20. *The Production of Organo-metalloidal Compounds by Micro-organisms. Part II. Dimethyl Selenide.*

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The formation of odorous compounds in the breath of animals treated with inorganic derivatives of tellurium was first observed by Gmelin (*Wirkungen . . . auf den tierischen Organismus*, Tübingen, 1824, p. 43). Hansen (*Annalen*, 1853, **86**, 213), on administration of potassium tellurite to dogs or men, detected a garlic odour, similar to that of diethyl telluride, in the breath after a few minutes. A similar effect was observed by Japha (*Dissert.*, Halle, 1842) with inorganic selenium compounds. The odours have also been attributed to hydrogen telluride or selenide (Rabuteau, *Gazz. hebdom. med. chirurg.*, 1869, **6**, 194, 241; Filippi, *Lo Sperimentale*, Firenzi, 1913, **67**, 565).

Hofmeister (*Arch. exp. Path. Pharm.*, 1894, **33**, 198) passed the expired air from dogs which had received injections of sodium tellurite or tellurate, through iodine in aqueous potassium iodide. Removal of iodine with potassium hydroxide and addition of sodium sulphide gave an odour resembling that of dimethyl sulphide. Tellurium was obtained from the solution on reduction. Solely from this evidence, Hofmeister concluded that the volatile compound was dimethyl telluride, hydrogen telluride being excluded owing to its different odour.

Maassen (*Arb. Kaiserl. Ges. Amt.*, 1902, **18**, 475) repeated Hofmeister's work, making parallel experiments on dogs injected with selenium. He also studied the odorous products evolved when *Penicillium brevicaule* was cultivated on media containing inorganic compounds of selenium and tellurium. By absorption in iodine as before, Maassen concluded that dialkyl selenides and tellurides were responsible for the odours, and that the animal body exerts a methylating and the mould fungi an ethylating action. This opinion was doubtless strengthened by Biginelli's work on the arsenical (Gosio) gas produced by *P. brevicaule*, which he believed to be diethylarsine, but which was recently shown (Part I; J., 1933, 95, where references are given) to be trimethylarsine. Almost simultaneously Rosenheim (*Proc.*, 1902, 138) showed that selenium and tellurium compounds interfered with the biological test for arsenic in which the garlic-smelling Gosio-gas is evolved. With selenium the odour is fæcal or leek-like, but tellurium gives an odour resembling that of trimethylarsine (see Abel and Buttenberg, *Z. Hyg.*, 1899, **32**, 499; Cevey, *Dissert.*, Lausanne, 1902; Gosio, *Atti R. Accad. Lincei*, 1904, **13**, I, 414, 642).

In view of some of the above statements and the insufficient evidence upon which they are based, we have examined the volatile product from pure cultures of two strains of *P. brevicaule* (*Scopulariopsis brevicaulis*) on sterile bread-crumbs containing sodium selenite or selenate. The products were separately aspirated in a stream of sterile air through (a) Biginelli's solution (J., 1933, 99), (b) neutral mercuric chloride, (c) neutral mercuric bromide, (d) nitric acid, (e) potassium platinochloride, (f) benzyl chloride. The products obtained were: in (a) and (b) *dimethyl selenide mercurichloride*, $(CH_3)_2Se,HgCl_2$; in (c) *dimethyl selenide mercuribromide*; in (d) dimethylhydroxyselenonium nitrate (Jackson, *Annalen*, 1875, **179**, 1); in (e) dimethyl selenide platinochloride, $PtCl_2,2(CH_3)_2Se$ (Fritzmann, *Z. anorg. Chem.*, 1912, **73**, 244); and in (f) dimethylbenzylselenonium chloride, isolated as the picrate (Baker and Moffitt, J., 1930, 1728). These compounds were identified by analysis and mixed m. p. determinations. Diethyl selenide mercurichloride, $(C_2H_5)_2Se,HgCl_2$, m. p. 92·5°, was also prepared and found to be different from the mould product. The isomeric platinochlorides of diethyl selenide melt at 55° and 73° (Fritzmann, *loc. cit.*). The mould gas is therefore dimethyl selenide and, in view of the numerous methylated products elaborated by animals and the conversion of pyridine into methylpyridinium hydroxide (His, *Arch. exp. Path. Pharm.*, 1887, **22**, 253) and of nicotinic acid to trigonelline (Ackermann, *Z. Biol.*, 1912, **59**, 17) in the dog, it is extremely probable that the selenium and tellurium derivatives formed in the body are also dimethyl selenide and telluride. Dimethyl selenide is also produced, and detected as the mercurichloride, when *P. brevicaule* (Strain D) is grown on Czapek–Dox solution containing sodium selenate with glucose (2%) as the sole source of carbon (for composition, see Raistrick, *Phil. Trans.*, 1931, *B*, **220**, 7). With selenite and bread cultures, considerable reduction to red selenium occurs; this is less readily methylated (Rosenheim, Maassen, *locc. cit.*). This does not occur with selenate in bread or glucose cultures, and better yields of dimethyl selenide are obtained.

Attempts were made to obtain dimethyl sulphide by addition of sulphur or certain of its compounds to bread cultures of Strains A and C of *P. brevicaule.* Negative results were obtained with sulphur, sodium sulphite, sodium thiosulphate, thiourea (compare the animal experiments of Pohl, *Arch. exp. Path. Pharm.*, 1904, **51**, 341), sodium ethylsulphonate, " rongalite " and thiodiglycollic acid and its sodium salt.

Failure with the last two compounds would suggest that the mould does not readily decarboxylate the group $CH_2{\cdot}CO_2H$, and that therefore methylation of arsenic and selenium does not proceed by the reactions $As(OH)_3 + 3H{\cdot}CH_2{\cdot}CO_2H = As(CH_2{\cdot}CO_2H)_3 + 3H_2O$ and $SeO(OH)_2 + 2H{\cdot}CH_2{\cdot}CO_2H = SeO(CH_2{\cdot}CO_2H)_2 + 2H_2O \longrightarrow Se(CH_2{\cdot}CO_2H)_2$ (see Part I, *loc. cit.*).

Diethyl sulphide is, however, produced when diethyl sulphoxide, but not the sulphone, is added to bread cultures of the mould. It was characterised as the dimercurichloride (Faragher, Morrell, and Comay, *J. Amer. Chem. Soc.*, 1929, **51**, 2779).

That *Penicillium brevicaule* can exert a strong reducing action is further shown by the formation in bread cultures of trimethylarsine from many derivatives of quinquevalent arsenic (J., 1933, 95), and by unpublished observations on tertiary arsine oxides. Dimethylhydroxyselenonium nitrate is similarly reduced to the selenide when added to cultures of the mould on bread.

EXPERIMENTAL.

The strains of *Penicillium brevicaule* used in this work were *P. brevicaule* Saccardo, *P. brevicaule* Saccardo (Strain Derx), and *P. brevicaule* Saccardo (Strain Washington 2), designated (A), (C), and (D) in an earlier investigation (J., 1933, 95). They were occasionally sub-cultured as before. The cultures on sterile bread were prepared in conical flasks (1 litre) and incubated as previously described. The sterilised solutions of sodium selenate or selenite were added, the flasks (usually eight) connected as before to the absorption vessels, and a stream of sterile air continuously passed through at room temperature. The average content of the selenate and selenite was 0·35 g. per 100 g. of fresh crumbs. The liquid medium occasionally employed was Czapek–Dox solution (2% glucose as sole carbon source), the inorganic selenium compound being added when growth of the mould was well established.

Mould Products.—Dimethyl selenide mercurichloride. The action of strains A and D on media containing sodium selenite or selenate produces a volatile compound giving a white precipitate in Biginelli's solution which on crystallisation from acetone has m. p. and mixed m. p. (with synthetic *dimethyl selenide mercurichloride*) 153—154° (Found: C, 6·6; H, 1·6; Hg, 53·15. $C_2H_6Cl_2SeHg$ requires C, 6·3; H, 1·6; Hg, 52·7%).

Dimethyl selenide mercuribromide. This separated from the aqueous mercuric bromide in the absorption flasks as a white solid, m. p. 99—100° after recrystallisation from acetone and mixed m. p. 99—100° with a synthetic specimen (Found: C, 4·7; H, 1·2. $C_2H_6Br_2SeHg$ requires C, 5·1, H, 1·3%). Mercuric bromide in hydrobromic acid is not a suitable absorbent for small quantities of the mould gas, owing to the partial decomposition of the mercuribromide by the acid.

Dimethylhydroxyselenonium nitrate was obtained from the nitric acid absorption liquors in (d) by evaporation under diminished pressure. The residue had m. p. 88—89·5°, but after recrystallisation from alcohol it melted at 87·5—88·5° alone or in admixture with the synthetic product, also of m. p. 87·5—88·5° (Found: C, 13·0; H, 3·7. Calc.: C, 12·75; H, 3·7%).

Dimethyl selenide platinochloride. This was obtained with Strain D, sodium selenate or selenite being used, and the product absorbed in 2% aqueous potassium platinochloride. The orange-yellow precipitate dissolved almost completely in cold chloroform and is apparently the α-form (Fritzmann, *loc. cit.*), m. p. and mixed m. p. after recrystallisation from chloroform 163—164° [Found: C, 10·0; H, 2·3. Calc. for $PtCl_2,2(CH_3)_2Se$: C, 9·9; H, 2·5%].

Benzyldimethylselenonium picrate. The mould gases were passed into alcoholic benzyl chloride, and the picrate isolated as in the case of trimethylarsine (J., 1933, 99), and recrystallised from water or alcohol. M. p., mixed m. p., and m. p. of an authentic specimen 117—118° (Found: C, 41·5; H, 3·5. Calc.: C, 42·0; H, 3·5%).

The absence of other volatile selenium compounds such as hydrogen selenide or methyl selenomercaptan was indicated by the absence of any coloured precipitate when the mould gases were passed into aqueous mercuric chloride or lead acetate.

Diethyl Sulphide Dimercurichloride.—Diethyl sulphoxide was obtained from the sulphide and "perhydrol" with or without heptane as diluent. It is described by Pummerer (*Ber.*, 1910, **43**, 1407) as an oil, m. p. 4—6°, but was obtained as odourless white crystals, m. p. 14° (compare Strecker and Spitaler, *Ber.*, 1926, **59**, 1765).

25 C.c. of a 1% aqueous solution were added to each of eight flasks containing bread cultures of Strain A. A strong odour of diethyl sulphide was apparent in 1—2 days. Aspiration through aqueous mercuric chloride gave a crystalline solid, m. p. 116—117°. It did not depress the constant m. p. (116—117°) of a specimen of diethyl sulphide dimercurichloride. Faragher and others (*loc. cit.*) give m. p. 119·5°. The possible presence of a trace of the monomercurichloride (m. p. 77°) in both preparations is indicated (Found, for synthetic specimen: Hg, 62·8. Calc., 63·3%). Addition of the sulphoxide to sterile uninoculated bread crumbs gave no sulphide odour.

Preparation of Reference Compounds from Synthetic Selenides.—Dimethyl and diethyl selenide were prepared by the method of Jackson (*Annalen*, 1875, **179**, 1).

Dimethyl selenide mercurichloride was obtained from the selenide and excess of cold Biginelli's solution (J., 1933, 99) or saturated aqueous mercuric chloride. The colourless product was crystallised from acetone; m. p. 153—154°. It is decomposed into its components by hot hydrochloric acid. With sodium hydroxide, mercuric oxide and dimethyl selenide are obtained, no reduction occurring (compare the trimethylarsine mercurichlorides, J., 1933, 99) (Found: Hg, 52·9. $C_2H_6Cl_2SeHg$ requires Hg, 52·7%).

Dimethyl selenide mercuribromide was obtained from aqueous mercuric bromide in neutral or in hydrobromic acid solution; m. p. 99—100°. It is much more unstable than the mercurichloride, smells strongly of dimethyl selenide, and must be kept in a closed vessel (Found : C, 4·7; H, 1·2; Br, 34·8; Hg, 43·6, 43·4. $C_2H_6Br_2SeHg$ requires C, 5·1; H, 1·3; Br, 34·0; Hg, 42·7%).

Dimethylhydroxyselenonium nitrate was prepared by the method of Jackson (*loc. cit.*), m. p. 87·5—88·5° after repeated crystallisation from alcohol (Found : Se, 41·7. Calc., 42·1%).

Dimethylbenzylselenonium picrate (Baker and Moffit, J., 1930, 1728) was kindly supplied by Dr. J. W. Baker. On recrystallisation from aqueous alcohol it had m. p. 117—118° (Found : Se, 17·8. Calc., 18·0%).

Diethyl selenide mercurichloride was obtained from its ingredients and recrystallised from acetone; m. p. 92·5° (Found : Hg, 49·1. $C_4H_{10}Cl_2SeHg$ requires Hg, 49·1%).

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