Table I. Liver

sulfamethazine added	N	av value calcd	SD	coeff of variation	qualitative identification requirements[a] a	b	c
0.00	12	ND[b]			–[c]	–	–
0.05	6	0.050	0.003	6.09	+[d]	+	+
0.10	9	0.104	0.005	4.58	+	+	+
0.20	6	0.211	0.009	4.19	+	+	+

[a] As stated under Results and Discussion. [b] ND, not detected. [c] (–) Implies requirements were not met. [d] (+) Implies requirements were met.

Table II. Muscle

sulfamethazine added	N	av value calcd	SD	coeff of variation	qualitative identification requirements[a] a	b	c
0.00	12	ND[b]			–[c]	–	–
0.05	6	0.053	0.007	12.29	+[d]	+	+
0.10	9	0.098	0.005	4.65	+	+	+
0.20	6	0.208	0.011	5.21	+	+	+

[a] As stated under Results and Discussion. [b] ND, not detected. [c] (–) Implies requirements were not met. [d] (+) Implies requirements were met.

that the 1:1 ratio of [^{12}C]sulfamethazine to [^{13}C]sulfamethazine as measured by the 227/233 ion mass ratio does not yield the theoretically calculated value of 1.82 (1/0.5494). As previously stated (see Materials) 54.94% of the isotopically enriched sulfamethazine internal standard is labeled at all six positions of the phenyl moiety while 34.60% is labeled at only five positions of the phenyl moiety. It is obvious that a positive contribution to m/e 233 (M – 65 fragment labeled at all six positions with ^{13}C) will occur when m/e 228 (M – 64 fragment) is labeled with ^{13}C at only five positions. Furthermore, less obvious positive contributions also occur. These positive contributions to the m/e 233 ion affect the 227/233 quantitative ion ratio. A typical 227/233 ion mass ratio from a 1:1 [^{12}C]sulfamethazine/[^{13}C]sulfamethazine standard when determined experimentally in our laboratory was 1.37.

Swine liver and muscle tissue previously determined to contain less than 0.01 ppm of sulfamethazine by the method of Tishler (Tishler et al., 1968) were fortified with 0.05, 0.10, and 0.20 ppm of [^{12}C]sulfamethazine. In addition, each sample was fortified with 0.10 ppm of [^{13}C]-sulfamethazine internal standard. Tables I and II present qualitative and quantitative data collected by using the conditions as stated under Instrumentation. It is apparent from the data presented that GC/MS in conjunction with an internal standard provides a very powerful technique for the detection and quantitation of sulfamethazine in swine liver and muscle tissue.

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High-Pressure Liquid Chromatographic Determination of Fungicidal Dithiocarbamates

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A specific method for determination of thiram, salts of alkylenebis(dithiocarbamic acids), and *N,N*-dimethyldithiocarbamic acid is presented. Iron, zinc, and manganese salts were transformed into water-soluble sodium salts with an alkaline EDTA solution. The extract was subjected to ion-pair methylation at pH 6.5–8.5 in chloroform–hexane. The organic phase was concentrated and analyzed by HPLC and UV detection at 272 nm. Thiram was reduced by nabam into *N,N*-dimethyldithiocarbamate, a reaction which was avoided by extracting with chloroform. However, extraction with chloroform of apple samples drastically reduced the recovery of ethylenebis(dithiocarbamates). Thiram was preferably determined after extracting with chloroform and purifying the extract on a silica gel column. The limit of detection in water solutions for zineb, ziram, and thiram was 0.05, 0.01, and 0.01 ppm, respectively, and the recovery from apple samples fortified at the 1.0-ppm level was 61, 88, and 88% in the order mentioned.

Salts and disulfides of mono- and dialkyldithiocarbamic acids are widely used as pharmaceuticals, rubber vulcanizers, and fungicides (Thorn and Ludwig, 1962). The polymeric salts of ethylenebis(dithiocarbamic acid) (the EBDC's zineb, maneb, and mancozeb, VII–IX; Figure 1) form the most important class of pesticides for broad spectrum control of a variety of fungal diseases on growing crops (Engst, 1977).

Many different types of methods have been developed for the analysis of the dithiocarbamates. Most of these are based on degradation of the dithiocarbamates prior to detection. These methods include hydrolysis to carbon disulfide and amines (Cullen, 1964; McLeod and McCully, 1969; Newsome, 1974; Greve and Hogendoorn, 1978; Uno et al., 1979) reduction (Domar et al., 1949; Rangaswamy

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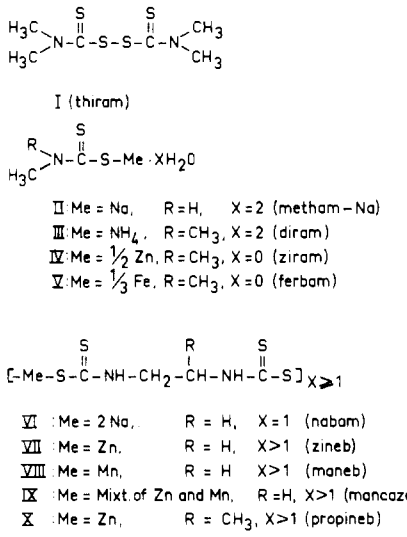

Figure 1. Fungicidal dithiocarbamates.

et al., 1970), and oxidation (Rangaswamy and Vijayashankar, 1975; Kurzawa and Krzymińska, 1977). Determination, without prior degradation of the dithiocarbamate structure, has been made by volumetric (Lakshminarayana, 1976), polarographic (Nangniot et al., 1978), and chromatographic methods (Fishbein and Fawkes, 1965; Smith et al., 1980; Pflugmacher and Ebing, 1980).

One of the most commonly used methods for determining residues of dithiocarbamate fungicides in foodstuffs is that of Keppel (1971). In this method, the dithiocarbamates are hydrolyzed with hot mineral acid to carbon disulfide, which is then determined spectrophotometrically as cupric complexes of *N,N*-bis(2-hydroxyethyl)dithiocarbamic acid. In a modification of this method (Chmiel, 1979), the dithiocarbamate content is determined spectrophotometrically as methylene blue after several transformations. This method is more sensitive and less vulnerable to plant colorant interference than Keppel's method. However, methods based on carbon disulfide evolution are neither specific for individual dithiocarbamates nor very accurate (Häfner, 1977; Thier et al., 1977). Furthermore, cupric salts sometimes used as fungicides in combination with EBDC reduce the recovery of the latter (Lesage, 1980). Some crops (Keppel, 1971), as well as some captan preparates and perchloromethylmercaptan normally present in technical captan, give rise to false response (Gustafsson and Thompson, 1979) in both the method of Keppel (1971) and that of Nangniot et al. (1978).

A specific and reliable method for the determination of EBDC's in foodstuffs has long been desired since this group of dithiocarbamates degrades to ethylenethiourea, a compound which has shown teratogenic (Khera, 1973), goitrogenic (Graham and Hansen, 1972), and carcinogenic properties (Seiler, 1974) in animal experiments.

The polarographic method of Nangniot et al. (1978) enables specific determination of EBDC's and thiram (I) in foodstuffs, but the presence of I interferes with EBDC's, giving rise to reduced EBDC levels. Furthermore, with this method it is not possible to distinguish between I and the salts of the *N,N*-dimethyldithiocarbamic acid (III–V, Figure 1). EBDC residues in foodstuffs have also been determined gas chromatographically after acid hydrolysis to 1,2-diaminoethane and subsequent pentafluorobenzoylation (Greve and Hogendoorn, 1978), methanesulfonylation (Uno et al., 1979), and trifluoroacetylation (Newsome, 1974). However, errors arising due to 1,2-diaminoethane already present, and side reactions occurring during the hydrolysis, cannot be excluded. In the method of Pflugmacher and Ebing (1980), the polymeric VII–X are transformed to sodium salts and specifically determined by means of gel permeation chromatography and UV detection.

EXPERIMENTAL SECTION

Apparatus. A liquid chromatograph (Spectra-Physics Model 3500) was used with a stainless steel column, 200 × 4 mm i.d., packed with Nucleosil RP-18, 5 μm. The mobile phase was water–acetonitrile (7:3) with a flow rate of 0.8 mL/min. The detection was made with a variable ultraviolet detector (Spectra-Physics, Model 770 spectrophotometric detector) run at 272 nm.

The chloroform extracts were purified on a 10-g silica gel column (i.d. 18 mm, packed in hexane with silica gel 60, 0.063–0.200 mm; E. Merck, Darmstadt, West Germany) with loosely packed glass wool on the top. The column was repacked before every run. The EDTA–sodium hydroxide extracts were filtered through Whatman GF/B (5.5 cm) glass microfiber filters.

Reagents. Thiram (98%; Fluka AG, Switzerland), ziram (98%; EPA, Research Triangle Park, NC), zineb (80.5%; EPA), methan-Na (Metam-Fluid, 380 g/L; BASF, West Germany), and 1,2-propanediol (E. Merck) were used. Otherwise, reagent-grade chemicals and solvents were used when possible.

Preparation of Methyl Mono- and Dimethyldithiocarbamates. Methyl *N,N*-dimethyldithiocarbamate. A solution of 0.036 mol of carbon disulfide in 15 mL of 96% ethanol was slowly added under stirring during 10 min to 6.91 g of a 40% aqueous dimethylamine solution in 15 mL of ethanol (0.061 mol of the amine). The temperature was kept below 30 °C. An excess of the amine was maintained throughout the reaction. Methyl iodide (0.037 mol) in 10 mL of ethanol was added and stirred for 15 min. The mixture was transferred to a separatory funnel and 150 mL of water was added. The oily product obtained was extracted from the water phase with two 50-mL portions of chloroform. The chloroform extract was washed with 3 × 5 mL of water and dried over anhydrous sodium sulfate. The solvent was stripped off under reduced pressure without warming, and the residue, 4.03 g, was dissolved in 25 mL of hexane and filtered hot. The filtrate was concentrated to ~20 mL and refrigerated, giving 3.42 g (70%) of a crystalline product. The product was recrystallized from hexane: mp 44–44.5 °C; λ_{max} 272 nm [log ε 4.04 in water–acetonitrile (7:3)]. The yield was not optimized. The MS, the IR, and the melting point data were consistent with those reported in the literature (Onuska and Boos, 1973; Konečný and Halgaš, 1977).

Methyl *N*-methyldithiocarbamate (the ester of II) was prepared in the corresponding way.

Dimethyl Ethylenebis(dithiocarbamate). A solution of 0.05 mol of 1,2-diaminoethane in 25 mL of 96% ethanol was slowly dropped under constant stirring into a 0.05 mol solution of carbon disulfide in 25 mL of 96% ethanol. The temperature was kept below 35 °C. The white precipitate formed was pulverized and 0.05 mol of methyl iodide was added to the mixture. After stirring for 2 h only a minor part of the precipitate remained. This was filtered off. Water (150 mL) was added and the precipitate formed was collected on a glass filter funnel, washed with water, and dried at room temperature overnight. The product (1.62 g; 27%) was recrystallized twice from chloroform: mp 105–105.5 °C [lit. mp 104–106 °C (Marshall, 1978)]. The melting point, the MS, and the IR data of the product are consistent with those reported for the compound. λ_{max} was 272 nm [(log ε 4.35 in water–acetonitrile (7:3)]. The yield was not optimized.

Analytical Procedure. *(I) Determination of IV–X (or I) but Not VI–X and I in Combination.* Unground commodity (100 g) was extracted for 15 min with 100 mL of 0.25 M EDTA in 0.45 M sodium hydroxide (pH 9.5–9.6) in a glass jar with a glass lid. The extract was filtered through a glass fiber filter. The extraction vessel and the filter were rinsed with 20 mL of water. The pH of the solution was adjusted to 6.5–8.5 by the addition of 8 mL of 2 M hydrochloric acid and 5 mL of a 0.41 M aqueous solution of tetrabutylammonium hydrogen sulfate. The water layer was stirred during the addition. The mixture was shaken in a separatory funnel for 5 min at room temperature with 30 mL of 0.05 M methyl iodide in chloroform–hexane (3:1). The phases were separated and the aqueous layer was rinsed with another 10 mL of the methyl iodide solution. The organic phase was then allowed to stand for 30 min. A 20% solution (5.0 mL) of 1,2-propanediol in chloroform was added. The volatile part of the mixture was stripped off at 30 °C in a rotatory evaporator. The residue was diluted with 1.0 mL of methanol and 10 μL was analyzed by high-pressure liquid chromatography. The recovery of I, IV, and VII added as talc mixtures to apple samples at the 1.0-ppm level was 71, 88, and 61%, respectively.

(II) Determination of Thiram. The sample (100 g) was extracted with 50 mL of chloroform for 3 min. The extract was concentrated to ~2 mL in a rotatory evaporator at 30 °C. The residue was cleaned on a silica gel column by eluting with 100 mL of hexane, followed by 120 mL of chloroform. The first 150 mL of the eluate was discarded. The following 60 mL was collected and concentrated to dryness. The residue was dissolved in 2 mL of methanol and analyzed by high-pressure liquid chromatography. The recovery of I at the 1-ppm level from a water solution and from an apple sample fortified with I mixed with talc was 87 and 88%, respectively.

RESULTS AND DISCUSSION

The pH of the EDTA–sodium hydroxide solution should not be lower than 9.5 in order to obtain an efficient transformation of the complex EBDC salts into the water-soluble VI within 15 min. On the other hand, I is not stable in alkaline media (Nangniot et al., 1978). At the pH used in procedure I, less than 1% of I in EDTA–sodium hydroxide was decomposed within 15 min. However, in an apple sample fortified at the 1-ppm level with a mixture of I in talc, 9% of *N,N*-dimethyldithiocarbamate was formed. An increased breakdown of I was not observed in procedure II. This method was therefore preferred for determination of I. I was found to readily react with VI in aqueous solution to give *N,N*-dimethyldithiocarbamate salt. If the latter and I are found, a new aliquot of the sample should be analyzed according to procedure II. Recent experiments in our laboratory point out a way of analyzing EBDC's in presence of I.

The ion-pair extraction was rapid and efficient. No residues of EBDC's were found in the aqueous phase after addition of the quaternary ammonium salt and extraction with chloroform–hexane (3:1). With the method used to check the aqueous phase (Nangniot et al., 1978), less than 0.05 μg/mL EBDC can be determined.

The yield of dimethyl EBDC obtained from VII in the subsequent phase-transfer alkylation (Figure 2) was constant within the pH range 6.5–8.5 (Figure 3). Salts of dithiocarbamic acids decompose rapidly at low pH (Thorn and Ludwig, 1962).

Several alkylating agents were examined. Methyl iodide, however, was preferred since it is easy to remove and reactive enough at room temperature. Hexane, benzene,

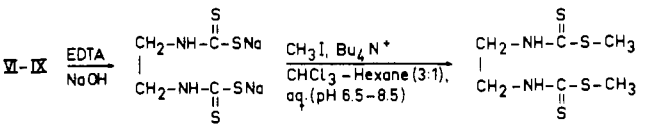

Figure 2. Extraction and phase-transfer methylation of ethylenebis(dithiocarbamate) salts.

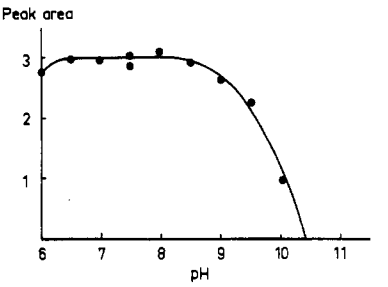

Figure 3. Influence of pH on the yield of dimethyl ethylenebis(dithiocarbamate) from VII in the phase-transfer methylation.

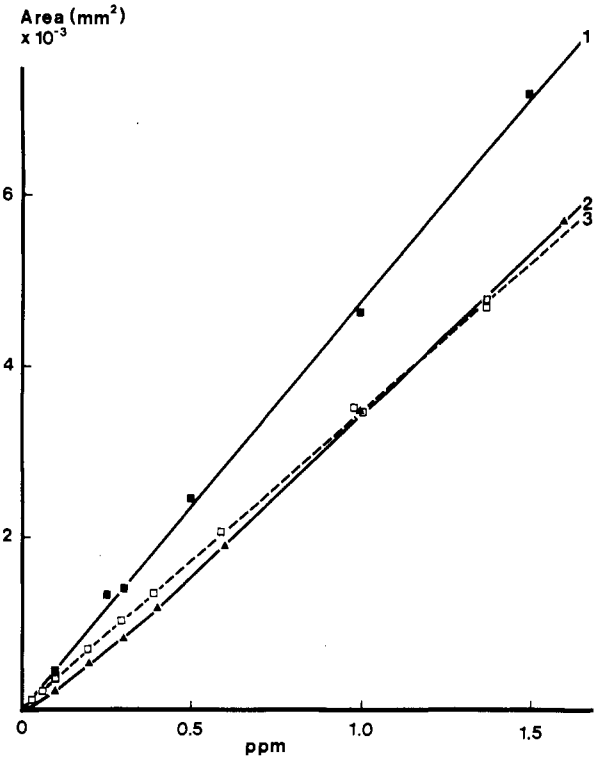

Figure 4. Calibration curves of (1) IV, (2) VII, and (3) I for procedure I.

dichloroethane, dichloromethane, chloroform, and mixtures of some of these were examined as a solvent for the ion-pair alkylation. Tetrabutyl-, tetrapentyl-, and tetrahexylammonium salts were tried as phase-transfer reagents. The best results were obtained with tetrabutylammonium hydrogen sulfate in chloroform–hexane (3:1).

A constant recovery of EBDC was obtained after 30 min of alkylation at room temperature. The yield did not change when the alkylation time was extended to 90 min. At 30 °C the maximum yield was obtained after 15 min. It was not necessary to dry the organic layer after alkylation.

1,2-Propanediol or 1,2-ethanediol was added as a keeper in order to avoid losses of the somewhat volatile methyl *N,N*-dimethyldithiocarbamate during evaporation. 1,2-Propanediol was preferred to 1,2-ethanediol since it is more soluble in chloroform.

Methanol solutions of I, the methyl ester of IV, and dimethyl EBDC were stable for at least 3 days, whereas

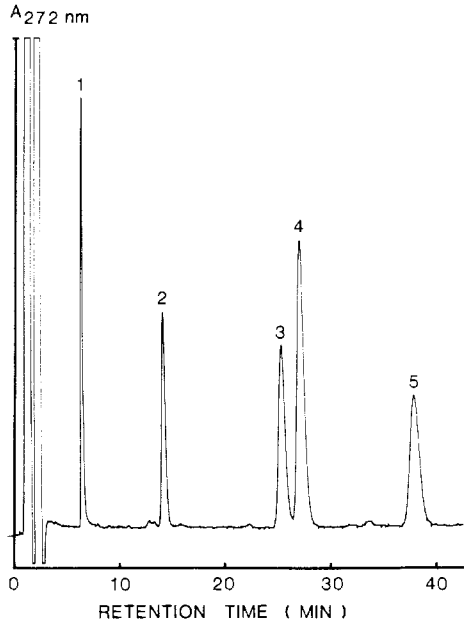

Figure 5. Chromatogram of (1) methyl *N*-methyldithiocarbamate (ester of II) (11 ng), (2) methyl *N,N*-dimethyldithiocarbamate (ester of III–V) (63 ng), (3) thiram (I) (170 ng), (4) dimethyl ethylenebis(dithiocarbamate) (ester of VI–IX) (150 ng), and (5) dimethyl propylenebis(dithiocarbamate) (ester of X) (~200 ng). Full scale corresponds to 0.04 absorbance unit.

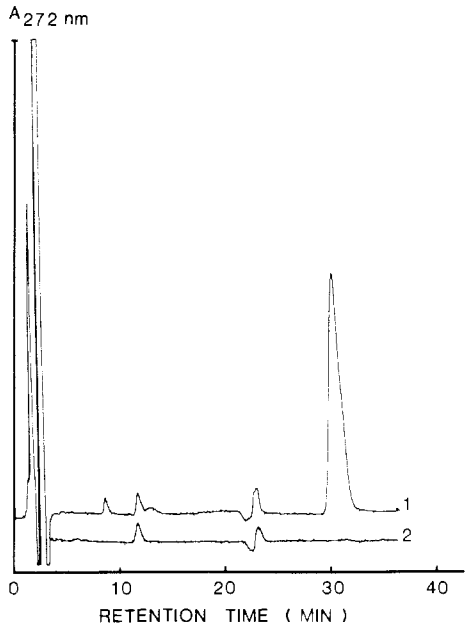

Figure 6. Chromatogram of apple samples (1) with VII added (0.8 ppm) and (2) without VII. Full scale corresponds to 0.04 absorbance unit.

methanol solutions of VI and VII were stable for only some hours. No decomposition of the dithiocarbamate esters in the solid form was observed after more than 1 year.

The methods chosen for the preparation of the dithiocarbamate esters were preferred to that of an ion-pair alkylation process since the tetrabutylammonium iodide formed in the latter method was difficult to separate from the esters formed.

The calibration curves for I, IV, and VII are given in Figure 4. A chromatogram of I and methyl esters of the acids corresponding to II, IV, VI, and X is presented in Figure 5. Figure 6 shows chromatograms of an apple sample and an apple sample fortified with VI before extraction with the EDTA solution.

In the present method unground samples of the commodities were analyzed and not homogenates for the following reasons: dithiocarbamates are not systemic and therefore residues will be found only on the surface (Engst and Schnaak, 1974); homogenization starts a rapid breakdown of the dithiocarbamates (Thier et al., 1977) and gives rises to more coextractives which interfere in the determination.

The recovery of I in procedure II and of IV and VII in procedure I from apple samples fortified at the 1-ppm level was 88, 88, and 61%, respectively. The corresponding recoveries from water solutions were 88, 88, and 64%. The limit of detection in water solutions was 0.01, 0.01, and 0.05 ppm, respectively. These limits of detection are well below 0.3 ppm, the lowest Swedish residue tolerance for dithiocarbamates.

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