

used. This method is specific for the analysis of compounds that can be converted to a volatile aldehyde or ketone. FMC 25213 is an example of this type of compound since it hydrolyzes to propionaldehyde.

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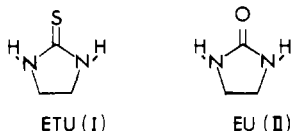
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Oxidation of Ethylenebisdithiocarbamate Fungicides and Ethylenethiuram Monosulfide to Prevent Their Subsequent Decomposition to Ethylenethiourea

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Oxidation of zineb in basic medium consumed approximately 16 equiv of hypochlorite and resulted in the production of 4 equiv of sulfate and 1 equiv of carbon dioxide but less than theoretical yield of ethyleneurea (EU), due in part to further oxidation of this product. Ethylenethiuram monosulfide (ETM) was oxidized by approximately 12 equiv of hypochlorite, which resulted in 3 equiv of sulfate and 0.45 equiv of EU. The degree of inactivation (to prevent subsequent decomposition to ethylenethiourea) of formulated zineb, maneb, mancozeb, and of ethylenethiuram monosulfide was found to be directly proportional to the quantity of oxidant added. The oxidative inactivation was equally effective for the three formulations and was complete in 5 min. No promotion of ETU formation was observed even when small quantities of oxidant were used. Hypochlorite oxidation in strong base is proposed as a technique for removal of residues of ethylenebisdithiocarbamates and ETM.

Ethylenebisdithiocarbamate (EBDC) fungicides (maneb, mancozeb, and zineb) provide broad spectrum control of fungal diseases and as such are widely used on a variety of crops. Concern for residues of these fungicides centers on the possibility that they may be converted to ethylenethiourea (ETU) (I), which is a potent tumorigen and teratogen to rats and mice (Graham et al., 1975; Graham, 1973; Graham and Hansen, 1972; Seiler, 1974; Khera, 1973; Ulland, 1972; Innis et al., 1969). Thus injection of ETU over long periods of time may be harmful to human health.



ETU formation from EBDCs is promoted by heat treatment (Marshall, 1977; Watts et al., 1974; Newsome and Laver, 1973) and has been demonstrated from EBDC field residues during normal food processing procedures (Baron, 1976). Thus a preprocessing technique whereby residues of these fungicides could be inactivated to avoid further decomposition to ETU would be of considerable utility.

The action of chloramine-T ($p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{N}\cdot\text{NaCl}$) on hydrogen sulfide (Bendall et al., 1942; Murthy and Rao,

1952), on carbon disulfide (Rao and Murthy, 1960), and on zineb (Lakshminarayana, 1976) has been studied. The active agent of this oxidant in basic solution has been suggested to be hypochlorite (Afans'ev, 1948). The oxidation of aqueous sulfide solutions by hypochlorite has also been investigated (Choppin and Faulkenberry, 1937). These latter authors have demonstrated a complex reaction pathway in which the ratio of products formed (sulfate and elemental sulfur) was a function of the concentration of reactants, the temperature, and the pH of the reaction medium.

Recently it was reported that ETU is oxidized to ethyleneurea (II) and sulfate using hypochlorite, a potential preprocessing water wash additive (Marshall and Singh, 1977). This paper describes the action of hypochlorite on EBDC fungicides and on ethylenethiuram monosulfide (ETM) (III) and suggests optimal conditions under which this oxidation may be performed.

EXPERIMENTAL SECTION

Materials. Ethylenethiourea (ETU) (I) (2-imidazolidinethione) and ethyleneurea (II) (2-imidazolidinone) were purchased from Fisher Scientific Co. ETU was crystallized from methanol-water (1:1) containing 5% hexane, while EU was sublimed at 105 °C (10^{-3} mm) and recrystallized from methanol prior to use.

Nabam (disodium ethylenebisdithiocarbamate) was synthesized according to the method of Engst and Schnaak (1967), while zineb was obtained by adding nabam to aqueous zinc chloride (Ludwig et al., 1955) or by purifying

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technical material according to the procedure of Lakshminarayana (1976). Ethylenethiuram monosulfide (ETM) (III) (5,6-dihydro-3*H*-imidazo[2,1-*c*]-1,2,4-dithiazole-3-thione) was obtained by vigorous aeration of nabam according to the method of Thorn and Ludwig (1962).

Ethylenebisdithiocarbamate dimethyl ester (IV) was synthesized by adding dimethyl sulfate to a solution of nabam in 1% sodium bicarbonate. The rapidly formed precipitate was recrystallized from chloroform to furnish an analytical sample: mp 104–106 °C; NMR (CDCl₃) δ 4.06 (NCH₂, m, 4 H), 2.63 (C(S)SCH₃, s, 3 H); IR (KBr) 3225, 3170 (NH), 2980, 2955, 2910, 2880 (CH₃), 1540 (dithiocarbamoyl ester), 1135, 1090, and 950 cm⁻¹; MS M⁺ 240, *m/e* 193 (M - SCH₃), 144 (CH₂NCS)₂, 133 (CH₂CHNHCS₂CH₃), 91 (-CS₂CH₃), and 86 (-CH₂CH₂NCS). Anal. Calcd for C₆H₁₂N₂S₄: C, 29.97; H, 5.03; N, 11.65; S, 53.34. Found: C, 30.24; H, 5.20; N, 11.66; S, 53.45.

Methyl (2-thioxo-1-imidazolidinyl)carbodithioate (V) isolated from nabam oxidations was purified by liquid chromatography to furnish an analytical sample as yellow crystals: mp 171–173 °C; NMR (CDCl₃) δ 4.73 (NCH₂, t, *J* = 9 Hz, 2 H), 3.75 (NCH₂, t, *J* = 9 Hz, 2 H), 2.61 (CS₂CH₃, s, 3 H); IR (KBr) 3210 (NH), 2970, 2920 (CH₃, CH₂), 1545 (dithiocarbamoyl ester) 1460, 1385 (CH₂), 1274; MS M⁺ 192, *m/e* 177 (M - 15), 144 (CH₂NCS)₂, 133 (CH₂CHNHCS₂CH₃), 91 (-CS₂CH₃), 86 (-CH₂CH₂NCS), and 72 (-CH₂NCS). Anal. Calcd for C₅H₈N₂S₃: C, 31.23; H, 4.19; N, 14.57; S, 50.02. Found: C, 31.48; H, 4.27; N, 14.52; S, 50.32.

Technical formulations Dithiane M22 and M45 were kindly supplied by Rohm and Haas Co. while Polyram 80WP was supplied by FMC Corp. Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) was purchased from Aldrich Chemical Co.

Method. Iodometric determinations of the amount of hypochlorite consumed were performed using procedures previously described (Marshall and Singh, 1977). Carbon dioxide evolution was determined using an absorption train consisting of successive traps containing magnesium perchlorate, anhydrous calcium sulfate, and magnesium perchlorate, silver gauze heated to 200 °C, and finally an absorption tube containing Ascarite followed by magnesium perchlorate. Oxidations were performed in a closed system (which had been purged previously with nitrogen). After 2 h the reaction vessel was attached to an absorption train and excess sulfuric acid was added to the oxidized solution. The reaction mixture was gently boiled for 5 min and volatiles were entrained in the absorption tubes using nitrogen until constant weight was achieved.

Sulfate determinations were performed by passing an aliquot of oxidized solution through a strong cation-exchange resin (Dowex 50W-X8), boiling the eluate for 10 min, adjusting the pH to 3.0 ± 0.2, diluting to volume, and finally titrating with 0.01 M barium perchlorate using dimethylsulfonazo-III indicator in acetone.

EU levels were measured photometrically. An aliquot of oxidized solution was passed through a short column of mixed-bed ion-exchange resin (DOWEX 50W-X8 and DOWEX 1-X4) and eluted with distilled water. The eluate was diluted to volume and an aliquot was reacted with an excess of *N,N*-dimethylaminocinnamaldehyde (Aldrich Chemical Co.) in methanol-4 N hydrochloric acid (3:1). The quantity of EU present was then determined photometrically at 530 nm by comparison with standards.

Inactivation studies (formulations) were performed by allowing a swirled suspension (100 mg) of the formulation in 1 N sodium hydroxide (50 mL) or 1% sodium bicarbonate to react with hypochlorite (0 to 20 equiv) for 15

min. The reaction was quenched by adding excess sodium sulfite, then boiled gently for 2 h to convert unreacted residues to ETU. The resulting suspensions were filtered and the filtrate diluted to volume and analyzed for ETU by reverse phase liquid chromatography using a 25 cm × 4 mm i.d. column packed with 10 μ silanized silica gel (RP-2, E.M. Merck Co.) and 0.05 M borate buffer (adjusted to pH 8) as eluent.

Equipment. IR spectra were determined as KBr pellets using a Beckman IR-20A spectrophotometer and UV spectra were recorded on a Pye-Unicam SP 800 instrument. Proton NMR spectra were obtained in CDCl₃ solution with Me₄Si as internal standard on a Varian T-60 NMR spectrometer. The MS were determined on a Finnigan 3100 GCMS coupled to a 6100 computer controlled data acquisition system. Samples were determined either as direct inlet probes or on a 5 ft × 4 mm i.d. glass column packed with 3% OV-17 on 100/120 mesh Gas-Chrom Q.

Thin-Layer Chromatography (TLC). Compounds were spotted on silica gel plates (0.25 mm Polygram-Machery Nagel and Co.) containing a fluorescent indicator and developed in either methanol or chloroform-methanol 1:1. Products were visualized using fluorescence quenching, iodine vapors, or one of the following chromagenic sprays: Dragendorff reagent, Grote's reagent, iodine-sodium azide, dimethylaminocinnamaldehyde, 2,6-dichloro-*N*-bromo-*p*-benzoquinoneimine, or ninhydrin.

RESULTS AND DISCUSSION

A preliminary investigation of the products from the reaction of hypochlorite with nabam in 1% sodium bicarbonate revealed the presence of ethyleneurea (by TLC) and sulfate (by precipitation with barium carbonate). No ETU or ETM could be detected by TLC of the crude reaction mixture and moreover the reaction appeared to be rapid and complete. As a probe of reaction stoichiometry, nabam, dissolved in 1% sodium bicarbonate, was oxidized in the dark with excess hypochlorite. Excess oxidant was then determined iodometrically using potassium iodide and sodium thiosulfate. Results are reported in Table I. There was excellent agreement between the predicted 4 equiv of oxidant required to oxidize each sulfur equivalent in the substrate and the observed 15.96 equiv which were consumed. It is assumed that each sulfur atom of the substrate (oxidation state -2) is oxidized to sulfate (oxidation state +6), while chlorine of the oxidant (oxidation state +1) is reduced to chloride (oxidation state -1). Hence 4 equiv of hypochlorite are consumed per equivalent of sulfate produced.

To determine the scope and efficacy of the reaction, zineb, which has been purified by successive washes with ethanol, chloroform, and dilute acetic acid (6 N) (Lakshminarayana, 1976), was dissolved or suspended in various buffer systems and reacted with excess oxidant (which had been adjusted to the pH of the buffer). Results are included in Table I. In 1 N sodium hydroxide, zineb was found to consume more than 16 equiv of hypochlorite whereas in less basic media, in which the zineb (1.3 mM) was not totally dissolved, values while less than predicted, did approach the predicted value with longer reaction times. A possible explanation for the larger than expected values in sodium hydroxide was suggested by the following results. EU (II) was oxidized and found to consume up to 8 equiv over 20 h. As the pH of the EU oxidation medium was varied, it was observed that at pH < 10 the oxidation of EU was relatively slow whereas at pH > 10 the reaction was considerably more rapid. A plot of the consumption of oxidant vs. the pH of the oxidizing me-

Table I. Variation in the Consumption of Hypochlorite with Time and pH of the Oxidation Medium

Substrate	No. of sulfur atoms	Oxidation conditions ^a							
		1 N NaOH		Borate buffer, pH 9.0		1% NaHCO ₃		Phosphate buffer, pH 8.0	
Zineb	4	16.71	30 min	14.81	90 min	14.31	60 min	13.82	60 min
		16.47	30 min	15.47	17 h				
		16.80	30 min						
Nabam	4	17.23	45 min			15.96	30 min		
						16.03	30 min		
						15.89	60 min		
Ethylene urea	0	2.64	90 min	0.46	90 min	0.0	60 min	0.15	90 min
		8.0	20 h						
ETM	3	12.40	120 min			10.92	120 min		
		12.18	120 min			10.67	120 min		
CS ₂	2	6.37	180 min			6.81	60 min		
		6.99	18 h			7.28	18 h		
CS ₂ + molybdate	2	6.66	180 min			6.30	60 min		
		7.07	18 h			7.19	18 h		

^a Oxidations were performed using excess hypochlorite. Results are expressed as equivalents of oxidant/equivalents of substrate.

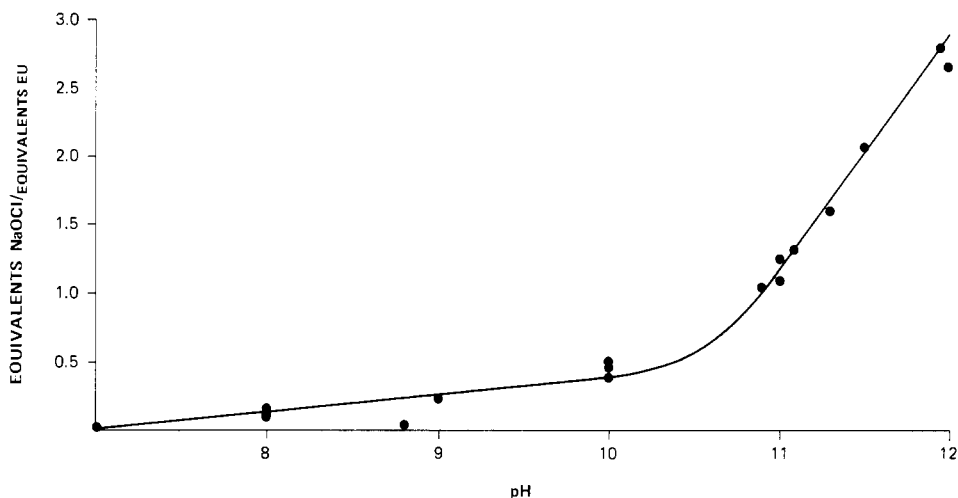


Figure 1. Variation in equivalents of hypochlorite consumed per equivalent of EU with pH (90 min at room temperature).

dium for this substrate is reproduced in Figure 1.

Reaction products were measured in the following series of experiments. Purified zineb in 1.0 N sodium hydroxide was oxidized with excess hypochlorite for 30 min. An aliquot of oxidized solution was purified on a cation-exchange column (to remove interfering zinc ions) and analyzed for sulfate content by titration with barium perchlorate. Oxidation of zineb resulted in values of 4.24, 4.33, and 4.28 equiv of sulfate. Blank determinations for the same product resulted in values of 0.19 and 0.24 equiv. Thus 4 equiv of sulfate was generated per equivalent of zineb.

The generation of carbon dioxide was also monitored. Triplicate analysis by this procedure using 200 mg of zineb resulted in weight gains of 48, 44, and 46 mg, whereas blanks (employing a similar volume of oxidant but no substrate) resulted in absorption tube weight gains of 12, 15, and 17 mg. Thus 1 equiv of carbon dioxide was generated per equivalent of zineb oxidized. When EU was similarly oxidized for 2 h, weight gains of the absorption tube were not significantly different from the blank determinations. This analysis was repeated using a phosphate buffer (adjusted to pH 8) and a similar quantity of zineb. After 2 h reaction and correcting for blank, 0.94 equiv of carbon dioxide was generated per equivalent of zineb.

In the analysis for EU a linear relationship (coefficient of correlation $r = 0.996$) between absorbance at 530 nm and concentration of standard EU was observed for

Table II. Variation in the Yield of EU from the Oxidation of Zineb in Various Media

Oxidizing medium	pH	Re-action time, min	Equivalents of EU/ equiv of zineb
Phosphate buffer	7.0	90	0.377
1% Bicarbonate	8.8	90	0.323
1% Bicarbonate	8.8	120	0.366
Phosphate buffer	10	60	0.323
Phosphate buffer	10	60	0.314
Phosphate buffer	10	90	0.313
1 N NaOH		30	0.04
		30	0.06

concentrations between 5 and 50 $\mu\text{g}/\text{mL}$. Oxidized solutions of zineb were found to contain small but reproducible quantities of EU as recorded in Table II. Recovery of standard EU from buffered solutions containing 1 equiv of zinc chloride was quantitative. Thus formation of a zinc chloride EU complex $[(\text{C}_3\text{H}_6\text{N}_2)_2\text{ZnCl}_2]$, Berni et al., 1963] is not responsible for recoveries of less than 1 equiv of EU per equivalent of zineb, nor does further oxidation of EU once formed account for low recoveries. Ethylenediamine (EDA) was formed in only trace amounts and no formaldehyde was observed under these oxidation conditions. EDA was measured by reaction with fluorescamine and steam distillation of the oxidized solution, followed by reaction with purpald (4-amino-3-hydrazino-5-mercap-

Scheme I

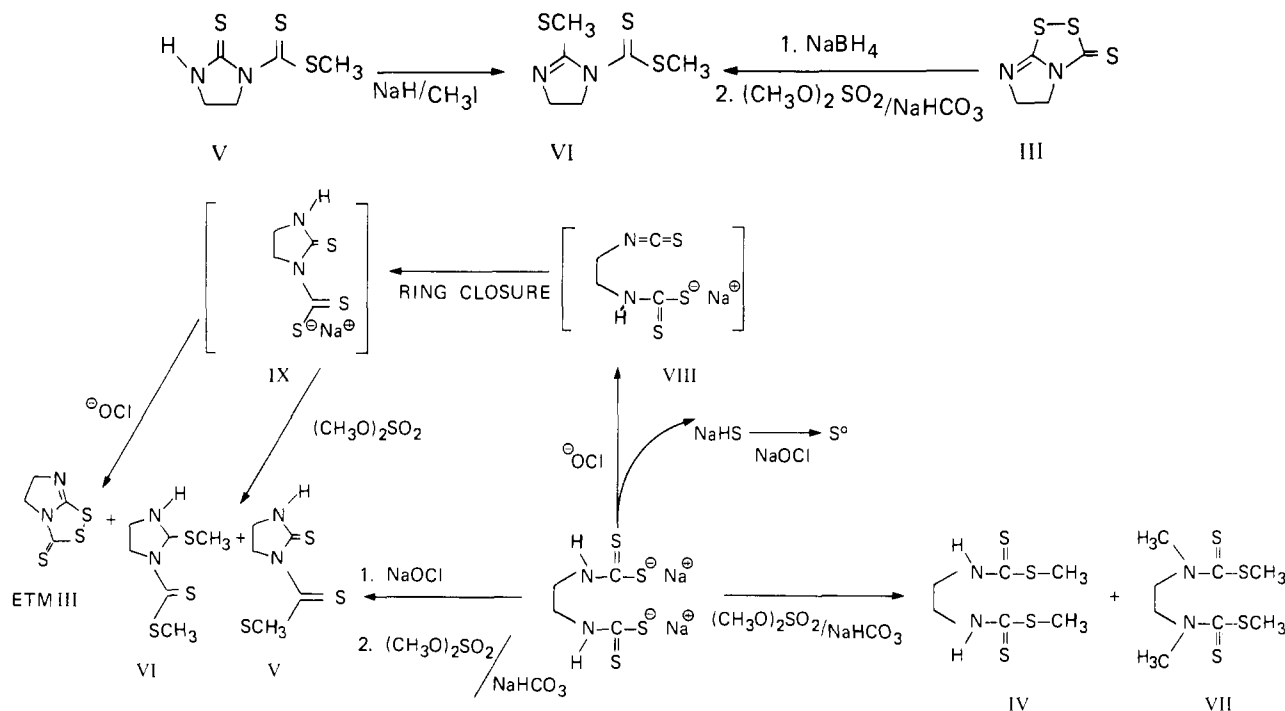


Figure 2. Partial oxidative decomposition scheme for nabam in basic solution.

to-1,2,4-triazole) gave a negative test for aldehydes.

An attempt to isolate oxidation intermediates (by trapping) with a view to further defining the oxidative pathway was only partially successful. Nabam in 1% bicarbonate solution was observed to react rapidly with excess dimethyl sulfate. The product, which precipitated immediately, was identified spectroscopically as ethylenebisdithiocarbamate dimethyl ester (IV) and confirmed by elemental analysis of the isolate recrystallized from chloroform. When nabam, in the same medium, was oxidized with 1 to 2 equiv of hypochlorite the reaction solution rapidly deepened in color to orange. Extraction with chloroform resulted in a mixture of products containing (IV), ETM (III), and elemental sulfur. Treatment of the aqueous fraction with excess dimethyl sulfate for 30 min followed by extraction with chloroform led to two other products, which were separated by washing with dilute hydrochloric acid.

The neutral oxidized and methylated fraction (insoluble in dilute acid) contained one major product, which was purified by preparative liquid chromatography to yield yellow crystals, mp 171–173 °C. On the basis of spectroscopic studies this product was assigned the structure methyl (2-thioxo-1-imidazolidinyl)carbodithioate (V). This assignment was corroborated by the following chemical transformations. Treatment of V with sodium hydride-methyl iodide in benzene (Scheme I) resulted in a new derivative (VI) having a broad multiplet δ 4.17 (NCH₂, 4 H) and sharp singlets 2.61 (C(S)SCH₃, 3 H) and 2.42 (N=C(S)SCH₃, 3 H). Amino absorptions in the IR were absent; however, a prominent absorption at 1545 (dithiocarbamoyl ester) remained. The product VI displayed a molecular ion M⁺ 206 and prominent ions at m/e 191 (M - 15), 159 (M - SCH₃), 91 (·C(S)SCH₃), and 86 (·CH₂CH₂N-C-S) and was assigned the structure methyl (2-methylthio-3-imidazolin-2-yl)carbodithioate. Derivative VI was found to be identical with the product obtained when ETM (III) in chloroform was treated with ethanolic sodium borohydride and subsequently methylated with dimethyl sulfate-sodium bicarbonate. The products

obtained by both methods were found to cochromatograph on TLC chloroform, R_f 0.13; methanol, 0.63; chloroform-1-butanol-methanol-water (100:5:1:0.5) (0.68).

The basic oxidized and methylated fraction (soluble in dilute acid) contained a mixture of products. Spectroscopic analysis of the major isolated product [NMR δ 3.77 (NCH₂, s, 4 H), 2.58 (C(S)SCH₃, s, 3 H), 2.40 (N(CH₃)C(S)); IR 2930, 2880, 2820 (CH₃, CH₂), 1540 (thiocarbamoyl ester) 1360, 1190 (C-S), and 930 cm⁻¹; UV 238 and 305 nm; MS M⁺ 268 (very weak), m/e 221 (M - SCH₃), 191, 159, 148, 134, 94, 86, 75] suggested that it was *N,N*-dimethylethylenebisdithiocarbamate dimethyl ester (VII). Also present in this fraction were small quantities of derivative VI. These results are summarized in Figure 2, together with a possible mechanistic route to the formation of the observed products. Oxidative loss of sodium hydrogen sulfide from nabam results in β -dithiocarbamoyl ethylisothiocyanate (VIII), which may subsequently rearrange via ring closure to (IX). Intermediate IX may be subsequently oxidized to the level of a disulfide resulting in ETM or may be trapped by reaction with dimethyl sulfate resulting in V and VI. Oxidation of hydrogen sulfide results in elemental sulfur.

Because field residues of EBDCs may also contain ETM, a decomposition product that may further degrade to ETU in vitro (Marshall, 1977) or in vivo (Iverson et al., 1977), the fate of this product on hypochlorite oxidation was also studied. Suspensions of ETM in 1% bicarbonate were observed to consume less than 4 equiv of hypochlorite per sulfur equivalent in the substrate (Table I) and when repeated in 1 N sodium hydroxide, ETM was found to consume 12.29 equiv. When sulfate production is measured, ETM was oxidized to 3.15 and 3.18 equiv of sulfate. Blank determinations resulted in 0.13 and 0.16 equiv. Thus 3 equiv of sulfate is generated per equivalent of ETM. Analysis of ethyleneurea from an oxidation in phosphate buffer (pH 10) resulted in 0.45 equiv of this product per equivalent of ETM. Thus it is suggested that hypochlorite oxidation of ETM consumes up to 12 equiv of oxidant and results in 3 equiv of sulfate. It appears

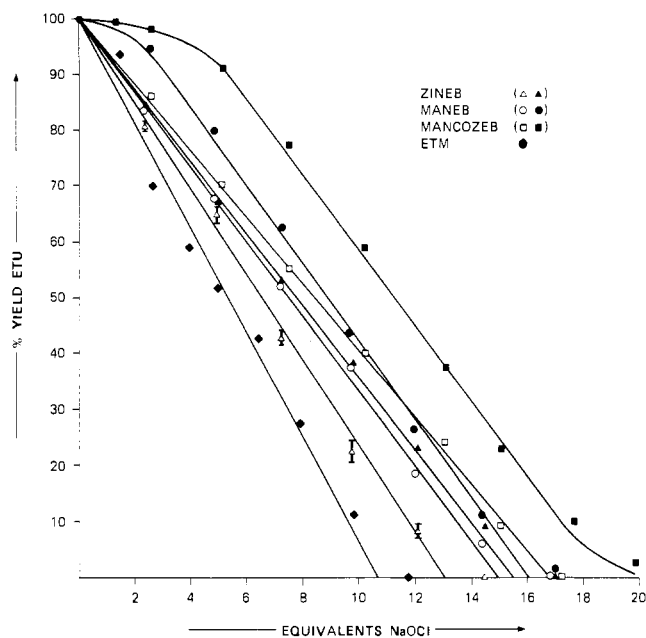


Figure 3. Variation in inactivation of EBDC formulations and of ETM (N NaOH, \blacklozenge) with increasing oxidation by hypochlorite, zineb (N NaOH, \blacktriangle , 1% bicarbonate \blacktriangle), maneb (O, \bullet), mancozeb (□, \blacksquare). Error bars represent the standard error for inactivation runs in triplicate while each point represents the average for analyses in duplicate.

likely that the thiocarbonyl carbon of the substrate is oxidized to the level of CO_2 ; however, this was not verified experimentally.

The oxidation of carbon disulfide suspended in basic medium was also monitored in the presence and absence of molybdate (an additive known to increase sulfate formation in oxidations of carbon disulfide by chloramine-T, Rao and Murthy, 1960). This substrate consumed less than the theoretical amount of oxidant (Table I); however, results were variable due to problems of solubility. Within experimental error no increase in sulfate formation was observed when 1% ammonium molybdate was added to the oxidation medium. The use of methanol as a substrate carrier in this oxidation is precluded by the fact that it too reacts with the oxidant.

In a final series of experiments an attempt was made to measure how well an incomplete oxidation would inactivate EBDC formulations so as to prevent subsequent decomposition to ETU. Swirled suspensions (0.2% weight) of EBDC formulations in 1 N sodium hydroxide or in 1% sodium bicarbonate were treated with hypochlorite (0 to 20 equiv) for 15 min. The reaction was quenched with a suitable antichlor (sodium sulfite) and the suspensions were boiled gently for 2 h on a steam bath to ensure complete conversion of unreacted residues to ETU. Suspensions were filtered, diluted to volume, and analyzed for ETU. Results, which are recorded in Figures 3 and 4, are expressed as percent yield of ETU relative to the controls in which no hypochlorite was added. Results obtained when the contact time of the formulation (zineb or maneb) with hypochlorite was reduced from 15 to 5 min are recorded in Figure 4.

These curves suggest that three formulations are all susceptible to oxidative inactivation. Moreover a linear dependence of the degree of inactivation upon the number of equivalents of oxidant was observed. Specifically no promotion of ETU formation was evidenced even when small quantities of oxidant were used. That the inactivation is rapid is demonstrated by the curves of Figure 4.

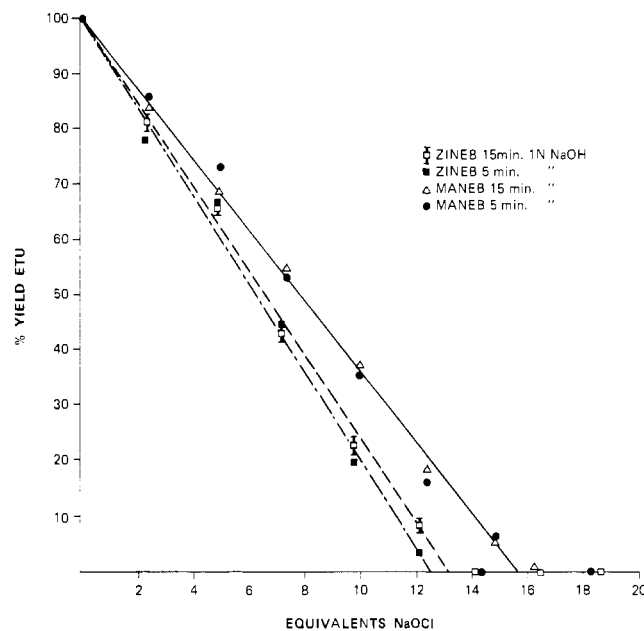


Figure 4. Variation in inactivation of zineb (15 min \square , 5 min \blacksquare) and of maneb (\triangle , \bullet) with increasing oxidation by hypochlorite in N NaOH.

Within experimental error no differences were observed between reaction times of 5 and 15 min for either zineb or maneb in 1 N sodium hydroxide. A decomposition study where ETM in 1 N sodium hydroxide was similarly inactivated is included in Figure 3 for comparison.

The rates with which these reactions proceed is dependent upon the solubility of the substrate and of elemental sulfur in the oxidation medium and on the pH of that medium. It has been demonstrated previously (Choppin and Faulkenberry, 1937) that when sulfide is oxidized in basic medium the ratio of sulfate to elemental sulfur observed reaches a minimum at pH 10. In any event oxidative inactivation of the three formulations studied and of ETM is rapid and does not seem to be pH dependent over the range studied.

These results suggest that it should be possible to inactivate surface residues of EBDCs, ETM, and ETU on fresh produce prior to industrial processing by adding hypochlorite to an alkaline water wash. Although EU represents an innocuous oxidation product (Sander, 1971), in the presence of nitrite, it readily forms a nitroso derivative, which has been demonstrated to be a potent tumorigen to rats (Sander, 1971). Since EU is oxidized at high pH and for reasons of cost strong base would appear to be the medium of choice for this inactivation.

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Heliocide H₁. A New Insecticidal C₂₅ Terpenoid from Cotton (*Gossypium hirsutum*)

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Two new C₂₅ terpenoids, heliocide H₁ and heliocide H₄, have been isolated from domestic cotton (*Gossypium hirsutum*). They were synthesized by the Diels-Alder reaction of hemigossypolone and *trans*- β -ocimene. The stereochemical configuration of these compounds was deduced from the ¹³C NMR spectrum. Heliocide H₁ is toxic to *Heliothis virescens* and appears to be involved in the host-plant resistance of glanded cotton to this insect pest. Heliocide H₄ shows a very low level of toxicity to *H. virescens*.

Some primitive and wild strains of cotton (*Gossypium hirsutum* L.) are more resistant to bollworms and tobacco budworms (*Heliothis* spp.) than cultivated cottons (Lukefahr et al., 1969; Shaver and Lukefahr, 1971). This resistance was correlated with high concentrations of five terpenoids isolated from flower bud extracts (Stipanovic et al., 1976; Seaman et al., 1977). One of these compounds was the sesquiterpenoid, hemigossypolone (HGQ, **5**) (Stipanovic et al., 1976; Gray et al., 1976). Two other compounds were identified as C₂₅ terpenoid derivatives of hemigossypolone, heliocide H₂ (H₂, **2**) (Stipanovic et al., 1977) and heliocide H₃ (H₃, **3**) (Stipanovic et al., 1978). We now report the identification and synthesis of two new C₂₅ terpenoids, heliocide H₁ (H₁, **1**) and heliocide H₄ (H₄, **4**).

EXPERIMENTAL SECTION

Isolation of Heliocide H₁. Young bolls (2–3 days old) and bracts of the *Heliothis* resistant commercial variety, HG-6-1N, were collected in the field, stored over ice, frozen to -20 °C, and lyophilized. This material was ground to a powder in a blender, extracted, and chromatographed as previously reported (Stipanovic et al., 1978). The heliocides gave bright yellow-orange spots when sprayed with concentrated HCl and 5% ethanolic phloroglucinol (1:1). HGQ and gossypol gave magenta spots. Heliocide H₁ was obtained as a dark oil, that eventually crystallized on scratching and cooling (mp 110–112 °C/hexane).

Isolation of Heliocide H₄. Although heliocide H₄ was present in *G. hirsutum* it was more easily isolated from *G. barbadense* (Seabrook Sea Island). Young bolls (2–3 days old) of Seabrook Sea Island 12B2 were collected and extracted as in the isolation of heliocide H₁. The residue from 100 g of freeze-dried powder was chromatographed over 20 g of Si gel using EtOAc-hexane-HOAc (10:90:0.25, solvent 1) as the developing solvent. The first 20 mL of colored material eluting from the column was rechromatographed on 20 g of Si gel using CHCl₃-HCOOH (99.5:0.5) as the eluting solvent. The first 80 mL of colored material was then chromatographed over 30 g of Si gel eluting with Et₂O-hexane-HCOOH (10:90:0.5). Fractions (15 mL) were collected and heliocide H₄ was found in fractions 2–4. These fractions were combined and chromatographed over Si gel plates developing with cyclohexane-acetone-HCOOH (95:5:0.5). Heliocide H₄ (R_f 0.35) gave a yellow color with phloroglucinol reagent. Heliocide H₄ was finally purified by TLC on Si gel with two successive developments in solvent 1. Heliocide H₄ was very unstable and was isolated as a dark oil.

Synthesis of Heliocides H₁ and H₄. HGQ (50 mg) was dissolved in benzene (1 mL) and *trans*- β -ocimene (**6**) (90% *trans* and 10% *cis*, provided by International Flavors and Fragrances, Inc.) (10 mL). The reaction mixture was protected from light and stirred under nitrogen for 5 days. The excess ocimene was removed as an azeotrope with methanol (4 × 300 mL) under reduced pressure. The product was chromatographed over Si gel (10 g) using hexane-EtOAc-HOAc (89:10:1). The first yellow band (H₁ and H₄) was clearly separated from the trailing orange

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