8. *Studies on Biological Methylation. Part XVI.* Natural Sulphonium Compounds. The Alkyl Methyl Sulphides evolved from the Urine of Dogs by Boiling Alkali.*

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The urine of dogs, when boiled with alkali, evolves a sulphide which previous workers have incorrectly assumed to be diethyl sulphide arising by fission of diethylmethylsulphonium hydroxide. Chromatographic examination of the sulphidimine $RR^1S{\rightarrow}N{\cdot}SO_2{\cdot}C_6H_4Me$-*p* and the methylsulphonium hydroxides $SRR'Me\}OH$ prepared from the natural sulphide has shown it to be a mixture of methyl *n*-propyl sulphide and probably *n*-butyl methyl sulphide, the former preponderating. The methyl *n*-propyl sulphide was also identified by the m. p. and mixed m. p. of its sulphidimine and mercurichloride.

ABEL [1] stated that when the urine of dogs was warmed with alkali diethyl sulphide was evolved, but no proof of identity was given. The m. p. of the mercurichloride of the sulphide was given as 145° and also as 150°. Diethyl sulphide mercurichloride has m. p. 119°. Nevertheless later workers [2,3,4] accepted Abel's statement and Neuberg and Grosser [4] claimed to have isolated the precursor of the sulphide as the phosphotungstate and iodobismuthate, and to have identified it as diethylmethylsulphonium hydroxide. No experimental details were supplied and no further communication appeared.

Diethylmethylsulphonium iodide with sodium hydroxide at 100° gives mainly ethyl methyl sulphide, characterised as mercurichloride. The work of Ingold and Kuriyan [5] also suggests that this sulphide is the main product of the alkaline decomposition of this sulphonium ion.

A re-investigation of the sulphide from dog's urine was therefore initiated. Dr. Margaret Whitaker passed the evolved vapours through (*a*) mercuric cyanide and (*b*) mercuric chloride. Thiols were absent. The m. p.s of the mercurichloride, mercuribromide, and benzylsulphonium picrate approximated to those of corresponding derivatives of methyl *n*-propyl sulphide. Methyl *n*-propyl sulphide was not, however, the only component because, even after crystallisation, the m. p.s of these derivatives were usually slightly lower than those of the authentic compounds.

The identity of the sulphide has been established by paper chromatography, the sulphidimine and the methylsulphonium hydroxide being examined. The sulphidimines were detected by spraying with acidified potassium iodide and heating at 80°, to give brown spots of iodine. The methylsulphonium hydroxides were detected by bromophenol-blue.

Chromatography of the sulphidimine of the natural sulphide produced only one spot,

* Part XV, *J.*, 1955, 1153.

[1] Abel, *Z. physiol. Chem.*, 1894, **20**, 253.
[2] Christomanos, *ibid.*, 1931, **198**, 185; 1933, **217**, 177; 1934, **225**, 211; *Klin. Woch.*, 1932, **11**, 177.
[3] Wohlgemuth, *Z. physiol. Chem.*, 1933, **221**, 207.
[4] Neuberg and Grosser, *Centralblatt Physiol.*, 1905—6, **19**, 316.
[5] Ingold and Kuriyan, *J.*, 1933, 991.

with the same R_F value as methyl *n*-propyl sulphidimine. On repetition of the chromatogram, cutting out the appropriate area, reduction with tin and hydrochloric acid [6] to the sulphides, and reconversion into the mercurichloride, gave a material whose m. p. was still lower than that of methyl *n*-propyl sulphide mercurichloride. The impurity was therefore another sulphide of similar R_F value. Five sulphidimines possessed approximately this R_F value, namely, (1) diethyl (2) methyl *iso*propyl, (3) *n*-butyl methyl, (4) *iso*butyl methyl, and (5) *sec.*-butyl methyl sulphidimine.

When specimens of the methylsulphonium hydroxide from the " natural " sulphide were chromatographed, two spots were produced. The stronger had the R_F value of dimethyl-*n*-propylsulphonium hydroxide; that of the weaker was slightly higher. Both diethylmethyl- and dimethyl*iso*propyl-sulphonium hydroxide had lower R_F values than the dimethyl-*n*-propyl compound [eliminating possibilities (1) and (2) for the second sulphide]. The three butyldimethylsulphonium hydroxides (*n*-, *iso*-, and *sec.*-) possessed higher R_F values than the dimethyl-*n*-propyl compound, but a mixture of the *sec.*-butyl compound and excess of the *n*-propyl compound was not separable on paper [eliminating possibility (5)].

The pattern of the natural mixture could be reproduced almost exactly by a mixture of dimethyl-*n*-propyl- and *n*-butyldimethyl-sulphonium hydroxides (by adjusting the relative proportions of the components) but not by a mixture containing the *n*-propyl and *iso*butyl compounds. This suggested that the second component of the natural sulphide was probably *n*-butyl methyl sulphide.

A sample of the " natural sulphidimine " was recrystallised, finally giving almost pure methyl *n*-propyl sulphidimine. The material from the mother-liquors was chromatographed alongside a synthetic mixture of methyl *n*-propyl and *n*-butyl methyl sulphidimine. The two spots from the natural mixture agreed in R_F value with those from the synthetic mixture. The remainder of the natural sulphidimine was also chromatographed, the appropriate zones were cut out, and the sulphidimines so separated reduced to sulphides and converted into the mercurichlorides. That from the band of higher R_F value had m. p. and mixed m. p. identical with that of methyl *n*-propyl sulphide mercurichloride. That from the other band had a rather unsharp m. p. which was, however, identical with that of *n*-butyl methyl sulphide mercurichloride.

These results indicate that the sulphide evolved from dog's urine is a mixture of methyl *n*-propyl and *n*-butyl methyl sulphide, the former predominating.

A study of the parent compound (or, more probably, compounds) in the urine was undertaken and a method of isolation devised. This compound is removed from solution by sulphonic acid cation-exchange resins and can be displaced from these by treatment with stronger bases. Its behaviour during isolation is similar to that of a sulphonium compound bearing an acidic group. The investigation is being continued.

The isolation of dimethyl-β-propiothetin chloride from the red marine alga *Polysiphonia fastigiata* by one of us and (Miss) Simpson [7] was the first authenticated example of the natural occurrence of a sulphonium compound, unless we regard sulphoraphen $Me{\cdot}SO{\cdot}CH{:}CH{\cdot}CH_2{\cdot}CH_2{\cdot}NCS$, isolated from radish by Schmid and Karrer,[8] as a potential sulphonium compound.

This propiothetin was also found in other marine algæ (*Enteromorpha intestinalis* and *Spongomorpha arcta*) by Bywood and Challenger.[9,10] Maw and du Vigneaud [11] showed that it supports the growth of rats on a methionine-choline-free diet [12] containing homocystine, and Dubnoff and Borsook [13] found that methionine is formed from the thetin and

[6] Ash, Challenger, and Greenwood, *J.*, 1951, 1881.
[7] Challenger and Simpson, *J.*, 1948, 1591.
[8] Schmid and Karrer, *Helv. Chim. Acta*, 1948, **31**, 1017, 1087, 1497.
[9] Bywood and Challenger, *Biochem. J.*, 1953, **53**, xxvi.
[10] Bywood, Thesis, Leeds, 1953.
[11] Maw and du Vigneaud, *J. Biol. Chem.*, 1948, **174**, 381.
[12] du Vigneaud, Moyer, and Chandler, *ibid.*, p. 477.
[13] Dubnoff and Borsook, *ibid.*, 1948, **176**, 789.

homocystine in enzyme preparations from rats. Cantoni then isolated "active methionine" [14] (the ionic *S*-adenosinylmethionine) from preparations of kidney and liver containing methionine, and McRorie *et al.*[15] showed that cabbage, lettuce, and other vegetables contain the methylmethioninesulphonium ion. The same ion occurs in asparagus.[16]

The occurrence of dimethylsulphonium compounds in numerous marine and some fresh water algæ, in bracken, and in several species of *Equisetum* was established in the Leeds laboratories.[17] Ericson and Carlson [18] showed by paper chromatography that dimethyl-β-propiothetin and β-alanine were present in all marine algæ examined. In *P. fastigiata* and *Ulva lactuca* where the thetin was present in large amount, the quantity of β-alanine, though relatively much lower, was at a maximum.

Challenger [19] suggested that there may be a biogenetic relation between the thetin and β-alanine, or (possibly) its betaine, similar to that established by Woolley [20] between quaternary ammonium compounds of the type of thiamine and various amines.

Experimental

Preparation of the Mercurichloride of the Sulphide from Dog's Urine.—The urine was made strongly alkaline with sodium hydroxide and boiled; the volatile products were aspirated through (*a*) dilute sulphuric acid to absorb volatile bases, (*b*) 4% mercuric cyanide, and (*c*) 3% mercuric chloride. No precipitate was obtained in the mercuric cyanide, indicating the absence of an alkanethiol. The sulphide was precipitated as its mercurichloride, m. p. (crude) ~156° (sintering from 145°) but melting was not complete below about 159°. This behaviour was characteristic, varying only slightly with different samples of urine. The yield of mercurichloride per l. was about 0·02—0·04 g. Recrystallisation from benzene containing mercuric chloride raised the m. p. to 158—160° and the mixed m. p. with authentic methyl *n*-propyl sulphide mercurichloride (m. p. 165°) was 159—162° [Found: Hg, 65·7. Calc. for $(CH_3{\cdot}S{\cdot}C_3H_7)_2,5HgCl_2$: Hg, 65·5%)].

Conversion of the Crude Mercurichloride into Other Derivatives.—The sulphide was regenerated from its mercurichloride by warm dilute sodium hydroxide, and separately aspirated into (*a*) saturated mercuric bromide and (*b*) 10% v/v alcoholic benzyl bromide. In (*a*) a mercuribromide was obtained, which, on recrystallisation from benzene, had m. p. 113° and mixed m. p. 115—116° with authentic methyl *n*-propyl sulphide mercuribromide (m. p. 116°). The alcoholic solution from (*b*) was left for 2 days, diluted with ether, and extracted with water, from which a precipitate was obtained with sodium picrate. This, when recrystallised from alcohol, had m. p. 93—94° and mixed m. p. 94—95° with benzylmethyl-*n*-propylsulphonium picrate, m. p. 95—96° (Challenger and Rawlings [21] give m. p. 95—95·5°). For other data see Table 1.

Chromatographic Methods.—The chromatograms were developed at about 20° in glass tanks by the descending front method.

(1) *Chromatography of sulphidimines: method* (*a*). The sulphidimines were applied to the paper (Whatman No 1) in acetone. The best solvent systems were *cyclo*hexanol–water and butan-1-ol–*iso*propyl ether–water (5 : 2 : 4 by vol.). The papers were irrigated with the top (organic) layer in both cases. With butanol–*iso*propyl ether, development was complete in 16 hr. but the chromatograms developed with *cyclo*hexanol required 3—5 days. The R_F values for a number of sulphidimines are given in Table 2.

Although producing less effective separations of the lower sulphidimines, *cyclo*hexanol gave more compact spots and greater separations of the higher sulphidimines than butanol–*iso*propyl

[14] Cantoni, *J. Amer. Chem. Soc.*, 1952, **74**, 2942; Conférences et Rapports, 3 Congrès Intern. Biochimie, 1956, p. 233; Challenger, *Quart. Rev.*, 1955, **9**, 274, 279.
[15] McRorie, Sutherland, Lewis, Burton, Glazener, and Shive, *J. Amer. Chem. Soc.*, 1954, **76**, 115.
[16] Challenger and (Miss) Hayward, *Chem. and Ind.*, 1954, 729.
[17] Challenger, Leaver, and (Mrs.) Whitaker, *Biochem. J.*, 1953, **56**, ii; Leaver, Thesis, Leeds, 1953.
[18] Ericson and Carlson, *Arkiv Kemi*, 1954, **6**, 511.
[19] Challenger, Conférences et Rapports, 3 Congrès Intern. Biochimie, 1956, p. 239.
[20] Woolley, *Nature*, 1953, **171**, 323.
[21] Challenger and Rawlings, *J.*, 1937, 868.

ether, and it was the preferred solvent. The chromatograms were sprayed with 1% potassium iodide in 0·2N-hydrochloric acid and heated at 80°. Brown spots were produced by the sulphidimines, presumably owing to hydrolysis to a sulphoxide which oxidises iodide to iodine. After a few hours the whole paper became brown.

TABLE 1. *Comparison of the m. p.s of corresponding derivatives of the crude sulphide from dog's urine, of methyl* n-*propyl sulphide and of diethyl sulphide.*

Derivative	Natural sulphide	$MePr^nS$	Mixed m. p.	Et_2S
Mercurichloride	145—159°	165°	159—162° *	119°
Mercuribromide	113	116	115—116	—
Sulphidimine (from chloramine-T)	91—93	104—105	—	146
Dialkylbenzylsulphonium picrate	93—94	95—96	94—95	113
Dialkylbenzylsulphonium styphnate	85—86	77	80—85	129

The derivatives of the natural sulphide had undergone only the minimum of recrystallisation.
* The recrystallised natural sulphide mercurichloride, m. p. 158—160°, was used for this mixed m. p. determination.

TABLE 2.

Sulphidimine	R_F value: In Bu^nOH–Pr^i_2O	R_F value: In *cyclo*hexanol	Sulphidimine	R_F value: In Bu^nOH–Pr^i_2O	R_F value: In *cyclo*hexanol
Me_2	0·73	0·75	$MeBu^n$	—	0·83—0·84
MeEt	0·83	0·81	$MeBu^i$	—	0·83—0·84
Et_2	0·90	0·83	$MeBu^s$	—	0·83—0·84
$MePr^n$	0·90	0·83	Pr^n_2	0·96	0·87
$MePr^i$	—	0·83	Bu^n_2	0·96	0·91

Method (*b*). In " reversed phase " chromatography of sulphidimines Whatman No. 1 paper was rendered water-repellent by treatment with a 0·75% w/v solution of Perspex (methyl methacrylate polymer) in chloroform. After application of the sulphidimines, in acetone solution, the papers were suspended in the tank for 12 hr. without solvent in the trough, so allowing them to come to equilibrium with both phases of the solvent system which were placed in separate vessels at the bottom of the tank. The trough was then filled with the aqueous phase which was allowed to move down the paper for a further 12 hr. The most satisfactory solvent system was water–ethyl acetate. The positions of the sulphidimines were revealed as in method (*a*). The order of R_F values was the reverse of that obtained by (*a*), thus affording a better separation of the higher sulphidimines.

(2) *Chromatography of bases.* Whatman No. 1 paper was washed by irrigation with 2N-hydrochloric acid (1 ml. per sq. in.), followed by distilled water until free from acid. (The use of unwashed paper resulted in poor and irregular background colours.) The solvent system used was *n*-butanol–acetic acid–water (5 : 1 : 4 by vol.) and development was complete in about 17 hr. The papers were dried and sprayed with a 0·1% solution of bromophenol-blue in alcohol containing acetic acid (0·2—0·5%). The bases gave blue spots on a yellow ground.

Preparation of the " Natural Sulphidimine."—The " natural sulphide " mercurichloride (0·3 g.) was decomposed by sodium hydroxide solution, and the sulphide aspirated through saturated aqueous chloramine-T. The sulphidimine was extracted with several small volumes of chloroform and the extracts were dried ($CaCl_2$) and evaporated, leaving the sulphidimine as a viscous residue.

Paper Chromatography of the " Natural Sulphidimine " by Method (*a*).—A trace of the sulphidimine (before recrystallisation) was chromatographed in acetone by method (*a*), with *cyclo*hexanol as solvent, alongside a mixture of authentic dimethyl and methyl *n*-propyl sulphidimine, which are readily separated. The " natural sulphidimine " produced only one spot of which the R_F value was the same as that of the methyl propyl sulphidimine. No dimethyl sulphidimine was present in the natural compound.

The rest of the sulphidimine was crystallised three times (m. p. 93—94°), dissolved in acetone (0·5 ml.), and applied as a band to the base line. After development with *cyclo*hexanol and drying, narrow strips were cut from the edges and treated with acidified potassium iodide to reveal the position of the sulphidimine. The area containing the sulphidimine on the unsprayed portion of the paper was then cut out and the sulphidimine removed with acetone. It was

reconverted into the mercurichloride by tin in boiling 2N-hydrochloric acid, the sulphide so produced being aspirated into 3% mercuric chloride solution.[6] The resulting mercurichloride X (m. p. 159°, sintering at 150°) was recrystallised twice from alcohol–benzene containing a little mercuric chloride, but its m. p. could not be raised above 159—160° with sintering from 150°. The components of the natural sulphide had not been separated by chromatography of the sulphidimine.

Preparation of the Methylsulphonium Hydroxide from the Natural Sulphide.—The mercurichloride of the natural sulphide was warmed with sodium hydroxide solution, and the sulphide aspirated through two tubes containing alcohol. To the resulting solution an excess of methyl iodide was added and, after 2 days, the solution was evaporated. The syrupy residue was dissolved in water, treated with silver oxide, and evaporated to 1 ml. in a vacuum-desiccator, giving an aqueous solution of the methylsulphonium hydroxide. The preparations A and B were carried out with two specimens of mercurichloride; (A) was obtained from the mercurichloride sample X, which, after recrystallisation, had been recombined with its mother-liquors; (B) was obtained from an untreated sample (0·1 g.) of the natural sulphide mercurichloride.

Chromatography of Synthetic Sulphonium Hydroxides.—The R_F values of a number of trialkyl sulphonium hydroxides were: $SMe_3\}OH$, 0·30; $SMe_2Et\}OH$, 0·40; $SMe_2Pr^n\}OH$, 0·44; $SMe_2Pr^i\}OH$, 0·37; $SMe_2Bu^n\}OH$, 0·51; $SMe_2Bu^i\}OH$, 0·48; $SMe_2Bu^s\}OH$, 0·46; $SMePr^n_2\}OH$, 0·55.

Each of the butyldimethylsulphonium hydroxides was mixed with about five parts of dimethyl-*n*-propylsulphonium hydroxide, and the three mixtures were chromatographed on the same paper. That containing the *sec.*-butyl compound was not separated. In accordance with the R_F values the mixture (N) containing the *n*-butyl compound separated more clearly than that (I) containing the *iso*butyl compound.

Chromatography of the Methylsulphonium Hydroxide of the Natural Sulphide.—Preparation A (see p. 43), when chromatographed, separated into two distinct zones, a strong one and a weaker one of higher R_F value. The same result was obtained with B and the stronger spot in each case had the R_F value of dimethyl-*n*-propylsulphonium hydroxide run as a control.

A second chromatogram was prepared in which B was run alongside mixtures N and I (see above). The relative proportions of the two components in the synthetic mixtures were not quite the same as those in the natural mixture (estimated from the intensities of the coloured spots) and consequently neither N nor I gave patterns exactly the same as preparation B, although N resembled it more closely than I. It was possible, by altering slightly the proportions of the ingredients, to make the pattern given by N almost identical with that of the natural mixture, but this was not possible with I which always remained chromatographically distinct.

Preparation and Chromatography of Methyl n-*Propyl Sulphidimine from the Natural Sulphidimine.*—The mercurichloride of the natural sulphide (0·42 g.) was converted into the sulphidimine and purified by precipitation three times from acetone with a mixture of light petroleum (b. p. 40—60°) and ether and then by recrystallisation three times from alcohol–light petroleum (b. p. 60—80°). The m. p. became constant at 103—104° and was 104·5° when mixed with authentic methyl *n*-propyl sulphidimine (m. p. 104—105°). The mother-liquors from all six crystallisations were combined and evaporated (see p. 40), and the residue crystallised from acetone–ether, to remove any toluene-*p*-sulphonamide. The sulphidimine (R) so obtained was dissolved in acetone (0·5 ml.). A sample was chromatographed by method (*b*) alongside a mixture of methyl *n*-propyl (2 parts) and *n*-butyl methyl sulphidimine (1 part). The two spots produced by the natural sulphidimine had the same R_F values as those produced by the synthetic mixture.

The remaining natural sulphidimine (R) was applied in acetone as a band to the base line of a large chromatogram on Perspex-coated paper and developed by method (*b*). Narrow strips were cut from the edges and, after treatment with potassium iodide, were used as guides to the positions of the separated sulphidimines on the main unsprayed chromatogram. The bands were cut out and boiled, separately, with aqueous sodium hydrogen sulphite, and volatile products aspirated through water, to absorb sulphur dioxide, and 3% mercuric chloride solution. The mercurichloride from the band of higher R_F value melted at 164—165° alone, and at 165° when mixed with authentic methyl *n*-propyl sulphide mercurichloride (m. p. 165°). That from the band of lower R_F value melted at 110—114°, sintering at 90°. This behaviour was identical with that of *n*-butyl methyl sulphide mercurichloride prepared from pure *n*-butyl

methyl sulphidimine by boiling with sodium hydrogen sulphite and aspiration of the sulphide into mercuric chloride.

Alkaline Decomposition of Sulphonium Iodides.—(1) *Diethylmethylsulphonium iodide.* The iodide (1 g.) was boiled with 2N-sodium hydroxide, and the vapours were aspirated through aqueous 3% mercuric chloride for 11 hr. Precipitation of mercurichloride (m. p. 117—119°, sintering from 100°) then ceased. This complex was recrystallised four times from ethanol as glistening white plates, having finally m. p. 126—127° (sintering at 122°) and mixed m. p. 126—127° with the mercurichloride of ethyl methyl sulphide (m. p. 127°). The m. p. of the uncrystallised product was near to that of diethyl sulphide mercurichloride (119°) but the substance was clearly a mixture. Challenger and Simpson [7] state (without further details) that the m. p. of the mercurichloride of the sulphide evolved from diethylmethylsulphonium iodide and 2N-sodium hydroxide is 127°. Ingold and Kuriyan [5] find that formation of alcohol represents 45% of the reactions in the thermal decomposition of the hydroxide, from which it appears that the sulphide mixture contains at least 55% of ethyl methyl sulphide.

(2) *Ethyldimethylsulphonium iodide.* The method was as in (1), and iodide (1 g.) and 2N-sodium hydroxide were used (time 10 hr.). The mercurichloride sintered slightly below 125° and melted at 127°. Recrystallisation from ethanol gave m. p. 127—129° (sintering 125°), unchanged on crystallisation. Ethyl methyl sulphide mercurichloride melts at 127°. Ingold and Kuriyan [5] find that the sulphonium hydroxide gives 73% of alcohol on thermal fission, and from the above results it appears that methanol predominates.

(3) *Dimethyl-*n*-propylsulphonium iodide.* Reaction was as in (1) but with (*a*) 2N- and (*b*) 6N-sodium hydroxide. In expt. (*a*) the unrecrystallised mercurichloride had m. p. 164—165° with slight sintering from 157—160°; in (*b*) the m. p. was 165°, fairly sharp with slight sintering from 159°. Methyl *n*-propyl sulphide mercurichloride melts at 165° and dimethyl sulphide mercurichloride at 157—158°. Ingold and Kuriyan [5] found 92% of alcohols on thermal fission of this sulphonium ion. Some of the unrecrystallised mercurichloride obtained in decomposition (*a*) was decomposed by sodium hydroxide and the sulphides were converted into the sulphidimine. This was a mixture since on paper chromatography [method (*a*)] with the sulphidimines of dimethyl and methyl *n*-propyl sulphides as controls the presence of both these sulphides was detected.

(4) *Methyldi-*n*-propylsulphonium iodide.* Decomposition of this iodide (1 g.) with 2N-sodium hydroxide for 11 hr. gave mercurichloride, m. p. 110—140° unrecrystallised. Crystallisation from ethanol gave a product having finally m. p. 163—164° (sintering at 160°). Methyl *n*-propyl sulphide mercurichloride melts at 165°. Ingold and Kuriyan [5] found 82% alcohol formation from the hydrochloride.

Preparation of Reference and Intermediate Compounds.—Alkyl methyl sulphides. The methyl *n*- and *iso*-propyl sulphide and *n*-butyl methyl sulphide were prepared from the alkanethiols by addition to sodium ethoxide (1 equiv.) in ethanol. Dimethyl sulphate (0·5 mol.) was added and the mixture boiled under reflux for 30 min. and poured into water. The separated sulphide was fractionated with a 12 in. column. Ingold, Jessop, Kuriyan, and Mandour [22] used methyl iodide.

iso- and *sec.*-Butyl methyl sulphide were prepared from methanethiol, sodium ethoxide (1 equiv.) in ethanol, and the alkyl bromide (1 equiv.), by the same procedure. Vogel and Cowan [23] prepared *iso*butyl methyl sulphide from *iso*butanethiol and dimethyl sulphate. sec.-*Butyl methyl sulphide* boils at 112—113° (Found: C, 57·5; H, 11·5; S, 30·3. $C_5H_{12}S$ requires C, 57·6; H, 11·6; S, 30·8%). The yields of these sulphides were usually 55—65%.

Sulphidimines. The sulphidimines were prepared from the sulphide and saturated chloramine-T and recrystallised to constant m. p. from alcohol–light petroleum (b. p. 60—80°). The following new compounds were prepared: *Methyl* n*-propyl sulphidimine*, m. p. 104—105° (Found: C, 50·9; H, 6·6; N, 5·3. $C_{11}H_{17}O_2NS_2$ requires C, 50·9; H, 6·6; N, 5·4%); *methyl* iso*propyl sulphidimine*, m. p. 116—117° (Found: C, 50·9; H, 6·6; N, 5·1%); iso*butyl methyl sulphidimine*, m. p. 122—123° (Found: C, 53·1; H, 7·1; N, 5·2. $C_{12}H_{19}O_2NS_2$ requires C, 52·7; H, 7·0; N, 5·1%); sec.-*butyl methyl sulphidimine*, m. p. 79—80° (Found: C, 52·6; H, 6·8; N, 5·1%).

Alkylsulphonium iodides (see Table 3). These were usually prepared by mixing the sulphide with methyl iodide in equivalent proportions and leaving them in a closed vessel. When the

[22] Ingold, Jessop, Kuriyan, and Mandour, *J.*, 1933, 533.
[23] Vogel and Cowan, *J.*, 1943, 21.

deposited syrup, frequently discoloured by iodine, did not increase in amount it was separated, washed with dry ether, and recrystallised from dry alcohol–ether. The iodides were deliquescent and were analysed by titration. They were converted into the sulphonium hydroxides in aqueous solution by a slight excess of silver oxide, filtered and used for chromatography.

TABLE 3. *Sulphonium salts*, SRR'R''}X.

No.	R	R	R''	X	M. p.	Solvent
1	Me	Et	Et	I	—	—
2	—	—	—	HgI_3	66—67°	—
3	—	—	—	HgI_4	150	$COMe_2$–Et_2O
4	Me	Pr^n	CH_2Ph	Picrate	96	—
5	—	—	—	*Styphnate*	77	—
6	Me	Pr^n	Pr^n	I	112	—
7	—	—	—	HgI_3	106	—
8	Me	Pr^n	*p*-NO_2·C_6H_4·CH_2	*Picrate*	155	$COMe_2$–EtOH
9	Et	Et	CH_2·Ph	*Picrate*	113	EtOH–ligroin
10	—	—	—	*Styphnate*	129	EtOH–ligroin
11	—	—	*p*-NO_2·C_6H_4·CH_2	*Picrate*	108	$COMe_2$–EtOH
12	Me	Me	Et	I	113	—
13	—	—	—	HgI_3	85	—
14	—	—	—	HgI_4	173	—
15	Me	Me	Pr^n	I	55—60	—
16	—	—	—	HgI_3	72—73	$COMe_2$–Et_2O
17	—	—	—	HgI_4	95	Sat. aq. KI
18	Me	Me	Bu^n	I	—	—
19	Me	Me	Bu^i	I	—	—
20	Me	Me	Bu^s	I	—	—

	Found (%)						Required (%)				
No.	C	H	N	Hg	I	Formula	C	H	N	Hg	I
1	—	—	—	—	54·7	$C_5H_{13}SI$	—	—	—	—	54·7
5	48·2	4·5	9·6	—	—	$C_{17}H_{18}O_8N_2S$	48·0	4·5	9·8	—	—
7	—	—	—	28·4	52·5	$C_7H_{17}SI_3Hg$	—	—	—	28·1	53·3
8	45·0	4·0	12·3	—	—	$C_{17}H_{18}O_9N_4S$	44·9	4·0	12·3	—	—
9	50·1	4·6	10·4	—	—	$C_{17}H_{19}O_7N_2S$	49·9	4·7	10·3	—	—
10	47·9	4·5	9·6	—	—	$C_{17}H_{18}O_8N_2S$	48·0	4·5	9·8	—	—
11	44·5	4·1	12·1	—	—	$C_{17}H_{18}O_9N_4S$	44·9	4·0	12·3	—	—
12	—	—	—	—	58·25	$C_4H_{11}SI$	—	—	—	—	58·2
15	—	—	—	—	54·6	$C_5H_{13}SI$	—	—	—	—	54·7
17	—	—	—	21·7	54·0	$C_{10}H_{26}S_2I_4Hg$	—	—	—	21·8	55·3
18	—	—	—	—	51·6	—	—	—	—	—	51·6
19	—	—	—	—	51·5	$C_6H_{15}SI$	—	—	—	—	51·6
20	—	—	—	—	51·5	—	—	—	—	—	51·6

Methyl *n*-propyl sulphide (3 g.) and *n*-propyl iodide (5·7 g.) were refluxed for 6 hr., a syrup separating. Nitromethane was then added. After some weeks, addition of ether gave a brown syrup. Dissolution in alcohol left white insoluble crystals, m. p. 215° (decomp.). Trimethylsulphonium iodide decomposes at 215°. With dry ether the alcoholic extract gave a syrup which, with aqueous potassium mercuri-iodide, gave a yellow paste. After five recrystallisations from acetone–ether this had m. p. 106°. Methyl di-*n*-propylsulphonium mercuri-iodide, prepared by use of methyl iodide, melted at 106°.

The benzyl- and 4-nitrobenzyl-sulphonium salts were prepared from the sulphide and the bromide in alcohol or, in the case of the diethyl-4-nitrobenzyl compound, without a solvent. Unchanged halide was removed by addition of water and ether, and the aqueous solution precipitated with sodium picrate or styphnate.

Methyl-n-propylacetothetin picrolonate. When methyl *n*-propyl sulphide (3 g.) and bromoacetic acid (4·5 g.) were mixed the temperature rose and a syrup separated; formation of this ceased after 2 days. It was removed and washed with dry ether; dissolution in ethanol and precipitation with dry ether did not effect crystallisation. It was therefore dissolved in water and passed through a column containing Zeo-Karb 225 resin (40—70 mesh; nominally 4·5% cross-linked; H^+ form). After being washed with water the column was developed with glycine (0·2M) until the amino-acid was detected in the effluent by ninhydrin. The effluent was evaporated, the residue extracted with ethanol, and the insoluble glycine removed. Evaporation of the ethanol left the syrupy thetin hydroxide. Picric and chloroplatinic acid gave oily

precipitates. Solid picrolonic acid gave a *picrolonate* which on crystallisation from ethanol–ligroin (b. p. 60—80°) had m. p. 140—141° (Found: C, 46·5; H, 4·8. $C_{16}H_{20}O_7N_4S$ requires C, 46·6; H, 4·9%).

*Methyl-*n*-propyl-α-propiothetin picrolonate.* From methyl *n*-propyl sulphide (3 g.) and α-bromopropionic acid (5·1 g.) a syrup slowly separated. It did not crystallise, so was converted as above into the hydroxide and the *picrolonate*, m. p. 135—136° (Found: C, 48·0; H, 4·9. $C_{17}H_{22}O_7N_4S$ requires C, 48·0; H, 5·0%).

Dimethylpyruvothetin bromide. From dimethyl sulphide (2·0 g.) and bromopyruvic acid (5·6 g.) in dry ether a white solid quickly separated and after 1—2 hr. was washed with dry ether. In a bath preheated to 120° the *bromide* had m. p. 143—144° (decomp.) (Found: Br, 34·7. $C_5H_9O_3SBr$ requires Br, 34·9%).

3-Carboxyallyldimethylsulphonium bromide. γ-Bromocrotonic acid (1·65 g., 0·01 mole) in dry ether (10 c.c.) and dimethyl sulphide (0·62 g., 0·01 mole) were mixed. After a week the colourless needles of the *sulphonium bromide* were washed with dry ether and recrystallised from ethanol–ether (Found: Br, 35·1. $C_6H_{11}O_2SBr$ requires Br, 35·2%).

α-Hydroxydimethyl-γ-butyrothetin and *α-chlorodimethyl-γ-butyrothetin picrolonates.* Methylmethioninesulphonium iodide [24] (5·8 g.) in water (150 c.c.) was shaken with excess of silver chloride, and the mixture filtered. Equal volumes (40 c.c.) of N-sodium nitrite and N-hydrochloric acid were slowly added simultaneously, with shaking. The mixture was then heated to 60° and left at room temperature for 2 hr., neutralised with silver oxide, filtered, and passed through a column of Zeo-Karb 225 (4·5% cross-linked; H^+ form; 6 g.). The column was developed with 0·2N-sodium hydroxide, and the effluent collected as fourteen fractions of 10 c.c. each. By chromatography of each fraction on No. 54 Whatman paper with bromophenol-blue (see p. 42) two bases were recognised; that of higher R_F value (*a*) in fractions 4—7 and the second (*b*) in fractions 6—14. Fractions 13 and 14 contained traces of methylmethioninesulphonium hydroxide and number 14 contained sodium. Glycine (1 g.) was added to fractions 6—7 and the solution refractionated on Zeo-Karb 225 as before. Of the 13 fractions of 10 c.c. which were obtained, numbers 2—10 contained glycine and base (*a*), number 11 contained glycine, base (*b*), and sodium, and number (8) only sodium. Fractions 2—9 were combined, and evaporated under reduced pressure, and the dry residue was extracted with ethanol, leaving glycine. The extract was combined with fractions 4—5 from the first fractionation. The resulting solution, free from base (*b*), contained halogen. Evaporation *in vacuo*, treatment with picrolonic acid as before, and crystallisation from alcohol–ligroin gave a *picrolonate*, m. p. 139—140° (Found: C, 43·2; H, 4·4; N, 12·6; Cl, 8·3. $C_{16}H_{19}O_7NSCl$ requires C, 43·0; H, 4·3; N, 12·5; Cl, 7·9%).

Fractions 8—13 from the first fractionation containing base (*b*) were halogen-free. Treatment as before gave a *picrolonate*, m. p. 147—148° (Found: C, 45·2; H, 4·7; N, 13·3. $C_{16}H_{20}O_8N_4S$ requires C, 44·9; H, 4·7; N, 13·1%).

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[24] Toennies and Kolb, *J. Amer. Chem. Soc.*, 1945, **67**, 849; Atkinson and Poppelsdorf, *J.*, 1951, 1378.