57. *The Formation of Organo-metalloidal Compounds by Micro-organisms. Part IV. Dimethyl*-n-*propylarsine and Methylethyl*-n-*propylarsine.*

By Frederick Challenger and Alan A. Rawlings.

Penicillium brevicaule (*Scopulariopsis brevicaulis*) *Saccardo* in bread cultures containing arsenious acid and its salts gives trimethylarsine (Challenger, Higginbottom, and Ellis, J., 1933, 95; Challenger and Higginbottom, *Biochem. J.*, 1935, **29**, 1757). With alkyl-arsonic and -arsinic acids, $R{\cdot}AsO_3H_2$ and $R_2AsO{\cdot}OH$,* mixed alkylmethylarsines, $AsRMe_2$ and AsR_2Me, are obtained (J., 1933, 97; 1935, 396). Sodium selenate and selenite give dimethyl selenide (Challenger and North, J., 1934, 68). The present communication describes two further instances of the methylating action of this mould as applied to methyl-*n*-propylarsinic and ethyl-*n*-propylarsinic acids (Wigren, *J. pr. Chem.*, 1930, **126**,

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* These acids would now be named dialkylarsonic acids.—Ed.

223) whereby dimethyl-*n*-propylarsine (J., 1935, 396) and *methylethyl*-n-*propylarsine* are obtained.

The analyses, m. p.'s, and mixed m. p.'s obtained for the derivatives of methylethyl-*n*-propylarsine produced by the mould leave no doubt regarding its identity. Any possibility that the mould might have removed an ethyl or a propyl group from ethylpropylarsinic acid, giving rise to dimethyl-*n*-propylarsine (J., 1935, 397) or dimethylethylarsine (J., 1933, 99), is eliminated by a comparison of the m. p.'s of the derivatives of these two arsines and those of methylethyl-*n*-propylarsine.

In view of the ease with which these biological methylations occur, it is somewhat surprising that the mould appears incapable of producing phenyldimethylarsine from phenylarsonic acid in the form of its monopotassium salt (approximate concentration in the medium, 0·5%) either in bread or glucose–Czapek-Dox cultures. That this failure is not due to any inhibition of the methylation processes of the mould exerted by the aromatic compound would appear from the fact that, when a slightly impure phenylarsonic acid (containing arsenious acid) is added to bread cultures, trimethylarsine is evolved and readily detected as the dimercurichloride (J., 1933, 99). The question is under further investigation.

The methylating action exerted on arsonic and arsinic acids having now been established, it is not proposed to elaborate this reaction further as regards simple aliphatic compounds of this type containing only one arsenic atom, except in so far as is necessary to establish the mechanism of the reaction. In this connexion it was shown by one of us and Higginbottom (*Biochem. J.*, 1935, **29**, 1766) that with α-arsonopropionic acid * and bread cultures of the mould no dimethylethylarsine was produced and only the barest traces of trimethylarsine, which presumably arose from the presence of a trace of inorganic arsenic or from the hydrolysis to arsenic acid which the arsono-fatty acids undergo. Experiments with α-arsono-*n*-butyric acid (Backer and Mulder, *Proc. K. Akad. Wetensch. Amsterdam*, 1928, **31**, 301) in bread cultures have now yielded very similar results. Neither dimethyl-*n*-propylarsine nor trimethylarsine could be detected by Biginelli's solution in two experiments each lasting 42 days. This additional evidence of the failure of the mould to decarboxylate derivatives containing the group $\vdots CH\cdot CO_2H$ (compare J., 1934, 69) weakens still further the theory that acetic acid is the biological methylating agent.

EXPERIMENTAL.

P. brevicaule Saccardo (*Baarn Strain A*) *and Methyl*-n-*propylarsinic Acid*.—Three 1 l. flasks, each containing 150 g. of bread and 20—30 c.c. of water, were sterilised, inoculated, and incubated for 4 days at 30°, and 25 c.c. of a sterile 1% solution of methyl-*n*-propylarsinic acid (kindly supplied by Dr. Nils Wigren, of Uppsala) added to each. Concentration, approx. 0·2%. The flasks were connected in series, and volatile products aspirated in a stream of sterile air into Biginelli's solution (mercuric chloride in hydrochloric acid; J., 1933, 96). After 21 days, 0·03 g. of a white precipitate, m. p. 196° (decomp.), had formed in the solution. This was recrystallised once from hot water containing a little mercuric chloride and then had m. p. 194°, decomp. 199°, alone or in admixture with synthetic dimethyl-*n*-propylarsine dimercurichloride (m. p. 194°, decomp. 199°; Challenger and Ellis, J., 1935, 398). In admixture with a further specimen (m. p. 195°, decomp. 200°) obtained by the same authors from *n*-propylarsonic acid and bread cultures of the mould, the m. p. was 194°, decomp. 198—199°. The quantity of dimercurichloride thus obtained was insufficient for analysis, but the m. p.'s given above and the analyses recorded (J., 1935, 397) for the dimercurichloride of dimethyl-*n*-propylarsine (of both synthetic and biological origin) clearly establish its identity.

The Biginelli's solution was then replaced by nitric acid (*d* 1·41), and aspiration continued for a further 22 days. The acid was then evaporated, and the last traces removed by repeated evaporation with water. Saturated aqueous sodium picrate then gave a yellow precipitate (0·06 g.), m. p. 140—141°. On recrystallisation from hot water this had m. p. 142—143° alone and 142·5—144° in admixture with synthetic dimethyl-*n*-propylhydroxyarsonium picrate (m. p. 143·5—144·5°; J., 1935, 397) (Found: C, 34·0; H, 4·4; N, 10·9. Calc. for $C_{11}H_{16}O_8N_3As$: C, 33·6; H, 4·1; N, 10·7%).

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* There wrongly given (p. 1759) the formula of the β-compound.

P. brevicaule Saccardo *and Ethyl-*n*-propylarsinic Acid.—Methylethyl-*n*-propylarsine Dimercurichloride.* Four 1 l. flasks, each containing 150 g. of bread crumbs and 20—30 c.c. of water, were sterilised, inoculated as usual, and incubated for 4 days at 30° and for 1 day at room temperature. To each flask were added 25 c.c. of a sterile 1·2% aqueous solution of ethyl-*n*-propylarsinic acid. Concentration, 0·2%. A garlic odour was perceptible in the empty bottle (used as a trap and for observation) which was placed before the Biginelli's solution, after 3 days. A solid was deposited on the 5th day and increased very slowly; yield, 0·06 g. in 79 days. A second experiment yielded 0·05 g. in 73 days; and a third, 0·07 g. in 80 days. The deposits melted at 170°, decomposed at 186°, and after recrystallisation from hot water containing a little mercuric chloride melted at 171—172°, decomposed at 187°, and did not depress the m. p. of synthetic methylethyl-*n*-propylarsine dimercurichloride, m. p. 171—172°, decomp. 188° (Found : C, 9·8; H, 2·1; Cl, 20·0. Calc. for $C_6H_{15}Cl_4AsHg_2$: C, 10·2; H, 2·15; Cl, 20·1%).

*Benzylmethylethyl-*n*-propylarsonium Picrate.*—In a similar series of experiments the Biginelli's solution was replaced on the 20th day by alcoholic benzyl chloride. After a further 28 and 21 days, 0·04 and 0·02 g. of an arsonium picrate, m. p. 61—63°, were obtained by the usual method (J., 1935, 99). In a second experiment lasting 56 days, 0·08 g. of the picrate was obtained. Recrystallisation of the united products from methyl alcohol below 50° gave a picrate, m. p. 65—66° alone or in admixture with synthetic benzylmethylethyl-*n*-propylarsonium picrate, m. p. 65—66° (Found : C, 47·3; H, 5·0; N, 8·7. Calc. for $C_{19}H_{24}O_7N_3As$: C, 47·4; H, 5·0; N, 8·7%).

*Methylethyl-*n*-propylhydroxyarsonium Picrate.*—Absorption of the mould gas from a similar series of flasks by nitric acid (*d* 1·41) during 38 days, followed by its evaporation and complete removal, gave a slight residue, which, with sodium picrate, gave 0·04 g. of a precipitate, m. p. 103—106°. In a second experiment lasting 46 days, 0·07 g., m. p. 104—106°, was obtained. One recrystallisation from alcohol gave a picrate, m. p. 108—109°, which was unchanged on recrystallisation or in admixture with synthetic methylethyl-*n*-propylhydroxyarsonium picrate, m. p. 108—109° (Found : C, 35·1; H, 4·4; N, 10·2. Calc. for $C_{12}H_{18}O_8N_3As$: C, 35·4; H, 4·5; N, 10·3%).

*Preparation of Reference Compounds.—Methylethyl-*n*-propylarsine.* The arsine was prepared according to the general method of Jones (J., 1932, 2284). A solution of methyl iodide (48 g.) and magnesium (8 g.) in ether (80 c.c.) was cooled in ice and slowly treated in an atmosphere of nitrogen with ethyl-*n*-propyliodoarsine (61 g.) (Wigren, *J. pr. Chem.*, 1930, **126**, 223) in ether (50 c.c.). After the mixture had been heated at 40° for 30 minutes, ammonium chloride (60 g.) in water (300 c.c.) was slowly added, the flask being cooled in ice and salt. Separation of the ether, evaporation, and fractionation were carried out in carbon dioxide, giving the arsine as a colourless liquid, b. p. 129—130°. Yield, 37% (Found : As, 45·8, 46·1. Calc. for $C_6H_{15}As$: As, 46·3%).

*Methylethyl-*n*-propylarsine dimercurichloride.* The arsine (1 c.c.) was added to an excess (80 c.c.) of Biginelli's solution. A white precipitate formed immediately and the mixture was left over-night and filtered. The solid, m. p. 168—170°, was recrystallised three times from hot water containing a little mercuric chloride, the recorded m. p.'s being 170—171°, 171—172°, 171—172°. In each case decomposition occurred at 188—189°. Addition of potassium hydroxide to a boiling aqueous solution gave a precipitate containing mercuric and mercurous oxides and mercury. Chlorine was determined in the filtrate (Found : C, 9·9; H, 2·1; Cl, 20·4, 20·0; Hg, 57·2, 57·2. Calc. for $C_6H_{15}Cl_4AsHg_2$: C, 10·2; H, 2·15; Cl, 20·1; Hg, 56·9%).

*Methylethyl-*n*-propylarsine monomercurichloride.* The arsine (0·7 g.) and mercuric chloride (1·15 g.; 1 mol.) in alcohol gave a white solid, which was separated after 24 hours (m. p. 113—115°). On recrystallisation from hot water it formed colourless needles, m. p. 137—138°, which were unaltered on repeated crystallisation. The mother-liquors on concentration yielded a product of the same m. p. and mixed m. p. (Found : C, 16·6; H, 3·5; Cl, 16·2, 16·2; Hg, 46·6, 46·7. Calc. for $C_6H_{15}Cl_2AsHg$: C, 16·6; H, 3·5; Cl, 16·35; Hg, 46·3%).

*Methylethyl-*n*-propylhydroxyarsonium picrate.* When the arsine (0·5 c.c.) was added to dilute nitric acid (20 c.c.), it appeared to be unchanged, but on further addition of a little nitric acid (*d* 1·41) it dissolved to a clear solution evolving white fumes. Removal of nitric acid as usual left the crude nitrate. This may be conveniently obtained by absorbing the uncondensed vapours which escape during the preparation and distillation of the arsine, in nitric acid (*d* 1·41). This gave a picrate, m. p. 103—106°, which, after two recrystallisations from alcohol, melted constantly at 108—109° (Found : C, 35·5; H, 4·6; N, 10·3; picric acid, 56·1. Calc. for $C_{12}H_{18}O_8N_3As$: C, 35·4; H, 4·5; N, 10·3; picric acid, 56·3%).

*Benzylmethylethyl-*n*-propylarsonium picrate.* Treatment of the arsine (0·25 c.c.) with excess of benzyl chloride (1 c.c.) in alcohol in the usual manner gave a deliquescent quaternary salt, which yielded a picrate, m. p. 62—63°, and 65—66° after recrystallisation from warm methyl alcohol (Found : C, 47·1; H, 5·1; N, 8·9; picric acid, 47·4. Calc. for $C_{19}H_{24}O_7N_3As$: C, 47·4; H, 5·0; N, 8·7; picric acid, 47·6%).

Phenyldimethylarsine monomercurichloride. The arsine (b. p. 198—200°; Burrows and Turner, J., 1920, **117**, 1378) (1·1 g.) with mercuric chloride (1·64 g.) in alcohol gave a white solid. This melted from 183° to 189° and after recrystallisation from hot water at 193—194° with sintering from 188°. A second crystallisation did not alter these figures (Found : Hg, 44·4, 44·1; Cl, 15·45. Calc. for $C_8H_{11}Cl_2AsHg$: Hg, 44·2; Cl, 15·6%). The same product, m. p. 193—194°, is obtained from the arsine and excess (more than 3 mols.) of mercuric chloride. The majority if not all of the known phenylarsines form only monomercurichlorides (Jones, J., 1932, 2284).

Phenylbenzyldimethylarsonium picrate. This was prepared as usual from the arsine (0·5 c.c.) and excess of benzyl chloride (1 c.c.) in alcohol. The crude arsonium chloride gave a yellow picrate, m. p. 122—125°, and 125—126° after recrystallisation from hot water. Further recrystallisation from alcohol failed to alter this m. p. (Found : C, 50·2; H, 4·3; N, 8·7; picric acid, 45·6, 45·5. Calc. for $C_{21}H_{20}O_7N_3As$: C, 50·3; H, 4·0; N, 8·4; picric acid, 45·7%).

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