351. *The Formation of Organo-metalloidal and Similar Compounds by Micro-organisms. Part VI. Further Studies on the Fission of the Disulphide Link.*

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The methylating action of *Penicillium brevicaule* in bread cultures on di-*n*-butyl and di-*n*-amyl disulphides gives *n*-butylthiol and methyl *n*-butyl sulphide and *n*-amylthiol and methyl *n*-amyl sulphide respectively, but the yields are very low. Carefully purified dimethyl disulphide gives methylthiol and very small quantities of dimethyl sulphide in cultures of the mould. The ratio of methylated alkyl sulphide to thiol produced in the mould cultures increases on passing from dimethyl disulphide to di-*n*-amyl disulphide. The fission of the S–S link by *P. brevicaule* appears to be a general reaction of the simple aliphatic disulphides. *n*-Butylthiol and *iso*amylthiol occur in the anal secretion of the skunk.

The fission of diethyl disulphide by aqueous mercuric chloride gives $EtS\cdot HgCl,HgCl_2$ and ethanesulphinic acid $Et\cdot SO_2H$ (arising from the chlorothiol EtSCl by way of $EtS\cdot OH$). Dimethyl disulphide behaves in a similar manner, giving $Me\cdot SO_2H$ and $MeS\cdot HgCl,xHgCl_2$.

It was shown by one of us and Rawlings (J., 1937, 868) that the methylating action of *Penicillium brevicaule*, previously established in the case of arsenic and selenium compounds, is also exerted on aliphatic disulphides $RS\cdot SR$ (R = Et and *n*-Pr), which are converted into RSH and RSMe. These were removed in a sterile air stream and identified as $(RS)_2Hg$ and $RSMe,xHgCl_2$ by absorption in mercuric cyanide and mercuric chloride respectively.

The reaction has now been extended to di-*n*-butyl and di-*n*-amyl disulphides, which, in bread cultures of the mould, are converted into *n*-butylthiol and methyl *n*-butyl sulphide and *n*-amylthiol and methyl *n*-amyl sulphide respectively. These were removed and identified as before. The amount of methyl alkyl sulphides is larger than that of the alkylthiols, but the total yield is very low. With diethyl disulphide as substrate, much passes over unchanged and undergoes fission in presence of mercuric chloride, so that the product $EtS\cdot HgCl,HgCl_2$ accompanies the methyl ethyl sulphide mercurichloride $MeSEt,2HgCl_2$. In the case of di-*n*-propyl disulphide very little fission-product is formed in the mercuric chloride (J., 1937, 870). This is still more obvious with di-*n*-butyl and di-*n*-amyl disulphides, the methyl alkyl sulphide mercurichloride being entirely free from chloromercury alkylthiol derivatives.

The fission of the S–S link by *P. brevicaule* is therefore a general reaction of simple aliphatic disulphides and, although strictly quantitative experiments were not made, the ratio of methylated alkyl sulphide to thiol produced by the mould undoubtedly increases from dimethyl disulphide to di-*n*-amyl disulphide.

This biological conversion of di-*n*-butyl disulphide into the thiol is of interest in view of the occurrence of these two compounds and of *iso*amylthiol in the secretion of the skunk (Beckmann, *Pharm. Centr.*, 1896, **37**, 557; Aldrich *J. Exper. Med.*, 1897, **1**, 323; *Amer. J. Physiol.*, 1901, **5**, 457). Traces of methylthiol are also present.

Earlier workers also state that higher homologues of methylthiol are contained, along with nitrogen compounds, in the secretions of various animals allied to the skunk (Nencki and Sieber, *Monatsh.*, 1889, **10**, 526). The zorrino, a South American marten, appears to produce a thiol with four atoms of carbon and probably the corresponding disulphide (Fester and Bertuzzi, *Rev. Fac. Quim. Ind. Agrica. Santa Fé Argentina*, 1936, **5**, 85).

Nord (*Ber.*, 1919, **52**, 1207) obtained *n*-butylthiol by addition of *n*-butaldehyde and ammonium sulphide to a sugar solution undergoing fermentation by yeast (see also *Ber.*, 1914, **47**, 2264).

When dimethyl disulphide (carefully purified from hydrogen sulphide, methylthiol and from polysulphides) is added to bread cultures of the mould and the usual procedure is then followed, the majority volatilises, giving a compound $MeS\cdot HgCl,xHgCl_2$ with the mercuric chloride, but a small quantity of dimethyl sulphide, characterised as the mercurichloride, is formed. Methylthiol is also produced, the quantity being larger than that of the dimethyl sulphide. It was characterised as the mercury derivative $(MeS)_2Hg$.

Many workers refer to the formation of methylthiol by bacteria. Maassen (*Arb. Kais. Ges. Amt.*, 1899, **15**, 500) and Herter (*J. Biol. Chem.*, 1905—6, **1**, 421) obtained it from peptone, Wohlgemuth (*Z. physiol. Chem.*, 1905, **43**, 469) and Kondo (*Biochem. Z.*, 1923, **136**, 199) from cystine, but its identification was usually effected by colour reactions. Nencki and Sieber (*loc. cit.*) obtained it by the bacterial decomposition of meat and analysed the lead salt. Recently its occurrence in plants, apparently free or very loosely combined, has been reported by various observers. Nakamura (*Biochem. Z.*, 1925, **164**, 31) isolated it as the mercury derivative from the freshly crushed radish (*Raphanus sativus*) and Koolhaas, using a similar procedure, detected it in the freshly gathered leaves of several species of *Lasianthus* (*ibid.*, 1931, **230**, 446).

The fission of diethyl disulphide by aqueous mercuric chloride (J., 1937, 869) gives $EtS\cdot HgCl,HgCl_2$ and a soluble product. It was suggested that this might be ethanesulphinic acid, $Et\cdot SO_2H$, arising from the chlorothiol, EtSCl, by way of EtS·OH. This has now been isolated through the sodium salt as *p*-nitrobenzylethylsulphone. The fission of dimethyl disulphide by mercuric chloride is similar and the methanesulphinic acid has been identified as *p*-nitrobenzylmethylsulphone. The intermediate sulphenic acid R·S·OH could arise directly by incipient hydrolytic fission (compare Smiles and Stewart, J., 1921, **119**, 1794) thus: $R_2S_2 + HOH = R\cdot SH + RS\cdot OH$, the thiol being removed as the insoluble $R\cdot S\cdot HgCl,HgCl_2$. Such fission was demonstrated for diethyl disulphide with water at 170° by one of us and Taylor (J., 1937, 871).

Similar reactions have frequently been postulated as occurring when disulphides are treated with alkali, *e.g.*, by Otto and Rossing (*Ber.*, 1886, **19**, 1235) and by Fromm (*ibid.*, 1908, **41**, 3403) for diphenyl disulphide, and by Price and Twiss (J., 1910, **97**, 1178) in the case of dibenzyl disulphide. Much work has recently been published on the fission of cystine by salts of mercury, silver and copper. References are given in papers by Medes and Padis (*Biochem. J.*, 1936, **30**, 943) and by Lavine (*J. Biol. Chem.*, 1937, **117**, 309), who obtained a mercury derivative of cysteine and a slightly impure sulphinic acid $CO_2H{\cdot}CH(NH_2){\cdot}CH_2{\cdot}SO_2H$, from cystine and mercuric sulphate. Our results, however, afford the first instance of the detection of a sulphinic acid produced by fission of a simple aliphatic disulphide.

The insoluble product formed from dimethyl disulphide and aqueous mercuric chloride is also of the type $RS{\cdot}HgCl,xHgCl_2$, but the exact value of x has not been determined. A double compound of this type is also formed from methylthiol and excess of aqueous mercuric chloride. The same, or a similar compound was obtained by Emmett and Jones (J., 1911, **99**, 716) from " β-trimethyl thiophosphate," $CH_3{\cdot}S{\cdot}PO(O{\cdot}CH_3)_2$, and mercuric chloride. No melting point is recorded, but the analyses were in agreement with the formula $Hg(S{\cdot}CH_3)_2,2HgCl_2$. If this be written as $2(CH_3{\cdot}S{\cdot}HgCl),HgCl_2$ its analogy to the products obtained by us is evident.

From methylthiol and mercuric chloride (0·3 mol.) in water, mercury bismethylthiol, $(CH_3{\cdot}S)_2Hg$, and chloromercury methylthiol, $CH_3{\cdot}S{\cdot}HgCl$, were obtained (compare the behaviour of ethyl- and *n*-propyl-thiol; J., 1937, 869). Klason (*Ber.*, 1887, **20**, 3407) states that the product from the thiol and mercuric chloride is chloromercury methylthiol but gives no experimental details and his product may well have been a mixture.

When dimethyl trisulphide interacts with aqueous mercuric chloride, the product is a mixture of chloromercury methylthiol mercurichloride, $CH_3{\cdot}S{\cdot}HgCl,xHgCl_2$, and the double compound $HgCl_2,2HgS$ (Jolibois and Bouvier, *Compt. rend.*, 1920, **170**, 1497).

As unchanged di-*n*-butyl and di-*n*-amyl disulphides volatilising from the cultures would react with the mercuric chloride in the absorption bottles, their behaviour to this reagent was studied, in water for the di-*n*-butyl compound and in alcohol in the case of di-*n*-amyl disulphide. The products were chloromercury *n*-butylthiol, $C_4H_9{\cdot}S{\cdot}HgCl$, and chloromercury *n*-amylthiol respectively. Unlike the fission products of the first three dialkyl disulphides (J., 1937, 869; this vol., p. 1876), these contain no co-ordinated molecule of mercuric chloride.

The two thiols with mercuric chloride give mixtures of the mercury salt $Hg(SR)_2$ and the chloromercury alkylthiol, the former predominating in the case of the *n*-amyl derivative. The production of mixtures from alkylthiols and mercuric chloride appears usual (see J., 1937, 869) and mercuric oxide or cyanide must be used in order to obtain solely compounds of the type $Hg(SR)_2$.

Certain compounds obtained in the course of this work have not been described previously. These are *di-*n*-amyl disulphide, methyl* n-*amyl sulphide, chloromercury* n-*butylthiol, chloromercury* n-*amylthiol, methyl* n-*butyl sulphide mercurichloride,* $CH_3{\cdot}S{\cdot}C_4H_9,2HgCl_2$, and *methyl* n-*amyl sulphide mercurichloride*, (probably) $3CH_3{\cdot}S{\cdot}C_5H_{11},7HgCl_2$. The unusual composition of this *n*-amyl derivative, observed with both the synthetic and the "mould" preparations, is similar to that of methyl *n*-propyl sulphide mercurichloride (J., 1937, 875).

Ethyl ethanethiolsulphonate (diethyl disulphoxide), $C_2H_5{\cdot}SO_2{\cdot}S{\cdot}C_2H_5$, has hardly any action on aqueous mercuric chloride, but in presence of sodium acetate fission occurs, giving chloromercury ethylthiol mercurichloride, $C_2H_5{\cdot}S{\cdot}HgCl,HgCl_2$, and presumably ethanesulphonic acid formed through the chloride. This reaction confirms the thiolsulphonate structure of the disulphoxides (Smiles and co-workers, J., 1924, **125**, 176; 1925, **127**, 224, 1821).

EXPERIMENTAL.

*Preparation of Substrates and Reference Compounds.—Di-*n*-butyl disulphide.* The disulphide was prepared by Stutz and Shriner's method (*J. Amer. Chem. Soc.*, 1933, **55**, 1242); b. p. 101°/10 mm. Gilman, Smith, and Parker (*ibid.*, 1925, **47**, 851) give b. p. 110—113°/15 mm. and Hunter and Sorenson (*ibid.*, 1932, **54**, 3364) record 93—94°/7 mm. (Found: C, 54·0; H, 10·2; S, 35·9. Calc.: C, 53·9; H, 10·1; S, 35·9%). It was entirely free from the thiol and hydrogen sulphide, giving no precipitate with lead acetate or mercuric cyanide solutions.

Methyl n-*butyl sulphide mercurichloride.* Methyl *n*-butyl sulphide (Ingold, Jessop, Kuriyan, and Mandour, J., 1933, 533) (0·4 g.) and saturated aqueous mercuric chloride (300 c.c.) gave a white solid, m. p. 115—116°, and 115—116·5° on recrystallisation from benzene (Found: C, 9·6; H, 2·2; Cl, 22·3; S, 4·8. $C_5H_{12}S,2HgCl_2$ requires C, 9·3; H, 1·9; Cl, 21·9; S, 4·95%).

*Di-*n*-amyl disulphide.* *n*-Amyl bromide (100 g.), alcohol (300 c.c.), and sodium thiosulphate (200 g.) in water (250 c.c.) were heated for 2 hours. Potassium hydroxide (94 g.) in water (200 c.c.) was then added, and the mixture left overnight, refluxed for 30 minutes, diluted with water, and extracted with ether. Removal of solvent and fractionation gave a colourless oil (30 g., b. p. 140·5—142°/17 mm.) free from thiols and hydrogen sulphide. The odour is less unpleasant than that of the lower disulphides (Found: C, 58·3; H, 10·8; S, 30·7. $C_{10}H_{22}S_2$ requires C, 58·2; H, 10·75; S, 31·1%).

*Mercury di-*n*-amylthiol.* This was prepared from the thiol (Mann and Purdie, J., 1935, 1549) and excess of aqueous mercuric cyanide. It melted constantly at 66° after one crystallisation from benzene. Mann and Purdie (*loc. cit.*) also found m. p. 66°, but Wertheim (*J. Amer. Chem. Soc.*, 1929, **51**, 3661) gives m. p. 74—75° (Found: C, 29·7; H, 5·65; S, 15·3. Calc.: C, 29·5; H, 5·45; S, 15·75%).

Methyl n*-amyl sulphide.* A solution prepared from sodium (4·6 g.; 1 mol.) and *n*-amylthiol (20·85 g.; 1 mol.) in alcohol (125 c.c.) was slowly treated with methyl iodide (28·5 g.; 1 mol.). The mixture was left overnight, refluxed for 1 hour, and diluted with water. The slightly yellow oil, washed with dilute sodium hydroxide solution, then with water and dried, had b. p. 144·5—145·5° and was then colourless (Found: C, 60·7; H, 11·9; S, 27·2. $C_6H_{14}S$ requires C, 60·9; H, 11·9; S, 27·1%). The *mercurichloride*, precipitated with saturated aqueous mercuric chloride, melted at 125—127°, and at 126—127° after two crystallisations from benzene (Found: C, 9·3; H, 1·95; Cl, 21·4; S, 4·1. $C_6H_{14}S,2HgCl_2$ requires C, 10·9; H, 2·1; Cl, 21·4; S, 4·85%. $3C_6H_{14}S,7HgCl_2$ requires C, 9·6; H, 1·9; Cl, 22·0; S, 4·26%).

Dimethyl disulphide. This was required free from mono- and poly-sulphide. The method of Cahours (*Annalen*, 1847, **61**, 91) and Ray and Gupta (*Z. anorg. Chem.*, 1930, **187**, 33) was discarded in favour of that employed by Stutz and Shriner (*loc. cit.*) for di-*n*-butyl disulphide. Methyl iodide (250 g.), methyl alcohol (400 c.c.), sodium thiosulphate (525 g.) and water (500 c.c.) were refluxed for 3 hours. Potassium hydroxide (245 g.) in water (500 c.c.) was added, and heating continued for an hour. Dilution with water, extraction with ether, washing with water and drying gave a product which on fractionation gave dimethyl disulphide as a colourless oil (30 g.), b. p. 110°. This was free from methylthiol and from hydrogen sulphide, being unreactive to lead acetate or mercuric cyanide, and was used throughout the investigation (Found: C, 25·25; H, 6·5; S, 67·9. Calc.: C, 25·5; H, 6·4; S, 68·1%). Hunter and Sorenson (*loc. cit.*) give b. p. 108—108·5°/748 mm. and Riding and Thomas (J., 1923, **123**, 3271) give b. p. 110°.

Diethyl Disulphide and Aqueous Mercuric Chloride.—From a mixture of the disulphide (12 g.), mercuric chloride (108 g.), and water (2000 c.c.) the solid chloromercury ethylthiol mercurichloride (J., 1937, 868) was separated after 14 days, and mercury removed from the filtrate by hydrogen sulphide. Excess of the gas was removed under diminished pressure, and the filtrate neutralised with sodium hydrogen carbonate and evaporated on the steam-bath. The residue was extracted with boiling alcohol, the extract evaporated, and the residue again extracted with the minimum quantity of alcohol. Final evaporation left sodium ethanesulphinate. This gave no precipitate with barium chloride, gave a red colour with ferric chloride, decolourised iodine in potassium iodide and acidified potassium permanganate solutions, and with zinc and hydrochloric acid gave a strong odour of a thiol. 0·6 G. (50% excess) was heated with *p*-nitrobenzyl bromide (0·76 g.) in alcohol (5 c.c.) for 24 hours under reflux. Dilution with water gave a deposit which, after two recrystallisations from alcohol, melted at 133—134° alone and in admixture with authentic *p*-nitrobenzylethylsulphone of the same m. p. (D.R.-P. 623,883). With warm sodium hydroxide it gave a deep red colour (Waldron and Reid, *J. Amer. Chem. Soc.*, 1923, **45**, 2408) (Found: C, 47·9; H, 4·8; N, 5·7, 5·8; S, 13·7. Calc.: C, 47·1; H, 4·8; N, 6·1; S, 14·0%). That the formation of ethanesulphinic acid was not due to the use of hydrogen sulphide for removing the mercury was shown by an experiment in which the mercury was precipitated as oxide with sodium carbonate. Treatment as before again yielded *p*-nitrobenzylethylsulphone.

Dimethyl Disulphide and Aqueous Mercuric Chloride.—The disulphide (12·4 g.) and mercuric chloride (160 g.) in water (3 l.) were left for 2 days. The white precipitate (X, 69·7 g.), which sintered from 141° and decomposed about 156°, was separated (see p. 1878), and the filtrate treated as for the ethyl compound. Extraction of the final residue with boiling alcohol yielded an oil which slowly solidified. This product (1·21 g.) was boiled with *p*-nitrobenzyl bromide (1·0 g.) and alcohol (5 c.c.) for 7 hours. Dilution with water gave a solid, m. p. 169—

170° after two crystallisations from alcohol (Found : C, 44·6; H, 3·95; S, 14·95; N, 6·6. Calc.: C, 44·65; H, 4·2; S, 14·9; N, 6·5%). *p*-Nitrobenzylmethylsulphone melts at 171—172° (D.R.-P. 623,883). Ingold, Ingold, and Shaw (J., 1927, 820) give m. p. 167—168°.

Methylthiol and Mercuric Chloride.—The thiol (0·5 c.c.) and saturated aqueous mercuric chloride (150 c.c., excess) were left for 15 hours. The product melted about 141° and decomposed at 156°, resembling the precipitate obtained from dimethyl disulphide and mercuric chloride (see p. 1875). Extraction with hot water left a residue, unmelted at 260°, presumably chloromercury methylthiol.

When the thiol (0·5 c.c.; 3 mols.) was added to aqueous 3% mercuric chloride (25 c.c.), the pink precipitate became white after 1 minute. Separation and extraction with hot ethyl acetate gave the mercaptide, m. p. 176°, and 176—177° in admixture with a specimen, m. p. 177°, obtained from mercuric cyanide. The insoluble portion was unmelted at 260°, contained halogen, sulphur and mercury, and evolved methylthiol with hydrochloric acid. It was presumably the compound CH_3·S·HgCl. The pink colour of the original precipitate was due to a trace of hydrogen sulphide in the thiol (see J., 1937, 873).

Dimethyl trisulphide. This was prepared from sodium trisulphide and methyl sulphate by Strecker's method (*Ber.*, 1908, **41**, 1105) and fractionated five times, giving a slightly yellow oil, b. p. 54°/13 mm., free from hydrogen sulphide and methylthiol. Strecker gives b. p. 60—62°/14 mm. (Found : C, 19·05; H, 4·85; S, 75·7. Calc.: C, 19·0; H, 4·8; S, 76·2%).

Dimethyl Trisulphide and Aqueous Mercuric Chloride.—The trisulphide (1·57 g.) was added to mercuric chloride (25 g.) in water (500 c.c.). The odour slowly disappeared and the solid (D; 11·0 g.) was separated after 10 days. The filtrate contained traces of sulphuric acid. D was insoluble in most solvents, but hydrochloric acid dissolved a large portion, a strong odour of methylthiol being produced. When D was boiled with water, mercuric chloride was removed, indicating the presence of a compound of the type CH_3·S·HgCl,xHgCl$_2$. Extraction of 7·4 g. of D with boiling pyridine (70 c.c. in all) and dilution of the extracts with alcohol (250 c.c.) gave a white solid (E). This was unmelted at 260°, gave methylthiol with hydrochloric acid, and was practically unchanged by cold sodium hydroxide solution but was decomposed on warming. After extraction of 1 g. with boiling alcohol (200 c.c.) for some hours the insoluble residue was analysed (Found : C, 5·1; H, 1·1; S, 11·3. Calc. for CH_3·S·HgCl : C, 4·2; H, 1·1; S, 11·3%). The pyridine extract of D contained mercuric chloride. The portion insoluble in pyridine darkened after each extraction and was finally reddish-brown and insoluble in dilute nitric acid. It was soluble in potassium sulphide solution and was shown to be mercuric sulphide. The residue obtained from D by boiling with water also blackened gradually with boiling pyridine, as did also the white double compound HgCl$_2$,2HgS.

Diethyl Disulphoxide (Ethyl Ethanethiolsulphonate), C_2H_5·SO$_2$·S·C_2H_5, *and Mercuric Salts.*—The disulphoxide was prepared by Kopp's method (*Annalen*, 1840, **35**, 343) (Found : C, 31·35; H, 6·6; S, 41·2. Calc.: C, 31·1; H, 6·55; S, 41·6%).

Mercuric chloride (7·5 g.), sodium acetate (16 g.), water (200 c.c.), and the disulphoxide (0·95 g.) were shaken for 2 days. As very little reaction had occurred, the mixture was left for a further 23 days; 0·98 g. of a white solid, m. p. 147—149° (decomp.), had then separated. This melted at 149—150° in admixture with the compound C_2H_5·S·HgCl,HgCl$_2$, m. p. 151°, and exhibited its properties (J., 1937, 869). The original reaction mixture slowly deposited a further 0·32 g. of solid, m. p. 146° (decomp.). The filtrate contained traces of sulphuric acid.

*Di-*n*-butyl Disulphide and Aqueous Mercuric Chloride.*—The disulphide (2·67 g.) and mercuric chloride (20 g.) in water (450 c.c.) were shaken at intervals. A white solid formed very slowly and was separated (2·03 g.) when unchanged disulphide was still present. It had m. p. 177—177·5° (decomp.), alone and in admixture with (C) obtained from *n*-butylthiol and aqueous mercuric chloride. The substance was unchanged in m. p. after boiling for some hours with water (250 c.c.). The product was used in the analysis. It gave practically no mercuric oxide with cold sodium hydroxide solution, but this was produced with the warm reagent. These reactions indicate that the *compound* has the composition R·S·HgCl and not R·S·HgCl,HgCl$_2$. It was insoluble in most solvents, but hydrochloric acid gave *n*-butylthiol (Found : C, 15·1; H, 3·1; S, 9·45. C_4H_9·S·HgCl requires C, 14·8; H, 2·8; S, 9·9%).

n-*Butylthiol and Aqueous Mercuric Chloride.*—The thiol (0·85 g.) with mercuric chloride (10 g.; large excess) in water (200 c.c.) gave a white solid (A) (2·25 g.) which was separated after a few days. It gave scarcely any reaction with cold sodium hydroxide solution and melted indefinitely at 80—90°. The solid was repeatedly boiled with alcohol, the extract depositing white crystals (B). After two crystallisations from the same solvent these melted at

84° and at 84—85° in admixture with authentic mercury di-*n*-butylthiol, m. p. 85° (see below), prepared from the thiol and mercuric cyanide. That portion of (A) which was insoluble in alcohol (C) melted at 177—177·5° (decomp.), was soluble in hydrochloric acid, evolving the thiol, and was shown to be chloromercury *n*-butylthiol (Found: C, 14·1; H, 2·8; S, 9·5, 9·4. Calc. for $C_4H_9{\cdot}S{\cdot}HgCl$: C, 14·8; H, 2·8; S, 9·9%).

*Di-*n*-amyl Disulphide and Mercuric Chloride.*—The disulphide (1·74 g.) with a 10% alcoholic solution of mercuric chloride (200 c.c.) gave white crystals after about 24 hours. The amount increased slowly during 9 days to about 0·1 g. This was separated and washed with alcohol; m. p. 180·5° (decomp.) (Found: S, 9·15. Calc. for $C_5H_{11}{\cdot}S{\cdot}HgCl$: S, 9·45%). After 53 days a further deposit was removed, boiled for several hours with water (300 c.c.), and separated. The residue melted at 180·5° (decomp.), alone and in admixture with chloromercury *n*-amylthiol, m. p. 180·5° (decomp.), obtained from the thiol and mercuric chloride. It was insoluble in most solvents (Found: C, 17·15; H, 3·3; S, 9·3. $C_5H_{11}{\cdot}S{\cdot}HgCl$ requires C, 17·7; H, 3·3; S, 9·45%). A further quantity separated from the original filtrate on standing.

n-*Amylthiol and Mercuric Chloride.*—The thiol (1·67 g.) and mercuric chloride (18 g.) in water (400 c.c.) gave a white solid (4 g.) during 4 days. It melted very unsharply and was almost unaffected by cold sodium hydroxide solution. Boiling alcohol left chloromercury *n*-amylthiol, m. p. 180·5° (decomp.), not depressing the m. p. of the product obtained from di-*n*-amyl disulphide and alcoholic mercuric chloride. The alcoholic extracts gave a deposit which on crystallisation from benzene had m. p. 65—66°, alone and in admixture with authentic mercury di-*n*-amylthiol, m. p. 66°. Interaction in alcohol gave similar results.

P. brevicaule Saccardo *(Baarn strain A) and Di-*n*-butyl Disulphide.*—Six 1 l. flasks, each containing 150 g. of bread and 20—30 c.c. of water, were sterilised, inoculated, incubated for 4 days at 30°, and left for 1 day at room temperature. 25 C.c. of a sterile 2% suspension of the disulphide were then added to each flask, and sterile air (J., 1933, 96) aspirated over the cultures through two bottles of aqueous 4% mercuric cyanide and two of aqueous 3% mercuric chloride. Several experiments, each with six flasks, were set up. Precipitates formed very slowly in both solutions; that in the cyanide was in two cases just sufficient for a m. p. determination, the values being 84° and 84—85°. Mercury di-*n*-butylthiol melts at 85° (Mann and Purdie, *loc. cit.*). Wertheim (*loc. cit.*) gives m. p. 85—86°. The small amount of precipitate in the mercuric chloride solution was usually confined to the first bottle and deposition generally ceased after 3—4 weeks. Precipitates formed after this time usually consisted of an ammonia–mercury chloride complex resulting from the breakdown of protein in the cultures. The mixed mercurichlorides from five experiments (30 bread flasks) were extracted with hot benzene (the residue had no m. p. and appeared to be mainly inorganic), which then deposited the pure mercurichloride of methyl *n*-butyl sulphide, m. p. 115—116·5° alone and in admixture with an authentic specimen of the same m. p. (Found: C, 8·5, 8·75; H, 1·9, 1·8; Cl, 21·5; S, 5·0, 4·65. Calc. for $C_5H_{12}S,2HgCl_2$: C, 9·3; H, 1·9; Cl, 21·9; S, 4·95%).

Control experiment. Di-*n*-butyl disulphide (25 c.c. of a sterile 2% aqueous suspension) was added to each of four sterilised uninoculated bread flasks. Sterile air was aspirated through the system into one bottle of aqueous mercuric cyanide and two of aqueous 3% mercuric chloride. No precipitate formed in any of the bottles during 14 days, but a trace of crystalline deposit, insufficient for a m. p. determination, was produced in the mercuric chloride after 29 days. It probably consisted of chloromercury *n*-butylthiol arising from traces of disulphide carried over in the air stream. This result may be compared with that of the analogous blank experiment carried out with the more volatile and more reactive diethyl disulphide (J., 1937, 874), where several deposits all consisting of $EtS{\cdot}HgCl,HgCl_2$ were obtained in the mercuric chloride.

P. brevicaule Saccardo *and Di-*n*-amyl Disulphide.*—This disulphide (25 c.c. of a sterile 2% aqueous suspension) was added to each of four 1 l. bread flasks previously sterilised, inoculated and incubated as usual. After 1 day at room temperature, volatile products were absorbed as before. The deposit in the cyanide solution melted at about 58°, but there was not sufficient for recrystallisation. Mercury di-*n*-amylthiol melts at 66° (see p. 1875).

The deposits in the mercuric chloride solutions were united (0·5 g. from 24 bread cultures) and recrystallised from benzene, a small amount of amorphous material (resembling that obtained in the corresponding experiment with di-*n*-butyl disulphide) remaining undissolved. The benzene deposited crystals, m. p. 126—127° alone and in admixture with authentic methyl *n*-amyl sulphide mercurichloride of m. p. 126—127° (Found: C, 9·4; H, 1·6, 2·1; S, 4·2. Calc. for $C_6H_{14}S,2HgCl_2$: C, 10·9; H, 2·1; S, 4·85%. Calc. for $3C_6H_{14}S,7HgCl_2$: C, 9·6; H, 1·9; S, 4·26%).

Control experiment. This was carried out exactly as with di-*n*-butyl disulphide. After

66 days no precipitate had formed in the mercuric cyanide solution and only a minute precipitate, too small for examination, in the first mercuric chloride bottle.

P. brevicaule *and Dimethyl Disulphide.*—The disulphide (25 c.c. of a sterile aqueous 2% suspension) was added to each of four bread cultures prepared as usual. After 1 day volatile products were absorbed as before. Precipitates formed rapidly. That in the mercuric cyanide (about 0·3—0·4 g. from one experiment) was confined to the first bottle. It was pure white, indicating the absence of hydrogen sulphide from the mould gas (see J., 1937, 873). On recrystallisation from much ethyl acetate it had m. p. 177°, alone and in admixture with authentic mercury bismethylthiol of the same m. p. obtained from mercuric cyanide. Klason (*loc. cit.*) gives m. p. 175° (decomp.).

The deposits in the mercuric chloride bottles from seven experiments, involving in all 28 1-l. bread flasks and a total of 14 g. of dimethyl disulphide, melted at various temperatures between 135° and 141° and decomposed at 156°. They consisted mainly but not entirely of the double compound $CH_3{\cdot}S{\cdot}HgCl,xHgCl_2$ (see p. 1875). They were united and treated with potassium hydroxide solution; a distinct odour of dimethyl sulphide was then noticed. Volatile products were removed in a stream of air and passed through one bottle of 4% mercuric cyanide solution and 3 of 3% mercuric chloride solution, the reaction mixture being gently warmed for several hours (see J., 1937, 870). No deposit formed in the mercuric cyanide at any time, but white crystals, too small in amount for convenient recrystallisation, formed in the first mercuric chloride bottle. These melted at 152—153° alone and when mixed with authentic dimethyl sulphide mercurichloride (m. p. 152—153°) recrystallised from benzene. Published details regarding the m. p. and composition of this mercurichloride are somewhat conflicting. Loir (*Annalen*, 1853, **87**, 369) gives the composition $(CH_3)_2S,HgCl_2$ and no m. p. Phillips (*J. Amer. Chem. Soc.*, 1901, **23**, 250) and Faragher, Morrell, and Comay (*ibid.*, 1929, **51**, 2774) find that the compound has the formula $2(CH_3)_2S,3HgCl_2$, the m. p. being 150—151° on rapid heating.

After 8—9 days reduction and methylation ceased, precipitation occurring only in the mercuric chloride bottles owing to fission of unchanged disulphide carried over with the air stream.

Control experiment. The formation of methylthiol in the above experiment can only be due to the action of the mould, as the disulphide was free from this substance. That the small amount of dimethyl sulphide was not present in the original disulphide was shown by examination of the 69·7 g. of chloromercury methylthiol mercurichloride obtained from 12·4 g. of dimethyl disulphide as described on p. 1875. The precipitate (X) (69·7 g.) was left in contact with 600 c.c. of saturated aqueous mercuric chloride for 3 days and separated. 29 G. were mixed with excess of sodium hydroxide solution, and air aspirated through the gently warmed mixture into one bottle of 4% mercuric cyanide solution and 2 bottles of 3% aqueous mercuric chloride. No precipitate formed in the absorption bottles during 5 days. The remainder of the precipitate was treated similarly, aspiration being continued through the same absorption liquors. After 7 days a trace of white deposit, barely sufficient for filtration, was visible in the first mercuric chloride bottle. It blackened with sodium hydroxide solution, but gave no odour of an alkyl sulphide. This procedure should have detected any dimethyl sulphide in the dimethyl disulphide, since this forms a mercurichloride which, unlike chloromercury methylthiol mercurichloride ($CH_3{\cdot}S{\cdot}HgCl,xHgCl_2$), readily liberates dimethyl sulphide with alkali.

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