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A Gas Chromatographic Method for the Determination of Residues of Bitertanol

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A specific gas chromatographic procedure is described for the determination of residues of bitertanol in plants. Following initial extraction, the extract is acetylated with acetic anhydride to convert bitertanol to the bitertanol acetate. Cleanup is performed with a florisil column. The resultant derivative is stable, has good gas–liquid chromatography characteristics, and can be detected by selective nitrogen–phosphorus detector. Nuclear magnetic resonance and infrared spectral data are used to deduce the structure of the derivative. Recoveries of the acetylated analogue of bitertanol from apples, peaches, watermelons, mangoes, guavas, onions, and beans at fortification levels of 0.1–0.6 ppm averaged 85 ± 14%.

Bitertanol (1-(biphenyl-4-yloxy)-3,3-dimethyl-1-(1*H*)-1,2,4-triazol-1-yl-2-butanol, Figure 1) is a fungicide with activity on a wide variety of crops. Specht (1977) developed a method using alkali flame ionization detector (N-FID) to determine residues of some fungicides in extracts of plant and soil samples cleaned up by column chromatography on florisil.

The present procedure was developed for the analysis of residues of bitertanol that are measured quantitatively as acetyl derivatives (Figure 1) by gas–liquid chromatography (GLC) equipped with a nitrogen–phosphorus thermionic detector (NPD). The steps of this procedure are the extraction of fungicide, solvent evaporation, and derivatization of bitertanol for determination with a thermionic detector. The extraction procedure is similar to the one developed by Specht (1977) although we have detected bitertanol as an acetyl derivative following cleanup on a florisil column.

More recently Specht and Tillkes (1980) reported on the determination of bitertanol by using gel permeation chromatography for cleanup.

EXPERIMENTAL SECTION

Chemicals. Acetic anhydride was obtained from Reagen and was used as received. Bitertanol was a product of Bayer and was stated to be 99.0% pure. Pyridine was also used without purification.

Equipment. NMR spectra were obtained in $CDCl_3$ solution with Me_4Si as an internal standard on a Bruker 80-MHz spectrometer. IR spectra were obtained in KBr disks on a Perkin Elmer 467.

Gas–Liquid Chromatography. A Varian 3700 gas chromatograph equipped with thermionic detector and a 1.8m × 2mm i.d. glass column packed with 1.5% OV-101 on 100–120 mesh H.P. Chromosorb W was used. The operating parameters were injection port 230 °C, column 220 °C, and detector 270 °C. The column flow was 50 mL/min of nitrogen, and the air and hydrogen flow to the detector were selected for optimum response. With a column temperature of 220 °C, the retention time of acetylated bitertanol was 7.08 min. Other operating parameters tested were injection port 240 °C, column 240 °C, and detector 270 °C with column packed with 3% OV-17 on 100–120 mesh H.P. Chromosorb W and a 1.8m × 2mm i. d. glass column. The column flow was 60 mL/min of nitrogen. In this condition the retention time of acetylated bitertanol was 7.4 min.

Thin-Layer Chromatography. This technique was used only to confirm the product of esterification reaction of bitertanol. The standard of bitertanol and the product of derivatization were spotted onto silica gel GF plates, 250 mm. The chromatograms were developed in a (4:1) benzene–ethyl acetate solvent system, the solvent front travelling 13 cm from the origin. Blue spots with different R_f values from those of the pure bitertanol appeared on the plate when visualized by ultraviolet (UV).

Derivative Preparation. The bitertanol (200 mg) was dissolved in 2 mL of pyridine and 2 mL of acetic anhydride and added to a 25-mL screw-capped vial with a Teflon-lined cap. The vial was wrapped in aluminum foil to protect the solution from light and allowed to stand at room temperature for 72 h. Then the vial was cooled and

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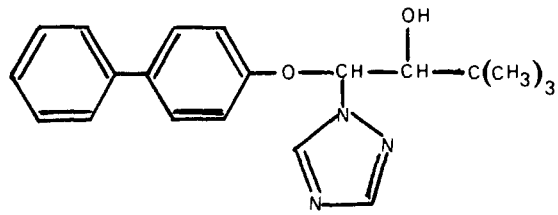

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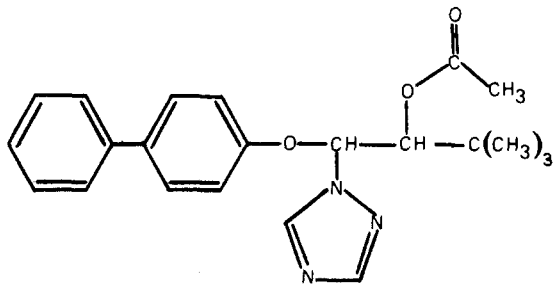

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Figure 1. (I) Bitertanol; (II) bitertanol acetate.

it was added slowly to 15 mL of water. Next the product was extracted successively with three 25-mL portions of chloroform. The phases of chloroform were combined and rinsed with 20 mL of HCl–water (2:1). The water phase was drawn off and the organic phase was rinsed twice with water (25 mL). The chloroform phase was collected in a 500-mL flask following passage through anhydrous sodium sulfate contained in a funnel. Removal of the chloroform on a rotary evaporator gave the residue that was dissolved in 4 mL of acetone and was spotted onto silica gel GF with standard of bitertanol. Two blue spots, with different R_f values, appeared on the plate. One spot was the product of the reaction and the other was the standard of bitertanol; they were visualized by ultraviolet radiation. The TLC analysis indicated complete conversion to acetylated bitertanol. No decomposition was observed in the gas chromatographic systems employed, even at a column temperature of 240 °C. The yield for the derivative of bitertanol was 95%. The NMR spectrum confirmed the acetyl derivative with signals at δ 2.13 (3 H, singlet), 5.3 (1 H, doublet, J = 2 Hz), and 6.5 (1 H, doublet, J = 2 Hz), the latter two being attributed to the shifts of methine protons bonded to the carbon atom attached to the acetate group and to the carbon atom attached the nitrogen and oxygen atoms, respectively. The IR spectrum of the acetylated bitertanol showed absorption at 1735 cm^{-1}, which was attributed to the C=O band of the C(=O)O group. In addition, the O–H band at 3180 cm^{-1} of bitertanol had disappeared in the derivative of bitertanol.

Analytical Procedures. Extraction. Samples of plant material (100 g) were homogenized with 10 g of Celite and 200 mL of acetone for 3 min in the blender and the homogenate was filtered through Whatman no. 1 in a Büchner funnel. The filtrate was transferred to a 1-L separatory funnel and shaken vigorously with 100 mL of dichloromethane for 2 min. The two layers were allowed to separate, 10 mL of 10% sodium chloride solution and 600 mL of distilled water were added, and the funnel was shaken again. The aqueous phase was discarded. The dichloromethane phase was washed twice with 100-mL portions of distilled water. The emulsion was dispersed

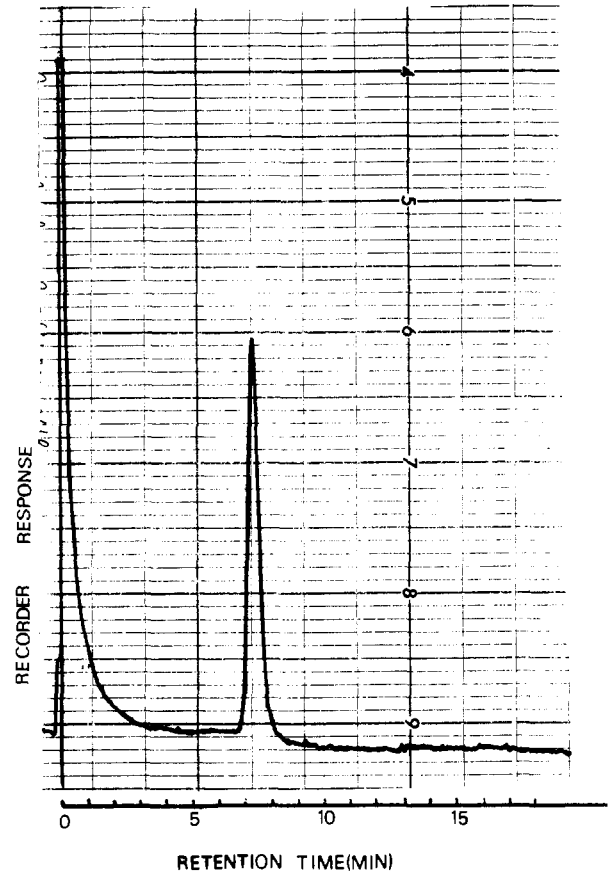

Figure 2. Gas chromatogram of the derivative of bitertanol, 2.08 ng, with a 3% OV-17 column at 240 °C and thermionic detector.

by adding a few milliliters of saturated sodium chloride solution. The organic phase was drawn off into a flask containing approximately 15 g of anhydrous sodium sulfate and it was dried for at least 30 min. The solution was filtered through a cotton wool plug covered with 3 cm of sodium sulfate in a funnel and was collected in a 500-mL round-bottomed flask. The former flask was rinsed, the filter was rinsed twice with 20-mL portions of dichloromethane, and the extracts concentrated to approximately 3 mL at 40–50 °C on the rotary vacuum evaporator. The residue was transferred to a 25-mL vial. The round-bottomed flask was rinsed with 10 mL of dichloromethane and the rinse added to the vial. The vial was heated at 40 °C in a water bath and the solution was concentrated to ca. 3 mL. The last traces of solvent were removed with a gentle stream of nitrogen.

Derivatization. The residue was redissolved in 0.5 mL of pyridine and 0.5 mL of acetic anhydride in a vial sealed with a Teflon-lined cap. The vial was wrapped in aluminum foil to protect the solution from light and allowed to stand at room temperature for 72 h for complete derivation. At the completion of the reaction, the reaction mixture was diluted with 50 mL of cool water to destroy the unreacted acetic anhydride and it was extracted three times with 25 mL of chloroform. The organic phase was combined and rinsed with 30 mL of hydrochloric acid–water (2:1) and two times with 25 mL of distilled water.

The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness on the rotary vacuum evaporator. A petroleum ether–diethyl ether (94:6) solvent system (2 mL) was added to the concentrated sample in preparation for the column chromatography cleanup.

Cleanup. A plug of glasswool was placed in a chromatographic tube (300 × 18 mm) which was equipped with

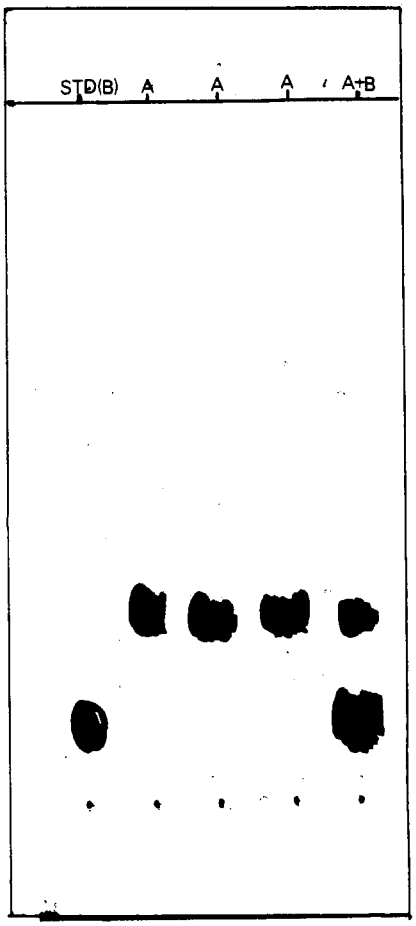

Figure 3. Typical thin-layer chromatogram of the acetate derivatization of bitertanol (A) and bitertanol standard (B).

a Teflon stopcock. The column was filled with petroleum ether and 20 g of (60–100 mesh) Florisil deactivated to a moisture content of 5% weight was added in small portions through a funnel and topped with 2 cm of dried sodium sulfate. The supernatant solvent was drained just level with the top of the column packing. The solution derived from derivatization was transferred by pasteur pipet to the column. The extract container was washed with small portions of petroleum ether–diethyl ether (94:6) and these washings were successively transferred to the column. The column was eluted with 200 mL (eluate I) of petroleum ether–diethyl ether (94:6). The receiver was changed and 300 mL of a mixture of 60% petroleum ether and 40% ethyl acetate was collected (eluate II). The receiver was changed again and 200 mL of a mixture of 70% petroleum ether and 30% ethyl acetate was collected (eluate III). Thus three fractions were collected from the cleanup column. Each of the eluate fractions was evaporated on a rotary vacuum evaporator at 40 °C to 5 mL and quantitatively transferred to a ground-stoppered graduated test tube by using ethyl acetate to complete the transfer, and made up with ethyl acetate to a volume of 10 mL. The eluate II contained the acetate bitertanol.

RESULTS AND DISCUSSION

The derivative of bitertanol is stable under the GLC conditions employed and no decrease in peak height was observed when maintained at room temperature during the course of a working day. Figure 2 is a chromatogram of bitertanol acetate. Thin-layer chromatography of our product of acetate derivatization of bitertanol confirmed the presence of a compound with different retention onto silica gel GF compared with bitertanol standard (Figure 3). We isolated the derivative in preparative plate for

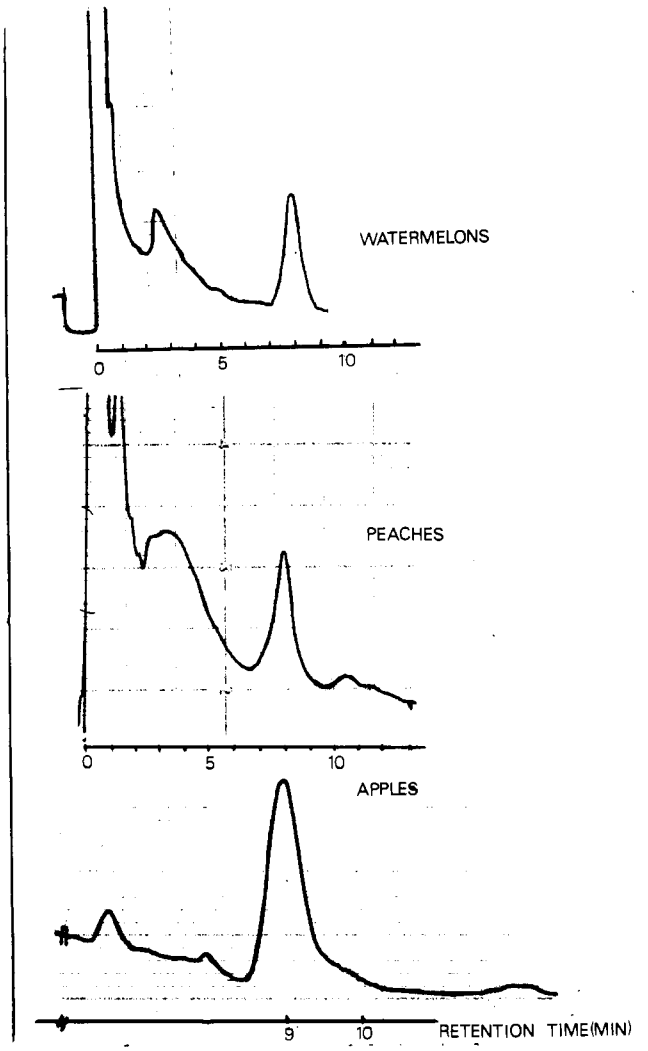

Figure 4. Gas–liquid chromatograms of derivatized watermelon, peach, and apple extracts fortified with bitertanol at 0.5, 0.6, and 0.1 ppm, respectively, with a 1.5% OV-101 column at 220 °C (watermelons and peaches, 20 mg injected) and at 190 °C (apples, 30 mg injected).

Table I. Percent Recovery of Bitertanol as Acetate Derivative from Samples Fortified with Bitertanol[a]

sample	added, ppm	recovery, % av	recovery, % range
apples	0.1	70	68–72
watermelons	0.5	101	98–105
mangoes	0.5	80	75–85
peaches	0.6	99	90–108
guavas	0.1	70	67–73
beans	0.1	100	95–105
onions	0.2	75	70–80

[a] The results are the average from two recoveries performed for each crop.

spectral analysis. The spectral properties of the derivative were found to be quite characteristic. A comparison of the infrared and NMR spectra of bitertanol and its acetate derivative serves to illustrate this point. The infrared spectra of bitertanol before and after derivatization show the appearance in the latter of carbonyl absorption at 1735 cm^{-1} characteristic of an ester group and the loss of O–H absorption at 3180 cm^{-1} serves to characterize the infrared spectrum. The NMR spectrum showed the appearance of a sharp singlet at δ 2.13 ppm in the derivative and a new chemical shift for the methine proton attached to the carbon atom next to the acetate group. The lower limit

tested for bitertanol sensitivity varied with the crop due to tolerance data. Samples of apples, watermelons, mangoes, peaches, guavas, and beans were fortified with bitertanol in the 0.1–0.6 ppm range. Figure 4 illustrates typical gas chromatograms of samples fortified at 0.6 ppm (watermelons), 0.5 ppm (peaches), and 0.1 ppm (apples). Table I shows the recovery of bitertanol as the acetylated bitertanol in fortified samples.

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Gas Chromatography–Mass Spectrometry of Acylalanine Fungicides

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Gas chromatographic–mass spectral data are presented for nine parent acylalanine fungicides and twelve metabolites or derivatives. Most compounds show similar fragmentation patterns indicating numerous single bond cleavages. For methoxyacetyl congeners, the base peak is usually m/z 45. The benzyl alcohol atropisomers of metalaxyl undergo thermal rearrangement in the gas chromatograph with loss of CH_3OH to produce a lactone that fragments with a base peak of m/z 146. All compounds have unique retention times on a SE-30 capillary column and common ions for most of the parent fungicides and their metabolites allow multiple-ion monitoring for confirmation.

The acylalanine fungicides are quickly becoming important in crop protection due to their systemic properties with both curative and protective activity against fungal pathogens of the Peronosporales. The general structure of the acylalanines is shown in Figure 1.

These compounds are structurally similar to the chloroacetamide (or acylanilide) herbicides such as alachlor and metolachlor. The term acylalanine was used by Ciba-Geigy (Staub et al., 1978) to describe their chemicals that had R_1 = alanine methyl ester but has become commonplace for all systemic fungicides of this general structure that are active against Oomycetes. Gisi and Wiedmer (1983) described these chemicals as phenylamides of three chemical subclasses differing in the R_1 substituent: the acylalanines (metalaxyl, furalaxyl, benalaxyl), the butyrolactones (ofurace, cyprofuram), and oxazolidinones (oxadixyl). The R_2 substituents include methoxymethyl, chloromethyl, as well as numerous other acyl derivatives. All the acylalanines described in this paper (Table I) are 2,6-dimethylaniline derivatives except cyprofuram which is a 3-chloroaniline derivative. Structure–activity relationships based on physicochemical properties have been discussed by Hubele et al. (1983).

This study was undertaken to obtain gas chromatographic-mass spectra of the parent fungicides, their metabolites, and derivatives, and to elucidate fragmentation patterns so that subsequent metabolic and residue studies could be conducted with MS confirmation or single- or multiple-ion quantitation.

EXPERIMENTAL SECTION

Gas chromatography–mass spectrometry (GC–MS) was conducted on a Hewlett-Packard 5970 mass selective detector (MSD). A Hewlett-Packard 5790 gas chromatograph with a 15 m × 0.25 mm fused silica capillary column with a 0.25-μm coating of SE-54 and He carrier gas flow of 2 mL min^{-1} was connected to the MSD with a direct capillary interface operated at 260 °C. One-microliter solutions were injected with a splitless injector operated at 220–250 °C with the bypass valve open for 1 min. The column oven was programmed for an initial 1-min hold at 90 °C, followed by a 10 °C min^{-1} rise to 270 °C and a final hold at this temperature to allow elution of late compounds.

The MSD was optimized by using Hewlett-Packard disk software under AUTOTUNE conditions with PFTBA (perfluorotributylamine) calibration. Mass spectra were acquired over the 40–400 amu range at 380 amu s^{-1} and normalized to DFTPP (decafluorotriphenylphosphine). Single- or multiple-ion monitoring was conducted with Hewlett-Packard software with up to 20 ions being monitored.

Retention time data were generated on a Hewlett-Packard 5880 capillary gas chromatograph with a 15 m × 0.25 mm fused silica capillary column with a 0.25-μm coating of SE-30 under the conditions described by Ripley and Braun (1983).

Parent acylalanines were analytical or technical grade and were prepared at 1 mg mL^{-1} in methanol and appropriately diluted to obtain 10 or 100 μg mL^{-1} solutions for GC–MS. Metabolites or derivatives were supplied by Ciba-Giegy (CGA) or synthesized by using standard procedures.

RESULTS AND DISCUSSION

The mass spectra of metalaxyl (Marucchini et al., 1983; Ripley, 1984) and ofurace (Cooke et al., 1982) have been reported previously and tentative fragmentation patterns have been made (Cooke et al., 1982; Ripley, 1984). This study confirms that all the examined acylalanines, their metabolites, and derivatives follow this general pattern as shown in Figure 2. The acylalanine compounds exhibit

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