at the lower gain appears as part of the solvent peak at the 10,000 gain setting.

Figure 2 shows a typical standard curve for amiben in which peak height in centimeters is plotted against nanograms injected using an electrometer gain setting of 10,000. Table I lists the recoveries of amiben added in an acetone solution to tomatoes. The method is sensitive to about 0.02 p.p.m. of the herbicide. This concentration would yield a peak height equal to about a 5% full-scale deflection when injecting 10 μl. of the sample and using an electrometer gain setting of 10,000. Analysis of tomatoes from plants which received the recommended 4 pounds per acre soil application of granular amiben on June 27, 1962, and which were harvested August 20 and September 15, showed replicate residue levels of 0.03 and 0.05, and 0.03 and 0.03 p.p.m., respectively. Tomatoes treated at twice the recommended rate (8 pounds per acre) showed replicate residue levels of 0.11 and 0.14 p.p.m. in August, and 0.05 and 0.06 p.p.m. in September.

Colby *et al.* (*2*) demonstrated that amiben forms plant conjugates with tomato substances which have R_f values by paper chromatography distinct from amiben. They showed that alkaline hydrolysis is necessary to regenerate free amiben.

Table I. Recovery of Amiben Added to Tomatoes

Amount Added, P.P.M.	Recovery, %
0.05	70, 110
0.1	85, 70, 75, 115
0.15	83
0.25	96
0.5	90
0.75	121
1.0	107
1.25	123

The ether and benzene extractions of the tomato extract (adjusted to pH 7.5) removed a considerable quantity of interfering substances which precipitated out if the extract solution was directly adjusted to pH 2 without extraction. Some of these interfering substances were acidic because the pH of the solution immediately after extraction was 8.5.

All of the solvents used in the procedure were distilled. The standard solution of amiben must be freshly prepared. Solutions of amiben in organic solvents turn yellow with time possibly because of oxidation of amine and further polymerization reactions leading to colored quinone-type compounds (*3*). Quantitative methylation of the herbicide required three treatments with BF_3-methanol because *ortho*-chloro substituted benzoic acids are less reactive due to stearic hindrance. Methanol distilled over KOH should be used for preparation of the boron trifluoride reagent.

When this method is used with a tritium electron affinity detector, the hexane should be dried over sodium sulfate before injection into the column (*7*).

A pH of 2 was optimum for extraction of amiben into benzene. In the determination of guthion residues, Adams (*1*) found an optimum pH of 4.1 for extraction of 2-aminobenzoic acid (anthranilic acid) into benzene. This pH apparently corresponded to a kind of "isoelectric point" for anthranilic acid. The presence of the two chlorines in amiben would make it a stronger acid and a weaker base. Therefore, a lower optimum pH (pH 2) is to be expected for amiben if, indeed, an isoelectric point can be said to exist for it.

Acknowledgment

The authors thank R. D. Sweet for making the amiben soil applications.

Literature Cited

(1) Adams, J. M., Chemagro Corp., Kansas City 20, Mo., personal communication.
(2) Colby, S. R., Baker, R. S., Warren, G. F., Purdue University, Lafayette, Ind., unpublished data.
(3) Fieser, L. F., Fieser, M., "Textbook of Organic Chemistry," p. 555, 1950.
(4) Goodwin, E. S., Goulden, R., Reynolds, J. G., *Analyst* **86,** 697 (1961).
(5) Goodwin, E. S., Goulden, R., Richardson, A., Reynolds, J. G., *Chem. Ind. London* **1960,** p. 1220.
(6) Metcalf, L. D., Schmitz, A. A., Chromatography Application Methods Bulletin WCA 1, Barber-Colman Co., Rockford, Ill., Sept. 22, 1960.
(7) Segal, H. S., Amchem Products Inc., Ambler, Pa., personal communication.

Received for review March 14, 1963. Accepted July 22, 1963.

DEFOLIANT RESIDUES

P^{32}- and S^{35}-Labeled *S,S,S*-Tributylphosphorotrithioate Defoliant Residue in Cottonseed

FRANK R. H. KATTERMAN and WAYNE C. HALL

Department of Plant Sciences, Agricultural and Mechanical College of Texas, College Station, Texas

Radioactive tributylphosphorotrithioate containing either the P^{32} or S^{35} isotopes was synthesized. The radioactive compounds were applied to intact cotton plants, and the distribution of the intact compound and its degradation products into the various sulfur and phosphorus fractions of the cottonseed kernel was traced by means of the P^{32} and S^{35} labels. Most of the radioactivity which was translocated to the seed resulted from the degradation products. Only a small amount of the total radioactivity present in the seed was represented by the unaltered thiophosphate compound.

THE FIRST organic thiophosphate compound reported to induce and accelerate abscission was *S,S,S*-tributylphosphorotrithioate (*6*, *14*). During the same period, Goyette (*5*) tested homologous trialkyl phosphorotrithioites and the corresponding trialkyl phosphates for abscission-inducing activity in the greenhouse, and noted that only the former were active. Hall *et al.* (*7*) studied a wide array of phosphorus- and sulfur-containing compounds for abscission properties and confirmed Goyette's findings. Both groups of workers concluded that the sulfur moiety was essential for effective defoliation.

The organic phosphorus insecticides, in general, are cholinesterase inhibitors, and some are extremely toxic to warm-blooded mammals. Little is known about the mammalian toxicity of organic phosphorus defoliants except that these compounds did not appear to be cholinesterase inhibitors in a preliminary study (*11*).

Because of the great potential of thiophosphate compounds in defoliation, this present study was primarily concerned with the residue of the intact P^{32}- or S^{35} - labeled *S,S,S* - tributylphosphorotrithioate and its degradation products in cottonseed.

Experimental

Preliminary results indicated that in vivo degradation of the trithioite and trithioate defoliants did not differ from each other because the relatively unstable trithioite is converted to the trithioate in the presence of oxygen (*9*). Therefore, *S,S,S*-tributylphosphorotrithioate (DEF) was used throughout the study and was synthesized with either the P^{32} or the S^{35} label as described previously (*13*).

P^{32} and S^{35} activity in the kernels, as well as in subsequent liquid and solid fractions, was assayed by means of a gas