Reduction of the Formation of Ethylenethiourea from Ethylenebis(dithiocarbamates) by Cupric Ions in Aqueous Media

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The formation of ethylenethiourea by the thermal degradation of ethylenebis(dithiocarbamates) in aqueous media was greatly reduced by the addition of copper salts. The evolution of CS_2 and the decomposition of either nabam or maneb were decreased in the presence of $CuSO_4$ and Copper-Count-N. The formation of a stable cupric ethylenebis(dithiocarbamate) complex is postulated as the reason for this effect.

The base-catalyzed degradation of ethylenebis(dithiocarbamate) fungicides (EBDC) to ethylenethiourea (ETU) during processing of food has been a matter of concern in recent years (Engst et al., 1977). While the parent compound is relatively nontoxic to humans, its degradation product ETU has been found to have carcinogenic and teratogenic effects in laboratory animals (Larsson et al., 1976; Graham et al., 1975; Graham, 1973; Graham and Hansen, 1972; Khera, 1973; Innis et al., 1969). Although fresh produce may not have detectable residues of ETU, residues of EBDC's constitute a potential source when the vegetable is heated during processing. The degradation was shown to be pH dependent (Marshall, 1977), and the use of a hypochlorite wash prior to processing to oxidize the EBDC to innocuous metabolites was suggested (Marshall and Singh, 1977).

EBDC fungicides are applied to tomatoes to prevent diseases such as early blight (*Alternaria solani*), late blight (*Phytophthora infestans*) and anthracnose (*Collectotrichum phomoides*), while other diseases such as bacterial speck (*Pseudomonas tomato*) and bacterial spot (*Xanthomonas vesicatoria*) are controlled by the application of copper salts (Kocide, Bordeaux) (Ontario Ministry of Agriculture and Food, 1979). Mixed sprays of EBDC's and copper have also been found to give better results against bacterial speck than copper alone (Pitblado, 1978).

EBDC's are applied as their manganese and zinc complex (maneb or mancozeb) on the crop. The solubility, activity, and stability of the dithiocarbamate complex are dependent on the metal ion (Eckert, 1957; Regenass et al., 1955). In the presence of copper ions, it seemed probable that these three characteristics could be altered. The study of the influence of cupric ions on the in vitro thermally induced decomposition of EBDC was thus undertaken.

MATERIALS AND METHODS

The EBDC (0.80 mg of active ingredient) was added to 200 mL of distilled water with various amounts of copper (as $CuSO_4$ or Copper-Count-N fungicide, 0.08–8.0 mg). The mixture was boiled for 1 h, and the gases formed were entrained by suction through two traps: the first contained 10 mL of aqueous NaOH (6.5%) and 5 mL of toluene to trap any H_2S evolved, while the second contained 12.5 mL of diethanolamine (1.25 g) and cupric acetate (0.6 mg) in ethanol which reacted with the CS_2 evolved. The resulting dithiocarbamate was assayed spectrophotometrically at 435 nm (Keppel, 1969). Each analysis was done in triplicate.

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Table I. Degradation of Maneb in the Presence of $CuSO_4$

Cu^{2+}/maneb, molar ratio	$CuSO_4$/maneb, wt ratio	CS_2, mg	ETU, mg	ETU, % conversion
16.6	10	0.01 ± 0.01	Tr	
5.0	3	0.01 ± 0.01	0.03 ± 0.03	10
1.7	1	0.01 ± 0.01	0.04 ± 0.03	13
1.1	0.66	0.06 ± 0.01	0.11 ± 0.05	36
0.5	0.33	0.15 ± 0.02	0.17 ± 0.04	55
0.17	0.1	0.27 ± 0.06	0.16 ± 0.04	52
0	0	0.31 ± 0.17	0.18 ± 0.06	58

The aqueous residue was then evaported under vacuum at 60 °C to 20 mL and transferred to a 25-mL volumetric flask. The presence of ETU was verified by liquid chromatography (50 ng → 4-cm deflection at 0.08 auf).

The materials used were as follows: LC assay, Tracor Model 5000 liquid chromatograph, Spectromonitor I UV detector at 230 nm; column, Partisil SCX 10 μm; eluant, 20% MeOH in water at 1 mL/min; ETU, practical grade from Eastman Kodak Ltd., Rochester, NY, recrystallized from 95% ethanol; manganese EBDC, as Dithane-M22 (80% maneb); disodium EBDC (nabam), prepared from CS_2 and ethylenediamine dihydrochloride (Engst and Schnaak, 1967); copper EBDC, prepared by addition of a solution of $CuSO_4$ to the disodium EBDC and collecting the precipitate by centrifugation; Copper-Count-N (8% copper as cupric ammonium carbonate), obtained from Mineral Research and Development, Charlotte, NC.

RESULTS

The degradation of maneb in the presence of $CuSO_4$ was first attempted with weight ratios of the two compounds ranging from 0.1 to 10 (Table I). Both ETU and CS_2 were measured because EBDC's may be degraded to different products, depending on the pH (Figure 1) (Marshall, 1977).

Monitoring only CS_2 was not sufficient because in a mildly acidic medium (pH 5–7) the two degradative pathways may occur concomitantly and an intermediate yield of CS_2 would be expected. By the same token, failure to detect any ETU would not preclude decomposition via an alternate pathway. Also, the assay of ETU by liquid chromatography, using a UV detector, is not as sensitive as the CS_2 evolution assay (~1 ppm ETU vs. 0.01 ppm CS_2).

As can be seen from Table I, increasing the amount of $CuSO_4$ reduced drastically the conversion of maneb to ETU, down to trace amounts when an excess (ten times) of $CuSO_4$ was present. Although the evolution of CS_2 and the presence of ETU followed the same downward trend, they did not parallel each other. Indeed, when small amounts of $CuSO_4$ were present, the amount of CS_2 evolved was greater than would be expected from the degradation to ETU. It is thus probable in this case that

0021-8561/80/1428-0787$01.00/0

Table II. Degradation of Maneb in the Presence of Copper-Count-N

Cu^{2+}/maneb, molar ratio	Copper-Count-N/ maneb, wt ratio[a]	CS_2, mg	ETU, mg	ETU, % conversion	pH, final
5	20	Tr	Tr		6.5
1	4.2	Tr	Tr		
0.66	2.8	Tr	0.10 ± 0.01	32	5.6
0.33	1.4	0.03 ± 0.02	0.13 ± 0.01	42	5.7
0.1	0.4	0.15 ± 0.03	0.27 ± 0.02	88	5.2

[a] Weight of copper; formulation contained 8% copper.

Table III. Degradation of Nabam in the Presence of $CuSO_4$

Cu^{2+}/nabam, molar ratio	$CuSO_4$/nabam, wt ratio	CS_2, mg	ETU, mg	ETU, % conversion	pH initial	pH final
8.0	5	Tr	Tr		5.1	4.3
1.6	1	0.01 ± 0.00	0.08 ± 0.05	25	4.8	4.6
1.1	0.66	0.10 ± 0.02	0.16 ± 0.02	50	5.0	5.0
0.50	0.33	0.13 ± 0.02	0.18 ± 0.01	56	5.0	5.9
0.16	0.10	0.14 ± 0.02	0.22 ± 0.03	69	5.3	6.3
0	0	0.15 ± 0.02	0.23 ± 0.02	72	5.1	6.4
0[a]		0.30 ± 0.14				

[a] 4 N HCl and $SnCl_2$ added.

Table IV. Degradation of Nabam in the Presence of Copper-Count-N

Cu^{2+}/nabam, molar ratio	Copper-Count-N/ nabam, wt ratio	CS, mg	ETU, mg	ETU, % conversion	pH initial	pH final
20	5	Tr	Tr		6.0	5.6
2.7	0.66	Tr	Tr		5.9	5.7
1.3	0.33	0.01 ± 0.01	0.04 ± 0.01	13	5.6	5.8
0.4	0.10	0.09 ± 0.01	0.13 ± 0.03	41	5.4	5.9

the EBDC is transformed to both ETU and ethylenediamine (EDA). But then, as the proportion of $CuSO_4$ became greater, the CS_2 evolved was not sufficient to account for the ETU present, which may be attributed to the fact that ETU can be found in the formulation (5–7% in ours) or to the formation of ETU after the assay for CS_2, during the concentration of the solution. The latter possibility is unlikely in view of the fact that the concentration of the solution by evaporation was performed under reduced pressure at a lower temperature (60 °C) than that at which the assay was performed (100 °C). However, the removal by vacuum of any CS_2 evolved could be considered as a driving force in favor of the decomposition to ETU. In any case, when a large excess of $CuSO_4$ was present in the solution, no ETU and only trace amounts of CS_2 could be detected. It is possible under such conditions that ethylenethiuram monosulfide was the final product, although this is not very likely because of the high temperature (Marshall, 1977).

To verify whether cupric ions were indeed responsible for this drastic reduction in the formation of ETU and to possibly extend these observations to actual agricultural practice where a copper containing pesticide would be applied in lieu of $CuSO_4$, we repeated the experiment, using Copper-Count-N as a source of Cu^{2+}. The pH of the solution was also monitored after the assay. The results are listed in Table II. As it appears that the reduction in the formation of ETU could be attributed to the presence of cupric ions, the comparisons between molar ratios of Cu^{2+} to fungicide becomes significant. For this purpose, Cu^{2+} was considered the active ingredient, and $CuSO_4$ was therefore treated as a solid with 40% Cu^{2+} as the active ingredient. By comparison of the results in Table II and Table I, a similar decrease in the evolution of CS_2 as the concentration of Cu^{2+} was increased is observed. The amount of ETU present was also much larger than would be predicted from the CS_2 evolved. It would seem that the

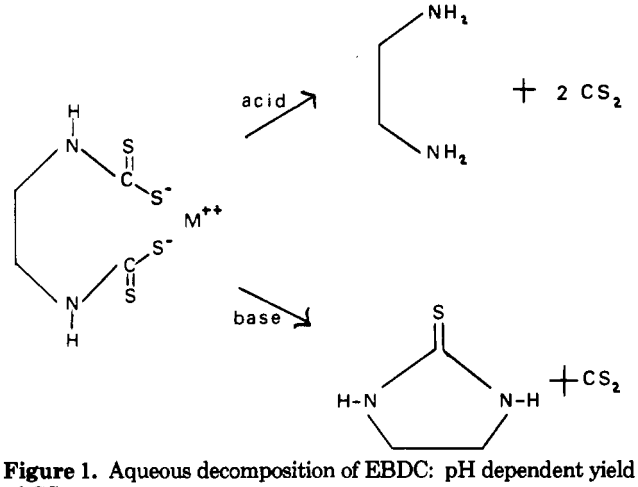

Figure 1. Aqueous decomposition of EBDC: pH dependent yield of CS_2.

evolution of CS_2 was prevented by the Copper-Count-N fungicide where copper is present as cupric ammonium carbonate, a salt more basic than cupric sulfate. One would expect it to act as a buffer and keep the pH higher. However, the decreasing trend in CS_2 evolution here seems to be the reverse of what would be expected on the basis of pH alone (Marshall, 1977). Only slight variations of pH were observed, but the amount of ETU found did not increase accordingly. The effect of cupric ions seems to be of a greater magnitude than that of pH.

If, as to be expected, the effect of Cu^{2+} was the result of competition between cupric and manganese ions for the EBDC moiety, then a similar effect, but maybe of different magnitude, would be observed if manganese was replaced by another ion. To verify this hypothesis, we repeated the experiment with the disodium EBDC (nabam), both with $CuSO_4$ and Copper-Count-N. The results are listed in Tables III and IV, respectively. As observed in the pre-

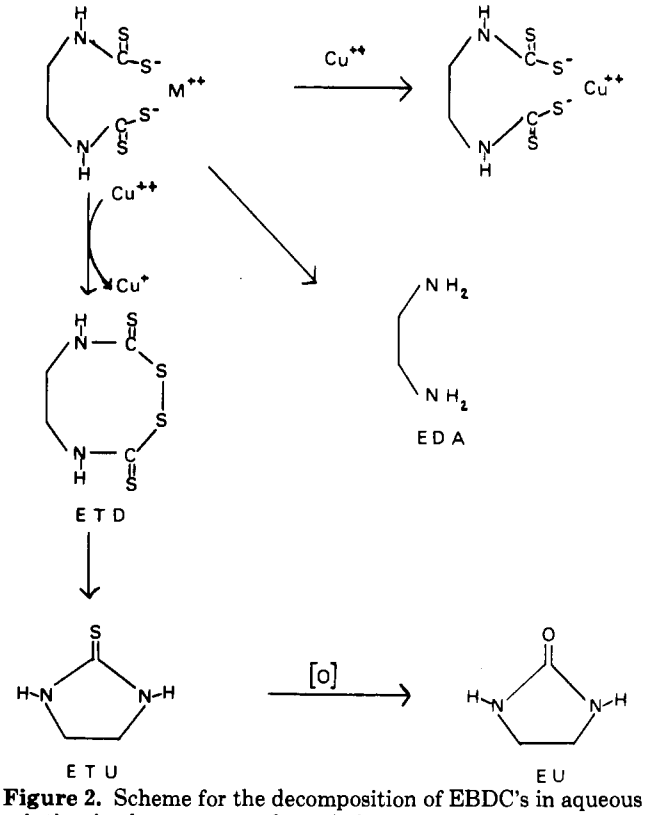

Figure 2. Scheme for the decomposition of EBDC's in aqueous solution in the presence of cupric ions.

vious experiment, an increase in the concentration of Cu^{2+} was accompanied by a decrease in ETU formation and CS_2 evolution. In this case, however, since pure nabam was used, higher amounts of ETU than those predicted by the CS_2 evolved cannot be accounted for by impurities, as in the maneb formulation. The half-life of nabam in aqueous solution is quite short (4–5 h at RT), and losses of CS_2 before the assay were almost inevitable. Indeed, when the degradation was carried out in acidic medium, so that the formation of 2 mol of CS_2 from the EBDC would be expected, about twice as much CS_2 was obtained, but it represented about half of the theoretical yield.

The pH of the solutions was meaured before and after boiling. Only minor variations were noted throughout and the decrease in formation of ETU cannot solely be attributed to pH. In the case of $CuSO_4$, the pH at the beginning was mildly acidic (pH 4.8–5.3) at all concentrations, and the formation of ETU seemed to be accompanied by an increase in pH. When the Copper-Count-N was used, the production of ETU was also accompanied by a slight increase in pH (5.4–5.9). Although the initial pH of the solution was higher in the experiments where Copper-Count-N was used than in those where $CuSO_4$ was used, the amount of ETU formed, at the equivalent molar content of Cu^{2+}, was similar. Therefore it appears that the concentration of Cu^{2+} has a stronger influence than pH has on the conversion of EBDC's to ETU.

In consideration of the substrates again, it seems that both nabam and maneb behave similarly, except for a slightly greater degradation of nabam. This is as expected on account of the difference in the relative stability of the two compounds. This does not seem to apply when Copper-Count-N was used as a source of Cu^{2+} as more decomposition product was obtained from maneb. If a stable copper complex were formed, it would be formed more readily from the soluble sodium salt and therefore less decomposition would be observed. However, the magnitude of these effects remains comparatively smaller than that of Cu^{2+}. The extent of the degradation was also independent of the matrix. When 0.10 mg of maneb was boiled in tomato juice to which 1 molar equiv of Cu^{2+} (as $CuSO_4$) was added, 0.01 mg of CS_2 was recovered as compared to 0.06 mg of CS_2 obtained from 0.80 mg at 1.1 $CuSO_4$/maneb ratio (Table I).

An attempt was made to elucidate the mechanism by which the formation of ETU is prevented. The most obvious interaction is the replacement of the metal ion by Cu^{2+} to form a new complex, a cupric EBDC. This cupric EBDC was first mentioned by Barratt and Horsfall (1947), who noted its low fungicidal activity. It is extremely insoluble and very stable. If it were formed, it would not likely be degraded in boiling water during the same time period as maneb. A cupric EBDC complex (0.52 mg) submitted to the same degradation produced only 0.02 mg of CS_2 compared to 0.31 mg of CS_2 from 0.80 mg of maneb in the absence of Cu^{2+} (Table I).

Another possibility is the cupric ion catalyzed oxidation of the substrate or some of its metabolites. If the EBDC were first oxidized, the production of ETU would be increased since the direct pathway to ethylenediamine would be shunted in favor of the formation of ethylenethiuram disulfide (EDT) (Figure 2). This was not however the case since the amount of ETU was decreased. Further oxidation to EU could be postulated, but this mode is usually prevalent in basic medium. The possibility of the reduction of Cu^{2+} to Cu^{+} was verified by titration of the solution with sodium thiosulfate prior to which residual Cu^{2+} was complexed with sodium citrate (Quadir and Kundar, 1953). No Cu^{+} could be detected after 1 h of boiling in an aqueous solution of maneb, nabam, or ETU.

Interactions with copper may be part of the reason why field weathered residues were showing a lower conversion rate to ETU than material freshly spiked with the EBDC's (Engst et al., 1977).

CONCLUSION

The thermal degradation of EBDC's in aqueous media diminished in the presence of cupric ions. This could have implications in the canning industry since the formation of ETU from EBDC during heating of the produce could be prevented as long as the concentration of copper exceeded that of the EBDC residues. This could be easily achieved since copper-containing compounds are already applied as bactericides to the same crops as the EBDC fungicides.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the excellent technical assistance of D. Pohlman.

LITERATURE CITED

Barratt, R. W., Horsfall, J. G., *Bull.—Conn. Agric. Exp. Stn., New Haven* **508**, 1 (1947).
Eckert, G., *Z. Anal. Chem.* **155**, 23 (1957).
Engst, R., Schnaak, W., *Z. Lebensmitt.-Unters.-Forsch.* **134**, 216 (1967).
Engst, R., et al., *Pure Appl. Chem.* **49**, 675 (1977).
Graham, S. L., *J. Agric. Food Chem.* **21**, 324 (1973).
Graham, S. L., Davis, K. J., Hansen, W. H., *Food Cosmet. Toxicol.* **13**, 493 (1975).
Graham, S. L., Hansen, W. H., *Bull. Environ. Contam. Toxicol.* **7**, 19 (1972).
Innis, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchel, I., Peters, J., *J. Natl. Cancer Inst. (U.S.)* **42**, 1101 (1969).
Keppel, G. E., *J. Assoc. Off. Anal. Chem.* **52**, 162 (1969).
Khera, K. S., *Teratology* **7**, 243 (1973).
Larsson, K. S., Arnander, C., Cekanova, E., Kjellberg, M., *Teratology* **14**, 171 (1976).

Marshall, W. D., *J. Agric. Food Chem.* **25**, 357 (1977).
Marshall, W. D., Singh, J., *J. Agric. Food Chem.* **25**, 1316 (1977).
Ontario Ministry of Agriculture and Food, "Vegetable Production Recommendations", Publication 363, Ontario, Canada, 1979, pp 64–65.
Pitblado, R. E., "Fungicides and Insecticides Trials Results", Ridgetown College of Agricultural Technology, Ridgetown, Ontario, Canada, 1978.
Quadir, S. J., Kundar, M. H., *J. Indian Chem. Soc.* **30**, 846 (1953).
Regenass, W., Fallab, S., Erlenmeyer, H., *Helv. Chim. Acta* **38**, 1448 (1955).

Received for review December 21, 1979. Accepted March 18, 1980. This paper was presented at the 178th National Meeting of the American Chemical Society, Washington, DC, Sept 1979.

Gas–Liquid Chromatographic Determination of Tyramine in Fermented Food Products

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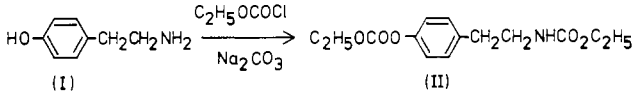
A simple and precise gas–liquid chromatographic method for the determination of tyramine in fermented food products has been described. Tyramine separated from foods, with purification by eluting through an Amberlite CG-120 column, was readily converted into the *N,O*-bis(ethyloxycarbonyl) derivative by the reaction with ethyl chloroformate, which was analyzed by gas–liquid chromatography, using 3,4-dimethoxyphenethylamine as an internal standard. Tyramine was clearly separated from other constituents in foods on a 1.5% OV-17, 0.2% SP-1000 mixed-phase column. The calibration curve for tyramine in the range of 2–100 μg is linear and sufficiently reproducible for quantitative determination. The coefficient of variation (n = 5) of the values determined is below 6.2%, and the average recovery rate throughout the procedure, including ion-exchange column chromatographic purification, derivatization, and gas–liquid chromatography, was above 94% in some kinds of foods investigated. The derivative preparation is simple and rapid, and the resulting stable derivative allowed us to develop a more precise and accurate method.

Tyramine is an indirectly acting sympathomimetic amine which releases norepinephrine from a sympathetic nerve ending, and it has been reported that tyramine-containing foods can cause unnatural and toxic effects (Stockley, 1973) when injested in large quantities. Blackwell et al. (1967) have suggested that foods containing tyramine may be particularly dangerous for patients receiving monoamine oxidase inhibitor. Tyramine and tyramine-containing foods can also cause dietary migraine patients to suffer a classical migraine attack (Hanington, 1967; Smith et al., 1971) and they produce vasoconstriction and consequently a rise of blood pressure in patients with carcinoid syndrome (Waldenstrom et al., 1956; Levine and Sjoerdsma, 1963) and with phaechromocytoma (Engelman and Sjoerdsma, 1964).

From this point of view, tyramine has been determined in foods in a variety of ways, including fluorometric (Spector et al., 1963; Price and Smith, 1971; Horwitz et al., 1964) and gas–liquid chromatographic (GLC) (Sen, 1969; Kaplan et al., 1974) methods. The presence of tyramine in foods has been reviewed (Lovenberg, 1974). In general, the fluorometric method is very sensitive but is not specific enough to determine tyramine in complex samples such as foods; the GLC technique offers a more specific method. In the GLC method described above, tyramine was analyzed as its trifluoroacetyl derivative. For sound quantitative routine GLC determination, it is desirable to convert tyramine into a more stable derivative.

We have reported that both phenolic hydroxyl and amino groups can be readily alkyloxycarbonylated with alkyl chloroformates in aqueous alkaline medium, and this reaction can be applicable to a derivatization method for microscale GLC analysis (Makita et al., 1976). On the basis of this observation, we have developed a simple and precise GLC method for the determination of tyramine in foods, and this method has been successfully applied to some Japanese fermented foods. Tyramine (I), separated from foods by using a Amberlite CG-120 column, was converted into the *N,O*-bis(ethyloxycarbonyl) (EOC) derivative (II)



(I) (II)

by reaction with ethyl chloroformate at room temperature, and the resulting derivative was determined by GLC using 3,4-dimethoxyphenethylamine as an internal standard.

EXPERIMENTAL SECTION

Materials. Each sample was purchased over the counter at local supermarkets and was treated for analysis on the same day.

Reagents. Tyramine hydrochloride and 3,4-dimethoxyphenethylamine hydrochloride, used as an internal standard, were obtained from Sigma Chemical Co. Standard solutions of tyramine (10 and 100 μg/mL, as free base) were prepared in water, and aliquots were taken for the preparation of the calibration curve and for the calculation of the recovery rate from food samples. Solutions of the internal standard in water were prepared at concentrations of 20 and 100 μg/mL, as free base, respectively. These solutions were stored in capped glass bottles at 4 °C. Ethyl chloroformate (Tokyo Kasei Kogyo, Tokyo, Japan) was used without further purification. Amberlite CG-120 resin (100–200 mesh) in the H^+ form was treated before use as follows: the resin was washed twice with 4 N HCl in a beaker and then covered with 2 N NaOH and swirled for 3 h at 70 °C after successive washing with water until approximately neutral. The resin was regenerated by washing three times with 4 N HCl and subsequently washed with water until neutral. With the use of resin as

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0021-8561/80/1428-0790$01.00/0