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Evaluation and Confirmation of Acetylation—Gas–Liquid Chromatographic Method for the Determination of Triadimenol in Foods

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An acetylation technique using acetic anhydride followed by gas–liquid chromatography is evaluated for a triadimenol fungicide. Identity of the derivative was confirmed by nuclear magnetic resonance and infrared spectral data. Recoveries of the acetylated analogue of triadimenol from foods studied at fortification levels of 0.1–0.2 mg/kg were usually >80%.

Triadimenol [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol] and bitertanol [1-(biphenyl-4-yloxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol] (Figure 1) are fungicides with activity on a wide variety of crops. Both chemicals have similar structures, with a functional group (–OH) in the secondary carbon, a phenoxy group, and a triazolyl ring present.

The first and usual method of residue determination for both fungicides is that from Specht (1977), in which the fungicides are extracted from the matrix with methylene chloride and determined after Florisil column cleanup by gas chromatography with nitrogen-selective detection.

Recently, a new method was reported for the determination of bitertanol in foods (Mendes, 1985), which consists of acetylation with acetic anhydride to convert bitertanol to the bitertanol acetate, which is measured quantitatively by gas chromatography with a nitrogen–phosphorus thermionic detector.

This paper includes evaluation of the acetylation method and the feasibility of using acetylation as a general reaction for the confirmation of the identity of other triazoid fungicides with structures similar to that of bitertanol.

EXPERIMENTAL SECTION

Equipment. NMR were obtained in $CDCl_3$ solution with Me_4Si as internal standard on a Bruker AC 300 spectrometer. IR spectra were obtained in KBr disks on a Bruker IFS 85. GC–MS were obtained on an HP 5970 and GC 5880A. A Varian Model 3700 gas chromatograph equipped with a thermionic detector (TSD) was used with a 1.80 m × 2 mm glass column.

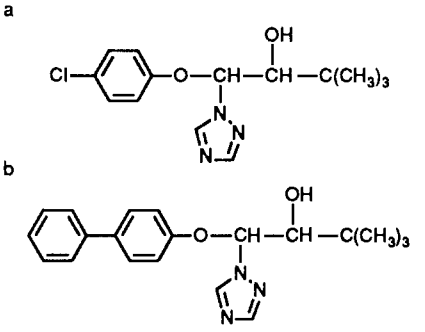

Figure 1. Structures of triadimenol (a) and bitertanol (b).

Reagents. Acetic anhydride was obtained from Merck and was used as received. Bitertanol and triadimenol were products from Bayer and were stated to be 99.0% pure. Pyridine from Grupo Química was also used without purification. Anhydrous sodium sulfate, petroleum ether, and acetone were pesticide-grade materials. All other solvents were analytical grade and were used as obtained from the suppliers. Silica gel GF (Merck, Art. 7730) and Celite 545 (Grupo Química) were used as received.

Thin-Layer Chromatography (TLC). This technique was used to confirm the product of esterification of triadimenol. The standard of fungicide and the product of derivatization were spotted onto silica gel GF plates. Blue spots with different R_f values appeared when visualized by ultraviolet after the plates were developed in a 4:1 benzene–ethyl acetate solvent system.

Derivative and Standard Preparation. Pyridine solution (2 mL) of triadimenol (50 mg/mL) and acetic anhydride (2 mL) were placed in 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 tempera-

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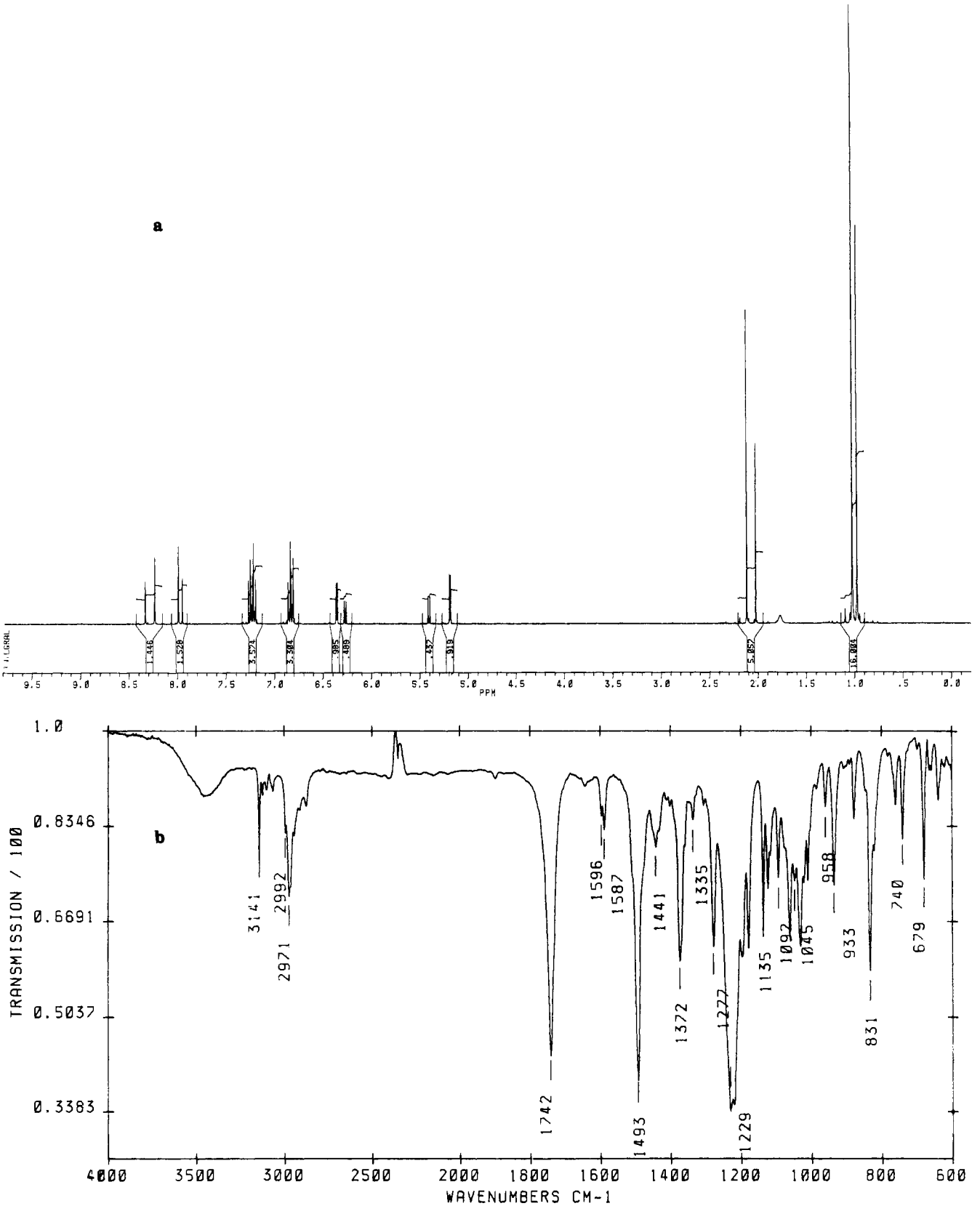

Figure 2. Spectra of acetylated triadimenol: (a) NMR; (b) IR.

ture for 72 h. Carefully, 15 mL of water was added to destroy the excess anhydride. 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 organic phase was rinsed twice with water, (25 mL) and 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 a residue that was dissolved in 3 mL of acetone and spotted onto silica gel GF.

The TLC analysis indicated complete conversion to acetylated triadimenol. Derivative yield was approximately 97%. The NMR spectrum confirmed the acetyl derivative with signals at δ 2.10 (3 H, s) and 2.03 (3 H, s) attributed to methyl groups of acetate of two diastereomeric forms of acetylated triadimenol. The signals at δ 5.15–5.45 and 6.25–6.35 were attributed to shifts of methine protons. These signals have two different values of J due to the presence of diastereomers (J = 6, 9 Hz) (Figure 2a).

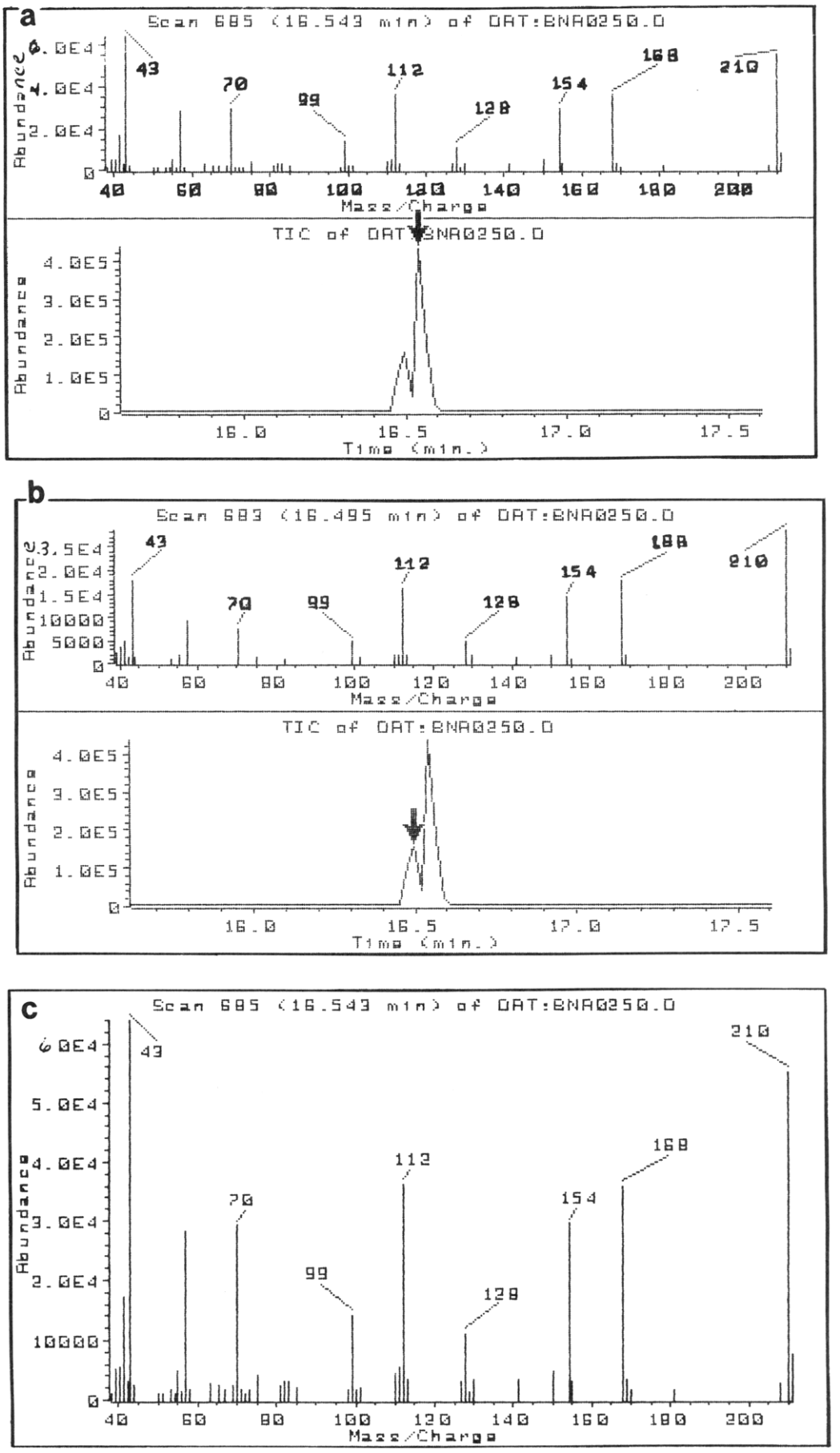

Figure 3. (a and b) GC/MS of the acetylated derivative separated in two diastereomeric forms. (c) MS of acetylated triadimenol.

The IR spectrum of the triadimenol acetate showed absorption at 1742 cm^{-1}, which was attributed to the C=O band of the acetate group (Figure 2b). The gas chromatographic–mass spectrometric (GC–MS) analysis showed two peaks correspond-

Table I. Recoveries of Triazoid Fungicides (%) from Foods[a]

	food (fortification, mg/kg)			
fungicide	coffee (0.1)	barley (0.1)	peanut (0.2)	garlic (0.1)
triadimenol	83	96		93
bitertanol			100	

[a] Average of duplicate extractions.

ing to the two isomers, but one of them was bigger than the other. The resulting mass spectra of the derivative, identified by arrows, are identical (Figure 3a,b). The mass spectrum of the acetylated product of triadimenol indicated the following: m/e 210 (M^+ – (thiazolyl)(CHCH(OC(O)CH_3)C(CH_3)$_3$), 127 (ClPhO), 43 (fragment as $CH_3C{=}O$) (Figure 3c).

Sample Extraction. Samples of fruits or cereal (100 g) were blended with 10 g of Celite 545 and 200 mL of acetone for 3 min. The filtered extract was transferred to a 1-L separatory funnel and shaken vigorously with 100 mL of dichloromethane. After phase separation, 10 mL of 10% sodium chloride solution and 600 mL of distilled water were added and the funnel was shaken again. The organic phase was washed with water (2 × 100 mL) and drawn off into a flask containing 15 g of anhydrous sodium sulfate. The dried solution was filtared and the extract concentrated to approximately 3 mL at 40–50 °C on the rotary vacuum evaporator. The last traces of solvent were removed under a gentle stream of nitrogen.

Acetylation. Sample residue from the previous steps was dissolved in 0.5 mL of pyridine and 0.5 mL of acetic anhydride. The reaction was carried out according to the procedures outlined in Derivative Standard Preparation. The residue obtained was dissolved in a petroleum ether–diethyl ether (94:6) solvent system (2 mL) as preparation for the column chromatography cleanup.

Liquid Column Chromatography for Cleanup and Fractionation. Glass columns (300 × 18 mm) with Teflon stopcocks were packed from the bottom with a glass wool plug. Petroleum ether and 20 g of Florisil (60–100 mesh) were deactivated to a mixture content of 5% by weight. The Florisil was added in small portions through a funnel and topped with 2 cm of anhydrous sodium sulfate. The supernatant solvent was drained just level with the top of the column packing. The solution derived from acetylation was transferred to the column. After the extract had entered the packing, the column was eluted with 200 mL of 94:6 petroleum ether–diethyl ether. This eluate was collected in a round-bottom flask as fraction 1. The receiver was changed, and 300 mL of a mixture of 60% petroleum ether and 40% ethyl acetate was collected (fraction 2). Both fractions were evaporated just to dryness on a rotary vacuum evaporator at 45 °C. The residue was dissolved in a 10 mL of ethyl acetate for gas chromatographic analysis. Fraction 2 contained the acetate triadimenol.

Gas Chromatographic Analysis. The standard prepared according to the procedures outlined in Derivative Standard Preparation was dissolved in acetone, and an appropriate aliquot was injected into the gas chromatograph maintained at the following conditions: column packed with 100–120-mesh Chromosorb W (HP) coated with 5% OV-101; gas flow, nitrogen carrier gas at 50 mL/min, hydrogen at 4.5 mL/min, Ar at 175 mL/min; temperatures, column at 210 °C, injection port at 250 °C, detector at 300 °C. For confirmation, the column packed with 100–120-mesh Chromosorb W (HP) coated with 1.5% OV 17 + 1.95% QF-1 was used. The column flow gas was maintained at 40 mL/min of nitrogen. The peak of triadimenol acetate was identified by its retention time, and the area or peak height produced was obtained on the reporting integrator. For both compounds analyzed here (triadimenol and bitertanol acetates) only one GC peak was detected with these columns.

RESULTS AND DISCUSSION

Percent recoveries obtained for the foods examined at 0.1 and 0.2 mg/kg are listed in Table I. No interferences were encountered for either fungicide studied. We

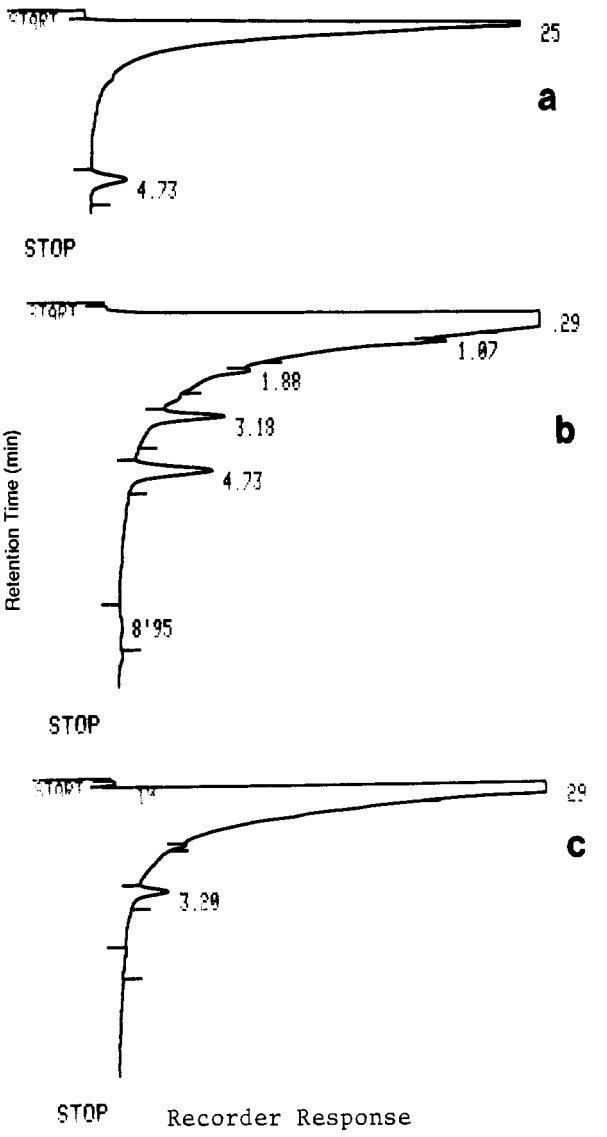

Figure 4. Gas chromatograms: (a) a derivative of triadimenol (2.22 ng) with a 5% OV-101 column at 210 °C; (b) barley fortified with 0.1 mg/kg of triadimenol; (c) barley control.

isolated the derivative standards on preparative plates for spectral analysis and for later preparation of standards solutions of the acetate. The OV-101 column performed well for all samples but can be changed to 1.5% OV-17 + 1.95% QF 1 also with good resolution. Since analysis can be carried out on other kinds of columns that resolve the diastereomeric forms, the total residue must be determined as the sum of two diastereomers without taking into account the percentage distribution of the two forms.

Analysis of this triazoid fungicide as GLC-stable derivatives showed good chromatograms more complex matrices such as coffee and peanut.

Figure 4 illustrates typical chromatograms of barley fortified at 0.1 mg/kg. The method of acetylation appears to offer some real advantages for the routine screening, quantitation, and confirmation of triadimenol.

The methodology described may prove useful in the analysis of fungicides with structures similar to that of Bitertanol.

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Derivative Spectrophotometric Determination of Vanillin and *p*-Hydroxybenzaldehyde in Vanilla Bean Extracts

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Vanillin (4-hydroxy-3-methoxybenzaldehyde) and *p*-hydroxybenzaldehyde were determined by spectrophotometric derivative measurements in vanilla bean extracts. The method tolerates *p*-hydroxybenzaldehyde to vanillin ratios of 3:1 (w/w) with relative error below 5% in the determination of vanillin. This method compares favorably with the official AOAC spectrophotometric procedure. Relative errors of 0.70% and RSD's of 1.0% are obtained.

The importance of vanillin for the flavor industry and, consequently, its economic implications justify the numerous analytical criteria proposed for the determination of this and other compounds present in it. Moreover, the amount of vanillin found in vanilla extracts is an index as much of the product quality as of its origin. One author showed a correlation between the vanillin and *p*-hydroxybenzaldehyde ratio contained in vanilla beans in relation to their geographical origin (Juergens, 1981a).

Most of the analytical methods previously published use one of several chromatographic methods: thin-layer chromatography (Meili and Chaveron, 1976; Courcelles et al., 1978; Rey et al., 1980; Jiang et al., 1987), gas chromatography (Lhuguenot, 1978; Tabacchi et al. 1978), liquid chromatography (Guarino and Brown, 1985; Fayet et al., 1987), and HPLC (Juergens, 1981b; Dalang et al., 1982; Herrmann and Stoeckli, 1982). Particularly remarkable is the work of Fraisse et al. (1984) who reported a comparative study of three different analytical techniques, liquid chromatography, gas chromatography, and mass spectrometry, for the quantitative determination of the vanillin contained in vanilla beans.

On the other hand, the fraudulent adulteration of vanilla with synthetic vanillin (4-hydroxy-3-methoxybenzaldehyde), derived from wood pulp lignin, has been shown by a method consisting of the determination of the relative isotopic composition of ^{13}C (Krueger and Krueger, 1983; Fayet et al., 1987).

The current AOAC method (1984), mainly used by the flavor industry to determine vanillin, consists of the spectrophotometric determination of the alkaline extract solution at 348 nm, where *p*-hydroxybenzaldehyde interferes. This interference leads to greater vanillin values that those expected.

The aim of the present work is the determination of vanillin in the presence of *p*-hydroxybenzaldehyde without a previous separation. To achieve it, a graphic model based on the interference-free character of the derivative amplitudes measured from an isodifferential point in the base line to the break with the actual derivative curve is proposed. This graphic model has been previously used with satisfactory results for the spectrofluorometric and spectrophotometric determination of metal chelates (Gracia Sánchez et al., 1987; Márquez Gomez, 1988), pesticides (García Sánchez and Cruces, 1988), and phenolic acids (García Sánchez et al., 1988).

This paper describes a rapid assay for vanillin and *p*-hydroxybenzaldehyde extracted from vanilla beans. The quantitative determination of vanillin and *p*-hydroxybenzaldehyde in binary mixtures and comparative data of the results of the determination of vanillin in vanilla beans by the AOAC method are also given.

EXPERIMENTAL METHODS

Apparatus. Spectral measurements were made with a Shimadzu UV-240 Graphicord recording spectrophotometer in 1-cm quartz cells. Instrument parameters: slit, 2 nm; scanning speed, 3 nm/s; recording chart speed, 10 nm/cm. First-derivative ultraviolet spectra were obtained with a Shimadzu derivative spectrum with optional program/interface (Model OPI-2), giving first to fourth derivatives, and λ values of 1, 2, and 4 nm.

Reagents and Solvents. Vanillin and *p*-hydroxybenzaldehyde were obtained from Sigma Chemical Co. Stock solutions were prepared in ethanol and stored in amber bottles at 4 °C. Dilute aqueous solutions were prepared daily from these solutions. Water was both distilled and demineralized. The $NaHCO_3$–NaOH buffer solution (pH 11.0) was prepared by mixing appropriate volumes of 0.1 M $NaHCO_3$ and 0.1 M NaOH. The pH 5 buffer solution was prepared from 1 M acetic acid and 1 M sodium acetate. All solvents used were of analytical reagent grade.

Analytical Procedure. An aliquot of standard solution containing 1–5 μg/mL of vanillin and 0.5–4 μg/mL of *p*-hydroxybenzaldehyde was added to a 10-mL calibrated flask, followed

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