39. *The Formation of Organo-metalloidal and Similar Compounds by Micro-organisms. Part VII. Dimethyl Telluride.*

By MARJORIE L. BIRD and FREDERICK CHALLENGER.

When three different strains of *Scopulariopsis brevicaulis* (*Penicillium brevicaule*), one of *P. Chrysogenum*, and one of a mould closely related to *P. notatum* are grown upon bread crumbs containing potassium tellurite, dimethyl telluride is evolved. This has been characterised as the mercurichloride, the dibromide and di-iodide and as benzyldimethyltelluronium picrate. Similar results are obtained on glucose–Czapek–Dox medium. From these results and by analogy with other methylations effected by the animal body the garlic odour exhaled by persons after ingestion of tellurite is almost certainly due to dimethyl telluride. *P. chrysogenum* and *P. notatum* evolve dimethyl selenide in bread cultures containing sodium selenate or selenite.

IN previous communications (Challenger and North, J., 1934, 68; Challenger, *Chem. and Ind.*, 1935, **54**, 657) an account was given of the observations of earlier workers on the production of a garlic odour resembling that of an alkyl telluride when potassium tellurite is administered orally to men or animals or added to cultures of *Scopulariopsis brevicaulis* (*Penicillium brevicaule*). Blyth (" Poisons: Their effects and detection," 1884, 588) refers to the case of a student who swallowed " a dose of tellurium " and had to be segregated. He also mentions the phenomenon of " bismuth breath," formerly well known to pharmacists and attributed to the presence of traces of tellurium in medicinal preparations of bismuth. Further details are given by Brownen (*Pharm. J.*, 1876, **6**, 561), Letts (*ibid.*, 1878, **9**, 405, 417) and by Reissert (*Amer. J. Pharm.*, 1884, **56**, 177). During a recent investigation of inorganic derivatives of tellurium in Leeds the odour could easily be detected in the vicinity of those engaged in the work, although they had never come into contact with organic derivatives of tellurium.

On the basis of work which is discussed in our earlier papers (see above) Maassen (*Arb. Kaiserl. Ges. Amt.*, 1902, **18**, 475) concluded that the animal body elaborated dimethyl selenide and dimethyl telluride, and *Scopulariopsis brevicaulis* the corresponding diethyl derivatives. (Maassen's conclusion is incorrectly quoted in *Abstracts*, 1902, ii, 629, and in Mellor's " Treatise on Inorganic and Theoretical Chemistry," Vol. XI, p. 30—the contrary view being attributed to him.) In the case of the mould this was disproved by one of us and North (*loc. cit.*), the product from cultures on bread or glucose–Czapek–Dox medium being shown to be dimethyl selenide. Difficulty was, however, experienced with similar cultures containing potassium tellurite. Aspiration of the volatile products through Biginelli's solution (mercuric chloride in dilute hydrochloric acid) gave traces of precipitate which decomposed without melting. Other absorbents gave equally unsatisfactory results.

Several factors appeared to contribute to this lack of success. Soluble tellurites are readily reduced to black amorphous tellurium by cultures of the mould. Maassen (*loc. cit.*) states that this is unavailable for conversion into the volatile product, a conclusion confirmed by Blackburn in Leeds. Furthermore the alkyl tellurides readily undergo atmospheric oxidation, giving complex products (Vernon, J., 1920, **117**, 894; Balfe, Chaplin, and Phillips, J., 1938, 341).

Success has now been achieved by growing *Scopulariopsis brevicaulis* upon bread crumbs in test-tubes and absorbing the volatile product in about 5 c.c. of Biginelli's solution or other reagent. In this way contact of the mould gases with large volumes of air was some-

what diminished and dimethyl telluride mercurichloride (Carr and Pearson, J., 1938, 282) was obtained. As this can be recrystallised from hot Biginelli's solution, some may have been lost in earlier experiments where 50 c.c. of solution were used.

With sodium hydroxide the mercurichloride gave mercury and soluble dimethyl telluride dihydroxide or oxide or a compound of this with some other methyl derivative of tellurium (compare Balfe, Chaplin, and Phillips, *loc. cit.*). The alkaline solution with hydrobromic acid gave dimethyl telluride dibromide (Vernon, J., 1920, **117**, 86), thus confirming the identification of the mould gas. Furthermore, by absorption in alcoholic iodine, dimethyl telluride di-iodide was obtained. The recorded m. p.'s of the dibromide (24°) and di-iodide (57°) of diethyl telluride (Lowry and Gilbert, J., 1928, 3181) differ widely from those of the corresponding dimethyl derivatives.

The mould gas is therefore dimethyl telluride and Maassen's statement (*loc. cit.*) that it consists of the diethyl compound is incorrect. This conclusion was also confirmed by the use of cultures on 2% glucose–Czapek–Dox medium. The behaviour of tellurium compounds in cultures of *S. brevicaulis* thus falls into line with that of inorganic derivatives of arsenic (J., 1933, 95) and selenium (see above) and of aliphatic disulphides R_2S_2 (J., 1937, 868; 1938, 1872) which give $(CH_3)_3As$, $(CH_3)_2Se$, and RSH and $RS{\cdot}CH_3$ respectively. In this connection it is of interest that arsenic resembles selenium and tellurium in its toxicological properties much more than it resembles antimony (Czapek and Weil, *Arch. exp. Path. Pharm.*, 1893, **32**, 438).

Vernon (*loc. cit.*) states that dimethyl telluride gradually deposits colourless crystals. By atmospheric oxidation we obtained a white solid (X) decomposing at about 240° without melting. This resembles the product obtained by addition of the telluride to water and evaporation of the resulting solution which contains dimethyl telluride dihydroxide (Vernon, *loc. cit.*). We find that the white solid gives the corresponding dichloride and dibromide with the appropriate acid. Addition of the telluride to water, followed by aspiration through Biginelli's solution, showed only a 15% recovery as the mercurichloride. Considerable loss by oxidation of the telluride produced in the cultures must, therefore, occur. Addition of dimethyl telluride to bread or liquid cultures of *S. brevicaulis* or of its oxidation product (X) to liquid cultures caused no deposition of tellurium.

In order to discover whether the deposition of tellurium in tellurite cultures of *S. brevicaulis* was due to a reducing action of the bread or of some product elaborated by the mould, bread crumbs moistened with a tellurite solution were left in a corked test-tube. Practically no deposition of tellurium occurred but after some days a green mould appeared and a strong odour of dimethyl telluride was noticed. A culture of this organism was sent to Dr. Thom of the U.S. Department of Agriculture, Washington, through the courtesy of Dr. St. John-Brooks of the Lister Institute: extracts from his report are as follows: "Routine examination of freshly-made transfers indicated *Penicillium notatum*, Westling." Dr. Thom and Dr. Raper made up tellurium culture media and checked the organism against "Westling's type culture, a type culture of *Penicillium chrysogenum* and the Fleming Penicillium." The culture of the green mould on both tellurite and tellurate "produced violent odours. The other three species produced the same odour but in less intense form. . . . To summarise, then, I place your organism near *P. notatum*, not necessarily identical with Westling's strain of *P. notatum*, since biochemical differences between strains are the rule rather than the exception."

Bread cultures of our "green mould" containing tellurite were then examined, and the evolved dimethyl telluride identified as before and as benzyldimethyltelluronium picrate. There was only very slight formation of tellurium, which would appear to be the special advantage of this particular organism. Dimethyl telluride was also produced in cultures on 2% glucose–Czapek–Dox medium.

In view of Dr. Thom's results pure cultures of *P. chrysogenum* Thom (Washington 26) and *P. notatum* were obtained from the Lister Institute. In tellurite–bread cultures the former gave dimethyl telluride, identified as the mercurichloride and the dibromide, but only a very faint odour could be observed when *P. notatum* was used. Both organisms readily gave dimethyl selenide in bread cultures containing sodium selenite or selenate. This was also produced in bread–selenate cultures by the "green mould" isolated by us.

None of these green *Penicillia* gave any odour of trimethylarsine in bread cultures containing arsenious oxide.

Bearing in mind the methylating powers of the animal body (see J., 1934, 69; *Biochem. J.*, 1935, **29**, 1757) there can be no doubt that men and animals also evolve dimethyl telluride after administration of tellurium. This has already been stated both by Hofmeister (*Arch. exp. Path. Pharm.*, 1894, **33**, 198) and by Maassen (*loc. cit.*), but their conclusions, which have been widely quoted, were based on considerations of odour and there exists no proof of the nature of the alkyl telluride evolved by experimental animals.

Dudley (*Amer. J. Hyg.*, 1936, **23**, 179, 183) refers to the garlic odour of the breath of persons suffering from selenium poisoning and to the presence of a volatile, ether-soluble selenium compound in the urine of a horse after ingestion of sodium selenite. As concluded by one of us and North from analogous experiments with *S. brevicaulis* (J., 1934, 69), the exhaled product is almost certainly dimethyl selenide.

It would therefore appear that arsenic also should be methylated in the animal body and exhaled as trimethylarsine. That no odour comparable in intensity with that produced by tellurite follows administration of medicinal doses of inorganic compounds of arsenic is well known (see also Reissert, *loc. cit.*), but occasional references to the presence of a garlic odour in the perspiration following upon arsenical poisoning occur (Blyth, *op. cit.*, 1895, 544, 545).

Pleschtizer and Preobrajensky (*Arch. Gewerbe Path. und Gewerb Hyg.*, 1935, **6**, 80) passed the breath from patients in receipt of inorganic arsenic through bromine water. Treatment with ammonia and evaporation gave a slight residue in which the presence of arsenic was detected by addition to cultures of *S. brevicaulis*, the garlic odour of " Gosio-gas " being obtained. The presence of some volatile compound of arsenic in the breath was thus rendered extremely probable, but the quantity was too small for identification. These authors, at that time unaware of the identification of Gosio-gas as trimethylarsine (Challenger, Higginbottom, and Ellis, J., 1933, 95), merely quote Biginelli's statement (*Gazzetta*, 1901, **31**, 58) that it consists of diethylarsine.

Keeser [Heffter's " Handbuch der exp. Pharmakologie (Ergänzungsband)," 1937, **3—4**, 176], however, while citing this later work, states without further comment that according to the Russian workers the gas from cultures of *S. brevicaulis* on arsenical media is diethylarsine and not trimethylarsine.

Carlson (*Z. physiol. Chem.*, 1906, **49**, 431), Montgomery (*J. Amer. Med. Assoc.*, 1916, **66**, 491), and Puntoni (*Annali d'Igiene*, 1917, **27**, 293) refer to the production of a garlic odour in the breath after ingestion or injection of cacodylic acid or its sodium salt. Bloemendal (*Arch. Pharm.*, 1908, **246**, 599) passed the exhaled air of a rabbit, which had received 20 mg. of sodium cacodylate, through alkaline potassium permanganate solution, which then contained arsenic. The odorous product was not identified. Montgomery suggested that it is cacodyl. From the behaviour of sodium cacodylate in cultures of *S. brevicaulis* (Challenger *et al.*, J., 1933, 99) the formation of trimethylarsine would be expected by reduction and further methylation. However, methylation does not occur readily in animals receiving arsenious oxide (see above), and trimethylarsine, if formed from cacodylic acid in the animal body, might conceivably arise by dismutation of cacodyl oxide: $2(CH_3)_2AsO = (CH_3)_3As + CH_3{\cdot}AsO_2$ (compare the action of diethylchloroarsine with alkali; Grischkewitsch-Trochimovski, *Rocz. Chem.*, 1928, **8**, 423; see also Baeyer, *Annalen*, 1858, **107**, 285). Such dismutations are well established in the case of the alkyl derivatives of tellurium (Drew, J., 1929, 566).

Puntoni attributed the garlic odour after oral administration of cacodylate to the effect of intestinal organisms, some of which he cultivated on cacodylate media, obtaining a similar odour. Using strains of the same and two other bacteria, Challenger and Higginbottom (*Biochem. J.*, 1935, **29**, 1762) were unable to detect any odour in media containing arsenious oxide, sodium arsenate, sodium methylarsonate or sodium cacodylate.

Experimental.

Preparation of Reference Compounds.—Dimethyl telluride was prepared by a modification of the method of Balfe, Chaplin, and Phillips (*loc. cit.*) for the di-*n*-butyl compound. Methyl

iodide (25 c.c.) was slowly added to a warm mixture of sodium formaldehydesulphoxylate (" rongalite," 54 g.), sodium hydroxide (42 g.), and precipitated tellurium (24 g.) in water (180 c.c.). White crystalline trimethyltelluronium iodide was deposited in the neck of the flask and in the condenser (Found : I, 42·5. Calc. : I, 42·5%). The mixture was finally heated for some minutes at 100°; the deep purple colour then disappeared. Distillation yielded an orange-coloured oil, which, on being dried over sodium sulphate and redistilled, gave dimethyl telluride as a pale yellow oil, b. p. 93·5°/749 mm. Very little oxidation (see p. 163) occurred during distillation, although air was not excluded. Extraction with ether was impracticable owing to the difficulty of separating the resulting mixture by distillation.

Dimethyl telluride mercurichloride, $(CH_3)_2Te,HgCl_2$, separated as a white solid, m. p. 174° (decomp.), when the telluride was mixed with excess of Biginelli's solution (mercuric chloride, 10 g.; concentrated hydrochloric acid, 20 c.c.; water, 80 c.c.). One recrystallisation from boiling Biginelli's solution gave small plates, m. p. 179° (decomp.), the value given by Carr and Pearson (J., 1938, 282) for a specimen prepared in acetone. Addition of dilute sodium hydroxide solution gave yellow mercuric oxide and an odour of dimethyl telluride, but immediate oxidation of the liberated telluride occurred and black mercury was deposited. The filtered alkaline solution on addition of hydrobromic acid gave dimethyl telluride dibromide, m. p. 92—94° after drying in a vacuum (Found : Br, 50·5. Calc. : Br, 50·5%).

This recalls the behaviour of trimethylarsine mono- and di-mercurichlorides (Challenger, Higginbottom, and Ellis, J., 1933, 99) with sodium hydroxide. Dimethyl selenide and sulphide when liberated from their mercurichlorides by alkali are not oxidised and the mercuric oxide remains yellow (Challenger and North, *loc. cit.*).

The *ethyl* ester of dimethyltelluretine bromide was rapidly precipitated as small white crystals on addition of an ethereal or alcoholic solution of ethyl bromoacetate to dimethyl telluride, or after a few minutes by aspiration of air over the telluride into the ester solution. These were very soluble in water or alcohol, but insoluble in ether; m. p. 137·5° (Found : Br, 24·5. $C_6H_{13}O_2BrTe$ requires Br, 24·7%).

Phenacyldimethyltelluronium bromide was precipitated by interaction of phenacyl bromide and the telluride in ether or alcohol. It formed white crystals soluble in water or alcohol and could be recrystallised from alcohol–ether; m. p. 90—91° Found : Br, 22·4. $C_{10}H_{13}OBrTe$ requires Br, 22·5%).

Benzyldimethyltelluronium picrate was obtained by passing the telluride (evolved by warming the di-iodide, sodium sulphite, and sodium carbonate) into alcoholic benzyl chloride. Dilution with water and extraction with ether removed the excess of benzyl chloride. Sodium picrate then gave yellow crystals, m. p. 117—118°, and 121° on recrystallisation from alcohol (Found : C, 37·8; H, 3·3; N, 9·4. $C_{15}H_{15}O_7N_3Te$ requires C, 37·75; H, 3·2; N, 8·8%).

Mould Experiments.—A comparison of the four strains of *Scopulariopsis brevicaulis* (*Penicillium brevicaule*) employed by Challenger *et al.* (*loc. cit.*) showed that *S. brevicaulis* Saccardo (Strain Washington 2), designated (D) in these investigations, gave the strongest odour on addition of potassium tellurite to its cultures, and this strain was used for the work described in I and II below.

Bread crumbs, slightly moistened with water and almost filling test-tubes of about 40 c.c. capacity, were used as the solid medium. The liquid medium was always 200 c.c. of 2% glucose–Czapek–Dox solution in 1 l. flasks. Sterilisation of the media, stoppers, connecting tubing, and the air stream was ensured as before (J., 1933, 98). The mould was always allowed to form a good mycelium before the potassium tellurite was added. The strains of *S. brevicaulis* usually required 2 days at 32° and 5 days at room temperature when grown on bread and 2—3 days longer on liquid media.

Judged by the intensity of odour produced in cultures at the same stage of growth, the most efficient concentration of potassium tellurite was 0·5—1·0 g. in 200 c.c. of liquid. Actually 0·5 g. was always employed. Each tube received 0·2 g. of potassium tellurite in 1·5 c.c. of sterile water. Aeration began when the cultures had acquired a distinct odour.

As small quantities of dimethyl telluride were occasionally used in the laboratory and the odour was then perceptible, the air drawn through the cultures was obtained by means of a pipe from outside the building except in one experiment (see below). Passage of laboratory air through Biginelli's solution (5 c.c.), however, never produced a precipitate during several weeks. Moreover, when dimethyl telluride was detected in the cultures by chemical means, the tubes or flasks had an intense odour. Addition of potassium tellurite to sterile, uninoculated bread crumbs and aspiration through Biginelli's solution gave no precipitate.

I. S. brevicaulis *on a liquid medium with potassium tellurite.* Twelve flasks were used, much

deposition of tellurium occurred on the mycelium, and an odour was apparent after 4 days. Volatile products were then aspirated through Biginelli's solution (5 c.c.). A solid formed in the absorption tube after several hours and gradually increased during 10 days. This melted at 173—174° (decomp.), at 177° (decomp.) on recrystallisation from hot Biginelli's solution, and at 177° (decomp.) in admixture with authentic dimethyl telluride mercurichloride of the same m. p. A portion was converted into the dibromide as described on p. 166. This melted at 92—93° (Found : Br, 49·75. Calc. : Br, 50·45%).

An experiment with 5 flasks, laboratory air being used, gave similar results. The recrystallised mercurichloride had m. p. 176° (decomp.) and mixed m. p. 177° (decomp.). The dibromide melted unrecrystallised at 92—93° (slightly sintering from 83°) and at 92—94° in admixture with authentic dibromide, m. p. 95°.

II. S. brevicaulis *on a bread medium with potassium tellurite.* Eighteen test-tube cultures were employed in series. The odour of dimethyl telluride was noticed after 48 hours, but deposition of tellurium occurred much earlier. After 10 days about 0·01 g. of mercurichloride, m. p. 178° (decomp.), had separated in the Biginelli's solution. Conversion into the dibromide gave a product of m. p. and mixed m. p. 94—95°. After 17 days the odour of telluride was very faint.

In a second experiment, seven tube cultures being used and the product being aspirated through alcoholic iodine solution, red needles, m. p. 125° (decomp.), were deposited. Dimethyl telluride di-iodide melts at 130° (decomp.) (Vernon, *loc. cit.*).

III. Similar results were obtained with two other strains of the same mould, namely, *S. brevicaulis* (Sacc.) Bainier and *S. brevicaulis* (Strain Derx), briefly designated Strains A and C (J., 1933, 98). Bread cultures in test-tubes were employed and with Strain A cultures on 2% glucose–Czapek–Dox medium also. In each case dimethyl telluride was identified as the mercurichloride.

IV. *The green* Penicillium *on a bread medium with potassium tellurite.* Four tube cultures in series gave an odour of dimethyl telluride in 24 hours and very little deposition of tellurium occurred. A solid formed in Biginelli's solution after 1 hour's aeration. This increased more rapidly than when *S. brevicaulis* was used, about 0·15 g. being obtained in 10 days. This had m. p. 172—173° (decomp.), and 178° (decomp.) on recrystallisation. Conversion into the dibromide gave a product, m. p. 91—92°, and mixed m. p. 93·5°.

The Biginelli's solution was then replaced by alcoholic benzyl chloride (5 c.c.), and aspiration continued. After 7 days this was diluted with water, extracted with ether, and treated with sodium picrate; the precipitated product had m. p. 117—118° (without recrystallisation) and 119—120° in admixture with authentic benzyldimethyltelluronium picrate, m. p. 121°.

When eleven tube cultures were aerated immediately after addition of tellurite, very little odour was perceptible and no precipitate appeared in the Biginelli's solution during 36 hours. The tubes were therefore closed for 24 hours; the odour was then very decided. Aeration quickly gave a mercurichloride, m. p. 177° (decomp.) and mixed m. p. 175° (decomp.). When benzyl chloride was used as absorbent, benzyldimethyltelluronium picrate was obtained as before, m. p. and mixed m. p. 118°.

V. *The green* Penicillium *in liquid cultures with potassium tellurite.* In three separate experiments this gave dimethyl telluride, characterised as the mercurichloride. The quantity was smaller, however, than in the experiments described in sections I to III.

VI. *The green* Penicillium *in bread culture with sodium selenite.* The mould was grown on bread crumbs in a 250 c.c. flask for 1 day at 32° and 2 days at room temperature. Sodium selenite (0·2 g. in 10 c.c. of sterile water) was then added. Aspiration into Biginelli's solution quickly gave dimethyl selenide mercurichloride, m. p. 152—153° and mixed m. p. 153—154° with an authentic specimen of the same m. p. The odour in the culture flask was very strong.

VII. Penicillium chrysogenum *and potassium tellurite.* Twelve bread cultures were grown in tubes for 3 days at room temperature, and potassium tellurite (0·3 g.) in a little sterile water added to each. A strong odour was produced and some tellurium was deposited in 48 hours. Absorption as before yielded dimethyl telluride mercurichloride, m. p. 170—172°, which was converted into the dibromide, m. p. 93° and mixed m. p. 94°. A second experiment with four tubes gave similar results.

VIII. P. notatum *and potassium tellurite.* An exactly similar experiment to VI was set up, but only a very slight odour was noticed after 48 hours and no deposit formed in Biginelli's solution (1—2 c.c.). A second experiment with 9 tube cultures gave similar results. Rather more tellurium was deposited than in VI.

IX. P. chrysogenum *and* P. notatum *with sodium selenite and selenate.* Both substances

were used with each organism in a bread culture contained in a 250 c.c. flask. An odour was observed 2 hours after addition of the salt (0·5 g. in 10 c.c. of sterile water). Aspiration through Biginelli's solution gave dimethyl selenide mercurichloride in each case. With *P. notatum* and sodium selenate, this had m. p. 149—151° and mixed m. p. 153—154°. In the other cases the deposits melted either at 153°, 154° or 154—155°, giving entirely satisfactory mixed m. p.'s with an authentic specimen of dimethyl selenide mercurichloride, m. p. 153—154°.

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