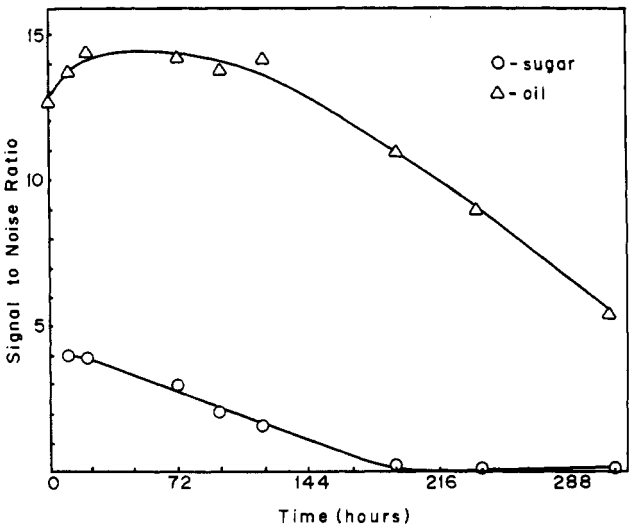

Figure 2. Relative intensities of oil and sugar peaks obtained from signal-to-noise ratios.

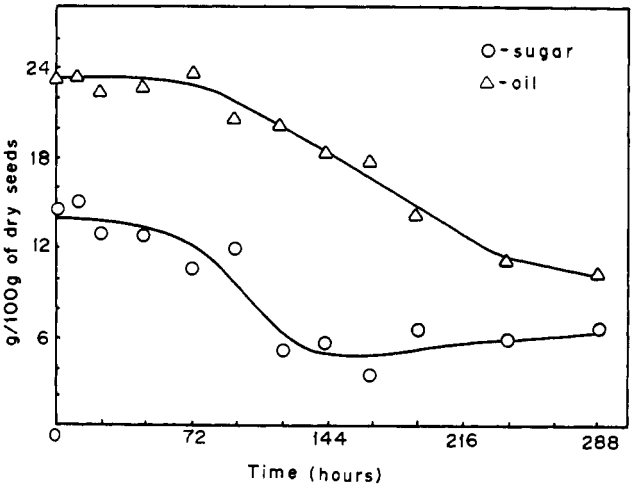

Figure 3. Relative change in oil and sugar proportions during the various stages of germination. Values are given in g/100 g of dry seeds.

consumed after the sugars reach a certain concentration (at approximately 72 h). This suggests the presence of an autofeedback mechanism where the enzymatic system used in degradation of the oil is activated when the sugars reach a low concentration.

Application of the ^{13}C NMR technique to this type of study has inherent limitations in the semiquantitative nature of the technique itself and in the difficulty presented by attempting to make a seed germinate in an NMR tube, where it is compressed and periodically soaked in deuterated water. On the other hand it is both fast and clean, allowing variables to be measured simultaneously and in a continuous fashion on the same specimen. Although results agree qualitatively with those obtained through the more lengthy multistep purification and characterization procedure, there appear to minor discrepancies. These, along with the adaptation of the technique to monitoring a wider range of variables, are presently under investigation.

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Determination of Dithiocarbamate Fungicides in Vegetable Foodstuffs by High-Performance Liquid Chromatography

A high-performance liquid chromatographic (HPLC) method for analyzing residues of thiram and salts of *N,N'*-alkylenebis(dithiocarbamic acids) and *N,N*-dimethyldithiocarbamic acid in vegetable foodstuffs is presented. Iron, zinc, and manganese salts of dithiocarbamic acids were transformed into readily water-soluble sodium salts in an alkaline solution of EDTA and L-cysteine. The dithiocarbamate anions were extracted into an organic solvent as ion pairs of tetrabutylammonium and S-alkylated with methyl iodide in one process at room temperature. The methyl esters formed were analyzed by HPLC with UV detection at 272 nm. The average recoveries of zineb, ziram, and thiram added as talc mixtures to six different food crops at the 0.5 mg/kg level were within the ranges 58.7–70.0, 69.4–84.8, and 61.5–78.2%, respectively. The limits of detection are below 0.02, 0.01, and 0.01 mg/kg, respectively.

A new method for high-performance liquid chromatographic (HPLC) determination of dithiocarbamates was described earlier by Gustafsson and Thompson (1981). In that method the sample was treated with an alkaline EDTA solution in order to transform iron, zinc, and manganese salts of dithiocarbamic acids into their readily

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water-soluble sodium salts. The dithiocarbamate anions were extracted at pH 6.5–8.0 to chloroform–hexane (3:1) as ion pairs of tetrabutylammonium and alkylated with methyl iodide to form *S*-methyl dithiocarbamates. However, disodium *N,N'*-ethylenebis(dithiocarbamate) formed from zineb, maneb, or mancozeb by the treatment with EDTA reacted with coextractives from the sample and with thiram. This problem has now been overcome by the addition of L-cysteine to the EDTA solution. Sodium *N-tert*-butyldithiocarbamate produced the same effect, but the recovery of zineb was slightly lower for some kinds of sample. In addition, sodium *N-tert*-butyldithiocarbamate gives rise to an extra peak on the chromatogram.

EXPERIMENTAL SECTION

Apparatus. A liquid chromatograph (Spectra-Physics Model 3500) with a stainless steel column, 200 × 4 mm i.d., and a precolumn, 50 × 4 mm i.d., was used. Both columns were packed with Nucleosil RP-18, 5 μm. The mobile phase was water–acetonitrile (3:2) with a flow rate of 1.2 mL/min. The detection was carried out at 272 nm with a variable-wavelength ultraviolet detector (Spectra Physics, Model 770 spectrophotometric detector). An ultrasonic bath (Bransonic 220, Bo Philip Instumentation, Stockholm) and a centrifuge (Wifug Clinic, Kebo, Stockholm) were used. Extracts were filtered through glass fiber filters (Whatman GF/D, 5.5 and 9 cm in diameter).

Reagents. Thiram (98%, Fluka AG, Switzerland), ziram (98%, EPA, Research Triangle Park, NC), zineb (80.5%, EPA), L-cysteine (99%, E. Merck, Darmstadt, West Germany), and Na_2EDTA (ethylenediaminetetraacetic acid disodium salt, p.a., E. Merck) were used. Otherwise, reagent-grade chemicals and solvents were used when possible.

Analytical Procedure. An aliquot (100 g) of the sample was cut into 10–15 pieces and analyzed immediately. The outer pieces of apples, pears, potatoes, and tomatoes were analyzed. Lettuce and strawberries were not chopped. The aliquot was shaken with 0.5 g of L-cysteine and 100 mL of 0.2 M EDTA in 0.4 M sodium hydroxide for 10 min in a closed glass jar. The jar was placed in an ultrasonic bath for 5 min and the extract was filtered through a glass fiber filter. Extracts which were too viscous to be filtered directly (e.g., those of strawberries) were centrifuged for 10 min at 1700*g* before filtering. The jar and the filter were rinsed with 20 mL of the EDTA solution (for lettuce 40 mL). A solution of tetrabutylammonium hydrogen sulfate (0.41 M, 5 mL) was added while stirring. The pH was cautiously adjusted to ca. 7.0 with 2 M hydrochloric acid and 30 mL of 0.1 M methyl iodide in chloroform–hexane (3:1) was added. The mixture was stirred for 10 min with a magnetic stirrer and then filtered through a glass fiber filter. The two phases were separated and an additional 20 mL of the methyl iodide solution was added to the aqueous layer. After the solution was stirred for 5 min, the organic layer was separated and added to the first one. The organic extract was allowed to stand for 25 min. Then a 20% solution (5 mL) of 1,2-ethanediol in methanol was added. The solvent and the excess of methyl iodide were stripped off at 30 °C in a rotatory evaporator. The residue was diluted with 1.0 mL of methanol or acetonitrile and 10 μL was analyzed by HPLC using UV detection at 272 nm.

RESULTS AND DISCUSSION

Thiram was quantitatively transformed into sodium *N,N*-dimethyldithiocarbamate by the addition of L-cysteine or sodium *N-tert*-butyldithiocarbamate during the EDTA extraction. When a sample is free from *N,N*-dimethyldithiocarbamic acid salts (e.g. ziram or ferbam), this reaction can be used also for quantitative determination of thiram. However, so that one can distinguish between ziram and thiram, half of the sample should be analyzed with the method outlined above and the other half by extracting with chloroform followed by cleanup and HPLC analysis according to Gustafsson and Thompson (1981). The latter method is specific for thiram and gives a somewhat higher recovery than the former one.

Table I. Percentage Recovery of Zineb, Ziram, and Thiram Added to Water or Food Samples at the 0.5-ppm Level (Mean and Standard Deviation; Number of Samples Is Shown in Parentheses)

	zineb	ziram	thiram
apples	64.9 ± 3.0 (8)	80.2 ± 2.2 (11)	78.2 ± 3.3 (4)
lettuce	61.8 ± 3.3 (6)	72.6 ± 3.6 (3)	61.5 ± 3.2 (3)
pears	63.1 ± 3.7 (5)	74.6 ± 2.0 (3)	63.9 ± 3.1 (3)
potatoes	70.0 ± 1.1 (6)	84.8 ± 1.1 (3)	77.2 ± 1.2 (3)
strawberries	58.7 ± 2.3 (4)	69.4 ± 2.7 (4)	64.1 (1)
tomatoes	65.6 ± 2.0 (7)	79.2 ± 0.5 (4)	72.1 ± 3.3 (3)
water	73.3 ± 2.9 (3)	91.2 ± 0.3 (3)	80.2 ± 0.2 (2)

The alkylation was performed in a flask with magnetic stirring. Less emulsions were formed in this way than when shaking in a separatory funnel. Addition of sodium chloride, potassium carbonate, or silicone defoamers (e.g., Antifoam A) did not prevent foaming.

Maximum recoveries of zineb for the foods tested were obtained when the extractive methylation was performed within the pH range 7–8 [cf. Gustafsson and Thompson (1981)].

Standard solutions of methyl *N,N*-dimethyldithiocarbamate and dimethyl *N,N'*-ethylenebis(dithiocarbamate) in methanol were used. These solutions were stable for at least 3 days when stored at +4 °C. However, acetonitrile was later found to be a more appropriate solvent, giving more stable solutions. The esters in the solid state were stable at room temperature for at least 2 years. The methyl iodide solution used was protected against direct sunlight.

1,2-Propanediol in chloroform or 1,2-ethanediol in methanol was added in order to avoid loss of methyl *N,N*-dimethyldithiocarbamate during the evaporation. 1,2-Ethanediol was preferred as a keeper since 1,2-propanediol retained a small amount of chloroform which could give rise to an interfering peak on the chromatogram. No keeper was needed when analyzing *N,N'*-ethylenebis(dithiocarbamates).

The retention times of methyl *N,N*-dimethyldithiocarbamate and dimethyl *N,N'*-ethylenebis(dithiocarbamate) were ca. 7 and 11 min, respectively.

GLC experiments with the dithiocarbamate esters using a SE-30 capillary column (25 m, 120 °C, temperature programming 4 °C/min) and an EC detector showed that GLC analysis would be possible.

The samples were not homogenized, since homogenization starts a rapid breakdown of dithiocarbamates (Thier et al., 1977). The inner parts of apples, pears, potatoes, and tomatoes were not analyzed in order to further minimize breakdown. This procedure is further justified by the fact that fungicidal dithiocarbamates are not systemic (Engst and Schnaak, 1974).

Recoveries of zineb, ziram, and thiram from water and food samples were determined by adding talc mixtures of zineb and ziram or zineb and thiram. No decomposition of those compounds in the talc mixtures was observed after 3 months. Zineb was analyzed as dimethyl *N,N'*-ethylenebis(dithiocarbamate) and ziram and thiram as methyl *N,N*-dimethyldithiocarbamate. The recoveries are

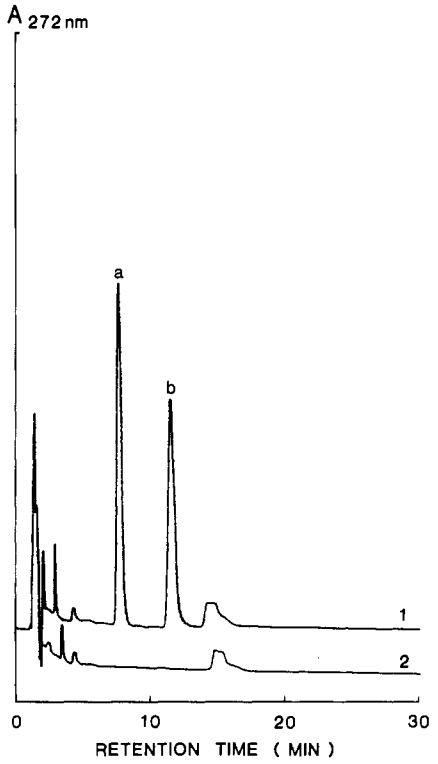

Figure 1. Chromatogram of an apple sample (1) with 1.0 mg/kg of both ziram (a) and zineb (b) added and (2) without. Full scale corresponds to 0.2 absorbance unit.

given in Table I. They are calculated with regard to the contents of active ingredients in the analytical standards used.

Water solutions of disodium *N,N'*-ethylenebis(dithiocarbamate) of pH 7.0 with and without EDTA added were analyzed in order to examine if the presence of EDTA influenced the recovery of zineb in a way other than by sequestering zinc cations. No such effect was found. The recoveries of zineb, ziram, and thiram from fortified food samples were linear within the concentration ranges studied, 0.01–1.5 mg/kg for ziram and thiram and 0.02–1.5 mg/kg for zineb when L-cysteine or sodium *N-tert*-butyldithiocarbamate was added. Figure 1 shows chromatograms of an apple sample free from dithiocarbamates and an apple sample fortified with ziram and zineb before extraction with the EDTA solution. The limits of detection for zineb, ziram, and thiram are below 0.02, 0.01, and 0.01 mg/kg, respectively. The limits of detection were not optimized.

Registry No. Thiram, 137-26-8; L-cysteine, 52-90-4; zineb, 12122-67-7; ziram, 137-30-4; EDTA, 60-00-4.

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The Volatile Constituents of *Schinus molle* L.

The volatile constituents of the fruit of *Schinus molle* L. have been investigated by gas chromatography–mass spectrometry. Forty-six compounds were identified or partially identified in the oil which was obtained from this fruit by a distillation–extraction method (Likens–Nickerson apparatus). The compounds reported here were 9 monoterpene hydrocarbons, 1 aromatic compound, 1 aliphatic acid ester, 2 monoterpene esters, 16 sesquiterpene hydrocarbons, and 17 other sesquiterpenoids. Major components of this oil were mcyrcene, α-phellandrene, δ-cadinene, limonene, α-cadinol, and β-phellandrene.

The California pepper tree (*Schinus molle* L.) is grown extensively as an ornamental plant in many areas of the Americas. Fruit from this tree yields a volatile oil that has been used as a substitute for black pepper, in flavor compositions, and in pharmaceutical products (Gonzales and Lombardo, 1946; Ottolino, 1948). In Greece the fruit serves for the preparation of certain beverages (Guenther, 1952).

A number of investigators have examined the physicochemical properties and chemical composition of the oil, but relatively few components have been identified and only tentative identification of some compounds has been accomplished (Brückner van der Lingen, 1930; Gonzales, 1931; Ottolino, 1948; Bernhard and Wrolstad, 1963). Cremonini (1928, 1930) indicated the presence of *trans*-terpin, ferrojone, and a sugar with properties similar to those of glucose. Bernhard and Wrolstad (1963) reported the presence of α-pinene, β-pinene, α-phellandrene, β-phellandrene, myrcene, D-limonene, camphene, *p*-cymene, and three unidentified constituents. More recently Jennings and Bernhard (1975) found, in addition to the above, sabinene, α-terpinene, γ-terpinene, terpinolene, methyl *n*-octanoate, bourbonene, α-*trans*-bergamotene, caryophyllene, α-terpineol, germacrene D, and δ-cadinene. Terhune et al. (1974) reported isolating and characterizing a new sesquiterpene, β-spathulene. Quite recently Pozzo-Balbi et al. (1976, 1978) have isolated several triterpenoid acids from an acidic fraction of an oleoresin obtained from the fruit.

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