powders, and thus to relate quantitatively surface area and the amount of DDT sorbed.

Comparison of the two sets of results in Tables 2 and 4 show that the rate at which the initial rapid sorption takes place depends on the concentration of the suspension used. This is further borne out by the results set out in Table 3, which records the amount of sorption by a fixed amount of chitin in a fixed time from colloidal suspensions of differing concentrations.

Consideration of the amount of DDT sorbed by a given mass of chitin (Tables 2 and 4) suggests that sorption takes place rapidly at first, and then either proceeds much more slowly or ceases altogether. Although the rate of sorption of DDT by chitin is partly dependent on the concentration of the suspension used, the apparent cessation of sorption (Tables 2 and 4) can be explained on the basis of the chitin becoming saturated with DDT, which again is the only possible explanation for the apparently constant amount of DDT sorbed by a unit weight of chitin in some experiments (Tables 1 and 2).

Although the molecular amounts of DDT analogues sorbed by a given weight of chitin from equimolecular suspensions (Table 4) are not significantly

different, there appears to be a tendency for the less toxic analogues to be sorbed to a greater extent than the more toxic analogues. The rates at which the various analogues are sorbed appear to be approximately equal, the differences being well within experimental error.

SUMMARY

1. DDT and its analogues are readily sorbed by chitin from colloidal suspension. The sorptive power appears to be peculiar to chitin since cellulose, wool (protein fibre) and silica powder do not possess this power.

2. The rate of sorption depends on the concentration of the colloidal suspension.

3. The total amount sorbed by a given weight of chitin appears to depend on the surface area of the chitin.

4. The molecular amounts of DDT and its analogues sorbed from suspensions of equimolecular concentrations are approximately equal, as are apparently the rates of sorption.

5. There is no significant correlation between the sorption of DDT analogues on chitin and their toxicity.

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Studies on Biological Methylation

11. THE ACTION OF MOULDS ON INORGANIC AND ORGANIC COMPOUNDS OF ARSENIC

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Methylation by moulds: general and selective

The conversion of arsenites, selenites and tellurites to trimethylarsine, dimethyl selenide and dimethyl telluride in cultures of *Scopulariopsis brevicaulis* has been described by Challenger & Higginbottom (1935) and in Parts I-VII and IX of this series (for references see Challenger, 1945). The theoretical aspects of the subject are discussed in Part VIII (Challenger, 1942, 1945). This methylating action is also exerted on salts of alkyl- and dialkyl-arsonic acids, $RAsO(OH)_2$ and $R_2AsO.OH$ (Challenger, Higginbottom & Ellis, 1933; Challenger & Ellis, 1935; Challenger & Rawlings, 1936) giving $RAs(CH_3)_2$ and

R_2AsCH_3 , and on salts of the alkylseleninic acids, $RSeO_2H$ (Bird & Challenger, 1942) giving $RSeCH_3$. Thom & Raper (1932) reported that several other moulds volatilized inorganic arsenic, doubtless as trimethylarsine, although they did not examine the product. Some of these organisms have been studied by Bird & Challenger (1939, 1942) with similar results. *Penicillium chrysogenum*, *P. notatum* and *P. notatum* sp. produce dimethyl telluride from potassium tellurite and dimethyl selenide from both sodium selenite and methaneseleninic acid. The three last named moulds are, however, incapable of producing trimethylarsine from arsenious acid under the usual conditions. This recalls the statement of

Segale (1903) who had obtained a mould (unspecified) which produced a volatile unidentified selenium compound, but was inert towards arsenic. No other workers have recorded any examples of this selective methylation. Further instances are now presented, and the interesting fact has emerged that *P. chrysogenum*, *P. notatum*, *P. notatum* sp. and two strains of *Aspergillus niger* exert a selective action on compounds of arsenic and readily methylate sodium methylarsonate, $\text{CH}_3\text{AsO}(\text{ONa})_2$, and in most cases sodium cacodylate, $(\text{CH}_3)_2\text{AsOONa}$ to trimethylarsine, but are inert to arsenious acid. It could, however, be suggested that this conversion is not necessarily a methylation, but might involve a reduction followed by a disproportionation:

- (a) $\text{RAsO}(\text{OH})_2 \rightarrow \text{RAs}(\text{OH})_2$;
 (b) $3\text{RAs}(\text{OH})_2 \rightarrow \text{R}_3\text{As} + 2\text{As}(\text{OH})_3$.

Thus, Grischkewitsch-Trochimovski (1928) states that the oxide $\text{C}_2\text{H}_5\text{AsO}$ which results from the action of alkali on ethyldichloroarsine $\text{C}_2\text{H}_5\text{AsCl}_2$, is converted to triethylarsine and sodium arsenite. This possibility is, however, excluded by the behaviour of sodium ethylarsonate $\text{C}_2\text{H}_5\text{AsO}(\text{ONa})_2$ and sodium allylarsonate $\text{CH}_2\text{CHCH}_2\text{AsO}(\text{ONa})_2$ in bread cultures of *A. niger* and of *Penicillium chrysogenum* respectively, dimethylethylarsine and dimethylallylarsine being produced in a pure condition. If reduction and disproportionation only had occurred, the products would have been triethylarsine and triallylarsine respectively. Had methylation of any arsenious oxide, simultaneously produced (see equation (b)), taken place, trimethylarsine would have been formed in addition. Since only dimethylethylarsine and dimethylallylarsine are evolved from the cultures, the reaction with these three moulds is therefore a methylation, as in the case of the analogous experiments with *Scopulariopsis brevicaulis*.

Three of the moulds under discussion, *Penicillium chrysogenum*, *P. notatum* and *P. notatum* sp. exert no such selective action on selenium compounds, but convert sodium selenite and methane-ethane- and propane-1-seleninic acids to dimethyl-, methylethyl- and methyl-*n*-propyl selenides respectively (Bird & Challenger, 1942). The inability of the five organisms mentioned above to methylate inorganic arsenic must be associated with the earliest stages of the methylation process, which, according to any theory of biological methylation, involves the formation of methylarsonic acid $\text{CH}_3\text{AsO}(\text{OH})_2$. The green *Penicillia* and *Aspergillus niger* readily convert this compound to trimethylarsine. The selective action may be connected with the electron-releasing effect of the alkyl groups, which would render electrons more readily available on the arsenic atom and the oxygen atom to which it is attached, thus facilitating both reduction and attachment of a methyl group.

Aromatic arsonic acids and Scopulariopsis brevicaulis

Phenylarsonic acid yields no volatile arsine in cultures of *S. brevicaulis* (Challenger & Rawlings, 1936, 1937). It seemed possible that 2:4-xylylarsonic acid containing two electron-releasing methyl groups might be more reactive, but in bread cultures of *S. brevicaulis* no xylyldimethylarsine or other volatile arsine was produced.

We have now found that phenyldimethylarsine is not evolved from cultures containing phenylarsenoxide ($\text{C}_6\text{H}_5\text{AsO}$) or phenylmethylarsonic acid ($\text{C}_6\text{H}_5\text{As}(\text{CH}_3)\text{OOH}$). An explanation based on the difficult volatility of phenylmethylarsines would appear to be excluded, because it has now been shown that the nitrate of phenyldimethylarsine oxide ($\text{C}_6\text{H}_5\text{As}(\text{CH}_3)_2\text{O}$) is readily reduced by bread cultures of *S. brevicaulis* giving phenyldimethylarsine ($\text{C}_6\text{H}_5\text{As}(\text{CH}_3)_2$), which can be removed in sterile air and characterized as the mercurichloride. Were the mould able to methylate phenylarsonic acid to phenyldimethylarsine the oxide $\text{C}_6\text{H}_5\text{As}(\text{CH}_3)_2\text{O}$ would be an intermediate compound, on the basis of either the transmethylation or the formaldehyde theory of biological methylation (Challenger, 1942, 1945).

This easy reduction of phenyldimethylarsine oxide nitrate (phenyldimethylhydroxyarsonium nitrate) recalls the formation of trimethyl- and tri-*n*-propylarsines from trimethylhydroxyarsonium nitrate and tri-*n*-propylarsine oxide respectively (Challenger & Higginbottom, 1935), of diethyl sulphide from diethyl sulphoxide, and of dimethyl selenide from dimethylhydroxyselenonium nitrate (Challenger & North, 1934), in cultures of *S. brevicaulis*. On the other hand, trimethylamine oxide hydrate and trimethylhydroxyammonium chloride give no trimethylamine in bread cultures of this mould. In view of the enzymic formation of trimethylamine from its oxide in fish this negative result is interesting.

Attempt to test the formaldehyde hypothesis of biological methylation

This hypothesis assumes the formation of hydroxymethylarsonic acid ($\text{HOCH}_2\text{AsO}(\text{OH})_2$) from arsenious oxide and its subsequent reduction to $\text{CH}_3\text{AsO}(\text{OH})_2$. This acid has not been synthesized and a crucial test of the hypothesis has not been possible (Challenger *et al.* 1933; Challenger & Higginbottom, 1935). It appeared possible that the behaviour of the condensation product of phenylarsine ($\text{C}_6\text{H}_5\text{AsH}_2$) and acetaldehyde ($\text{C}_6\text{H}_5\text{As}(\text{CHOCHCH}_3)_2$) (Palmer & Adams, 1922) in cultures of *S. brevicaulis* might throw some light on the problem, since this should yield phenyldiethylarsine. In glucose-Czapek-Dox cultures of the mould, however, the compound underwent hydrolysis yielding acetalde-

hyde, its instability recalling that of the condensation product of ethylthiol and acetaldehyde (Levi, 1932; Challenger & Rawlings, 1937). The behaviour of this hydroxyarsine to mercuric chloride was studied in view of its possible volatilization from the cultures and reaction with this reagent in the absorption train. With excess of a saturated aqueous solution the arsine gave a white precipitate, which, on boiling with ethanol, gave mercury, mercurous chloride, phenylmercury chloride, arsenobenzene, acetaldehyde, and a trace of a phenylhalogenarsine, probably phenyldichloroarsine.

It is well known that choline and betaine can engage in transmethylation in rats (Simmonds, Cohn, Chandler & du Vigneaud, 1943; du Vigneaud, Simmonds, Chandler & Cohn, 1946), and mycological methylation may proceed by a similar process (Challenger, 1945). No appreciable effect on trimethylarsine production was observed by Challenger & Higginbottom (1935) on addition of choline or betaine to bread cultures of *S. brevicaulis* containing arsenious oxide. The effect of 'triethylcholine' chloride $[(C_2H_5)_3NCH_2CH_2OH]^+Cl^-$ and 'triethylbetaine' $^+(C_2H_5)_3NCH_2COO^-$ in similar cultures has now been investigated. Careful examination of the dimercurichloride so obtained failed to show the presence of an ethyl derivative. This recalls the work of du Vigneaud (1941), who showed that when ethionine (*S*-ethylhomocysteine) and choline are fed to rats on a methionine-free diet no growth resulted, indicating that de-ethylation of ethionine to homocysteine does not occur. Results of the present and earlier papers are summarized in Table 1.

EXPERIMENTAL

Behaviour of different moulds in bread cultures to various sources of arsenic

Unless otherwise stated the moulds were obtained from the National Collection of Type Cultures at the Lister Institute, London (N.C.T.C.).

Arsenious oxide. The following moulds were found to convert As_2O_3 to trimethylarsine when the oxide was present to the extent of about 0.2% in the bread: *Aspergillus glaucus* (Birkbeck) (N.C.T.C. no. 1216) and *A. versicolor* (N.C.T.C. no. 1883). The quantity produced was, however, much smaller than with the organisms and substrates described below. The trimethylarsine was absorbed in acid $HgCl_2$ (Biginelli's solution) (Challenger *et al.* 1933), and characterized as the dimercurichloride; the melting point (decomp.) of the unrecrystallized product varied from 255 to 260°. Satisfactory mixed melting points were carried out in these and most of the following experiments. Trimethylarsine dimercurichloride has been repeatedly examined during the course of these investigations (Challenger *et al.* 1933; Challenger & Higginbottom, 1935). We find that, using bread cultures of *Scopulariopsis brevicaulis* (Sacc.) Bainier (*Baarn List of Fungi*, 1932) containing about 0.1% of As_2O_3 , roughly 70% of the added arsenic may be recovered as trimethylarsine dimercurichloride during

Table 1. *Behaviour of different moulds to various sources of arsenic, selenium and tellurium in bread cultures*

Mould	N.C.T.C. no.	Compound added to culture					
		As_2O_3	Na_2HAsO_4	Na_2SeO_3	K_2TeO_3	$CH_3AsO(ONa)_2$	$CH_3)_2AsO(ONa)_2$
		Identified product					
<i>S. brevicaulis</i>	1362, 580	$(CH_3)_3As$	$(CH_3)_3As$	$(CH_3)_3Se$	$(CH_3)_3Te$	$(CH_3)_3As$	$(CH_3)_3As$
<i>P. notatum</i>	4222	None	—	$(CH_3)_3Se$	Doubtful $(CH_3)_3Te$, odour only	$(CH_3)_3As$	$C_2H_5As(CH_3)_2$
<i>P. chrysogenum</i>	589	None	—	$(CH_3)_3Se$	$(CH_3)_3Te$	$(CH_3)_3As$	$C_2H_5As(CH_3)_2$
<i>P. notatum</i> sp.	—	None	—	$(CH_3)_3Se$	$(CH_3)_3Te$	Odour of $(CH_3)_3As$, only trace of $(CH_3)_2As$, $2HgCl_2$	—
<i>A. niger</i>	3779	None	—	Na_2SeO_4	(No odour, no redn.)	$(CH_3)_3As$	—
<i>A. fischeri</i>	—	None	—	(No odour, no redn.)	(No odour, no redn.)	$(CH_3)_3As$	—
<i>A. niger</i> 17	1216	—	—	—	(No odour, no redn.)	$(CH_3)_3As$	—
<i>A. glaucus</i> (Birkbeck)	1883	$(CH_3)_3As$	—	(No odour, no redn.)	(No odour, no redn.)	$(CH_3)_3As$	—
<i>A. versicolor</i>	—	$(CH_3)_3As$	—	(No odour, no redn.)	(No odour)	$(CH_3)_3As$	—

24 months' aspiration. *S. brevicaulis* (strain Derx N.C.T.C. no. 1362) yielded about 80% in 10 months. An attempt was made by Dr C. Simons in these laboratories to increase the rate of trimethylarsine formation by this mould by cultivating the strain *S. brevicaulis* (Sacc.) Bainier (*Baarn List of Fungi*, 1932) for 33 generations on a potato-agar medium containing 0.12–0.15% of As_2O_3 . No appreciable alteration in the rate of methylation could be detected, when the 'trained' mould was inoculated on to sterile bread containing 0.1% of As_2O_3 .

Sodium methylarsonate ($\text{CH}_3\text{AsO}(\text{ONa})_2$). The following moulds gave trimethylarsine, characterized as the dimercurichloride (unrecryst.), the melting point (decomp.) varying from 252 to 263°: *Penicillium chrysogenum* (N.C.T.C. no. 589), *P. notatum* (N.C.T.C. no. 4222), *P. notatum* sp. (see Bird & Challenger, 1939), *Aspergillus niger* and 'A. niger 17' (obtained from Dr T. K. Walker of the Biochemistry Department, The College of Technology, Manchester), *A. fischeri* (N.C.T.C. no. 3779), *A. glaucus* (Birkbeck) (N.C.T.C. no. 1216) and *A. versicolor* (N.C.T.C. no. 1883). The concentration of the sodium methylarsonate in the bread was 0.2–0.25% except in the case of *A. niger*, where it was 0.07%. In the case of *Penicillium notatum* and *Aspergillus niger* 17 the trimethylarsine was also characterized as trimethylhydroxyarsonium nitrate ($(\text{CH}_3)_3\text{As}(\text{OH})\text{NO}_3$ (Challenger *et al.* 1933), m.p. and mixed m.p. 124 and 126° respectively).

Sodium cacodylate ($(\text{CH}_3)_2\text{AsOONa}$). Trimethylarsine was again obtained and characterized as before in cultures of the following: *Penicillium chrysogenum*, *P. notatum*, *Aspergillus niger* and 'A. niger 17'. The usual concentration was 0.2 and 0.25% in the bread. *Penicillium notatum* sp. gave only a trace of the dimercurichloride.

Ethylarsonic acid ($\text{C}_2\text{H}_5\text{AsO}(\text{OH})_2$) at a concentration of 0.13% in the bread gave with *Aspergillus niger* dimethylethylarsine, characterized as the mercurichloride, m.p. and mixed m.p. 238° (Challenger *et al.* 1933).

Sodium allylarsonate ($\text{CH}_2\text{CHCH}_2\text{AsO}(\text{ONa})_2$), concentration 0.2%, gave with *Penicillium chrysogenum* dimethylethylarsine, characterized as the mercurichloride, m.p. and mixed m.p. 209°. Here again no reduction of the double linkage in the allyl group occurs (Challenger & Ellis, 1935).

Ethyl-n-propylarsonic acid ($\text{C}_2\text{H}_5\text{C}_3\text{H}_7\text{AsO}(\text{OH})_2$), concentration 0.17% in the bread cultures, gave with 'Aspergillus niger 17' methylethyl-n-propylarsine, characterized as the dimercurichloride, m.p. and mixed m.p. 171–172°, decomp. between 189 and 192° (Challenger & Rawlings, 1936).

n-Propylarsonic acid ($\text{C}_3\text{H}_7\text{AsO}(\text{OH})_2$), concentration 0.34% in the bread cultures, gave with *Penicillium notatum* dimethyl-n-propylarsine dimercurichloride, m.p. and mixed m.p. 195–197°, decomp. 199–200° (Challenger & Ellis, 1935).

The non-occurrence of methylation of arsenious oxide with certain moulds

In preliminary experiments the following organisms appeared unable to convert As_2O_3 to trimethylarsine in bread cultures: *P. chrysogenum*, *P. notatum*, *P. notatum* sp., *Aspergillus niger*, 'A. niger 17' ($\text{As}_2\text{O}_3=0.02\%$) and *A. fischeri* ($\text{As}_2\text{O}_3=0.2\%$). Nevertheless, it was decided to treat five of these organisms with varying concentrations of As_2O_3 . Six cultures of each mould on 50 g. of bread without added water were incubated for 9 days, and a sterile aqueous solution of As_2O_3 (15 ml.; 20 ml. in F) added to each group of five flasks (groups A–F) to give concentrations in the bread of 0.02, 0.05, 0.10, 0.20, 0.30 and

0.40%. During the first 3–4 days the *Penicillium notatum* flasks D, E and F had a very faint odour, barely detectable on the 10th day. On the 14th day an odour was detected in flask C, though with difficulty. With *P. chrysogenum* a very faint odour appeared to be present in E and F. The two strains of *Aspergillus niger* gave no odour, nor did *Penicillium notatum* sp., until after about 2 months, by which time slight contamination may have occurred.

Appearance of the cultures. With the six cultures of *P. notatum* the bread was covered with mycelium about 3 days after addition of As_2O_3 . After 9 weeks the cultures showed almost black patches, especially flasks D, E and F. Subcultures from these on to wort agar failed to grow during 45 days, although they were reinoculated on the 5th day. All the cultures of *P. chrysogenum* and *P. notatum* sp. grew well. The strains of *Aspergillus niger* grew well in groups A and B, but in D, E and F very little growth occurred after addition of the As_2O_3 .

Penicillium notatum and arsenious acid. It was decided to test for trimethylarsine evolved by *P. notatum* by chemical means. Five 1 l. flasks, each containing 150 g. of bread, were sterilized, inoculated with spores of *P. notatum* and incubated for 7 days at room temperature. To each were added 45 ml. of sterile 1% (w/v) As_2O_3 solution (conc. in bread 0.3%), and aspiration commenced through two tubes of Biginelli's solution. No deposit appeared and no odour was detected during 30 days. The cultures in two flasks were then quite white, the green sporing area being completely covered. Subcultures were made from these two flasks, each to two wort-agar slopes, to see if this white growth was contamination or sterile mycelium due to the As_2O_3 . After 5 days' incubation at room temperature all four slopes showed only typical green colonies of *P. notatum*. One plate culture was also made from each flask with the same result. *P. notatum* would therefore appear not to produce trimethylarsine from As_2O_3 , in agreement with the original observation.

Growth of P. notatum on a liquid medium containing arsenious oxide from the outset. Seven 250 ml. conical flasks, each containing 100 ml. of 5% (w/v) glucose-Czapek-Dox solution with concentrations of As_2O_3 of 0.01, 0.02, 0.04, 0.08, 0.16, 0.32 and 0.64%, were sterilized, inoculated and incubated at room temperature. After 10 days all the flasks except the last showed a white surface growth which was compact, raised and abnormal. During another 4 days, growth continued in concentrations 0.01–0.16%; increase was slow with 0.32%.

Arsonic acids containing the phenyl group and Scopulariopsis brevicaulis

The strain used was designated *S. brevicaulis* (Sacc.) Bainier in the *Baarn List of Fungi* (1932), and was distinguished in the Leeds laboratories as strain A (Challenger *et al.* 1933). It was grown on 150 g. of bread in 1 l. flasks. Solutions containing the arsonic acids were made up thus: (a) Phenylarsonic acid (1.2 g.) carefully purified by three crystallizations from water, m.p. 161–162°, was dissolved in water (100 ml.) containing NaHCO_3 (1.1 g.) and sterilized. (b) 2:4-Xylylarsonic acid (0.6 g.) (Michaelis, 1902) was dissolved in sterile water (80 ml.) containing NaHCO_3 (0.65 g.). (c) Benzylarsonic acid (0.75 g.), three times recrystallized from water without change in m.p. 196–198° after air-drying (the acid readily loses water), was suspended in sterile water (80 ml.).

25 ml. of solutions (a) and (b) and of suspension (c) were added to bread cultures of the mould (three in each case), and volatile products aspirated through acid HgCl_2 solution. A precipitate formed after some weeks in each case, but was shown to be free from the mercurichloride of a tertiary arsine $\text{RAs}(\text{CH}_3)_2$ and to consist of the ammonia compound of HgCl_2 which was prepared for comparison.

Behaviour of arsenious oxide in bread cultures of Scopulariopsis brevicaulis containing 'triethylcholine' chloride and the triethylbetaine of aminoacetic acid

'Triethylcholine' chloride (25 ml. of a sterilized 8% (w/v) aqueous solution) was added to each of three flasks of sterile bread, which were then inoculated with the mould and incubated for 4 days at 32° and 4 days at room temperature. Each flask then received 25 ml. of a sterile aqueous 1.2% (w/v) solution of As_2O_3 . Aspiration through Biginelli's solution gave during 46 days only 0.22 g. of trimethylarsine dimercurichloride, m.p. $266\text{--}267^\circ$ decomp. A mixed melting point confirmed its identity. 'Triethylbetaine' (25 ml. of a sterilized 3% (w/v) aqueous solution) was added to each of four 1 l. flasks containing 150 g. of sterile bread, which were then inoculated and incubated for 4 days at 32° and 2 days at room temperature. Addition of 25 ml. of sterilized aqueous 1.2% As_2O_3 solution gave as before during 28 days 0.2 g. trimethylarsine dimercurichloride, m.p. $266\text{--}267^\circ$ with sintering from 263° . The mixed melting point with an authentic specimen of m.p. 267° was 266° . On the 40th day a further deposit was removed, m.p. 265 and 266° after recrystallization from very dilute HgCl_2 solution. Similar results were obtained with two cultures containing 2% and one with 1% of added 'triethylbetaine' connected in series.

Scopulariopsis brevicaulis and aromatic compounds of arsenic

S. brevicaulis and phenylarsonic acid. The acid (4 g.) and Na_2CO_3 (2 g.) were dissolved in water (150 ml.). 25 ml. of the solution were added to each of six 1 l. flasks containing 3% (w/v) glucose-Czapek-Dox medium (250 ml.) on which a good mycelium had formed. Sterile air was then drawn through the flasks in series with Biginelli's solution during 30 days, but no deposit formed, indicating the absence of phenyldimethylarsine. The whole of the medium was then filtered, concentrated to 400 ml. and a portion tested for phenyldimethylarsine oxide with picric acid, but no precipitate formed. The main bulk was then extracted ten times with ether, which only removed traces of phenylarsonic acid. A portion of the concentrated medium gave with HCl , SO_2 and I_2 (see p. 82) a yellow oil, which with ethanolic HgCl_2 yielded phenylmercury chloride, m.p. and mixed m.p. 247° in admixture with a specimen obtained in a similar manner from authentic phenylarsonic acid (see p. 83). From the bulk of the concentrated medium 30% of the total phenylarsonic acid was recovered as the thorium salt (Rice, Fogg & James, 1926). Quantitative recovery was not expected due to the material having been used for preliminary tests. Removal of thorium as oxalate, and of oxalic acid with H_2O_2 yielded phenylarsonic acid. It sintered at 158° , in agreement with an authentic specimen.

S. brevicaulis and phenylarsenoxide. Addition of 0.2% of the oxide to each of four cultures of the mould prepared as in Exp. 1 gave no odour during 6 months, and the insoluble

oxide appeared unchanged. Neither reduction to phenylarsine nor production of phenyldimethylarsine occurred.

S. brevicaulis and phenylmethylarsonic acid. For the preparation of the acid see Burrows & Turner (1920, 1921); Gibson & Johnson (1928). Five 1 l. conical flasks, each containing a good growth of the mould on 5% (w/v) glucose-Czapek-Dox solution (200 ml.), were treated with 25 ml. of sterile 2% aqueous sodium phenylmethylarsonate. There was no odour and no precipitate in aqueous HgCl_2 after aspiration for 5 weeks. Evaporation of the slightly acidified medium, extraction with ethanol and treatment with HCl , SO_2 and excess I_2 gave a yellow oil. This with ethanolic HgCl_2 yielded a white precipitate, which on recrystallization from ethanol sintered at 148° , but did not melt. Its behaviour was identical with that of the mercurichloride of authentic phenylmethyliodoarsine. (Found, for the specimen from the culture: C, 14.5; H, 1.8; halogen, 33.2. $\text{C}_6\text{H}_5(\text{CH}_3)\text{AsI} \cdot \text{HgCl}_2$ requires C, 14.9; H, 1.42; halogen 35.0%.) The medium therefore appeared to contain unchanged phenylmethylarsonic acid or phenylmethylarsenoxide.

S. brevicaulis and phenyldimethylhydroxyarsonium nitrate. Six 1 l. flasks, each containing 150–200 g. of bread slightly moistened with water, were inoculated with an aqueous spore suspension of the mould (strain *Saccharomyces* Bainier, see p. 80). After 4 days the odourless solution of the hydroxyarsonium nitrate in sterile water was added, the concentration in the bread being 0.17%. In a few hours an arsenical odour was apparent, and the flasks were connected with sterile bungs and delivery tubes as in earlier experiments (Challenger *et al.* 1933). A stream of sterile air was then aspirated through the cultures into saturated aqueous HgCl_2 . A deposit formed after 2 days, which when recrystallized from hot water had m.p. and mixed m.p. 196° with authentic phenyldimethylarsine mercurichloride, m.p. 196° . A second crop sintered from 185 to 195° . After one recrystallization the melting point rose to 196° . Later on, due to the production of NH_3 in the cultures, the precipitate increased rapidly. It was extracted with ethanol and then with boiling water leaving the ammonia compound of HgCl_2 . The phenyldimethylarsine mercurichloride obtained (0.62 g.) represented 23% of the original hydroxyarsonium nitrate. In a control experiment six test tubes, each containing approximately 7 g. of sterile bread and 0.17% of the hydroxyarsonium nitrate, gave no odour or any deposit in aqueous HgCl_2 during 28 days.

S. brevicaulis and phenylbis(1-hydroxyethyl)arsine. The arsine (3 g.) was suspended in sterile water (150 ml.), and 25 ml. added to each of six well-grown cultures on glucose-Czapek-Dox solution (200 ml.). Sterile air was drawn over the cultures into saturated HgCl_2 . After a few days the only odour recognizable was that of the original arsine. A slight precipitate in the absorption bottles was shown to be HgCl_2 . On placing aqueous 2:4-dinitrophenylhydrazine hydrochloride at the end of the absorption train a yellow solid formed. When recrystallized from ethanol this melted at 156° alone and in admixture with authentic acetaldehyde 2:4-dinitrophenylhydrazone of m.p. 156° .

Reactions of some aromatic compounds of arsenic

Conversion of phenylarsonic acid to phenylmercury chloride. The acid was converted to phenyldiiodoarsine. In acetone this gave a white addition product with aqueous HgCl_2 . Heating this with water gave a red solid and hot

acetone produced a red solution, doubtless due to HgI_2 . Crystallization from ethanol yielded white glistening plates, m.p. 242°. On successive recrystallizations from acetone, ethanol and acetone, the m.p. were 247, 246 and 247°, not depressing the m.p. (251°) of phenylmercury chloride.

Phenyldiethylarsine mercurichloride. The arsine (c. 1 g.) (Burrows & Turner, 1920) was shaken with excess of saturated aqueous HgCl_2 until the mixture was odourless. The white precipitate was recrystallized from ethanol (m.p. 127, 129, 129°). (Found: Cl, 15.2; Hg, 42.2. $\text{C}_{10}\text{H}_{15}\text{Cl}_2\text{AsHg}$ requires Cl, 14.74; Hg, 41.65%.)

Phenylbis(1-hydroxyethyl)arsine and mercurichloride. A concentrated ethanolic solution of the arsine (Palmer & Adams, 1922) was shaken with excess of saturated aqueous HgCl_2 for several hours. The white precipitate quickly became yellow. It still smelled slightly of the arsine and was washed with water and a little ethanol. It blackened and decomposed from 90°. Warming with ethanol gave Hg_2Cl_2 and mercury but no Hg_2O . The ethanolic filtrate (B) gave white crystals, m.p. 254°; an identical product also slowly separated from the aqueous filtrate (A) from the crude mercurichloride. It contained halogen and mercury but no arsenic, and was shown by mixed m.p. (255°) to be phenylmercury chloride (m.p. 252°). Concentration of the filtrate (B) gave a white crystalline material, m.p. 187–194°, which was twice recrystallized from benzene-ether, m.p. 212 and 213°. It contained arsenic but no halogen or mercury, and was unaffected by NaOH , NH_3 or HCl . It was readily attacked by HNO_3 , KMnO_4 , H_2O_2 and I_2 and was insoluble in water. (Found: C, 48.3; H, 3.5; mol.wt. (camphor), 491. Calc. for $\text{C}_{12}\text{H}_{10}\text{As}_2$: C, 47.4; H, 3.3%; mol.wt. 304.) Arseno-benzene, m.p. 212°, gives high values in ebullioscopic molecular weight determinations in benzene (Michaelis & Schäfer, 1913). Its identity was confirmed (1) by oxidation with HNO_3 when the product (phenylarsonic acid) gave a white gelatinous thorium salt (see p. 82); and (2) by treatment with Cl_2 giving a yellow oil ($\text{C}_6\text{H}_5\text{AsCl}_2$) which soon crystallized ($\text{C}_6\text{H}_5\text{AsCl}_4$). A portion of the filtrate (B) with 2:4-dinitrophenylhydrazine hydrochloride gave a

copious yellow precipitate, m.p. and mixed m.p. 159°, with authentic acetaldehyde 2:4-dinitrophenylhydrazone, m.p. 159°. This was also obtained from the aqueous filtrate (A). Complete evaporation of (B) left only a few drops of a yellow lachrymatory oil containing chlorine. It was probably phenyldichloroarsine.

SUMMARY

1. *Scopulariopsis brevicaulis* converts arsenious acid and methylarsonic acid to trimethylarsine; other alkylarsonic acids yield alkyltrimethylarsines. *Penicillium notatum*, *P. notatum* sp., *P. chrysogenum* and two strains of *Aspergillus niger* are inert to arsenious acid, but can methylate alkylarsonic acids. *A. glaucus* gives trimethylarsine with arsenious and methylarsonic acids. All cultures were on bread.

2. Phenylarsonic and phenylmethylarsonic acids in glucose-Czapek-Dox medium, and *m*-xylylarsonic and benzylarsonic acids in bread cultures give no volatile arsenic compounds with *Scopulariopsis brevicaulis*. Phenyldimethylhydroxyarsonium nitrate, however, is readily reduced in bread cultures to phenyldimethylarsine which readily volatilizes.

3. In cultures of *S. brevicaulis* on glucose-Czapek-Dox medium phenylbis(1-hydroxyethyl)arsine does not give phenyldiethylarsine, but is hydrolyzed to acetaldehyde. The behaviour of the hydroxy arsine to mercuric chloride has been studied.

4. In bread cultures of *S. brevicaulis* containing arsenious acid and 'triethylcholine' chloride or 'triethylbetaine' only trimethylarsine is evolved; trans-ethylation apparently does not occur.

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