Determination of Residues of Vitavax and Plantvax in Grains

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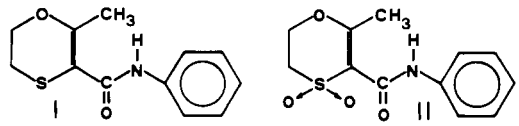
A gas–liquid chromatographic method using a nitrogen-selective detector is described for the determination of Vitavax and Plantvax residues in grains. Vitavax and Plantvax are extracted from the grains and the extract is cleaned up. Gas–liquid chromatography is applied to Plantvax following the formation of the reduced derivative. The structure of the reduced Plantvax is established by NMR and infrared spectra. The recoveries of Vitavax from fortified grains varied from 87.2 to 92.1% in the range 0.01–0.20 mg/kg. The sensitivity was such that Vitavax could be detected down to a level of 0.005 mg/kg. Results for Plantvax recoveries showed that the method can be used in grain extracts for levels down to 0.5 ppm for quantitative determination of Plantvax. Below this limit the reduced Plantvax peak rapidly disappeared as concentration diminished.

Carboxin (2,3-dihydro-6-methyl-5-phenylcarbamoyl-1,4-oxathiin, I) and oxycarboxin (2,3-dihydro-6-methyl-5-phenylcarbamoyl-1,4-oxathiin 4,4-dioxide, II), introduced under the trade names Vitavax and Plantvax, are systemic



fungicides: the first is mainly employed for the treatment of cereal seeds against smuts and bunts and the second for the treatment of cereals against rusts (Schlör, 1970). A small part of carboxin is oxidized to oxycarboxin in the plants (Chin et al., 1970).

Their use in agriculture has made it necessary to devise a sensitive and specific analytical procedure for determining residues in vegetable foods.

Lane (1970) developed a method for measuring the residue of carboxin in plant material by hydrolysis and the colorimetric determination of the aniline so formed by coupling with *p*-dimethylaminobenzaldehyde. Sisken and Newell (1971) increased the quantitative sensitivity required by a residue procedure from 1 to 0.2 ppm by gas chromatographic determination of aniline with a microcoulometric nitrogen detector.

These methods, which are subject to interference from various aniline-containing compounds, lacked specificity.

Chin et al. (1970) studied the metabolism of carboxin by barley and wheat plants and developed a method for its direct determination by using gas chromatography with a microcoulometric sulfur detector. Farrow et al. (1975) reported on the nitrogen-selective detector gas chromatographic determination of carboxin, after it had been extracted from the grain sample with acetone in a Soxhlet apparatus and concentrated.

In this last procedure the limit of detection was at best 0.025 ppm, but the recovery was only checked to 0.5 ppm. The grain extracts were not purified and this resulted in an increase in the standing current, a loss of resolution and a shorter column life. Therefore, Farrow et al. (1975) recommended that 20-g samples of the grain be employed for the analysis and that the first 10 cm of column packing be replaced after 50 sample injections. When this method was applied to oxycarboxin, it failed, because the response varied by ±25%. In our preliminary trials no peak for oxycarboxin was found when flame or nitrogen selective detectors were used.

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The following investigation was undertaken to establish a satisfactory procedure for grains.

EXPERIMENTAL SECTION

Apparatus. Gas-chromatographic analyses were performed isothermally using a Perkin-Elmer Model 900 gas chromatograph equipped with a nitrogen-selective detector and a Hitachi Perkin-Elmer 196 5-mV recorder.

Two glass columns were used: I (2 m × 6 mm) contained 80–100 mesh Chromosorb WHP coated with OV-1 (2% w/w) plus OV-210 (6% w/w); and II (0.8 m × 6 mm) was packed with 80–100 mesh Chromosorb WHP coated with OV-101 (3% w/w). Column I was maintained at a temperature of 210 °C for Vitavax determination and 200 °C for Plantvax; the temperatures for the second column were 160 °C for Vitavax and 140 °C for Plantvax. General operating conditions were as follows: carrier gas, helium; flow rate, 35 mL/min; hydrogen and air flow rates, 5 and 100 mL; injector temperature, 240 °C; chart speed, 5 mm/min.

Infrared spectra were determined on a Perkin-Elmer 21 spectrometer and the samples were run as KBr pellets.

NMR spectra were obtained with a Joel C-60 HL instrument at a frequency of 60 MHz. Samples were run in [^{2}H]chloroform using 1% tetramethylsilane as the internal standard.

Reagents. All organic solvents were of "pure" grade and were distilled before use. Polyethylene-coated alumina was obtained from Kesington Scientific Corporation; GC columns were from Perkin-Elmer Corporation; TLC plates were obtained from Merck.

Extraction. Samples (from 20 to 100 g) of ground grains were transferred to a Blendor jar and homogenized with chloroform (200 mL) for 15 min. The suspension was filtered under suction through a G 3 fritted glass filter (ϕ = 5.5 cm) in which 10 g of $CaCO_3$ was previously stratified. The residue was washed with two 50-mL portions of chloroform and the first portion was allowed to be sucked through before adding the second. The chloroform extracts, after 1 g of Celite 545 was added (Thornburg, 1963), were evaporated just to dryness in a rotary evaporator at 40 °C.

Clean-up Procedure. A chromatography column (ϕ = 2.5 cm) with a fritted disc (G 1) was filled with a slurry prepared by shaking 15 g of polyethylene-coated alumina with a water–acetonitrile mixture (4:1) and allowed to settle (Thornburg, 1963).

The extraction residue was taken up in three (25 mL) portions of a water–acetonitrile mixture (4:1) and transferred to the top of the column; the first portion was left to be adsorbed through before adding the other. The effluent was collected. The column was then eluted with a water–acetonitrile mixture (4:1). NaCl (0.5 g) was added

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to the combined effluent and the eluate allowed to stand for 30 min, with occasional mixing, and filtered through a G 3 fritted glass filter (ϕ = 3.5 cm) in which 4 g of $CaCO_3$ was stratified. The filtrate was collected in a 500-mL separating funnel, treated with saturated aqueous NaCl (150 mL), and extracted with benzene (4 × 50 mL). The combined benzene extracts were dried with anhydrous Na_2SO_4 and evaporated just to dryness using a rotary evaporator at 40 °C.

The residue was dissolved in methanol (2 mL) and the solution was concentrated in the graduated centrifuge tube to 0.1–1 mL using a gentle stream of nitrogen. Aliquots (0.5–1 μL) were injected into the gas chromatograph.

Derivatization Procedure on the Macroscale. Plantvax (0.5 g) dissolved in 15 mL of anhydrous tetrahydrofuran (THF) was added to 0.5 g of $LiAlH_4$ in 40 mL of the same solvent and placed in a 200-mL round-bottomed flask. The mixture was heated to boiling point under reflux for 24 h and then cooled and cautiously treated with ethyl acetate to destroy the $LiAlH_4$ surplus. After completion of the reaction, the content of the flask was transferred to a 500-mL separating funnel containing saturated aqueous NaCl (100 mL) and extracted with diethyl ether (3 × 100 mL). The combined diethyl ether extracts were dried (Na_2SO_4) and evaporated just to dryness in a rotary evaporator at 35 °C.

Purification and Analysis of the Plantvax Reduced Derivative. The residue from the hydrogenation was taken up in acetone (3.5 mL) and was purified by thin-layer chromatography (TLC). This was carried out on 2 mm layers of Kieselgel F 254 Merck, previously activated by heating at 110 °C for 40 min, using the following solvent mixture: diethyl ether–petroleum spirit (bp 40–60 °C) (3:1 by volume). The area equivalent to the spot (R_F 0.60) located by ultraviolet lamp on the chromatogram was removed by means of the Mottier apparatus (Bobbitt, 1963), which was also used directly as an elution tube. The reduced derivative was eluted from the adsorbent with small portions of ethyl acetate (up to 20 mL total volume) and the solvent was removed with a gentle stream of dry nitrogen.

The combined products from several derivatization experiments were analyzed by GLC, nuclear magnetic resonance (NMR) spectroscopy, and infrared spectroscopy. Aliquots of the residue dissolved in methanol were injected into the gas chromatograph.

Determination of Plantvax Residues in Grains. After Vitavax had been determined, the same methanolic solution was evaporated just to dryness with a gentle stream of dry nitrogen. The residue dissolved in 4 mL of anhydrous THF was hydrogenated by $LiAlH_4$ (80 mg) in 2 mL of THF and thereafter processed as described in the "Derivatization Procedure on the Macroscale" section. The reaction products were transferred to a 100-mL separating funnel containing saturated aqueous NaCl (20 mL) and extracted with diethyl ether (3 × 20 mL). The combined diethyl ether extracts were dried (Na_2SO_4) and evaporated to dryness in a rotary evaporator at 35 °C. The residue was dissolved in methanol (2 mL); the solution was concentrated in the graduated centrifuge tube to 0.1–1 mL using a gentle stream of nitrogen. Aliquots of this (0.5–1 μL) were injected into the gas chromatograph. Figure 1 is a schematic diagram of the method described above.

RESULTS AND DISCUSSION

To determine the recovery of Vitavax, various known quantities of this fungicide were added in solvent to grains. The solvent (chloroform) was evaporated, and samples were processed as outlined in Figure 1. Blank experiments

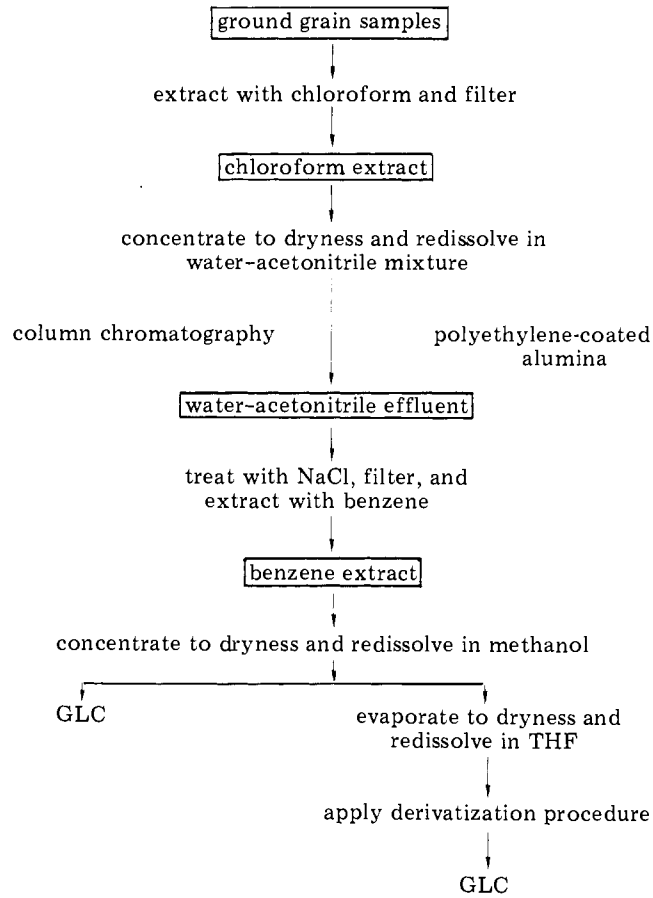


Figure 1. Schematic diagram for the analysis of Vitavax and Plantvax residues in grains.

Table I. Mean Recoveries of Vitavax Added to Wheat Grains before Extraction

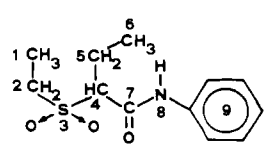
Vitavax added, mg/kg	recov. from wheat grains,[a] %
0.01	87.2 ± 2.45 (4)
0.05	91.5 ± 2.02 (5)
0.10	90.3 ± 1.73 (5)
0.20	92.1 ± 1.94 (3)

[a] ± standard error (number of determinations).

were carried out. The efficiency of the whole procedure was checked, and the mean recovery values are shown in Table I. The recoveries varied from 87.2 to 92.1% in the range 0.01–0.20 mg/kg and were satisfactory for the concentrations tested. In the clean-up procedure, the use of adsorbents other than polyethylene-coated alumina (Florisil, alumina, magnesium oxide, silica gel) does not permit quantitative recoveries. On the basis of the chromatograms of the numerous blanks tested (six from grains), a value of 0.005 mg/kg was fixed as the lower limit of determination.

As Plantvax did not show a peak in the chromatograms when the procedure described for Vitavax was used, we appealed to its derivatization.

Infrared (Figure 2) and NMR (Figures 3 and 4) spectra of Plantvax and its derivative indicate that the Plantvax reacts with hydrogen to give compound III.



III

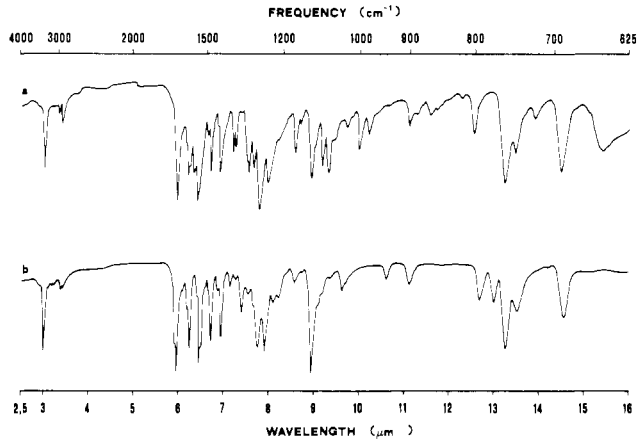


Figure 2. Infrared spectra of (a) Plantvax and (b) its reduced derivative as KBr pellets.

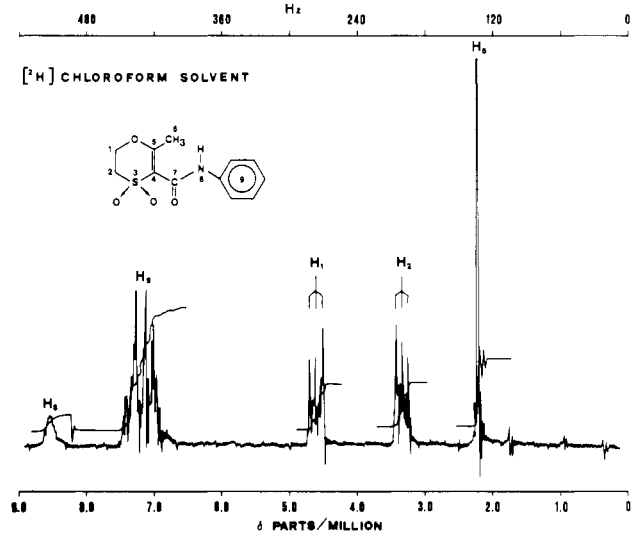


Figure 3. 60-MHz proton magnetic resonance spectrum of Plantvax.

In the derivatization procedure Vitavax was also reduced, but when the reduction method was applied to Vitavax residue determinations in grains, the peak of reduced Vitavax disappeared. It can be hypothesized that reduced Vitavax interacts with certain compounds extracted from grains.

The reduced Vitavax structure was not established, because it had no importance in the determination of Vitavax residues in grains.

After the structure of the reduced Plantvax was fixed by infrared and NMR spectra, this product was compared with the reaction product at the residue level using the two previously described chromatography columns. No retention time discrepancies were observed on either of the columns.

Recovery and control determinations were conducted on grains. The samples were fortified with chloroform

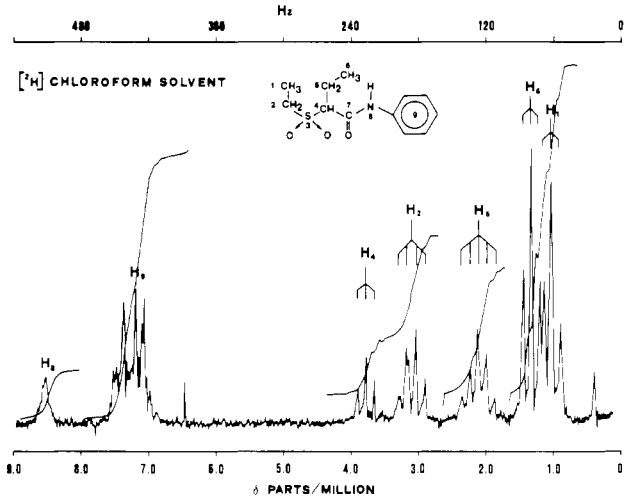


Figure 4. 60-MHz proton magnetic resonance spectrum of reduced Plantvax.

Table II. Mean Recoveries of Plantvax Added to Wheat Grains before Extraction

Plantvax added, mg/kg	recov. from wheat grains,[a] %
0.50	50.1 ± 3.65 (6)
0.75	78.6 ± 2.50 (5)
1.0	89.5 ± 1.95 (5)
2.0	90.0 ± 2.24 (4)

[a] ± standard error (number of determinations).

solutions of Plantvax at varying concentrations. The results of recovery are summarized in Table II and it will be seen that the method can be used in grain extracts at levels down to 0.5 ppm for the quantitative dosage of Plantvax; under this limit the reduced Plantvax peak rapidly disappears as concentration diminishes. An interaction of reduced Plantvax with co-extractives probably occurs; in fact it is possible to determine reduced Plantvax in quantities corresponding to 0.005 ppm when the grain is absent.

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