

THE AERATION PRODUCTS OF DISODIUM ETHYLENEBISDITHIOCARBAMATE¹

BY G. D. THORN² AND R. A. LUDWIG³

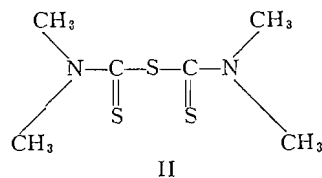
ABSTRACT

The aeration of dilute solutions of disodium ethylenebisdithiocarbamate yields hexahydro-1,3,6-thiadiazepine-2,7-dithione (I), polymeric I, 2-imidazolidinethione, and elemental sulphur. I with ammonia gives mainly hexahydro-1,3,5-triazepine-2,4-dithione. I and polymeric I have high antifungal activity.

The anomalous protective fungicidal action of the highly water-soluble disodium ethylenebisdithiocarbamate (nabam) has been investigated in this laboratory. It has been shown (9, 10) that the aeration of dilute solutions of nabam results in the formation of a yellow insoluble fungicidal material consisting mainly of a polymer, $(C_4H_6N_2S_3)_n$, together with up to 20% of the corresponding monomer and varying amounts of elemental sulphur. The filtrate of the reaction mixture yields further amounts of the monomer and nonfungicidal 2-imidazolidinethione (ethylenethiourea). The latter had been identified previously by Barratt and Horsfall (1) as a breakdown product of nabam.

The compound $C_4H_6N_2S_3$ (I) gives a positive sodium azide - iodine test (4) for thioketones and mercaptans. Reaction of I with Grote's reagent (5) gives a red-brown color. According to Grote, a red coloration indicates the presence of thiol, while a green or blue color is obtained with thioketones.

The ultraviolet absorption spectra of I and tetramethylthiuram monosulphide (II) are distinctly similar. For compound I, the peaks are at $280\text{ m}\mu$, $E_m = 19600$, and at $227.5\text{ m}\mu$, $E_m = 8800$; for tetramethylthiuram monosulphide maximum absorption is at $280\text{ m}\mu$, $E_m = 16300$, and at $210\text{ m}\mu$, $E_m = 17000$.



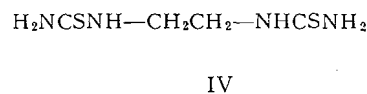
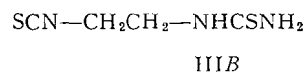
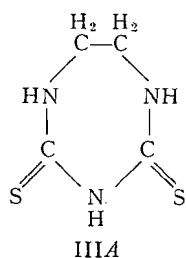
Reaction of I either with concentrated aqueous ammonia at room temperature or with liquid ammonia yields a compound $C_4H_7N_3S_2$, for which the structures IIIA and IIIB can be written. The reaction in aqueous ammonia

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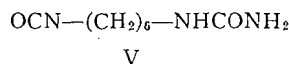
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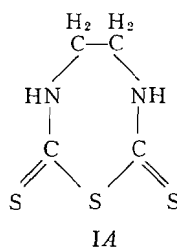


yields also a compound $\text{C}_4\text{H}_{12}\text{ON}_4\text{S}_2$, probably the monohydrate of the open-chain diamide, ethylenebisthiourea (IV). The attempted preparation of IV through reaction of excess concentrated aqueous ammonia with ethylenebis-isothiocyanate gave, surprisingly, the compound III in good yield. Petersen (13) has shown that the reaction of excess hexamethylenebis-isocyanate with ammonia yields 6-ureido-hexylisocyanate (V), which with excess ammonia is converted to the expected diurea. Structure III B for the compound

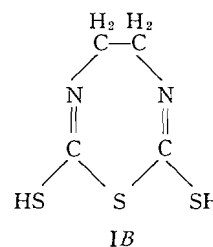


$\text{C}_4\text{H}_7\text{N}_3\text{S}_2$ is however ruled out, as the infrared spectrum of the compound shows no absorption in the 2100 cm^{-1} region attributable to the isothiocyanate grouping. Further, the spectrum in the "finger-print" region shows a very close similarity to that of ethyl dithiobiuret.⁴ The compound $\text{C}_4\text{H}_7\text{N}_3\text{S}_2$ must be therefore ethylenedithiobiuret (hexahydro-1,3,5-triazepine-2,4-dithione⁵) (III A).

From the foregoing, especially the reaction of I with ammonia and the independent synthesis of ethylenedithiobiuret, it can be concluded that the compound $\text{C}_4\text{H}_6\text{N}_2\text{S}_3$ (I) is ethylenethiuram monosulphide (hexahydro-1,3,6-thiadiazepine-2,7-dithione⁵):



or



The structure may be that of the thioamide, IA, or of the enethiol, IB. Our interpretation of the infrared spectrum favors the latter structure. The strong band at 1610 cm^{-1} is taken as indicative of $\text{C}=\text{N}$ (3); the bands attributable to the thioamide group (3, 14) are absent, and there is no evidence of $\text{N}-\text{H}$ stretching in the 3200 cm^{-1} region.

⁴Sample kindly supplied by Dr. G. Woolfe, Boots Pure Drug Co. Ltd., West Bridgford, Notts., England.

⁵Named in accordance with Patterson and Capell (12).

Ethylenethiuram monosulphide forms a hydrochloride, m.p. 210–211°C., a picrate, m.p. 171–172°C., a perchlorate, m.p. 182–183°C., and an acetyl derivative, m.p. 151–152°C.

The insoluble yellow polymeric ethylenethiuram monosulphide is obtained simply by removal of the more soluble components of the precipitate from the aeration of nabam by washing with water, drying the residue, and washing with cold chloroform. The material appears to be quite insoluble in all organic solvents tested. Its infrared spectrum shows the same bands of the monomer, some with slight shift in wave length. Digestion of the polymer with hot chloroform or acetone gives rise to a change in the elemental analysis (increased carbon and hydrogen values) of the residue together with the disappearance of the 1610 cm^{-1} band of the spectrum and the appearance of bands at 1475 cm^{-1} and at 3210 cm^{-1} (N—H), and also at 1705 cm^{-1} (C=O), when acetone is used as the digestion solvent. The nature of the materials formed was not investigated further.

The polymer can be obtained also in a crystalline form by allowing a 1000 p.p.m. solution of nabam to evaporate slowly (room temperature) in a shallow tray to about one half its volume, filtering, and washing as above. The material has a decomposition point of 145–147°C.

It was hoped that a convenient method of analysis of micro amounts of ethylenethiuram monosulphide and its polymer might be based on carbon disulphide evolution through acid hydrolysis (2). However, only roughly 10% of the theoretical amount of carbon disulphide was obtained on digestion with 1 *N* sulphuric acid for one hour. Lower recoveries resulted with stronger acid. The use of sodium bisulphite (6) was of no avail.

The synthesis of ethylenethiuram monosulphide was attempted using several methods. The reaction of ethylenethiourea with carbon disulphide at elevated temperatures and pressures (up to 20 atm.) yielded at best a small amount of odiferous brown material. More promising are the reaction of ethylenebisithiocyanate with hydrogen sulphide, of sodium β -aminoethyl-dithiocarbamate with thiophosgene, and of thiophosgene, sodium sulphide, and ethylenediamine. This work is as yet incomplete, but low yields have been obtained of yellow material possessing the required sulphur and nitrogen content, and showing a good measure of toxicity towards *Sclerotinia fructicola*.

The oxidation of nabam with the usual mild oxidizing agents—hydrogen peroxide, persulphate, iodine—resulted in the formation of the polyethylenethiuram disulphide (8).

EXPERIMENTAL⁶

Aeration of Nabam Solutions

The course of breakdown of nabam is affected markedly by solution concentration. It was found that 1000 p.p.m. nabam is optimal for the preparation of the fungicidal yellow precipitate without external control of pH. Accordingly, 150 liters of 0.1% nabam was aerated vigorously in a wooden cask.

⁶All melting points are uncorrected and were obtained on the Fisher-Johns block. E.D. 50 values were obtained by the spare drop technique using *Sclerotinia fructicola* as the test organism.

The pH, initially about 8.5, dropped rapidly to about 7.0, and slowly rose to between 8 and 9. The solution first turned orange in color, then became milky, and finally the yellow material separated out. The "reaction time" varied, but was usually three to four days. The solid was separated by basket centrifugation. The air-dried yield was 30 to 65 gm. The material obtained in this way analyzed 62% S (av.) and has been given the code G.D. 26 (10). It gave an E.D. 50 of 12-15 p.p.m.⁷

Polyethylenethiuram Monosulphide

The yellow material above was washed with water, dried, then allowed to stand with successive amounts of chloroform to remove the more soluble sulphur and ethylenethiuram monosulphide, to leave 70 to 80% by weight as insoluble material, m.p. 145-147°C. (decomp.). Found: C, 27.6; H, 3.27; N, 16.0; S, 54.6%. Calc. for $(C_4H_6N_2S_3)_n$: C, 27.0; H, 3.37; N, 15.7; S, 53.9%. The E.D. 50 value was 8-10 p.p.m.

Fractional crystallization of the chloroform soluble portion yielded elemental sulphur (up to 15% of total) and ethylenethiuram monosulphide.

Ethylenethiuram Monosulphide (I)

The filtrate from G.D. 26 was extracted by vigorous stirring with about 3 liters of chloroform. The lower layer was siphoned off, dried with anhydrous sodium sulphate, and the solvent removed *in vacuo* to leave a yellow residue, 6 to 15 gm., anal. 48 to 51% S. Repeated crystallization from chloroform-ethanol, and chloroform alone, yielded ethylenethiuram monosulphide, m.p. 125-6°C. Found: C, 27.3; H, 2.97; N, 15.9; S, 54.2%; mol. wt., 176 (isopiestic (11)). Calc. for $C_4H_6N_2S_3$: C, 27.0; H, 3.37; N, 15.7; S, 53.9%; mol. wt., 178. The E.D. 50 value was 1.5 p.p.m.

Concentration of the extracted aqueous portion to small volume and extraction with ether gave ethylenethiourea, 7 gm. (after removal of a small amount of ethylenethiuram monosulphide with chloroform and crystallization from ethanol), m.p. 197-198°C., not depressed on admixture with authentic ethylenethiourea. Calc. for $C_3H_6N_2S$: S, 31.4%. Found: S, 31.5%.

Ethylenedithiobiuret (Hexahydro-1,3,5-triazepine-2,4-dithione) (IIIA)

A. Ethylenethiuram monosulphide, 0.94 gm., was covered with about 5 ml. liquid ammonia and the mixture allowed to stand for 24 hr. Evaporation of the ammonia from the resulting solution left a pale yellow residue, which after crystallization from ethanol gave white material, m.p. 190-192°C. The compound can be sublimed at 140°C. under 0.001 mm. without apparent decomposition, but without sharpening of the melting point. Found: C, 29.9; H, 4.60; N, 26.1; S, 39.7%. Calc. for $C_4H_7N_3S_2$: C, 29.8; H, 4.35; N, 26.1; S, 39.8%. The material gave an E.D. 50 value of 75-80 p.p.m.

B. The reaction of ethylenethiuram monosulphide with aqueous concentrated ammonia at room temperature gave mainly the cyclic imide, ethylenedithiobiuret, with some open-chain amide, ethylenebisthiourea. The total yield was about 90%.

⁷E.D. 50—effective dosage required for 50% inhibition of spore germination.

On one occasion, the amide was the main reaction product. A constant melting point of 195–196°C. (decomp.) was obtained after two crystallizations from 95% ethanol. Found: C, 24.7; H, 6.25; N, 28.4; S, 32.6%. Calc. for $C_4H_{10}N_4S_2 \cdot H_2O$: C, 24.5; H, 6.13; N, 28.6; S, 32.7%. E.D. 50 value was above 100 p.p.m., the highest concentration tested.

C. The reaction of the polyethylenethiuram monosulphide with concentrated aqueous ammonia at room temperature also gave ethylenedithiobiuret in the same order of yield as in B; high analytical figures for N and low for S in the crude reaction product indicated, here also, the formation of some amide.

D. Ethylenebisisothiocyanate (8), 0.2 gm., was covered with 4 ml. conc. ammonium hydroxide and rubbed against the side of the flask with a glass rod. The white crystalline material formed was removed by filtration, washed with water, and crystallized from ethanol, giving 0.15 gm., m.p. 190–192°C., not depressed on admixture with material from A. Found: C, 30.2; H, 4.69; N, 26.1; S, 39.7%. Calc. for $C_4H_7N_3S_2$: C, 29.8; H, 4.35; N, 26.1; S, 39.8%.

Interaction of I with Hydrogen Chloride

A mixture of 0.95 ml. conc. hydrochloric acid and 0.10 gm. ethylenethiuram monosulphide in 25 ml. chloroform was shaken for five minutes. The solid was removed by filtration, washed with chloroform, and dried over sodium hydroxide *in vacuo*. The yield was 0.12 gm. of pale yellow material melting at 210–211°C. after crystallization from ethanol. The E.D. value was found to be 1–1.5 p.p.m. Found: C, 22.2; H, 3.01; N, 13.3; S, 44.6; Cl, 16.7%. Calc. for $C_4H_6N_2S_3 \cdot HCl$: C, 22.4; H, 3.26; N, 13.1; S, 44.7; Cl, 16.6%.

Addition of an equivalent of sodium hydroxide to a chloroform suspension of the compound gave back the ethylenethiuram monosulphide. The use of a greater excess of hydrochloric acid did not give a dihydrochloride.

Interaction of I with Acetic Anhydride

Ethylenethiuram monosulphide, 0.20 gm., was suspended in 8 ml. acetic anhydride and the mixture allowed to stand at room temperature for two hours. The resulting solution was poured over a small quantity of crushed ice. The orange precipitate was removed, washed well with water, and dried; yield, 0.16 gm. It melted at 151–152°C. (decomp.) after crystallization from chloroform. Found: C, 33.1; H, 3.23; N, 12.7; S, 43.2%. Calc. for the monoacetyl derivative, $C_6H_8ON_2S_3$: C, 32.7; H, 3.63; N, 12.7; S, 43.6%. The E.D. 50 value was above 100 p.p.m., the highest concentration tested.

The use of elevated temperatures for the reaction resulted in much decomposition with the formation of a tarry material.

I and Perchloric Acid

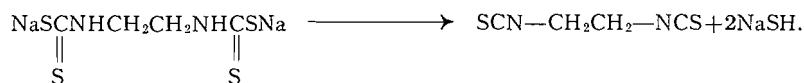
The addition of excess concentrated perchloric acid to a chloroform solution of ethylenethiuram monosulphide gave yellow material, which, when washed well with chloroform and ether, melted at 182–183°C. (decomp.). The yield was quantitative. Found: N, 10.1; S, 33.7; Cl, 12.8%. Calc. for $C_4H_6N_2S_3 \cdot HClO_4$: N, 10.1; S, 34.5; Cl, 12.8%.

I with Picric Acid

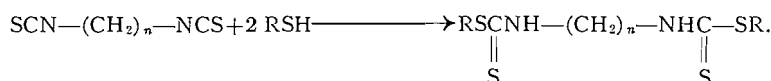
The addition of excess picric acid in ethanol to ethylenethiuram monosulphide in minimum chloroform solution gave on standing yellow crystals, m.p. 171–172°C. out of ethanol. Found: S, 24.2%. Calc. for C₄H₆N₂S₃·C₆H₃O₇N₃: S, 23.8%.

DISCUSSION

The formation of the yellow material, G.D. 26, would appear to explain the prolonged fungicidal activity when nabam is sprayed in the field. It does not however give any insight into the actual mode of fungicidal action. On the basis of fungistatic tests involving different mold species, Klöpping and van der Kerk (7, 8) concluded that "the antifungal activity of the bis-dithiocarbamates is due to their transformation into the corresponding diisothiocyanates":



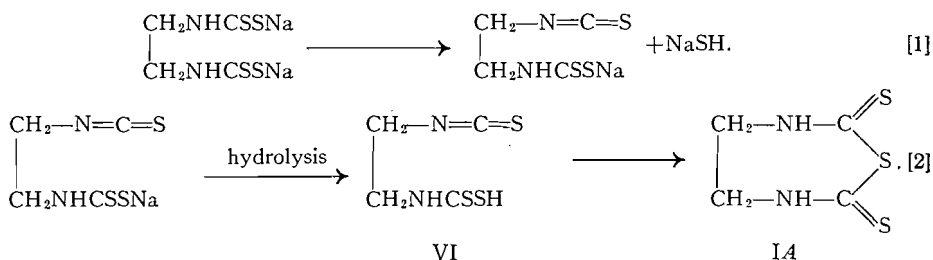
Sijpesteyn and van der Kerk (15) have shown that nabam and tetramethylenebis(iso)thiocyanate are rendered inactive by the addition of thiols to the culture medium. They believe this to be brought about by the formation of (stable) dithiocarbamate esters:



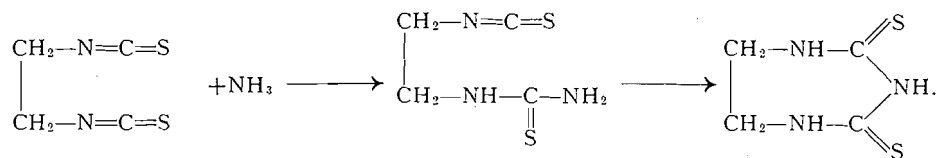
These authors have shown also that there is a marked decrease in toxicity for the reaction products of thioglycollic acid with the ethylene- and tetramethylenebis(iso)thiocyanates. They suggest therefore that the antifungal action of the bisdithiocarbamates and the bis(iso)thiocyanates is through reaction of these compounds with essential —SH compounds in the cells.

The present authors have found dimethyl ethylenebis(dithio)carbamate to possess an E.D. 50 value of 200 p.p.m. In contrast to this, nabam shows an apparent E.D. 50 value of 2–5 p.p.m., and ethylenebis(iso)thiocyanate an E.D. 50 value well below 1 p.p.m. This can be taken as evidence in support of the Dutch workers' theory.

Sijpesteyn and van der Kerk (15) consider that the formation of ethylenethiuram monosulphide serves as a direct indication for the transitory appearance of isothiocyanate groups. They offer the following equations:



The formation of ethylenedithiobiuret (IIIA) from ethylenebis(iso)thiocyanate and ammonia very likely proceeds by analogy with the equations above, and so lends support to the proposal of Sijpesteyn and van der Kerk:⁸



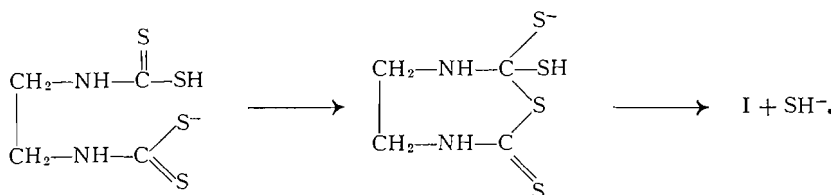
Moreover, the fungicidal action of ethylenethiuram monosulphide is antagonized by thiol compounds (15) in the same way as was demonstrated for nabam and tetramethylenebis(iso)thiocyanate. Sijpesteyn and van der Kerk state that this fact points to a common biochemical mode of action. They consider a reversal of the ring closure presented in equation [2] above (presumably induced by RSH) to form the isothiocyanate group (VI) requisite to their theory. Similarly, ethylenedithiobiuret should undergo the same ring fission and display the same order of fungitoxicity as ethylenethiuram monosulphide. The observed E.D. 50 value of ethylenedithiobiuret is rather high in comparison with that of the thiuram monosulphide. This would suggest that the modes of fungicidal activity are quite different, or perhaps that the dithiobiuret is more stable towards ring opening than is ethylenethiuram monosulphide. No chemical or physical evidence has yet been gathered to establish the formation of isothiocyanate through the proton-wandering postulated by Sijpesteyn and van der Kerk.

The actual demonstration of the presence of the isothiocyanate grouping is rendered difficult because of its high reactivity. This does not detract, however, from the possibility that the biochemical action of nabam and of the thiuram monosulphide is through transitory isothiocyanate which acts on —SH groups in the fungus cell.

ACKNOWLEDGMENTS

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⁸The referee has expressed skepticism of the formation of isothiocyanate in dilute aqueous solution, and suggested that the thiuram monosulphide could form as follows:



We tend to concur, however, with the proposal of van der Kerk and Sijpesteyn in the light of the considerable evidence, albeit mainly circumstantial, for the momentary existence of isothiocyanate.

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