320. *Studies on Biological Methylation. Part XII.* A Precursor of the Dimethyl Sulphide evolved by* Polysiphonia fastigiata. *Dimethyl-2-carboxyethylsulphonium Hydroxide and its Salts.*

By Frederick Challenger and Margaret Isabel Simpson.

On exposure to air the marine algæ *Polysiphonia fastigiata* and *P. nigrescens* evolve dimethyl sulphide. An alcoholic extract of *P. fastigiata* on concentration under diminished pressure finally yielded a brown solid. This, with cold sodium hydroxide, evolved dimethyl sulphide, characterised as the mercurichloride. With ammonium reineckate a precipitate was obtained which was converted successively through a crude chloride into the platinichloride, picrate, and a pure sulphonium chloride. This was optically inactive and evolved dimethyl sulphide with cold sodium hydroxide. By direct comparison of this chloride and various derivatives with synthetic specimens it was shown to be *dimethyl-2-carboxyethylsulphonium chloride*. This establishes for the first time the occurrence of sulphonium compounds in plants. The evolution of dimethyl sulphide from the alga probably depends on the enzymic decomposition of this compound or of a more complex derivative.

It was shown by Haas (*Biochem. J.*, 1935, **29**, 298) that the marine algæ *Polysiphonia fastigiata* and *P. nigrescens* when exposed to air evolve dimethyl sulphide. This was characterised as the dibromide, m. p. 93—94°, the mercurichloride, and the platinous chloride compound by aspiration through the appropriate reagents.

It appeared unlikely that the dimethyl sulphide was stored as such in the alga, and the possibility of its occurrence as a sulphonium compound suggested itself. Dr. Haas kindly allowed us to continue the investigation in order to identify the precursor from which it appeared probable (see below) that the sulphide was eliminated by enzyme action. Haas (*loc. cit.*) considered it unlikely that bacterial action was involved in view of the short interval elapsing between the time of collection of the weed and the appearance of the odour. Professor L. Newton of the Department of Botany, University College of Wales, Aberystwyth, has provided us with a regular supply of *P. fastigiata*. This collaboration on her part has been invaluable and we wish to record our sincere thanks. The alga, when received, was still attached to its host *Ascophyllum nodosum*, but was removed before investigation.

When the volatile products from the alga are aspirated through (*a*) mercuric cyanide and (*b*) mercuric chloride, no precipitate is formed in (*a*), indicating the absence of methylthiol, a conclusion in agreement with the relatively pleasant odour which is observed. The properties of the deposit in (*b*) show it to consist solely of the mercurichloride of dimethyl sulphide; mercurated fission products of dimethyl disulphide are absent (see p. 1593; and Challenger and Rawlings, *J.*, 1937, 870; and Blackburn and Challenger, *J.*, 1939, 1872). The volatile sulphur compound is, therefore, homogeneous. The dimethyl sulphide has now been further characterised as benzyldimethylsulphonium picrate. Addition of cold sodium hydroxide to the alga accelerates the elimination of dimethyl sulphide.

The question arises whether dimethyl sulphide evolution is an essential life-process of the alga or whether it is a sign of death or damage. The well-known occurrence of trimethylamine, probably arising from choline, in the living flowers of many trees and plants may be cited.

Two experiments carried out by one of us (M. I. S.) in the marine laboratory at Robin Hood's Bay, through the courtesy of Professor E. A. Spaul, showed that when the alga, still attached to its host, is removed from the rock-pools and immediately re-immersed in a jar of sea-water, no dimethyl sulphide is evolved for at least 24 hours, though after this time a very slow production occurs, possibly due to gradual damage under abnormal conditions. On the other hand the alga, detached from its host and immersed in tap or distilled water, evolves the sulphide in a few hours. When in the rock-pools the alga has no sulphide odour. The production of dimethyl sulphide would seem, therefore, to be an abnormal phenomenon. Further work along these lines is in progress.

Several observations suggest that an enzyme is concerned with the biological process. Grinding the weed with sand or immersing it in distilled water, whereby the cells are ruptured, facilitates the evolution. Haas (*loc. cit.*) states that immersion in boiling water inhibits the production of the odour. We find that weed so treated and allowed to cool evolves no dimethyl sulphide in an air stream (no formation of mercurichloride, or only a trace) but that prolonged boiling with water or addition of sodium hydroxide (purely chemical hydrolysis) causes a fresh evolution. If the alga is left in alcohol for a few hours, and then suspended in water, air removes no dimethyl sulphide but addition of alkali causes a ready evolution.

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* Part XI appears in *Biochem. J.*, 1948, **43**, 78.

In all experiments the use of a stream of nitrogen and air-free water made no difference to the result.

The alga exerts no methylating action on sodium arsenite, sodium selenite, or potassium tellurite when suspended in artificial sea-water containing 0·2 and 0·5% of these compounds. The dimethyl sulphide contained no trace of trimethylarsine, dimethyl selenide, or dimethyl telluride. When 1% and 10% sodium ethyl sulphate or 2% triethylsulphonium iodide was used in the "sea-water", methyl ethyl or diethyl sulphides were absent from the dimethyl sulphide.

The procedure leading to the identification of the precursor of the dimethyl sulphide (or its most important fragment) may now be outlined.

The red alga was left in absolute alcohol for 2—3 weeks, and the extract concentrated under diminished pressure to a syrup which yielded a brown solid on rubbing with alcohol. This, with cold sodium hydroxide, evolved dimethyl sulphide, characterised as the mercurichloride $2Me_2S,3HgCl_2$ (Blackburn and Challenger, *J.*, 1938, 1878; Challenger and Charlton, *J.*, 1947, 426). The solid gave a precipitate with aqueous ammonium reineckate, $NH_4[Cr(NH_3)_2(SCN)_4]$. The reineckate ion was removed by silver sulphate, and the sulphate with barium chloride (Kapfhammer and Bischoff, *Z. physiol. Chem.*, 1930, **191**, 179). Evaporation gave a residue which was converted into the platinichloride. This, with metallic silver (Dudley, *Biochem. J.*, 1929, **23**, 1071), gave silver chloride and platinum, and on evaporation as before, a solid chloride. This yielded a picrate which was carefully purified by recrystallisation from alcohol. Decomposition with hydrochloric acid finally yielded a *chloride*, $C_5H_{11}O_2ClS$. This was optically inactive, evolved dimethyl sulphide with cold sodium hydroxide, and was again converted into the *picrate* and also into the bromide, chloroplatinate, and *styphnate*.

The acidity of the chloride and the absence of reaction with 2 : 4-dinitrophenylhydrazine exclude such constitutions as $Me_2\overset{+}{S}(\overset{-}{Cl})\cdot CH_2\cdot CO\cdot CH_2\cdot OH$ or $Me_2\overset{+}{S}(\overset{-}{Cl})\cdot CH(OH)\cdot CH_2\cdot CHO$.

Two structural formulæ appeared probable according to which the chloride would be either an α- or a β-thetin derivative (I) or (II) (see Brown and Letts, *Trans. Roy. Soc. Edinburgh*, 1877—1878, **28**, II, 571).

$$Me_2\overset{+}{S}(\overset{-}{Cl})\cdot CHMe\cdot CO_2H \quad \text{(I.)} \qquad\qquad Me_2\overset{+}{S}(\overset{-}{Cl})\cdot CH_2\cdot CH_2\cdot CO_2H \quad \text{(II.)}$$

The corresponding bromides (Carrara, *Gazzetta*, 1893, **23**, 502, 506; Carrara and Rossi, *Atti R. Accad. Lincei*, 1893, **6**, II, 211; Biilmann and Jensen, *Bull. Soc. chim.*, 1936, **3**, 2306) were prepared and converted into the chlorides, picrates, styphnates, and platinichlorides. Mixed m. p. determinations carried out with these compounds and with the chloride from the alga and its corresponding derivatives left no doubt that the algal product is the chloride of the β-propiothetin derivative, dimethyl-2-carboxyethylsulphonium chloride (II).

Holmberg (*Arkiv Kemi, Min. Geol.*, 1946, **21** *B*, No. 7, 1) states that β-methylthiopropionic acid, $SMe\cdot CH_2\cdot CH_2\cdot CO_2H$ with 2½ equivs. of dimethyl sulphate in cold alkaline solution eliminates dimethyl sulphide giving sodium acrylate, characterised as the addition product with benzylsulphinic acid, $Ph\cdot CH_2\cdot SO_2\cdot CH_2\cdot CH_2\cdot CO_2H$. He observed the same reaction with the free dimethyl-2-carboxyethylsulphonium bromide. A similar instability was noticed with benzylmethyl-2-carboxyethylsulphonium bromide, $CH_2Ph\cdot\overset{+}{S}Me(\overset{-}{Br})\cdot CH_2\cdot CH_2\cdot CO_2H$, and cold alkali.

With sodium hydroxide and toluene-*p*-sulphinic acid we find that the synthetic β-thetin bromide (II), the chloride from the alga, and authentic acrylic acid give the same product, $CH_3\cdot C_6H_4\cdot SO_2\cdot CH_2\cdot CH_2\cdot CO_2H$, p-*tolyl-2-carboxyethyl sulphone*.

The α-thetin bromide is more stable and only evolves dimethyl sulphide when heated with sodium hydroxide.

Some interesting decompositions of substituted dimethylethylsulphonium compounds are described by Crane and Rydon (*J.*, 1947, 766). Dimethyl-2-benzoyloxyethylsulphonium iodide, $Ph\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot\overset{+}{S}Me_2\}\overset{-}{I}$, and $Ph\cdot O\cdot CH_2\cdot CH_2\cdot\overset{+}{S}Me_2\}\overset{-}{I}$ yield dimethyl sulphide, acetylene, sodium iodide, and sodium benzoate and phenoxide respectively with 10% sodium hydroxide at 100°, but in each case recognisable decomposition occurs in M/100-solution at 25° and pH 10.

It is possible that the carboxysulphonium chloride isolated from the alga arises from a more complex derivative, which, during the process of separation, may undergo hydrolysis. The carboxyl group may, in the living alga, exist as a peptide or ester linkage and the anion may be a polysaccharide sulphate ion. Such sulphuric esters occur in many algæ including *P. fastigiata*

(Haas and Russell-Wells, *Biochem. J.*, 1923, **17**, 696) as well as in agar (Neuberg and Ohle, *Biochem. Z.*, 1921, **125**, 311). Haas (*Biochem. J.*, 1931, **25**, 1472) isolated a polypeptide of glutamic acid from *P. fastigiata.*

Hydrolysis of such a linkage may have occurred during the evaporation of the alcoholic extract. This was slightly acid, probably owing to hydrolysis of a potassium or calcium salt of a polysaccharide sulphuric acid.

Only one other instance of the isolation of a sulphonium compound from a natural product is recorded. Neuberg and Grosser (*Centr. Physiol.*, 1905—1906, **19**, 316) state that the precursor of the diethyl sulphide evolved on warming the urine of dogs with alkali (Abel, *Z. physiol. Chem.*, 1894, **20**, 253; Christomanos, *ibid.*, 1931, **198**, 185; 1933, **217**, 177; *Klin. Woch.*, 1932, **11**, 177; Wohlgemuth, *Z. physiol. Chem.*, 1933, **221**, 207) is methyldiethylsulphonium hydroxide, which was isolated as a salt by successive precipitation with phosphotungstic acid and potassium bismuth iodide. They also consider that this hydroxide is formed on feeding diethyl sulphide to dogs. Experimental details are very scanty and no analyses are given.

It is difficult to regard a methyldiethylsulphonium salt as the parent of the diethyl sulphide, for we find that the iodide $\overset{+}{S}MeEt_2\overset{-}{I}$ with sodium hydroxide at 100°, but not below this temperature, gives methyl ethyl sulphide, characterised as the mercurichloride. Moreover, it is clear from Abel's paper (*loc. cit.*) that dogs' urine evolves diethyl sulphide at room temperature with calcium or sodium hydroxide and Christomanos (*loc. cit.*) employed only 60°. The nature of the sulphur compound in dogs' urine is now under investigation in these laboratories. We also find that dimethylethylsulphonium iodide gives dimethyl sulphide with alkali.

Allicin from garlic cloves (Cavallito *et al.*, *J. Amer. Chem. Soc.*, 1944, **66**, 1950, 1952; 1945, **67**, 1032), probably $C_3H_5{\cdot}\underset{\underset{O}{\downarrow}}{S}{\cdot}S{\cdot}C_3H_5$, may perhaps be regarded as of sulphonium type.

Toennies (*J. Biol. Chem.*, 1940, **132**, 455) and Toennies and Kolb (*J. Amer. Chem. Soc.*, 1945, **67**, 849) suggested that sulphonium compounds, *e.g.*, derivatives of methionine, may be involved in biological processes, *e.g.*, in the conversion of methionine into cystine (see also Binkley, Anslow, and du Vigneaud, *J. Biol. Chem.*, 1942, **143**, 59; Binkley, *ibid.*, 1944, **155**, 39).

The possibility that transmethylation from methionine may involve the intermediate formation of a sulphonium compound was suggested by du Vigneaud *et al.* (*ibid.*, 1941, **140**, 640; see also Bennett, *ibid.*, 1941, **141**, 573, and Handler and Bertheim, *ibid.*, 1943, **150**, 335).

One of us and Charlton (*J.*, 1947, 424) have shown that in cultures of *S. brevicaulis*, *dl*-methionine gives methylthiol and (presumably by further methylation) dimethyl sulphide. Apparently this fission does not involve the preliminary formation of a sulphonium salt because *dl*-methionine methiodide in cultures of *S. brevicaulis* evolves dimethyl sulphide and no trace of methylthiol.

The behaviour of various sulphonium compounds in mould cultures is being studied by Mr. Y. Cheng Lin, who finds that dimethyl-2-carboxyethylsulphonium chloride (II) readily evolves dimethyl sulphide in bread cultures of *Penicillium notatum* but much less readily with *S. brevicaulis.*

Added August 27th, 1948.—The biological importance of sulphonium compounds is emphasised by the recent announcement that dimethyl-2-carboxyethylsulphonium chloride and also dimethylcarboxymethylsulphonium chloride (dimethylthetin chloride), $(CH_3)_2\overset{+}{S}(\overset{-}{Cl}){\cdot}CH_2{\cdot}CO_2H$, possess a mobile methyl group, and can support the growth of rats on a diet containing no choline or methionine but containing homocystine (Maw and du Vigneaud, *J. Biol. Chem.*, 1948, **174**, 381; du Vigneaud, Moyer, and Chandler, *ibid.*, p. 477). In the case of the second thetin which has not yet been discovered in Nature, earlier work (du Vigneaud, "Harvey Lectures," 1942—43, **38**, 57) had led to the same conclusion. Dubnoff and Borsook (*Fed. Proc.*, 1948, **7**, 152) have shown that dimethylthetin chloride can methylate homocystine *in vitro*, using a liver preparation.

EXPERIMENTAL.

Further Characterisation of the Dimethyl Sulphide liberated from P. fastigiata. *Absence of Methylthiol and Dimethyl Disulphide.*—The alga was plucked from its host, suspended in water, and a stream of air passed through into two absorption bottles containing 4% mercuric cyanide and two or more containing 3% mercuric chloride. In numerous experiments with different batches of alga no precipitate was ever observed in the mercuric cyanide, indicating the absence of an alkylthiol. The deposit in the mercuric chloride always melted at 156—158°, not depressing the m. p. of an authentic specimen of the mercurichloride of dimethyl sulphide. Its identity was further confirmed by treating a quantity accumulated from several experiments with sodium hydroxide. The liberated dimethyl sulphide was (*a*) extracted with ether, which was then treated with aqueous potassium chloroplatinite, and (*b*) aspirated through alcoholic benzyl chloride. In (*a*) the solid which separated melted at 160° after two crystallisations. Enebuske (*J. pr. Chem.*, 1888, **38**, 358) gives m. p. 159°, and Challenger, Taylor, and Taylor (*J.*, 1942, 48) give m. p. 160°, for the compound $SMe_2,PtCl_2$. From (*b*), benzyldimethylsulphonium picrate, m. p. 134°, was obtained (Baker and Moffitt, *J.*, 1930, 1722, and Challenger and Charlton, *J.*, 1947, 427, give m. p. 134°). The corresponding *styphnate*, m. p. 114°, was also prepared (Found : C, 45·4; H, 3·85; N, 11·1; S, 8·2. $C_{15}H_{15}O_8N_3S$ requires C, 45·3; H, 3·8; N, 10·6; S, 8·1%).

It remained to be shown that no disulphide, *e.g.*, dimethyl disulphide, arising from the possible, though improbable, complete oxidation of traces of methylthiol was present in the dimethyl sulphide. A further quantity of the dimethyl sulphide mercurichloride, m. p. 157° (from the alga), was recrystallised five times from benzene. It was completely soluble each time and the m. p.s were 156—157°, 156—157°, 155°, 157°, and 156—157°. The compounds $SMe\cdot HgCl$ and $SMe\cdot HgCl,xHgCl_2$ which arise from dimethyl disulphide and mercuric chloride (Blackburn and Challenger, *J.*, 1938, 1872) are very insoluble. The first does not melt below 260° and the second has m. p. 151°. The absence of both these compounds was conclusively proved by treating the mercurichloride from the alga with diluted hydrochloric acid (1 : 2) and aspirating through mercuric cyanide and chloride as before. The dimethyl sulphide was readily evolved in the cold, the cyanide remaining quite clear even on heating the mixture to 60°. At the conclusion of the experiment a small quantity of authentic methylthiomercuric chloride mercurichloride, $SMe\cdot HgCl,xHgCl_2$, was added to the acid reaction mixture. At 60° methylthiol was evolved and a precipitate appeared in the mercuric cyanide.

Preparation of an Extract of the Seaweed.—The *Polysiphonia fastigiata* was picked from the *Ascophyllum nodosum*, washed with water, and dried as thoroughly as possible with towels. It was then left in cold absolute alcohol for periods which varied from 2 to 3 weeks, the alcohol decanted, and a fresh quantity added. After about three extractions the weed was found to evolve hardly any dimethyl sulphide on treatment with cold sodium hydroxide.

The united alcoholic extracts were then filtered and evaporated at 40—50°/15—25 mm. The greenish-brown semi-solid residue tended to crystallise slowly in a desiccator, but was usually triturated at once with absolute alcohol, giving a pale brown solid (P.B.S.). This was acid to litmus and contained organic and inorganic matter, nitrogen, sulphur, and chlorine. Chloride and sulphate ions were present, and the sulphate increased on warming with dilute hydrochloric acid. Warming with dilute sulphuric acid, followed by neutralisation, treatment with solid sodium acetate, glacial acetic acid, and phenylhydrazine, gave an osazone or phenylhydrazone, m. p. 192°, which has not been examined further. As would be expected, the P.B.S. gave a positive Molisch reaction for carbohydrate; the biuret reaction was negative. Amino-acids were present, however, since addition of a dilute solution of triketoindane hydrate (" ninhydrin ") to the neutralised P.B.S. gave a violet colour on warming. Ferric chloride gave a green coloration. (The original alcohol washings from the P.B.S. gave this reaction much more intensely and on evaporation gave a viscous residue similar in properties to the P.B.S. but containing much green colouring matter.)

On heating the dry P.B.S., ammonia and dimethyl sulphide were evolved. On treatment with cold sodium hydroxide and aspiration through aqueous mercuric chloride dimethyl sulphide mercurichloride, m. p. and mixed m. p. 157—158°, was obtained. When evolution of this ceased, the alkaline solution was gently warmed, and the vapours absorbed in dilute hydrochloric acid. Evaporation gave a non-deliquescent residue of ammonium chloride. Alkylamines were apparently absent.

On the assumption that it contained a sulphonium compound, concentrated solutions of various salts and acids were added to the P.B.S. in the hope of obtaining a solid derivative. No success was obtained with the sodium salts of styphnic, 2 : 4-dinitrobenzoic, 2 : 4 : 6-trinitrobenzoic, picrolonic, and α-naphthalenesulphonic acids or with potassium ferro- and ferri-cyanides, potassium bismuth iodide, ferric chloride in hydrochloric acid, and ammonium stannichloride in hydrochloric acid. Sodium picrate gave only a precipitate of potassium picrate, but with ammonium reineckate and phosphotungstic acid precipitates were obtained.

Examination of the Reineckate and Phosphotungstate obtained from the P.B.S.—These evolved dimethyl sulphide (characterised as before) on treatment with cold sodium hydroxide or on warming with water and lead hydroxide or baryta. Removal of lead and baryta from the filtered test portions left solutions which, on concentration, contained traces of nitrogen, but no sulphur. They were free from carbohydrate and protein (biuret reaction) but gave a positive ninhydrin reaction. Acidification gave a sharp acidic odour later shown to be due to acrylic acid (see p. 1595). Acidified permanganate was readily reduced in the cold. Ferric chloride gave no colour.

Conversion of the Sulphonium Reineckate into a Sulphonium Platinichloride.—The reineckate (50 g.) was suspended in 50% aqueous acetone (about 100 c.c.) and stirred while excess of saturated (N/25) aqueous silver sulphate solution was added from a burette. On complete precipitation of the reineckate as silver salt, the filtered solution was treated with N/12-barium chloride solution, in amount equivalent to the silver sulphate added. The liquid was filtered through a sintered-glass crucible, and the filtrate (acid to litmus) evaporated to dryness under diminished pressure at 50—60°, leaving a brownish viscous residue which showed signs of solidifying when kept in a desiccator. It evolved dimethyl sulphide with cold aqueous sodium hydroxide. On addition of a very concentrated aqueous solution of chloroplatinic acid, a platinichloride separated on rubbing and standing for a few minutes. This was separated and washed with successive small quantities of water–alcohol (30 : 70). The aqueous filtrate gave a further deposit on addition of alcohol. The platinichloride was recrystallised from water–alcohol (1 : 10), and then decomposed between 194° and 202° according to the rate of heating. It gave a negative Molisch reaction and evolved dimethyl sulphide with cold alkali or when heated alone.

Conversion of the Sulphonium Platinichloride into the Picrate.—The platinichloride, dissolved in the minimum amount of water, was treated with twice its weight of silver, prepared by Dudley's method (*loc. cit.*). (The silver had been very carefully washed, otherwise it was found to occlude ferrous sulphate which then contaminated the picrate.) The mixture was shaken vigorously for a few minutes until the solution was colourless. Silver, platinum, and silver chloride were separated, leaving a solution of the sulphonium chloride, which was evaporated to dryness at 50—60° under diminished pressure. Addition of saturated aqueous sodium picrate to the residue gave, after rubbing with a glass rod and standing for a few minutes, a yellow crystalline precipitate. This was separated, washed with absolute alcohol, and dried in a desiccator; m. p. 128°. · After recrystallisations from the same solvent the m. p. was 131° (decomp. 131·5°). On three further crystallisations the m. p. was on each occasion 131·5° (decomp. 132°). The m. p.s of these five specimens were taken simultaneously on the same thermometer. The picrate readily evolved dimethyl sulphide with cold sodium hydroxide.

In later experiments the picrate was prepared from the chloride resulting directly from the removal of the reineckate ion, without passing through the platinichloride stage. The product was at first somewhat discoloured but on recrystallisation from alcohol the pure picrate was obtained.

Conversion of the Sulphonium Picrate into the Chloride.—The picrate, three times recrystallised, was weighed, dissolved in 1 equiv. of dilute hydrochloric acid, and shaken repeatedly with successive quantities of ether till the extract was colourless. The aqueous solution was then faintly red and still acidic. Evaporation at 50—60° under diminished pressure gave a residue which gradually crystallised in almost colourless needles when left in a vacuum desiccator. Crystallisation from absolute alcohol gave colourless needles of constant m. p. 134° (decomp.). These contained no nitrogen and gave no Molisch reaction before or after removal of dimethyl sulphide with alkali. On exposure to air for a few minutes the m. p. quickly fell to below this value as the chloride is deliquescent (Found, by micro-analysis: C, 35·5, 35·7; H, 6·6, 6·45; Cl, 20·3, 20·6; S, 18·9, 19·0. Calc. for $C_5H_{11}O_2ClS$: C, 35·2; H, 6·5; Cl, 20·7; S, 18·7%). The compound contained ionic chlorine and was acid to litmus. This and the earlier experiments suggested the formula $\overset{+}{S}Me_2(\overset{-}{Cl})\cdot C_2H_4\cdot CO_2H$. The carboxyl group and the ionised chlorine were determined volumetrically.

The chloride (1·1308 g.) in 100 c.c. of water was titrated in 10-c.c. portions with 0·036N-sodium hydroxide, using phenolphthalein. When the carboxyl group was neutralised the indicator turned **but** the colour was not permanent, for the salt began to decompose thus: $\overset{+}{S}Me_2(\overset{-}{Cl})\cdot C_2H_4\cdot CO_2Na + NaOH = SMe_2 + C_2H_3\cdot CO_2Na + NaCl + H_2O$, and an odour of dimethyl sulphide was detected at the end-point. Serious error was avoided by shaking thoroughly during the titration and taking the reading when the pink colour was first produced (Found: CO_2H, 25·7%; equiv., 172).

The chloride ion in the titrated solutions was determined by Volhard's method (Found: Cl, 20·6). An earlier specimen of the sulphonium chloride gave by gravimetric analysis Cl, 20·5 (Calc. for $C_5H_{11}O_2ClS$: CO_2H, 26·3; Cl, 20·7%; equiv., 170·5).

The platinichloride, prepared from the pure chloride, melted at 193° or 200° according to the rate of heating. Carrara and Rossi (*loc. cit.*) give m. p. 184° (see also Billows, *Gazzetta*, 1893, **23**, I, 508) [Found: Pt, 29·5. Calc. for $(C_5H_{11}O_2ClS)_2PtCl_4$: Pt, 28·8%].

Properties of the Sulphonium Chloride: Reconversion into Picrate.—The chloride liberated carbon dioxide from sodium hydrogen carbonate and was optically inactive in water (*c*, 2·5, 5, or 10). When it was heated above its m. p., dimethyl sulphide (mercurichloride, m. p. 157—158°) and some hydrogen chloride were obtained. The residue had a sharp acidic odour (see below) and decolorised bromine water and potassium permanganate. It gave no colour with ferric chloride and no precipitate with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid.

As stated on p. 1592, these results indicate that the chloride is dimethyl-2-carboxyethylsulphonium chloride. It could be reconverted into the picrate of unchanged m. p. 131·5° (decomp. 132°). Specimens of the *picrate*, obtained from the chloride, with or without passing through the platinichloride, were analysed (Found: C, 36·8; 36·5; H, 3·65, 3·75; N, 11·3. $C_{11}H_{13}O_9N_3S$ requires C, 36·4; H, 3·6; N, 11·6%). Subtraction of the picrate ion $C_6H_2O_7N_3$ gives for the sulphonium cation $C_5H_{11}O_2S$, in agreement with the formula of the chloride $C_5H_{11}O_2ClS$. Determination of the picrate ion as nitron picrate gave for the molecular weight of the sulphonium ion 136·1 ($C_5H_{11}O_2S$ requires *M*, 135. The *styphnate*, crystallised from alcohol, had m. p. 148° (Found: C, 35·1; H, 3·4; N, 10·7. $C_{11}H_{13}O_{10}N_3S$ requires C, 34·8; H, 3·45; N, 11·1%).

The sulphonium bromide was prepared by repeated evaporation of the algal chloride with hydrobromic acid almost to dryness at 100° during 4—5 hours. The final residue solidified on treatment with absolute alcohol, from which it was recrystallised by addition of ether. It melted at 112—114°. Carrara and Rossi (*loc. cit.*) give m. p. 115°.

Decomposition of the Sulphonium Chloride with Sodium Hydroxide.—The algal chloride (1 g.) was treated with excess of sodium hydroxide, and the liberated dimethyl sulphide removed by a stream of air at room temperature. To the odourless solution toluene-*p*-sulphinic acid (1 g.) was added, and the mixture slightly acidified with hydrochloric acid. A white solid separated on standing which, after recrystallisation from water and drying in a desiccator, had m. p. 112°. A synthetic specimen of of dimethyl-2-carboxyethylsulphonium chloride (see p. 1596) on similar treatment gave a product of m. p. 112—113° and mixed m. p. 112—113° with the algal product.

The same compound, p-*tolyl* 2-*carboxyethyl sulphone*, was prepared from acrylic acid and the equivalent amount of toluene-*p*-sulphinic acid in dilute alkali. Acidification as before gave a product which, when crystallised from water and thoroughly dried in a desiccator, had m. p. and mixed m. p. 112° with the two specimens just described prepared from the algal and synthetic chlorides (Found: C, 52·0; H, 5·2. $C_{10}H_{12}O_4S$ requires C, 52·5; H, 5·25%).

For comparison, the m. p.s of toluene-*p*-sulphinic acid and its possible transformation products toluene-*p*-sulphonic acid and *p*-tolyl disulphoxide, $C_7H_7\cdot SO_2\cdot S\cdot C_7H_7$, are 90°, 92° and 104·5° (hydrated and anhydrous respectively), and 76°, respectively.

Preparation of Reference Compounds.—*Dimethyl-*2-*carboxyethylsulphonium derivatives.* (a) The bromide was prepared by the method of Carrara (*loc. cit.*), but as his procedure is not very clear the following details are given. Equimolecular quantities of β-bromopropionic acid and dimethyl sulphide were mixed. After 24 hours at room temperature a test portion yielded only a small quantity of sulphonium compound, insoluble in ether, giving a precipitate with aqueous ammonium reineckate. The reaction mixture was then refluxed at 55—60° for 6—8 hours. It solidified on cooling; washing with dry ether removed unchanged reactants, leaving a white solid which was recrystallised from absolute alcohol; m. p. 112—114°. Carrara (*loc. cit.*) gives m. p. 115° (decomp.). Under these conditions β-chloropropionic acid and dimethyl sulphide did not react, but (b) the *chloride* was obtained from the bromide and silver chloride. On recrystallisation from absolute alcohol it melted at 134° (Found : C, 34·7; H, 6·5; Cl, 20·3, 20·6. $C_5H_{11}O_2ClS$ requires C, 35·2; H, 6·45; Cl, 20·7%). When a mixture of equimolecular amounts of β-iodopropionic acid and dimethyl sulphide was left at room temperature only traces of a sulphonium compound could be detected by ammonium reineckate. After a few hours' refluxing a sulphonium compound was isolated as before. This gave no picrate and only evolved dimethyl sulphide on boiling with alkali. It melted at 202—204°, and at 200—202° in admixture with authentic trimethylsulphonium iodide, m. p. 202—204° (all with decomp.). Biilmann and Jensen (*loc. cit.*) obtained trimethylsulphonium bromide from dimethyl sulphide (2 mols.) and α-bromopropionic acid (1 mol.) or its ethyl ester (1 mol.) at 100°. With the acid and sulphide in equimolecular proportions at room temperature the thetin bromide was formed (see also Carrara, *loc. cit.*). Trimethylsulphonium iodide was formed from methyl iodide (2 mols.) and ethyl α-methylthiopropionate at 100°.

(c) The picrate, m. p. 132°, was prepared from the bromide (Found : C, 36·6; H, 3·6; N, 11·7; S, 9·0%).

(d) The styphnate had m. p. 148·5—149°, the m. p.s on recrystallisation from alcohol being 148—148·5°, 149°, 148·5—149°, 148·5—149°, 148·5° (Found : C, 34·9; H, 3·35%).

Dimethyl-1-*carboxyethylsulphonium derivatives.* (e) The bromide was prepared by Carrara's method (*loc. cit.*) at room temperature. The solid, crystallised from warm alcohol by addition of ether, had m. p. 94—95° and was very deliquescent. Carrara gives m. p. 84—85° (decomp.). (f) It was converted into the picrate. Five recrystallisations from alcohol only raised the m. p. from 151·5—152° to 152—152·5°. (g) The styphnate had m. p. 151—151·5°, the figures on recrystallisation from alcohol being 152°, 150—151°, 151·5—152°, 151—151·5°.

The chloride was prepared from the bromide and silver chloride. It was very deliquescent and was not crystallised. (h) It gave a platinichloride, m. p. 111°, on recrystallisation from absolute alcohol. Billows (*loc. cit.*) gives m. p. 105—106°.

Table of m. p. and mixed m. p. of derivatives of dimethyl-α- and -β-propiothetins.

	Chloride.	Bromide.	Picrate.	Styphnate.	Platinichloride.
α-Thetin deriv.	—	94—95°	152—152·5°	151—151·5°	111°
β-Thetin deriv.	134°	112—114	132	148·5—149	193 or 200 *
Mixed m. p.	—	74	124	139	111—200
Algal product	134	112—113	131·5	148	193 or 200 *
Algal product + α-thetin deriv.; mixed m. p.	—	75	122	138	111—200
Algal product + β-thetin deriv.; mixed m. p.	132—133	112—113	131—132	148	193 or 200 *

* Dependent on the rate of heating.

Preparation of Other Sulphonium Compounds.—(1) *Dimethyl*-n-*amylsulphonium iodide.* Methyl *n*-amyl sulphide (Blackburn and Challenger, *J.*, 1938, 1875) and its equivalent of methyl iodide were left at room temperature without a solvent for 24 hours. The resulting *iodide* was recrystallised from alcohol-ether; m. p. 102° (Found : I, 48·8. $C_7H_{17}IS$ requires I, 48·8%).

(2) *Dimethyl*-2-*hydroxyethylsulphonium iodide.* Methyl 2-hydroxyethyl sulphide (*Org. Synth.*, Coll. Vol. II, 346) gave a mercurichloride, m. p. 94°, from alcohol. A mixture of the sulphide and an equivalent of methyl iodide gradually became warm and a solid separated. This was recrystallised from absolute alcohol; m. p. 57—59°. It was extremely deliquescent.

(3) *Dimethyl*-2-*acetoxyethylsulphonium iodide.* The methyl 2-hydroxyethyl sulphide was refluxed with acetic anhydride for 30 mins., cooled, shaken with aqueous sodium carbonate solution, and the mixture extracted with ether, yielding 2-*acetoxyethyl sulphide* as a colourless oil having an odour resembling that of amyl acetate; b. p. 68—70°/15 mm. (Found : C, 44·5; H, 7·6; S, 24·7. $C_5H_{10}O_2S$ requires C, 44·7; H, 7·5; S, 23·8%). The mercurichloride, when recrystallised from alcohol, melted at 93°, and at 85° in admixture with the mercurichloride of the unacetylated sulphide, m. p. 94°.

With methyl iodide in the cold a syrup separated after a day. This was very deliquescent and could not be crystallised. The picrate recrystallised from alcohol, had m. p. 133—134°.

Methyl 2-hydroxyethyl sulphide (2·5 g., 1 mol.), tetra-acetyl glucosidyl bromide (11·0 g.; 1 mol.), and active silver oxide (3·15 g., 0·5 mol.), prepared by the method of Helferich and Klein (*Annalen*, 1926, **450**, 225), were shaken in dry benzene for an hour and heated under reflux for the same time. The mixture darkened, silver and silver bromide were deposited, and an odour resembling that of amyl acetate was observed. Removal of solvent and extraction with water and ether yielded an oil of b. p. 55—60°/15 mm. with the same odour. It gave a mercurichloride of m. p. and mixed m. p. 93° with the mercurichloride of methyl 2-acetoxyethyl sulphide. The attempt to prepare the acetate of a 2-methylthioethyl glucoside was therefore unsuccessful.

Decomposition of Some Sulphonium Compounds by Aqueous Sodium Hydroxide.—Each sulphonium compound was treated with excess of 2N-sodium hydroxide, and the temperature slowly raised in a water-bath. Volatile matter was aspirated into aqueous mercuric chloride. Trimethylsulphonium iodide, m. p. 203—206° (decomp.) (Steinkopf and Muller, *Ber.*, 1923, **56**, 1928, give m. p. 202°, decomp.), dimethylbutylsulphonium iodide, m. p. 81·5° (Ingold *et al.*, *J.*, 1933, 535, give m. p. 82°), and dimethylamylsulphonium iodide (see above) yielded dimethyl sulphide mercurichloride, m. p. 157—158°, with boiling sodium hydroxide, but no action was observed below 100°. Under exactly similar conditions methyldiethyl- and triethyl-sulphonium iodides yielded the mercurichlorides of methyl ethyl sulphide,

m. p. 127°, and diethyl sulphide, m. p. 118° (Challenger and Rawlings, *J.*, 1937, 868, give m. p. 127—128° and 119°, respectively). No change occurred below 100°.

Methylmethioninesulphonium iodide, $\overset{+}{S}Me_2(\overset{-}{I})\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot CO_2H$, evolved dimethyl sulphide with 2N-sodium hydroxide just below the boiling point. No decomposition occurred below 90°. Dimethyl-2-hydroxyethylsulphonium iodide and dimethyl-2-acetoxyethylsulphonium picrate were unchanged at room temperature, but liberated dimethyl sulphide at 55° and 60—70°, respectively. The m. p.s of the mercurichlorides were 156—157°, 157—158°, and 157°.

The authors thank the Royal Society and Imperial Chemical Industries Ltd., for grants, and the Department of Scientific and Industrial Research for a maintenance grant (to M. I. S.).

THE UNIVERSITY, LEEDS. [*Received, October 27th*, 1947.]