87. *The Formation of Organo-metalloidal Compounds by Micro-organisms. Part III. Methylated Alkyl- and Dialkyl-arsines.*

By Frederick Challenger and Louis Ellis.

The formation of dimethylethylarsine by the growth of *Penicillium brevicaule* (*Scopulariopsis brevicaulis*) *Saccardo* on sterile bread crumbs containing sodium ethylarsonate, $EtAsO(ONa)_2$, has been described (J., 1933, 95). This type of reaction, involving both methylation and reduction, has been further studied. By addition of (a) diethylarsinic acid, $Et_2AsO{\cdot}OH$ (Wigren, *Annalen*, 1924, **437**, 285; Grischkewitsch-Trochimowski, *Rocz. Chem.*, 1928, **8**, 423), (b) *n*-propylarsonic acid, and (c) allylarsonic acid, $CH_2{:}CH{\cdot}CH_2{\cdot}AsO(OH)_2$ (Quick and Adams, *J. Amer. Chem. Soc.*, 1922, **44**, 807) to similar cultures of the same strain (A) of the mould (J., 1933, 95) in concentrations varying from 0·2 to 0·5%, mixed methylated arsines are produced. These have been removed in a sterile air stream, and absorbed in suitable reagents.

Methyldiethylarsine is obtained from (a) and converted into (1) the *dimercurichloride* and (2) *hydroxymethyldiethylarsonium picrate*. Similarly (b) gives dimethyl-*n*-propylarsine, which has been identified as (3) the *dimercurichloride*, (4) *benzyldimethyl*-n-*propylarsonium picrate*, and (5) *hydroxydimethyl*-n-*propylarsonium picrate*.

In an analogous manner (c) gives rise to *dimethylallylarsine*, which has been characterised as (6) the *dimercurichloride* and (7) *benzyldimethylallylarsonium picrate*. Absorption in nitric acid is in this case unsuitable as indicated by preliminary experiments with the synthetic arsine. All the above compounds have been analysed [(4) excepted] and in all cases compared as regards m. p. and mixed m. p. with analysed specimens obtained from the synthetic arsines.

Growth of the mould on bread or on aqueous media containing potassium antimonyl tartrate with or without glucose gave no odour of an alkyl stibine. This is in agreement with the negative results obtained by Knaffl-Lenz (*Arch. exp. Path. Pharm.*, 1913, **72**, 224), Tiegs (*Kleine Mitth. Wasserversorg.*, 1925, **1**, 51), and Smith and Cameron [*Ind. Eng. Chem.* (*Anal.*), 1933, **5**, 401].

Experimental.

P. brevicaule Saccardo (*Baarn Strain A*) *and Diethylarsinic Acid*.—Five 1-l. flasks, each containing 150 g. of bread, were inoculated and incubated for 4 days at 30° and at room temperature for 1 day, and 25 c.c. of a sterile 1% solution of diethylarsinic acid added to each. Concentration = approx. 0·2%. The flasks were connected in two parallel sets of 3 in series and 2 in series, and the volatile matter passed into one bottle containing Biginelli's solution (mercuric chloride in hydrochloric acid; J., 1933, 96). A trace of precipitate formed in the solution after 3 days, but increased very slowly and after 27 days the small amount was removed. It had m. p. 202° (decomp.) and the same on recrystallisation. There was not sufficient for a second recrystallisation and it may have contained a trace of mercuric chloride. It did not depress the m. p. of synthetic methyldiethylarsine dimercurichloride, m. p. 196—198°, decomp. 201° (Found : C, 8·6; H, 1·8. Calc. for $C_5H_{13}Cl_4AsHg_2$: C, 8·7; H, 1·9%).

The poor yield of arsine from diethylarsinic acid is possibly due to the toxic action of this acid on the mould. Earlier experiments with sodium cacodylate (J., 1933, 98) gave much less trimethylarsine than was obtained with sodium methylarsonate.

Two experiments in which (a) disodium diethylarsinate and (b) monopotassium diethylarsinate were present in the medium in concentrations of about 0·4% were also made. In (a) after 60 days no garlic odour and no precipitate were obtained. In (b) after 93 days the amount of dimercurichloride obtained (2 deposits) was only sufficient for a determination of the m. p.'s, 199—200° and 200—202°.

Hydroxymethyldiethylarsonium picrate, $OH{\cdot}AsMeEt_2{\cdot}O{\cdot}C_6H_2(NO_2)_3$.—Absorption of the mould gas in nitric acid, evaporation, and precipitation with sodium picrate gave yellow crystals, m. p. 128—130°, and 134—135° in admixture with authentic synthetic picrate (m. p. 134—135°) (Found : C, 33·15; H, 4·4. Calc. : C, 33·6; H, 4·1%).

P. brevicaule Sacc. *and* n-*Propylarsonic Acid.—Dimethyl-*n-*propylarsine dimercurichloride,* $AsMe_2Pr,2HgCl_2$. Four 1-l. flasks (150 g. of bread) were inoculated and incubated at 30° for 4 days and at room temperature for 2 days. To each flask were added 25 c.c. of a sterile solution made from *n*-propylarsonic acid (2·23 g.), potassium bicarbonate (1·33 g.), and water (125 c.c.), giving a concentration of about 0·3% of the monopotassium salt in each flask. A garlic odour was detected after 2 days and was still perceptible after 12 weeks. A deposit formed in the Biginelli's solution after 3 days weighed 0·09 g. on the 23rd day, sintered at 187°, melted at 195°, and decomposed, evolving gas, at 200°. It was mixed with 0·11 g. of an exactly similar product obtained in a duplicate experiment and recrystallised (as in the case of all mercurichlorides prepared in this research) from hot water containing a little mercuric chloride; m. p. 195°, decomp. 200°. It did not depress the m. p. of synthetic dimethyl-*n*-propylarsine dimercurichloride, m. p. 195°, decomp. 200° (Found : C, 8·5; H, 1·8; Cl, 19·9. Calc. for $C_5H_{13}Cl_4AsHg_2$: C, 8·7; H, 1·9; Cl, 20·5%).

*Benzyldimethyl-*n-*propylarsonium picrate,* $CH_2Ph{\cdot}AsMe_2Pr{\cdot}O{\cdot}C_6H_2(NO_2)_3$. From the 23rd to the 51st day of the experiment the arsenical gas was absorbed in alcoholic benzyl chloride. The resulting quaternary salt was converted into the picrate in the usual way (J., 1933, 99). This was mixed with a further quantity obtained in a fresh experiment of 69 days' duration. The m. p. was 108—110° and 113—114° on recrystallisation from hot water. The product, which was obtained in yield barely sufficient for analysis, did not depress the m. p. (113—114°) of synthetic benzyldimethyl-*n*-propylarsonium picrate.

*Hydroxydimethyl-*n-*propylarsonium picrate,* $OH{\cdot}AsMe_2Pr{\cdot}O{\cdot}C_6H_2(NO_2)_3$. From the 51st to the 86th day nitric acid (*d* 1·4) was used as absorbent. Evaporation then gave a trace of semi-solid residue, which with aqueous sodium picrate gave a precipitate, m. p. 136—139°, and 142—143·5° after two recrystallisations from water. In admixture with synthetic hydroxydimethyl-*n*-propylarsonium picrate (m. p. 143—144°) the m. p. was 142—143·5° (Found : C, 33·8; H, 4·2; N, 10·5. Calc. : C, 33·6; H, 4·1; N, 10·7%).

P. brevicaule Saccardo *and Allylarsonic Acid.*—Four 1-l. flasks (150 g. of bread). Incubation 4 days at 30°, 1 day at room temperature. To each were added 25 c.c. of a solution of allylarsonic acid (5 g., m. p. 128—129°. Quick and Adams give m. p. 128—129°) and potassium bicarbonate (3 g.) in water (250 c.c.).

Concentration in the bread = 0·35%. There was a slight odour in 2 days and a slight deposit in Biginelli's solution in 3 days. After 21 days 0·1 g. of precipitate was removed, m. p. 200° (decomp.). With sodium hydroxide it turned yellow and then black, evolving an intense odour, the usual behaviour of an aliphatic arsine mercurichloride (J., 1933, 99).

Absorption in fresh Biginelli's solution then gave deposits of 0·1 g., 0·1 g., 0·35 g., and 0·09 g. after 9, 8, 8, and 12 days. Total, 0·74 g. in 58 days. Yield, about 9%. The combined precipitates had m. p. 209° (decomp.) before and after crystallisation in the usual way. In admixture with synthetic dimethylallylarsine dimercurichloride (m. p. 209° decomp.) the m. p. was unaffected. The m. p.'s of both the synthetic and the mould mercurichlorides depend on the rate of heating, variations of as much as 6° being observed with the same specimen on different occasions (Found : C, 8·1 ; H, 1·7 ; Cl, 20·9. Calc. for $C_5H_{11}Cl_4AsHg_2$: C, 8·7 ; H, 1·6 ; Cl, 20·6%).

Absorption of the mould gas in benzyl chloride gave a quaternary salt, which was converted into *benzyldimethylallylarsonium picrate*, $CH_2Ph{\cdot}AsMe_2(C_3H_5){\cdot}O{\cdot}C_6H_2(NO_2)_3$, m. p. 96—97° after recrystallisation, not depressing the m. p. of the synthetic picrate, 96—97° (Found : C, 46·2; H, 4·2; N, 8·9. $C_{18}H_{20}O_7N_3As$ requires C, 46·4; H, 4·3; N, 9·0%). It is clear from these results that the mould does not reduce the double linkage in the allyl group.

Preparation of Reference Compounds from Synthetic Arsines.—Dimethyl-*n*-propylarsine, b. p. 21°/22 mm., was prepared by the method of Jones (J., 1932, 2284), who gives b. p. 27°/17 mm. With excess of Biginelli's solution it gave the *dimercurichloride*, m. p. 195°, decomp. 200°, after recrystallisation (Found : C, 8·5; H, 1·7; Cl, 20·3. $C_5H_{13}Cl_4AsHg_2$ requires C, 8·7; H, 1·9; Cl, 20·5%).

*Dimethyl-*n*-propylarsine monomercurichloride*, $AsMe_2Pr,HgCl_2$, m. p. 126—128°, was obtained from the calculated quantities of the ingredients in dilute hydrochloric acid or in alcoholic solution. In the former case the reaction was gradual (Found : C, 14·2; H, 3·3; Cl, 16·9. $C_5H_{13}Cl_2AsHg$ requires C, 14·3; H, 3·1; Cl, 16·9%).

*Benzyldimethyl-*n*-propylarsonium picrate* was obtained from equal volumes (1 c.c.) of the arsine and benzyl chloride. The arsonium chloride was deliquescent and gave a picrate, m. p. 113—114° on recrystallisation (Found : C, 46·3; H, 4·6; picric acid, as nitron picrate, 49·3. $C_{18}H_{22}O_7N_3As$ requires C, 46·3; H, 4·75; picric acid, 49·0%).

*Hydroxydimethyl-*n*-propylarsonium picrate.* Addition of a few drops of nitric acid to the arsine caused immediate reaction and white fumes. Addition of water and evaporation gave an oily hydroxynitrate which did not crystallise. The *picrate* had m. p. 142—144°, and 143—144° on recrystallisation from hot water (Found : C, 33·3; H, 4·1; picric acid, 58·2. $C_{11}H_{16}O_8N_3As$ requires C, 33·6; H, 4·1; picric acid, 58·3%).

Methyldiethylarsine. The arsine was prepared following the general procedure of Jones (*loc. cit.*) from methyldi-iodoarsine (50 g.), ethyl bromide (60 g.), and magnesium (14 g.). It boiled at 112—114° in an atmosphere of carbon dioxide. Yield, 10 g. Cahours (*Annalen*, 1862, **122**, 220) gave no b. p.

Excess of Biginelli's solution gave the *dimercurichloride*, $AsMeEt_2,2HgCl_2$, which on recrystallisation had m. p. 196—198° and decomp. 201°. It was also obtained by slow passage of air over an *iso*amyl ether solution of the arsine into Biginelli's solution (see J., 1933, 97) (Found : C, 8·8; H, 2·2; Cl, 20·1. $C_5H_{13}Cl_4AsHg_2$ requires C, 8·7; H, 1·9; Cl, 20·5%).

Methyldiethylarsine monomercurichloride was formed (rather slowly) from the calculated amounts of its ingredients in dilute hydrochloric acid or alcoholic solution; m. p. 153—154° (Found : C, 14·3; H, 3·2; Cl, 17·2. $C_5H_{13}Cl_2AsHg$ requires C, 14·3; H, 3·1; Cl, 16·9%).

Benzylmethyldiethylarsonium picrate, $CH_2Ph{\cdot}AsMeEt_2{\cdot}O{\cdot}C_6H_2(NO_2)_3$, was obtained as in similar cases; m. p. 85—86° (Found : C, 46·4; H, 4·9; picric acid, 48·8. $C_{18}H_{22}O_7N_3As$ requires C, 46·3; H, 4·75; picric acid, 49·0%).

Benzylmethyldiethylarsonium styphnate, $CH_2Ph{\cdot}AsMeEt_2{\cdot}O{\cdot}C_6H(OH)(NO_2)_3$. The arsonium chloride was treated with a solution of sodium carbonate (anhydrous, 3·7 g.), and styphnic acid (8·5 g.) in water (250 c.c.). After recrystallisation from hot water the precipitate melted at 89—90° (Found : C, 44·7; H, 4·5. $C_{18}H_{22}O_8N_3As$ requires C, 44·8; H, 4·6%. The ingredients are united in equimolecular proportion).

Hydroxymethyldiethylarsonium picrate. The arsine (0·5 c.c.) was slowly added to dilute nitric acid (5 c.c.), but remained undissolved. Addition of the concentrated acid gave thick white fumes and a clear solution. This on evaporation left a colourless hydroxynitrate which did not solidify. The corresponding *picrate*, recrystallised from hot water, had m. p. 134—135° (Found : C, 33·6; H, 4·2; picric acid, 58·35. $C_{11}H_{16}O_8N_3As$ requires C, 33·6; H, 4·1; picric acid, 58·3%).

Dimethylallylarsine was prepared in the usual manner. The solution of allylmagnesium bromide was obtained by the method of Gilman and McGlumphy (*Bull. Soc. chim.*, 1928, **43**, 1322) as modified slightly by Shoemaker and Boord (*J. Amer. Chem. Soc.*, 1931, **53**, 1508). The arsine was fractionated in carbon dioxide at ordinary pressure. It boils at 108—110°, is colourless, has a very penetrating odour, and is spontaneously inflammable on filter-paper in air. Arsenic was determined by the iodometric method of Ewins (J., 1916, **109**, 1356) (Found : As, 51·1, 51·6. $C_5H_{11}As$ requires As, 51·3%).

Dehn and Wilcox (*Amer. Chem. J.*, 1906, **35**, 20) describe this arsine as a light yellow liquid, b. p. about 160°, obtained from methylarsine and allyl iodide in a sealed tube. They give no analysis of it or of the corresponding methiodide.

The *dimercurichloride*, m. p. 208—209° (decomp.), was obtained with Biginelli's solution (Found : C, 8·5; H, 1·4; Cl, 20·4. $C_5H_{11}Cl_4AsHg_2$ requires C, 8·7; H, 1·6; Cl, 20·6%).

Dimethylallylarsine monomercurichloride, $C_3H_5\cdot AsMe_2,HgCl_2$, prepared from its ingredients in alcohol or dilute hydrochloric acid, has m. p. 120—121° (Found : C, 14·3; H, 2·8; Cl, 17·3. $C_5H_{11}Cl_2AsHg$ requires C, 14·4; H, 2·7; Cl, 17·0%).

Benzyldimethylallylarsonium picrate was prepared in the usual way, the arsine and benzyl chloride being mixed in an atmosphere of carbon dioxide. The closed box described by Dyke and Jones (J., 1930, 1923) was used in this and several similar reactions. The picrate melts at 96—97° after crystallisation from hot water (Found : C, 46·3; H, 4·4; picric acid, 49·3. $C_{18}H_{20}O_7N_3As$ requires C, 46·4; H, 4·3; picric acid, 49·2%).

Trimethylallylarsonium iodide, $C_3H_5\cdot AsMe_3\cdot I$. Dimethylallylarsine (2 c.c.) was added to excess of methyl iodide in a carbon dioxide atmosphere. The mixture solidified in a few minutes; it was dissolved in methyl alcohol and concentrated to half its volume on the steam-bath, and the arsonium salt precipitated by ether. A second crystallisation from methyl alcohol–ether gave white crystals, m. p. 184—186° (Found : I, 44·4. $C_6H_{14}IAs$ requires I, 44·1%).

Atmospheric Oxidation of Aliphatic Tertiary Arsines.—Dimethylallylarsine. During the preparation of the arsine its ethereal solution was evaporated. The recovered ether, kept in a loosely corked vessel for some time, evaporated, leaving a residue. Solution in alcohol and addition of ether gave a white solid, m. p. 195—196°. In admixture with cacodylic acid (m. p. 195—197°) the m. p. of the latter was unchanged.

The filtrate with alcoholic picric acid gave a yellow precipitate, m. p. 155—157°. One recrystallisation from water gave the constant m. p. of 159—160° (Found : C, 33·6; H, 3·8; N, 10·7. $C_{11}H_{14}O_8N_3As$ requires C, 33·75; H, 3·6; N, 10·7%). The compound was therefore *hydroxydimethylallylarsonium picrate*. It could not be prepared from the arsine and nitric acid, since almost complete oxidation occurred (see p. 396).

Trimethylarsine. A similar behaviour was exhibited by trimethylarsine, which, with limited access of air, gave after 6 weeks a white powder, from which by an exactly similar process cacodylic acid (m. p. 197—199°, and 198—200° in admixture with an authentic specimen, m. p. 198—200°) and hydroxytrimethylarsonium picrate, m. p. 218—219° (J., 1933, 100), were isolated.

(Trimethylarsine is best prepared by the action of methylmagnesium iodide on dimethyliodoarsine rather than on arsenic trichloride. Reduction to elementary arsenic is thereby avoided and a much better yield is obtained.)

Triethylarsine with limited access of air gives diethylarsinic acid (Friedrich and Marvel, *J. Amer. Chem. Soc.*, 1930, **52**, 376).

Triethylarsine monomercurichloride, m. p. 161—162°, has been prepared from its ingredients in alcoholic solution (Found : C, 16·2; H, 3·4. Calc. for $C_6H_{15}Cl_2AsHg$: C, 16·6; H, 3·5%) Attempts to obtain a pure dimercurichloride by using excess of Biginelli's solution or of alcoholic mercuric chloride failed. The product melted at 130—140° and from its behaviour on crystallisation appeared to be a mixture.

Grischkewitsch-Trochimowski describes a compound $AsEt_3,HgCl_2$, m. p. 163—164°, and also mentions a monomercurichloride of trimethylarsine, $AsMe_3,HgCl_2$, decomposing at 256° (*Rocz. Chem.*, 1928, **8**, 423; 1926, **6**, 798), but gives no analysis in the latter case. The dimercurichloride of trimethylarsine melts at 264° (decomp.), and the monomercurichloride at 224—226° (J., 1933, 96, 97).

Derivatives of Trimethylstibine.—An ethereal solution of the stibine was prepared by Hibbert's method (*Ber.*, 1906, **39**, 160). The ethereal reaction mixture after treatment with ammonium chloride solution was distilled in carbon dioxide, giving an ethereal solution of the stibine.

A portion of this solution was treated with benzyl chloride and the resulting *benzyltrimethylstibonium picrate* isolated and recrystallised from hot water; m. p. 183—185° (Found : C, 39·3; H, 3·7; N, 8·7. $C_{16}H_{18}O_7N_3Sb$ requires C, 39·5; H, 3·7; N, 8·65%).

P. brevicaule *and Potassium Antimonyl Tartrate.*—To a culture of the mould on 50 g. of sterile bread in a 750 c.c. flask were added 25 c.c. of a sterile 5% tartar emetic solution. Aspiration through Biginelli's solution gave no precipitate after 30 days, and no odour was observed at any time.

Growth of P. brevicaule *on Liquid Media containing Potassium Antimonyl Tartrate.*—To two groups of three flasks containing 100 c.c. of Czapek–Dox medium (J., 1934, 65; inorganic salts only) 1 g., 2 g., and 3 g. of tartar emetic were added. Glucose (2 g.) was added to one group, and the sterilised media inoculated. Growth began in the glucose flasks after 7, in the others after 10 days. No odour was detected during 33 days and the cultures were then left for 9 months.

The flask containing 2% glucose and 3% tartar emetic was then examined. The filtered medium was free from antimony, hydrogen sulphide giving only a faint yellow colour. The washed mycelium contained opaque shining crystalline particles (observed in all six flasks), doubtless antimony trioxide, since on washing with tartaric acid large quantities of antimony were dissolved. The mould, therefore, consumes the tartrate and throws antimony trioxide out of solution.

Similar results were obtained with 250 c.c. Czapek–Dox medium (inorganic salts only) containing 12·5 g. of tartar emetic. Failure to detect trimethylstibine under these conditions might possibly be due to its ease of oxidation. On addition of 10 c.c. of the ethereal solution (see above) to a flask containing 50 g. of dry bread crumbs, the odour of the stibine, which was at first very pronounced, completely disappeared in 35 minutes. An attempt to isolate trimethylstibine oxide as the corresponding picrate from a liquid culture of the mould containing tartar emetic was unsuccessful.

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