

**181. The Formation of Organo-metalloidal and Similar Compounds by Micro-organisms. Part V. Methylated Alkyl Sulphides. The Fission of the Disulphide Link.**

By FREDERICK CHALLENGER and ALAN A. RAWLINGS.

EARLIER communications have established the methylating action of *Penicillium brevicaule* (*Scopulariopsis brevicaulis*) Saccardo on inorganic and organic compounds of arsenic (J., 1933, 95; 1935, 396; 1936, 264; *Biochem. J.*, 1935, **29**, 1757) and on inorganic compounds of selenium (J., 1934, 68). In the memoir last cited, it was mentioned that no methylation could be observed with sulphur or with several of its common inorganic and organic compounds, including thiourea.

This was somewhat surprising in view of the experiments of Pohl (*Arch. exp. Path. Pharm.*, 1904, **51**, 341), who noticed a leek-like odour in the expired air of animals receiving subcutaneous or intravenous injections of thiourea. The odorous product was non-reactive to sodium hydroxide or mercuric cyanide, and was therefore not a mercaptan. It was, however, absorbed by sulphuric acid and gave a precipitate with mercuric chloride which, on oxidation, yielded a sulphate. Pohl therefore concluded that the product was an alkyl sulphide. Hofmeister (*ibid.*, 1894, **33**, 198) observed the formation of an odorous tellurium compound, assumed to be dimethyl telluride, in the breath of dogs after injections of potassium tellurite. This work and the similar animal experiments of Maassen with selenites (*Arb. Kaiserl. Ges. Amt.*, 1902, **18**, 475) have been critically discussed by one of us (*Chem. and Ind.*, 1935, **54**, 657). Hofmeister was, however, unable to detect any odour in the expired air of dogs and rabbits fed with powdered sulphur or injected with sodium sulphide or thiosulphate.

Neuberg and Grosser (*Centr. Bl. Physiol.*, 1905-6, **19**, 316) stated that the precursor of the diethyl sulphide which was shown by Abel (*Z. physiol. Chem.*, 1894, **20**, 253) to be evolved on warming the urine of dogs with alkali is methyldiethylsulphonium hydroxide. They also state that administration of diethyl sulphide to dogs gives rise to this compound, but experimental details have not been published.

Particular interest attaches to the observation of Haas (*Biochem. J.*, 1935, **29**, 1298) that the seaweeds *Polysiphonia fastigiata* and *P. nigrescens* evolve dimethyl sulphide shortly after being gathered. The occurrence in nature of methylated compounds of sulphur such as cheirolin,  $\text{CH}_3\cdot\text{SO}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{NCS}$ , erysolin,  $\text{CH}_3\cdot\text{SO}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{NCS}$  (see Armstrong and Armstrong, "The Glycosides," 1931, p. 66, for references), and particularly methionine,  $\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{CO}_2\text{H}$ , demonstrates the possibility of a biological methylation of sulphur. The relation of methionine to cysteine and to cystine suggested that compounds containing the  $-\text{SH}$  or  $-\text{S}-\text{S}-$  links might be more amenable to the methylating action of the mould.

Neuberg and Schwenk (*Biochem. Z.*, 1915, **71**, 118) showed that, on addition to a solution of sugar undergoing fermentation by bottom yeast, diethyl disulphide is reduced to ethyl mercaptan.

Dr. H. E. North (unpublished observation) found that on addition of diethyl disulphide to bread cultures of *P. brevicaule*, ethyl mercaptan was evolved; it was identified as the mercaptide,  $\text{Hg}(\text{SEt})_2$ , by absorption in mercuric cyanide. The gases issuing therefrom reacted with mercuric chloride, giving a precipitate which was clearly a mixture. Diethyl disulphide, b. p. 153°, is volatile in a stream of air and, as it is inert to mercuric cyanide, any which escaped reaction in the culture flasks would reach the mercuric chloride.

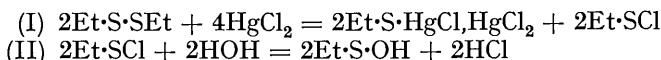
Morin (*Pogg. Annalen*, 1839, **48**, 483) and Otto (*Ber.*, 1882, **15**, 125) mention the formation of a heavy white crystalline precipitate from diethyl disulphide and alcoholic mercuric chloride, but give no further details.

The behaviour of aliphatic disulphides  $\text{R}\cdot\text{S}\cdot\text{S}\cdot\text{R}$  ( $\text{R} = \text{Et}$  or  $n\text{-Pr}$ ) with excess of saturated aqueous mercuric chloride was therefore examined. In each case a white insoluble precipitate of the composition  $\text{R}\cdot\text{S}\cdot\text{HgCl}_1\text{HgCl}_2$  was formed. Its weight accounted for only about 70% of the disulphide, the remainder having formed soluble products (see p. 869). The insoluble compounds were shown by analysis, m. p. and mixed m. p. to be identical with

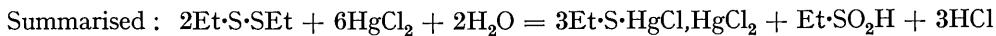
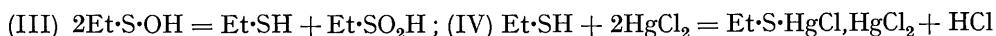
those obtained from the corresponding mercaptans and excess of aqueous mercuric chloride. The information obtainable from the literature regarding the last reaction is incomplete. Bertram (*Ber.*, 1892, **25**, 64) obtained  $\text{CH}_3\text{S}\cdot\text{HgCl}$  from methyl mercaptan. The product formed from ethyl mercaptan in alcoholic solution is stated by Debus (*Annalen*, 1849, **72**, 18) to be  $\text{Et}\cdot\text{S}\cdot\text{HgCl}$ , but no mention of a double compound with mercuric chloride is made. Sachs (*Ber.*, 1921, **54**, 1849), who obtained  $\text{Et}\cdot\text{S}\cdot\text{HgCl}$  from ethyl thioacetate,  $\text{CH}_3\text{CO}\cdot\text{SEt}$ , mercuric acetate, and sodium chloride, states that with mercuric chloride in ether it is converted into  $\text{Et}\cdot\text{S}\cdot\text{HgCl}, \text{HgCl}_2$ . We have observed that the same addition takes place slowly in aqueous solution.

We find that when ethyl or *n*-propyl mercaptan reacts with suitable quantities of aqueous mercuric chloride, any one of the three compounds  $(\text{RS})_2\text{Hg}$ ,  $\text{R}\cdot\text{S}\cdot\text{HgCl}$ , and  $\text{R}\cdot\text{S}\cdot\text{HgCl}, \text{HgCl}_2$  may be isolated at will. The formation of the mercaptides  $(\text{RS})_2\text{Hg}$  under these conditions does not appear to have been described, these compounds being usually obtained from mercuric oxide or cyanide.

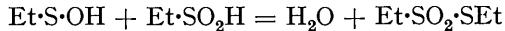
The soluble products from the interaction of diethyl disulphide and mercuric chloride, which will be described in a later communication, presumably arise from the decomposition of the sulphenic acid,  $\text{Et}\cdot\text{S}\cdot\text{OH}$ , produced thus :



The weights of chloromercury ethylthiol mercurichloride obtained in this reaction and the general properties of the aromatic chlorothiols  $\text{R}\cdot\text{SCl}$  (Zincke and Farr, *Annalen*, 1912, **391**, 57; the corresponding aliphatic compounds have not been isolated) are in agreement with the following scheme for the further reaction :



The sulphenic and the sulphinic acid may react, giving diethyl disulphoxide thus :



and the sulphinic acid may give disulphoxide and sulphonic acid (Otto and Rössing, *Ber.*, 1886, **19**, 1235; von Braun and Weissbach, *Ber.*, 1930, **63**, 2836).

Since the double compounds  $\text{R}\cdot\text{S}\cdot\text{HgCl}, \text{HgCl}_2$  would clearly be formed in the mercuric chloride absorption bottles during experiments with the mould and  $\text{R}\cdot\text{S}\cdot\text{SR}$ , their properties were studied.

On boiling with water a molecule of mercuric chloride is removed, and an insoluble solid  $\text{R}\cdot\text{S}\cdot\text{HgCl}$  (in the case of the ethyl derivative, unmelted at  $250^\circ$ ) obtained. This is almost unaffected by cold sodium hydroxide, but on warming, mercuric oxide and sodium alkyl mercaptide are produced. Hydrochloric acid gives the mercaptan at once, as also with  $\text{R}\cdot\text{S}\cdot\text{HgCl}, \text{HgCl}_2$ . Neither  $\text{R}\cdot\text{S}\cdot\text{HgCl}, \text{HgCl}_2$  nor  $\text{R}\cdot\text{S}\cdot\text{HgCl}$  liberates any mercaptan when warmed with sodium hydroxide in an air stream, aspiration through mercuric cyanide giving no precipitate.

The fission of the disulphides under the influence of mercuric chloride is in accord with many other of their reactions. For instance, alkyl iodides give trialkylsulphonium iodides and tri-iodides (Davies, *Ber.*, 1891, **24**, 3548; Hilditch and Smiles, *J.*, 1907, **91**, 1397; Steinkopf and Müller, *Ber.*, 1923, **56**, 1926); ethylmagnesium bromide with diethyl disulphide gives ethyl sulphide and ethyl mercaptan (Baroni, *Atti R. Accad. Lincei*, 1930, **11**, 905); nitrated aryl disulphides and chlorine yield the corresponding chlorothiols  $\text{R}\cdot\text{SCl}$  (Zincke *et al.*, *Annalen*, 1913, **400**, 6, 11; 1918, **416**, 95). The fission of alkyl and aryl disulphides by chloramine-T (Alexander and McCombie, *J.*, 1932, 2087) should also be mentioned.

The work of Schönberg (*Ber.*, 1935, **68**, 163 and earlier references) shows that the aromatic disulphides must be regarded as undergoing dissociation to the free radical  $\text{RS}$ .

The behaviour of disulphides to mercuric chloride having been established, diethyl and *n*-propyl disulphides were added in dilute aqueous suspension to the bread cultures.

In each case the products issuing from the culture flasks consisted of the mercaptan

$\text{R}\cdot\text{SH}$ , the unchanged disulphide  $\text{R}\cdot\text{S}\cdot\text{SR}$ , and the methyl alkyl sulphide,  $\text{R}\cdot\text{S}\cdot\text{CH}_3$ . In the case of the relatively non-volatile di-*n*-propyl disulphide very little of this came over. The precipitates in the mercuric chloride flasks consisted of mixtures of the mercuric chloride addition product of the methyl alkyl sulphide with various amounts of  $\text{R}\cdot\text{S}\cdot\text{HgCl}_2\text{HgCl}_2$ . On treatment of these mixtures with sodium hydroxide in a slow stream of air pure methyl alkyl sulphide was evolved and converted into the mercurichloride. No mercaptan volatilised in these circumstances (see p. 869). The methyl alkyl sulphides were also characterised as the benzylmethylalkylsulphonium picrates, and (in the case of methyl ethyl sulphide) as the double compound with platinous chloride.

All these derivatives were compared with authentic specimens. The compounds not previously described were : *chloromercury n-propylthiol mercurichloride*,  $\text{Pr}\cdot\text{S}\cdot\text{HgCl}_2\text{HgCl}_2$ ; *methyl ethyl sulphide dimercurichloride*,  $\text{Me}\cdot\text{SEt}_2\text{HgCl}_2$ ; *benzylmethylethylsulphonium picrate* and *benzylmethyl-n-propylsulphonium picrate*.

In view of the reducing properties of cultures of *P. brevicaule* (*Biochem. J.*, 1935, **29**, 1763; *J.*, 1935, 396) the formation of mercaptans from the disulphides was not surprising. Addition of ethyl and *n*-propyl mercaptans to bread cultures of the mould under the same conditions as obtained for the disulphides gave very similar results, methylation being observed in each case.

As regards the mechanism of the methylation and reduction of the disulphides, it appeared possible that some such reaction as  $\text{R}\cdot\text{S}\cdot\text{SR} + 2\text{CH}_2\text{O} = \text{R}\cdot\text{S}\cdot\text{CH}_3 + \text{R}\cdot\text{SH} + \text{CO}_2$  might occur. If so, the ratio of methyl alkyl sulphide to mercaptan should be at least 1 : 1; allowing for some possible methylation of the mercaptan, an even higher ratio might be expected. This, however, is not the case. By weighing the alkyl mercaptides  $\text{Hg}(\text{SR})_2$  and the methyl alkyl sulphide mercurichlorides it was shown in preliminary experiments that the mercaptan is produced in large excess. The higher proportion of methylation occurs with di-*n*-propyl disulphide, *i.e.*, with the least volatile substrate.

No allowance was made, however, for possible solubility of the mercury derivatives in the cyanide and chloride solutions. It is hoped to determine the ratio mercaptan : methyl alkyl sulphide more accurately. Methylation of ethyl and *n*-propyl mercaptans when added to the mould cultures (see p. 874) is also very incomplete.

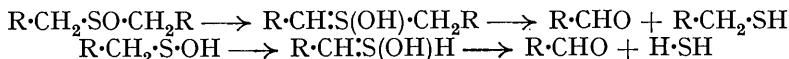
It appears probable, therefore, that mercaptan formation is independent of and precedes methylation. The formation of mercaptan does not necessarily, however, involve a preliminary reduction, thus :  $\text{R}_2\text{S}_2 + 2\text{H} = 2\text{R}\cdot\text{SH}$ . Mercaptan may arise by hydrolytic fission :  $\text{R}_2\text{S}_2 + \text{H}\cdot\text{OH} = \text{R}\cdot\text{SH} + \text{R}\cdot\text{S}\cdot\text{OH}$ .

Important results have been obtained by Schöberl and his co-workers (*Annalen*, 1933, **507**, 111; 1936, **522**, 97) in an examination of the hydrolysis, in alkaline or neutral solution, of a large number of carboxylic acids containing the disulphide link. They have shown that the sulphenic acid,  $\text{R}\cdot\text{S}\cdot\text{OH}$ , breaks down to hydrogen sulphide and an aldehydo- or keto-acid; thus dithiodiglycollic acid,  $(\text{CO}_2\text{H}\cdot\text{CH}_2\cdot\text{S})_2$ , gives thioglycollic and glyoxylic acids; disulphidosuccinic acid,  $[\text{CO}_2\text{H}\cdot\text{CH}_2\cdot\text{CH}(\text{CO}_2\text{H})\cdot\text{S}]_2$ , gives thiolactic and pyruvic acids. Hydrogen sulphide is obtained in each case. Reactions of this type are involved in the alkaline desulphurisation of cystine and the action of alkali on wool (Crowder and Harris, *J. Res. Nat. Bureau Standards*, 1936, **16**, 475).

This decomposition of the unstable intermediate sulphenic acids, giving aldehyde or ketone and hydrogen sulphide, is probably of the same type as that of the sulphoxides in presence of hydrochloric acid, which was described almost simultaneously by Smythe (*J.*, 1909, **95**, 349) and by Pummerer (*Ber.*, 1909, **42**, 2283), and further studied by Larsson and Jönsson (*Ber.*, 1934, **67**, 1263) and by Hellström and Lauritzson (*Ber.*, 1936, **69**, 2003) :



If the sulphenic acid as well as the sulphoxide be assumed to react in a tautomeric form, the analogy is readily seen :



This reaction does not appear, however, to have been demonstrated in the case of the

simple dialkyl disulphides. Price and Twiss (J., 1910, **97**, 1183) showed that diethyl disulphide is completely decomposed at 150—180° by alcoholic potash, but only partly so on boiling under reflux. The products were not identified.

Mr. B. Taylor has recently shown that when diethyl disulphide is heated with water in a copper autoclave at 200—210° for four hours, extensive decomposition takes place. The aqueous liquor contained acetaldehyde, which was isolated as the 2 : 4-dinitrophenylhydrazone. The autoclave was coated with a mixture of copper sulphide and copper ethyl mercaptide, Cu(SEt)<sub>2</sub>. This gave hydrogen sulphide and ethyl mercaptan on decomposition with acid, which were identified as lead sulphide and chloromercury ethylthiol mercurichloride, m. p. and mixed m. p. 150—151° with an authentic specimen of m. p. 151°. When heated in nitrogen, the deposit in the autoclave evolved ethyl sulphide : Cu(SEt)<sub>2</sub> = CuS + Et<sub>2</sub>S. The ethyl sulphide was identified as the dimercurichloride, m. p. and mixed m. p. 117—118° with an authentic specimen of the same m. p.

The same decomposition, though less extensive, was observed on heating the disulphide and water in a sealed tube at 170° for 5 hours. Hydrolytic fission of the —S—S— group in diethyl disulphide is therefore possible; it is not yet clear whether this change can be effected under the conditions obtaining in the mould cultures. No acetaldehyde could be detected on passing the volatile products from bread cultures containing diethyl disulphide through a solution of 2 : 4-dinitrophenylhydrazine in dilute hydrochloric acid. The minute amount of precipitate produced did not suffice for a determination of m. p.

The methylation of mercaptans described in this paper may be explained on the assumption that formaldehyde (probably arising from glycine; see *Biochem. J.*, 1935, **29**, 1761; *Chem. and Ind.*, 1936, **55**, 900) condenses to form compounds of the type R·S·CH<sub>2</sub>·OH, which then undergo reduction. Such compounds have been described by Levi (*Gazzetta*, 1932, **62**, 775). The product from formaldehyde and ethyl mercaptan is very unstable and we could not obtain it free from the odour of mercaptan. Consequently the capacity of *P. breviculae* to effect the change Et·S·CH<sub>2</sub>·OH → Et·S·CH<sub>3</sub> could not be tested, but the possibility of this type of biological reduction will be explored by the use of more stable compounds.

The discovery of djenkolic acid in the djenkol bean of Java (van Veen and Hyman, *Rec. trav. chim.*, 1935, **54**, 493; du Vigneaud and Patterson, *J. Biol. Chem.*, 1936, **114**, 533) lends further support to the formaldehyde theory of the methylation of mercaptans, as its constitution, CH<sub>2</sub>[S·CH<sub>2</sub>·CH(NH<sub>2</sub>)·CO<sub>2</sub>H]<sub>2</sub>, shows it to be derived from formaldehyde and cysteine.

#### EXPERIMENTAL.

*Preparation of Reference Compounds.*—*Methyl ethyl sulphide dimercurichloride.* The sulphide (1.26 g.) was added to saturated aqueous mercuric chloride (300 c.c.). The white solid, which separated immediately, was removed next day, m. p. 124—126°. Two crystallisations from acetone gave a product of constant m. p. 127—128° (Found : C, 5.9; H, 1.6; S, 4.8; Cl, 22.7; Hg, 64.6. C<sub>3</sub>H<sub>8</sub>S<sub>2</sub>HgCl<sub>2</sub> requires C, 5.8; H, 1.3; S, 5.2; Cl, 22.9; Hg, 64.8%). The *dimercurichloride* was also obtained, m. p. 126—127°, when the precipitate (m. p. 101°) resulting from the interaction of the sulphide (0.84 g., 1 mol.) and mercuric chloride (2.99 g., 1 mol.) in water (75 c.c.) was recrystallised twice from acetone. A pure monomericurichloride could not be isolated (compare Thierry, J., 1925, **127**, 2756, who obtained methyl ethyl sulphide from petroleum as the mercurichloride, which formed "rather unstable crystals," m. p. 101—102°).

*Benzylmethylethylsulphonium picrate.* Methyl ethyl sulphide (2.1 g.) was mixed with benzyl chloride (7 g.) in alcohol (15 c.c.). After several days water (20 c.c.) was added, and unchanged benzyl chloride removed by ether. Owing to the instability of sulphonium salts the solution was not concentrated but was treated with sodium picrate, and the resulting precipitate (m. p. 99—100°) recrystallised twice from alcohol (m. p. 100.5—101°) (Found : C, 48.3; H, 4.5; N, 10.9; picric acid, 58.1. Calc. for C<sub>18</sub>H<sub>17</sub>O<sub>7</sub>N<sub>3</sub>S : C, 48.6; H, 4.3; N, 10.6; picric acid, 58.0%).

*Methyl n-propyl sulphide mercurichloride.* The sulphide (0.5 c.c.) was shaken with an excess of saturated aqueous mercuric chloride, and the precipitate, m. p. 162—164°, recrystallised twice from benzene; it then had the constant m. p. 163.5—165°. Ingold, Jessop, Kuriyan, and Mandour (J., 1933, **533**) record the m. p. 164—166°, but give no analysis (Found : C, 6.7; H, 1.2; S, 4.11; Hg, 64.7. Calc. for C<sub>4</sub>H<sub>10</sub>S<sub>2</sub>HgCl<sub>2</sub> : C, 7.6; H, 1.6; S, 5.1; Hg, 63.4%. Calc. for

$2C_4H_{10}S \cdot 5HgCl_2$  : C, 6.2; H, 1.4; S, 4.2; Hg, 65.1%). A mercurichloride of the same m. p. and mixed m. p. and giving almost identical analyses was obtained from methyl *n*-propyl sulphide of biological origin (p. 875).

*Benzylmethyl-n-propylsulphonium picrate.* The sulphide (1 c.c.), benzyl chloride (5 c.c.), and alcohol (10 c.c.) were left for several days and diluted with water; after conversion into picrate without concentration, the crude product had m. p. 92—94° and 95—95.5° on recrystallisation from alcohol (Found : C, 49.9; H, 4.7; N, 10.4; picric acid, 55.8. Calc. for  $C_7H_{18}O_7N_3S$  : C, 49.9; H, 4.7; N, 10.3; picric acid, 56.0%).

*Diethyl Disulphide and Mercuric Chloride.*—When the disulphide (b. p. 153° after careful fractionation) and aqueous mercuric chloride were mixed in the molecular proportion 1 : 4 (A), or when the mercuric chloride was employed in excess (B), a white precipitate formed rather slowly. This melted at 151° and was insoluble in all the usual organic solvents. The same compound, m. p. 150° and mixed m. p. 151°, was obtained when ethyl mercaptan was added to an excess of saturated aqueous mercuric chloride (C). With hydrochloric acid it gave ethyl mercaptan. Sodium hydroxide gave yellow mercuric oxide but no odour of sulphide; no disulphide or mercaptan was obtained either in the cold or on warming. In an attempt to find a solvent for (A), about 0.1 g. was boiled with water (40 c.c.). The extract contained mercuric chloride, which suggested the presence of some loosely combined, probably co-ordinated, mercuric chloride in (A), (B), and (C). The residue (D) was then found to be unmelted at 260°, and still contained mercury and sulphur. With hydrochloric acid ethyl mercaptan was at once evolved.

The loss in weight on boiling (A) with three successive quantities of water and the mercury thus removed were determined and the insoluble residue (D) analysed [Found for (A) : C, 4.7; H, 1.4; Hg, 70.2, 70.25; loss in weight on extraction with water, 48.1, 47.9; Hg removed as  $HgCl_2$ , 35.6, 35.75. Calc. for  $C_2H_5S \cdot HgCl \cdot HgCl_2$  : C, 4.2; H, 0.9; Hg, 70.55; loss in weight, 47.8; Hg removable as  $HgCl_2$ , 35.3%. Found for (D) : Hg, 67.2, 67.8. Calc. for  $C_2H_5S \cdot HgCl$  : Hg, 67.5%]. Analyses of the products obtained from di-*n*-propyl disulphide with mercuric chloride were effected in a similar manner (see below).

Two preparations of (A) were carried out in which 12.05 and 11.95 g. of diethyl disulphide were treated with mercuric chloride (108 g.; 4 mols.) in water (2 l.). After 15 days, during which the bottles were frequently shaken, the yields of (A) were 79.1 and 78.3 g. No further deposit separated from the filtrate. These figures represent yields of 94% and 95% of the amounts required by the summarised equation on p. 869.

*Di-n-propyl Disulphide and Mercuric Chloride.*—Di-*n*-propyl disulphide was prepared by heating sulphur (5.3 g., 1 atom) and sodium sulphide (13 g., 1 mol.) in *n*-propyl alcohol (150 c.c.) till almost complete solution occurred. *n*-Propyl bromide (41 g., 2 mols.) was then added, and the mixture refluxed for 4 hours. The mixture was poured into water (500 c.c.) and repeatedly extracted with ether, the solvent removed, and the residue fractionated at 16 mm. A small fraction, b. p. 80° (di-*n*-propyl sulphide boils at 141.5—142.5°), and one, b. p. 129°, were obtained, together with a fraction, b. p. 100—102°, which was pure di-*n*-propyl disulphide. Spring and Legros (*Ber.*, 1882, **15**, 1939), who prepared it from sodium *n*-propylthiosulphate, give b. p. 192.5° at ordinary pressure. In our preparation the presence of the fraction b. p. 129° (presumably a polysulphide) caused decomposition on distillation of the reaction product at ordinary pressure.

The disulphide (0.9 g.) and 3% aqueous mercuric chloride (300 c.c.) were frequently shaken during 4 days, giving 3.9 g. of a white solid (E), m. p. 139°. 0.68 G. of this product was treated with an excess of cold aqueous sodium hydroxide in a stream of air, which was passed through aqueous mercuric cyanide into 3% aqueous mercuric chloride. No deposit formed in either solution during 2 hours or throughout a further 3 hours during which the mixture was heated at 100°. The mixture was then acidified with hydrochloric acid, liberating *n*-propyl mercaptan, and aspiration continued into mercuric chloride; 0.60 g. of the original compound of m. p. 139° was then re-formed (F).

When (E) (0.5 g.) was boiled with water (100 c.c.) for 6 hours, the aqueous extract contained mercuric chloride. The insoluble residue (G) after washing melted at 182°. It gave a strong odour of a mercaptan with hydrochloric acid [Found for (E) : C, 6.65; H, 1.4; S, 5.8; Hg, 68.6, 68.5; loss in weight on extraction with water, 47.0; 47.1; Hg removed as  $HgCl_2$ , 33.9, 34.1. Calc. for  $C_3H_7S \cdot HgCl \cdot HgCl_2$  : C, 6.2; H, 1.2; S, 5.5; Hg, 68.9; loss in weight, 46.6; Hg removable as  $HgCl_2$ , 34.4%. Found for (G) : C, 11.55; H, 2.3; S, 10.5; Hg, 64.7. Calc. for  $C_3H_7ClSHg$  : C, 11.55; H, 2.25; S, 10.3; Hg, 64.5%].

*Ethyl Mercaptan and Mercuric Chloride.*—Mercuric chloride (3 g.) in water (80 c.c.) was added to the mercaptan (2 g., 3 mols.). A red precipitate was immediately formed, quickly becoming pink and finally white. Next day the solid (m. p. 73°) was separated and after one

crystallisation from alcohol had m. p. 76—77° alone and in admixture with authentic mercury diethyl mercaptide.

Mercuric chloride (1·6 g.) in water (30 c.c.) was then added to the original filtrate, giving a white solid, m. p. 86°. Extraction of this with alcohol left a residue of ethylthiol mercury chloride,  $\text{Et-S-HgCl}_2$ , unmelted at 260°. The alcohol deposited a further quantity of the mercaptide, m. p. 76—77°.

Final addition of excess of aqueous mercuric chloride to the second main filtrate, which still contained mercaptan, gave a solid, m. p. 149° alone and 150° in admixture with the double compound  $\text{Et-S-HgCl}_2\text{HgCl}_2$ , m. p. 151°, obtained from diethyl disulphide and mercuric chloride.

Later work showed that the ethyl mercaptan used in this experiment must have contained traces of hydrogen sulphide. A specimen which gave a clean yellow precipitate of lead ethyl mercaptide with lead acetate gave no red precipitate with mercuric chloride. If, however, a few bubbles of hydrogen sulphide were passed through the mercaptan, addition of mercuric chloride then gave a red solid, which became almost white on shaking with excess of the mercury salt.

This red solid is presumably identical with or related to the red compound  $\text{Hg}(\text{SEt})_2\text{HgS}$  prepared by Sachs and Balassa (*Z. anorg. Chem.*, 1925, 145, 196), who also describe a yellow isomer (see below). The excess of mercuric chloride probably removes the mercuric sulphide as the double compound  $\text{HgCl}_2\text{HgS}$  (Jolibois and Bouvier, *Compt. rend.*, 1920, 170, 1497).

*n-Propyl Mercaptan and Mercuric Chloride.*—The mercaptan (Ingold *et al.*, *loc. cit.*) (0·5 c.c.) was mixed with an excess of saturated aqueous mercuric chloride and left overnight. The product had m. p. 138—139° (decomp.) alone and in admixture with (E) (m. p. 139° decomp.) and was therefore  $\text{C}_3\text{H}_7\text{S-HgCl}_2\text{HgCl}_2$ . Mercuric chloride (2 g., 1 mol.) in water (80 c.c.) was added to the mercaptan (1·5 g., 3 mols.). The resulting white solid was separated next day and found to sinter from 60° to 120°. 0·5 G. was refluxed with alcohol (20 c.c.) for 30 minutes; the filtered solution deposited mercury di-*n*-propyl mercaptide, m. p. and mixed m. p. 65—66° with a specimen obtained from the mercaptan and mercuric cyanide. The residue insoluble in alcohol had m. p. 182° alone and in admixture with authentic *n*-propylthiol mercury chloride,  $\text{C}_3\text{H}_7\text{S-HgCl}$  (see p. 872).

The mercaptan (0·5 g.) was added to mercuric chloride (1·8 g., 1 mol.) in alcohol (60 c.c.), the mixture left overnight, and the solid then separated. It consisted of the thiol mercury chloride  $\text{C}_3\text{H}_7\text{S-HgCl}$  and had m. p. and mixed m. p. 182°.

*P. brevicaule Saccardo (Baarn Strain A; see J., 1933, 95) and Diethyl Disulphide.*—Four 1 l. flasks each containing 150 g. of bread crumbs and 20—30 c.c. of water were sterilised, inoculated, and incubated for four days at 30°, and 1 day at room temperature. 25 C.c. of a sterile 2% aqueous suspension of diethyl disulphide were then added (final concentration in the bread, about 0·2%), and the flasks left for 1 day at room temperature. They were then connected in series (as described in Parts I—IV), and volatile products aspirated in a stream of sterile air through two bottles containing 4% aqueous mercuric cyanide, and then through five bottles containing 3% aqueous mercuric chloride. Four separate experiments, each with four bread flasks, were carried out, and in every case the precipitate of mercury diethyl mercaptide was confined to the first mercuric cyanide bottle. The observed m. p. varied from 71° to 74° and in one case a m. p. of 70—73° was obtained. The precipitates were often slightly yellowish-green. On recrystallisation from ethyl alcohol, only traces remained insoluble and the solution deposited the pure white mercury diethyl mercaptide  $(\text{C}_2\text{H}_5\text{S})_2\text{Hg}$ , m. p. 76—77° (Found : C, 14·85; H, 2·8. Calc.: C, 14·9; H, 3·1%).

The yellow colour was probably due to a small amount of the yellow compound  $\text{Hg}(\text{S-C}_2\text{H}_5)_2\text{HgS}$  (Sachs and Balassa, *Z. anorg. Chem.*, 1925, 145, 196) arising from the presence of a trace of hydrogen sulphide. The amount was, however, too small to be cited as evidence of the formation of hydrogen sulphide by hydrolytic fission (see p. 870). The specimen of diethyl disulphide had been well fractionated after purchase, but may have contained a slight trace of hydrogen sulphide. In later experiments the disulphide was washed with dilute sodium hydroxide solution before fractionation and addition to the mould cultures. The mercury diethyl mercaptide produced was then quite white. Similar yellow precipitates were obtained by Rübner (*Arch. Hygiene*, 1893, 19, 136) when methyl mercaptan containing hydrogen sulphide was passed into mercuric cyanide.

At the commencement of the experiment the m. p. of the deposit in the first mercuric chloride bottle is always below that (151°) of the pure compound  $\text{C}_2\text{H}_5\text{S-HgCl}_2\text{HgCl}_2$  (A), and it falls as the experiment proceeds. This is due to the admixture of (A) with the dimercurichloride of methyl ethyl sulphide (m. p. 127—128°). This monosulphide reacts rapidly with mercuric chloride and is mostly retained in the first bottle. The disulphide, on the other hand, reacts more slowly and

tends to pass over into the second and third bottles. At any given time with very few exceptions the m. p. of the deposit in the second absorption bottle is found to be higher than that in the first, due to the predominance of (A). Later on, methylation ceases and as unchanged disulphide is still present, the m. p. of the deposits rises again to that of (A). Almost at the same time the deposition of mercaptide in the mercuric cyanide bottle ceases. As ethyl mercaptan is probably the precursor of methyl ethyl sulphide (see p. 870), this simultaneous cessation of reduction and methylation would naturally be expected. It is not clear, however, why reduction (or possibly hydrolysis; see p. 870) should cease while diethyl disulphide is still available.

*Detection of Methyl Ethyl Sulphide in the Precipitate from the Mercuric Chloride Absorption Bottles.*—(1) *As dimercurichloride.* The deposits having the lowest m. p.'s were collected, and 1·2 g. treated with cold sodium hydroxide solution. The odour of an alkyl monosulphide was at once apparent. A slow stream of air was then passed through the mixture into (a) aqueous mercuric cyanide (4%) and (b) aqueous mercuric chloride (3%). No deposit formed in (a) throughout the experiment, but after 30 minutes a precipitate formed in (b), the first mercuric chloride bottle. Two such deposits were removed and the reaction flask was then heated, giving a third deposit. All these had m. p. 126—127° and when mixed and recrystallised from benzene melted at 127—128° alone and in admixture with authentic methyl ethyl sulphide dimercurichloride, m. p. 127—128° (Found : C, 6·05; H, 1·65; Cl, 23·1; S, 5·0. Calc. for  $C_3H_8S \cdot 2HgCl_2$  : C, 5·8; H, 1·3; Cl, 22·9; S, 5·2%).

(2) *As benzylmethylethylsulphonium picrate.* A further 1·19 g. of the crude deposit were decomposed with sodium hydroxide solution as before, and the evolved sulphide aspirated through mercuric cyanide (no precipitate) into 5% alcoholic benzyl chloride (15 c.c.) during 36 hours. Addition of the minimum of water and extraction with ether left a solution, which with sodium picrate gave a precipitate, m. p. 97—99°. Three recrystallisations from alcohol gave benzylmethylethylsulphonium picrate, m. p. and mixed m. p. 100·5—101° with an authentic specimen of m. p. 100·5—101° (Found : C, 48·2; H, 4·35; N, 10·85. Calc. for  $C_{16}H_{17}O_7N_3S$  : C, 48·6; H, 4·3; N, 10·6%).

(3) *As methyl ethyl sulphide platinochloride.* A further 1·28 g. of the crude mercurichlorides from the absorption bottles were mixed with sodium hydroxide solution and shaken with three successive quantities of ether. The combined extracts were then shaken with aqueous potassium platinochloride for 18 hours, separated, and evaporated. The oily residue solidified and after two crystallisations from alcohol melted at 121·5—122·5° alone and in admixture with synthetic methyl ethyl sulphide platinochloride, m. p. 124—125° (Mann, J., 1930, 1745). The  $\alpha$ - and  $\beta$ -modifications of the platinochloride of diethyl sulphide both melt at 108° (Klason, Ber., 1895, 28, 1494).

*Control experiment.* To each of four 1 l. flasks containing sterilised but uninoculated bread were added 25 c.c. of a sterile 2% aqueous suspension of diethyl disulphide, and aspiration commenced as usual. No precipitate formed in the mercuric cyanide during 17 days. Fourteen deposits were taken from the mercuric chloride bottles, five of m. p. 151°, seven of m. p. 150°, and two of m. p. 149°. In order to confirm that no methylation had occurred the entire product was made alkaline with potassium hydroxide. Aspiration for 24 hours gave no precipitate in either the mercuric cyanide or chloride absorption bottles, indicating the complete absence of any alkyl monosulphide. The non-formation of mercaptan on treatment with alkali confirms the result obtained with precipitate (A) (p. 872).

*P. brevicaule Saccardo and Ethyl Mercaptan.*—Four 1 l. flasks of sterile bread were inoculated, incubated as usual, and treated with 25 c.c. of 2% aqueous suspension of ethyl mercaptan. The products were aspirated through mercuric cyanide (5 bottles) and chloride (3 bottles) as before. After 11 days the deposits in the first two cyanide bottles had m. p. 71—72° and in the corresponding chloride bottles 132° and 140°; the higher m. p. of the deposit in the second bottle was to be expected (p. 873). It is clear from these m. p.'s that some disulphide had been formed from the mercaptan during aeration. After a further 13 days the deposits in the first cyanide and the first chloride bottle had m.p. 71—72° and 150° respectively. The mercury diethyl mercaptide was recrystallised from alcohol, m. p. 76—77°. The mercurichlorides were collected and treated with alkali, and volatile matter aspirated through mercuric cyanide (no deposit) into mercuric chloride, giving a product, m. p. 126—127° alone and in admixture with authentic methyl ethyl sulphide dimercurichloride, m. p. 127—128°. There was insufficient for recrystallisation (Found : C, 6·0; H, 1·3; S, 4·7. Calc. for  $C_3H_8S \cdot 2HgCl_2$  : C, 5·8; H, 1·3; S, 5·2%).

*P. brevicaule Saccardo and Di-n-propyl Disulphide.*—The conditions in these experiments as regards number of culture flasks, weight of bread, concentration of added disulphide and absorption solutions were the same as for diethyl disulphide. The deposit from the mercuric cyanide

(0.4 g.) was crystallised once from alcohol, giving mercury di-n-propyl mercaptide, m. p. and mixed m. p. 65—66° with an authentic specimen, m. p. 65—66° (Found : C, 20.8; H, 4.3; S, 18.6. Calc. for  $C_6H_{14}S_2Hg$  : C, 20.6; H, 4.0; S, 18.35%).

*Methyl n-propyl sulphide mercurichloride.* The crude mercurichloride (0.3 g., m. p. 158—162°) obtained directly by absorption of the mould gases from di-n-propyl disulphide in mercuric chloride was recrystallised twice from alcohol; m. p. 163—164° [Found : C, 6.5; H, 1.2; S, 4.1; Cl, 22.3. Calc. for  $C_3H_7S\cdot CH_3\cdot 2HgCl_2$  : C, 7.6; H, 1.6; S, 5.1; Cl, 22.4%. Calc. for  $2(C_3H_7S\cdot CH_3)\cdot 5HgCl_2$  : C, 6.2; H, 1.4; S, 4.2; Cl, 23.12%].

From the m. p. it would appear that this crude mercurichloride consisted almost entirely of methyl n-propyl sulphide mercurichloride, m. p. 163.5—165°, this being probably due to the relative non-volatility of di-n-propyl disulphide. The analytical results after recrystallisation moreover agree with those obtained for the synthetic product (see p. 872). Nevertheless in view of the somewhat unusual composition further purification was attempted by treatment of 0.86 g. with cold aqueous potassium hydroxide. Aspiration through mercuric cyanide gave no precipitate, but with mercuric chloride two deposits, both of m. p. 163°, were obtained. These were united and crystallised from benzene, giving a product of constant m. p. 163.5—165° alone and in admixture with a synthetic specimen. The analytical results were the same as before (Found : C, 6.6; H, 1.3; S, 4.0%).

*Benzylmethyl-n-propylsulphonium picrate.* The mercurichloride (m. p. 163—164°; 0.66 g.) from the absorption bottles was treated with cold sodium hydroxide solution, and the volatile sulphide aspirated through mercuric cyanide (no deposit) into alcoholic benzyl chloride during 48 hours. Conversion into picrate as before gave a solid, m. p. 91—93°, which, on recrystallisation from alcohol, gave benzylmethyl-n-propylsulphonium picrate, m. p. 95—95.5° alone or in admixture with an authentic specimen, m. p. 95—95.5° (Found : C, 49.3; H, 4.8; picric acid, 56.3. Calc. for  $C_{17}H_{19}O_7N_3S$  : C, 49.9; H, 4.7; picric acid, 56.0%).

*P. brevicaule Saccardo and n-Propyl Mercaptaian.*—The experimental details were identical with those employed in the case of ethyl mercaptan. A precipitate quickly formed in the mercuric cyanide and, after about 12 hours, in the chloride solution. After 3 days these were removed (mercury di-n-propyl mercaptide : 2.84 g., m. p. 62—64°; 0.10 g., m. p. 160—162° respectively). The mercurichloride after two crystallisations from alcohol had m. p. 163—165° alone and 163.5—165° in admixture with synthetic methyl n-propyl sulphide mercurichloride (see above and p. 871). During the next 7 days 0.2 g. of mercaptide and less than 0.05 g. of product, m. p. 159—161°, formed in the cyanide and chloride bottles. No further deposits were obtained.

*P. brevicaule Saccardo and Diethyl Sulphide.*—The sulphide (25 c.c. of a 2% aqueous suspension) was added to each of four bread cultures of the mould, and volatile products aspirated as usual through mercuric cyanide and chloride. No deposit formed in the cyanide bottles during 14 days, by which time no further precipitate formed in the chloride. Several deposits were removed and each had m. p. 113—115°, which rose to 118° after one crystallisation from alcohol. Diethyl sulphide dimercurichloride prepared for comparison melted at 118—119°. The mould, as was to be expected, has no reducing action on diethyl sulphide.

*P. brevicaule Saccardo with Dibenzyl Disulphide and Diphenyl Disulphide.*—Small flasks containing 50 g. of sterile bread were inoculated and incubated as usual and treated with (a) 10 c.c. of 3% dibenzyl disulphide and (b) 10 c.c. of 2% diphenyl disulphide, both in aqueous suspension. No odour was observed during 30 and 35 days respectively. In the case of diphenyl disulphide four 1-l. flasks of bread cultures were set up, each treated with 25 c.c. of a 2% aqueous suspension, and volatile products aspirated through mercuric cyanide and chloride for 15 days. No precipitate formed in either case. Similar negative results have been obtained with phenylarsonic acid (Challenger and Rawlings, J., 1936, 264) and with benzylarsonic acid (Taylor, unpublished observation).

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THE UNIVERSITY, LEEDS.

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