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SYNTHESIS AND FUNGICIDAL ACTIVITY OF ALKANE-α,ω-BIS(2-HYDROXY ETHYL DISULPHIDE)S

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ABSTRACT. The synthesis and fungicidal activity of alkane-α,ω-bis(2-hydroxyethyl disulphide)s are reported. The compounds are new, and were characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometric techniques. The results of the biological screening showed very high fungicidal activity of the synthesized compounds.

INTRODUCTION

The first synthetic organic disulphide was made by Zeise [1] who distilled ethyl disulphide from a mixture of potassium ethyl sulphate and barium disulphide. At a later date, the same product was obtained by other workers [2-4]. Methods for the synthesis of organic disulphides had been reviewed [5-7].

Sulphur and its compounds particularly disulphides continue to have applications in agrochemicals, as shown by large varieties of new sulphur-based crop protection chemicals development around the world [8-10].

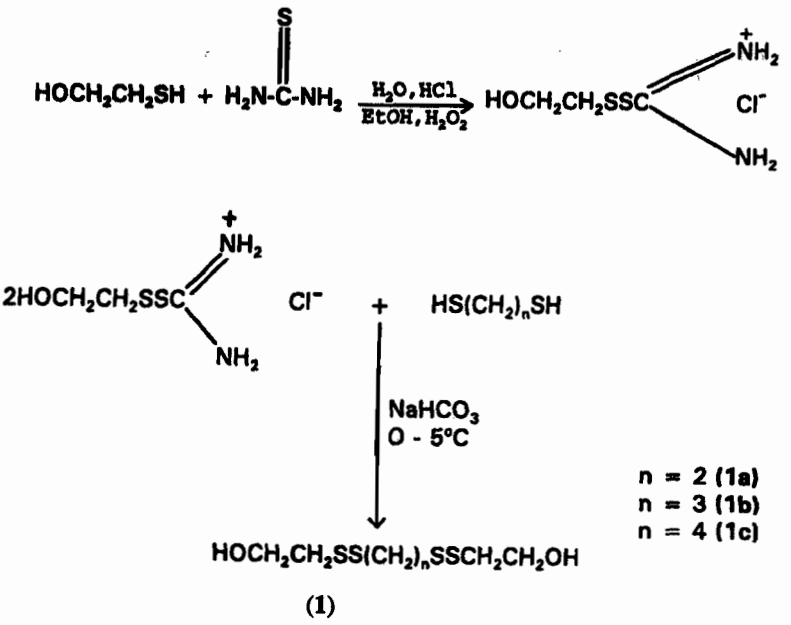
In the present study, the synthesis and fungicidal activity of alkane-α,ω-bis(2-hydroxyethyl disulphide)s (**1**, n = 2, 3, 4) are reported. The synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometric techniques. To our knowledge, no detailed study of the synthesis and fungicidal activity of these class of compounds have been reported in the literature.

$$HOCH_2CH_2SS(CH_2)_nSSCH_2CH_2OH$$

(1) n = 2 **(1a)**
n = 3 **(1b)**
n = 4 **(1c)**

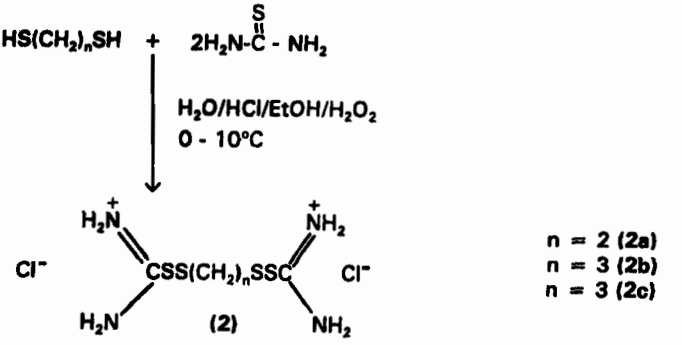
RESULTS AND DISCUSSION

Two methods were used to synthesize the novel compounds (**1**, n = 2,3,4), of which the first involved the reaction of *S*-(2-hydroxyethylthio)isothiouronium chloride with an α,ω-dimercaptoalkane in alkaline medium as shown in Scheme 1 [11].



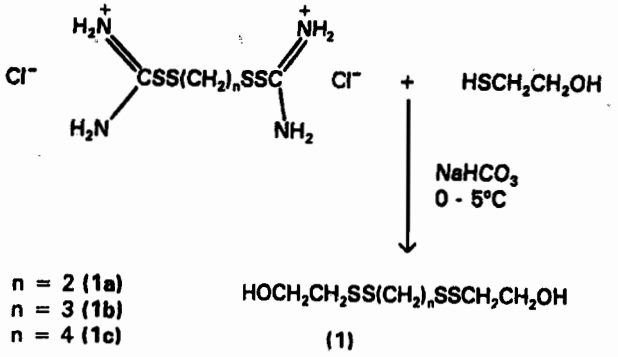
Scheme 1.

The second method involved two steps: step one was the reaction of the α,ω-dimercaptoalkane with thiourea as shown in Scheme 2 to give the alkane-α,ω-bis (thioisothiouronium chloride) (2).



Scheme 2.

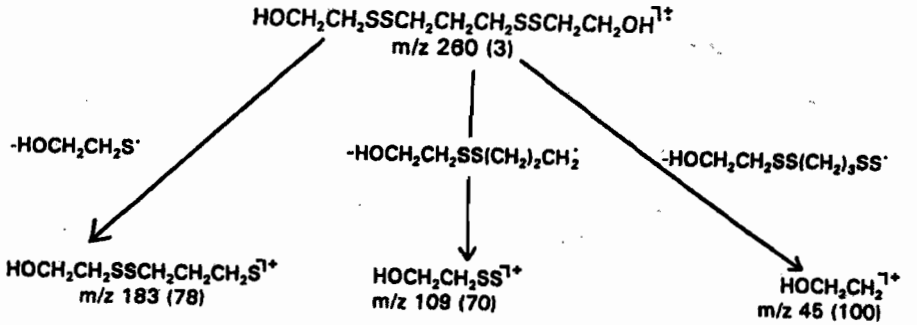
In step 2, the alkane-α,ω-bis(thioisothiouronium chloride) (2) was allowed to react with mercaptoethanol in alkaline medium (Scheme 3) to give product (1). Products were purified by recrystallization from dichloromethane.



Scheme 3.

The analytical and spectral results showed that all the synthesized compounds were pure, irrespective of the reaction route employed. Yields of product obtained is also comparable irrespective of the reaction route employed.

Mass spectrometry[2]. For all the synthesized compounds, the molecular ion peaks observed were weak except in the compound (**1**, n = 3). The compounds (**1**, n = 3 and 4), gave prominent peaks at m/z 183 and 197, respectively, corresponding to the loss of the radical $HOCH_2CH_2S^{\cdot}$ from their parent molecular ion. All the compounds (except **1**, n = 4) gave a prominent peak at m/z 109 assigned to $HOCH_2CH_2SS^+$. The compound (**1**, n = 4) gave a base peak at m/z 197, due to the molecular ion having lost the cation radical, $HOCH_2CH_2S^+$; the base peaks of other compounds were the hydroxyethyl cation $HOCH_2CH_2^+$. In the compound (**1**, n = 4), the strong peak at m/z 120 may be due to the molecular ion having lost the fragment ion $HOCH_2CH_2SSCH_2CH_2OH^+$. The fragmentation pattern of propane-1,3-bis(2-hydroxyethyl disulphide) as a representative of this class of compounds is shown in Scheme 4.



Scheme 4.

[2]Exact molecular mass m/z was determined for compound (**1**, n = 4): found M^+ = 274.0190. $C_8H_{18}O_2S_4$ requires 270.0186.

Fungicidal screening. Compounds (1, n = 2, 3, 4) were tested for fungicidal activity as described in the experimental section. Also tested for fungicidal activity were guazatine/imazalil and phenyl mercury acetate, the two well-established fungicides, so as to compare their activities with those of the synthesized compounds. The fungal organisms against which the synthesized compounds were tested are *Fusarium culmorum* (F1), *Fusarium oxysporum* (F2), and *Gaeumannomyces graminis* (F3). Germination of spores was assessed by measuring the diameter of growth of each plate and comparing it with the control. The percentage of inhibition (% I) of growth was calculated by using the following equation:

$$\% I = \frac{d_c - d}{d_c} \times 100$$

where d = diameter of growth for the plate treated with chemical and dc = diameter of growth for the control .

The percentage inhibition (% I) of growth were then ranked as given in Table 1. The results of *in vitro* test are given in Table 2.

Table 1. Ranking of the percentage inhibition (% I) of growth.

Rank	0	1	2	3	4
% I	10	11-20	21-49	50-79	80

Table 2. *In vitro* test results for $HOCH_2CH_2SS(CH_2)_nSSCH_2CH_2OH$ (1, n = 2,3,4), guazatine/imazalil and phenyl mercury acetate.

Compound	[a]C_{F1} (ppm)			C_{F2}(ppm)			C_{F3}(ppm)		
	10	100	1000	10	100	1000	10	100	1000
n = 2 (1a)	3[b]	3	4	3	3	4	3	3	4
n = 2 (1a)	2	3	4	2	3	4	2	3	4
n = 2 (1a)	3	4	4	2	3	4	3	4	4
Guazatine/imazalil	3	3	4	3	3	4	3	3	4
Phenyl mercury acetate	3	3	4	2	3	4	2	3	4

[a]Concentration (ppm) of fungal organism. [b]Rank for the percentage inhibition of growth (Table 1).

All the compounds tested (1, n = 2,3,4) showed very good activity and were able to control the fungi under investigation even at low compounds concentration. The activity results for these compounds were comparable with those of guazatine/imazalil and phenyl mercury acetate, which are the two well established fungicides. The results established that the synthesized compounds have a very high fungicidal activity; and thus useful as good fungicides.

EXPERIMENTAL

Reagents. Most reagents involved in this synthetic work were used without further purification. Diethyl ether was dried over sodium wire.

Analytical methods

Elemental analysis. All analyses for carbon, hydrogen, nitrogen and sulphur were carried out in the Department of Applied Chemistry, University of North London using a Carlo Erba 1106 Elemental Analyzer.

Nuclear magnetic resonance spectroscopy. Routine ^1H NMR spectra were obtained using a Perkin-Elmer R 12B continuos wave spectrometer at a field of 60 MHz and on a Bruker WP 80 instrument at 80 MHz. Higher field ^1H NMR and ^{13}C NMR were recorded on a Bruker AM 250 Fourier transform spectrometer at 250.13 MHz and 62.89 MHz, respectively. Chemical shifts for ^1H NMR and ^{13}C NMR spectra are given relative to internal standard tetramethylsilane (TMS).

Mass spectrometry. Electron impact (EI) mass spectra were recorded in the Department of applied Chemistry, University of North London, using a KRATUS 'PROFILE' high resolution double-focusing field instrument. Accurate mass spectra determination was carried out at SERC mass spectrometry service center at University College of Swansea.

Chemical synthesis

Preparation of S-(2-hydroxyethylthio)isothiouronium chloride [12]. A mixture of 2-mercaptoethanol (7.80 cm^3, 0.11 mol), thiourea (10.50 g, 0.13 mol), concentrated hydrochloric acid (17.5 cm^3), water (12.06 cm^3) and ethanol (180 cm^3) was kept at 0-10 °C by means of an ice-salt bath while hydrogen peroxide (14.0 cm^3) was added dropwise with vigorous stirring for a period of 2 h. The stirring was continued for additional 2 h. Dithioformamidine hydrochloride that was formed was filtered off and the solvent was removed from the filtrate by rotary evaporation to give a white solid. The solid was recrystallized from ethanol-ether solvent system and dried to give the desired *S*-(2-hydroxyethylthio)isothiouronium chloride (17.65 g, 85%) as a white crystalline solid. (Found: C, 19.07; H 4.82; N, 14.93; S, 33.89. Calc. for $C_3H_9ClN_2OS_2$: C, 19.09; H, 4.82; N, 14.86; S, 33.93); m.p. 105-106 °C (lit. [8], m.p. 106-107 °C). Similar procedures were used to prepare the following compounds.

Ethane-1,2-bis(thioisothiouronium chloride) (2a). Addition of 1,2-dimercapto ethane (4.4 cm^3, 0.05 mol), thiourea (10.50 g, 0.13 mol), hydrochloric acid (17.5 cm^3), and hydrogen peroxide (14.0 cm^3) gave ethane-1,2-bis(thioisothiouronium chloride) (2a) (9.14 g, 75%) as a white crystalline solid; (found: C, 19.63; H, 4.88; N, 22.89; S, 52.42; $C_4H_{12}N_4S_4$ requires C, 19.67; H, 4.92; N, 22.95; S, 52.46); m.p. 169-170 °C.

Propane-1,3-bis(thioisothiouronium chloride) (2b). Addition of 1,3-dimercapto propane (5.1 cm^3, 0.05 mol), thiourea (10.50 g, 0.13 mol), hydrochloric acid (17.5 cm^3), and hydrogen peroxide (14.0 cm^3) gave propane-1,3-bis(thioisothiouronium chloride) (2b) (8.77 g, 68%) as a white crystalline solid; (found: C, 23.20; H, 5.41; N, 21.69; S, 49.58; $C_5H_{14}N_4S_4$ requires C, 23.25; H, 5.43; N, 21.70; S, 49.61); m.p. 173-174 °C.

Butane-1,4-bis(thioisothiouronium chloride) (2c). Addition of 1,4-dimercapto butane (5.4 cm^3, 0.05 mol), thiourea (10.50 g, 0.13 mol), hydrochloric acid (17.5 cm^3), and hydrogen peroxide (14.0 cm^3) gave butane-1,4-bis(thioisothiouronium chloride) (2c) (9.79 g, 68%) as a

white crystalline solid; (found: C, 26.45; H, 5.85; N, 25.70; S, 46.97; $C_6H_{14}N_4S_4$ requires C, 26.47; H, 5.88; N, 25.74; S, 47.06); m.p. 183-185 °C.

Synthesis of ethane-1,2-bis(2-hydroxyethyl disulphide) (1a). [Method 1]. A solution of sodium hydrogen carbonate (6.00 g, 0.071 mol) in water (100 cm^3) was added to a solution of 1,2-dimercaptoethane (1.24 cm^3, 0.014 mol) and 2-hydroxyethylthioisothiouronium chloride (5.36 g, 0.028 mol) in methanol (100 cm^3) with vigorous stirring in an ice/salt bath. Stirring was continued for 2 h after which a white solid separated out. The solid was recrystallized from dichloromethane and dried to give ethane-1,2-bis(2-hydroxyethyl disulphide) (2.14 g, 62%) as a white powder solid. The thin layer chromatographic (tlc) result with 3:1 ether/dichloromethane (DCM) on silica gave a single spot with retention factor (R_f) of 0.53; (found: C, 29.27; H, 5.73; S, 51.99. $C_6H_{14}O_2S_4$ requires C, 29.26; H, 5.74; S, 52.04%); m.p. 38-39 °C; δ_H ($CDCl_3$): 2.32 (SCH_2CH_2S, t, 4H $^3J_{HCCH}$ = 6 Hz); 2.68 ($HOCH_2CH_2$, t, 4H, $^3J_{HCCH}$ = 6 Hz); 2.96 (OH, s, 2H); 3.84 ($HOCH_2$, t, 4H, $^3J_{HCCH}$ = 6 Hz); δ_C ($CDCl_3$): 38.23 (SCH_2CH_2S); 40.89 ($HOCH_2CH_2S$); 60.16 ($HOCH_2$); MS: m/z (%), 246 (M^+, 5), 197 (7), 165 (4), 137 (8), 109 (65), 45 (100). Similar procedures were used to synthesized the following compounds.

Propane-1,3-bis(2-hydroxyethyl disulphide) (1b). Addition of sodium hydrogen carbonate (3.41 g, 0.041 mol) to a solution of 1,3-dimercapto propane (1.43 cm^3, 0.014 mol) and *S*-2-hydroxyethylthioisothiouronium chloride (5.36 g, 0.028 mol) gave propane-1,3-bis(2-hydroxyethyl disulphide) (2.40 g, 66%) as a white amorphous solid; tlc (3:1 ether/DCM on silica) gave a single spot, R_f 0.49; (found: C, 32.31; H 6.18; S, 49.00. $C_7H_{16}O_2S_4$ requires: C, 32.31, H, 6.20, S, 49.17); m.p. 46-47 °C; δ_H ($CDCl_3$): 2.12 ($SCH_2CH_2CH_2S$, q, 2H); 2.87 ($-CH_2-S-S-CH_2-$, dt, 8H $^3J_{HCCH}$ = 6 Hz); 3.11 (OH, s, 2H); 3.87 ($HOCH_2$, t, 4H, $^3J_{HCCH}$ = 6 Hz). δ_C ($CDCl_3$): 28.14 ($SCH_2CH_2CH_2S$); 36.98 ($SCH_2CH_2CH_2S$), 41.13 ($HOCH_2CH_2S$); 60.14 ($HOCH_2$); MS: m/z (%), 260 (M^+, 3), 183 (78), 154 (9), 139 (4), 109 (70), 45 (100).

Butane-1,4-bis(2-hydroxyethyl disulphide) (1c). Addition of sodium hydrogen carbonate (3.41 g, 0.041 mol) to a solution of 1,4-dimercaptobuatne (0.014 mol) and *S*-2-hydroxyethyl-thioisothiouronium chloride (5.36 g, 0.028 mol) gave buatne-1,4-bis(2-hydroxyethyl disulphide) (2.65 g, 69%) as a white amorphous solid, tlc (3:1 ether/DCM on silica) gave a single spot, R_f 0.56; (found: C,35.03; H, 6.62; S, 46.67. $C_8H_{18}O_2S_4$ requires: C, 35.09; H, 5.70; S, 46.61%); m.p. 54-56 °C; δ_H ($CDCl_3$) 1.82 ($SCH_2CH_2CH_2CH_2S$, q, 4H, $^3J_{HCCH}$ = 6 Hz); 2.86 ($HOCH_2CH_2S$, t, 4H, $^3J_{HCCH}$ = 6 Hz); 3.87 ($HOCH_2$, t, 4H, $^3J_{HCCH}$ = 6 Hz); δ_C ($CDCl_3$); 27.72 ($SCH_2CH_2CH_2CH_2S$); 38.37 ($SCH_2CH_2CH_2CH_2S$); 41.09 ($HOCH_2CH_2S$); 60.26 ($HOCH_2$); MS: m/z (%), 274 (M^+, 2), 197 (78), 168 (8), 139(2), 109 (65), 45 (100).

Synthesis of ethane-1,2-bis(2-hydroxyethyl disulphide) (1a). [Method 2]. The procedure used were similar to that described above (method 1) except that sodium hydrogen carbonate (3.41 g, 0.041 mol) was added to a solution of ethane-1,2-bis (thioisothiouronium chloride) (**2a**) (7.81 g, 0.032 mol) and 2-mercaptoethanol (5.68 cm^3, 0.064 mol) to give ethane-1,2-bis(2-hydroxyethyl disulphide) (**1a**) (4.72 g, 60%) as a solid white powder.

Propane-1,3-bis(2-hydroxyethyl disulphide) (1b). Addition of sodium hydrogen carbonate (3.41 g, 0.041 mol) to a solution of propane-1,3-bis(thioisothiouronium chloride) (**2b**) (8.26 g, 0.032 mol) and 2-mercapto ethanol (5.68 cm^3, 0.064 mol) gave propane-1,3-bis(2-hydroxyethyl disulphide) (**1b**) (5.66 g, 68%) as a white amorphous solid.

Butane-1,4-bis(2-hydroxyethyl disulphide) (1c). Addition of sodium hydrogen carbonate (3.41 g, 0.041 mol) to a solution of butane-1,4-bis(thioisothiouronium chloride) (2c) (1.70 g, 0.032 mol) and 2-mercapto ethanol (5.68 cm^3, 0.064 mol) gave butane-1,4-bis(2-hydroxyethyl disulphide) (1c) (6.22 g, 71%) as a white amorphous solid

Biological screening experiments

In vitro screening of compounds. After a couple of trials that involved cultivation of the fungi *Fusarium culmorum*, *Fusarium oxysporum* and *Gaeumannomyces graminis* in different nutrients, it was found that the most suitable was Saboround Dextrose Agar (SDA). Therefore these fungi were cultivated in this nutrient for all the screening experiments.

A solution of the chemical to be tested (1000 ppm) in SDA medium was prepared by dissolving 0.2 g in acetone (20 cm^3), followed by the addition of distilled water (80 cm^3) at 60 °C. This mixture was then added to sterilize SDA solution containing agar (13 g) in water (100 cm^3). (Sterilization of the SDA solution had been previously carried out by placing it in an autoclave at 121 °C and leaving it at this temperature for 15 min). Addition of 20 cm^3 of the 1000 ppm solution to a solution containing SDA (13 g) in water (180 cm^3) gave a 100 ppm solution. Similarly, a 10 ppm solution was prepared by adding 20 cm^3 of the 100 ppm solution to a solution containing SDA (13 g) in water (200 cm^3). Also, solutions containing (a) guazatine/imazalil, and (b) phenyl mercury acetate at various concentrations of active ingredient (1000 ppm, 100 ppm, and 10 ppm) were prepared as standard.

The solutions were poured in Petri dishes and allowed to cool. Each plate was then inoculated with a 5 mm agar plug containing actively growing fungus. All plates were kept inside a sterilized incubator, maintained at 25 °C. The growth diameter of the fungal spore was measured every three days until there was complete growth on the control dish, i.e. until all the surface of the plate was covered with fungal spore (approximate diameter, 86 cm^3).

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