375. *The Fission of the Thioether Linkage in Certain β-Sulphonyl-sulphides.*

By A. H. Ford-Moore, R. A. Peters, and R. W. Wakelin.

Di-[2-(2′-amino-2′-carboxyethylthio)ethyl] sulphone (II) and *S*-2′-phenylsulphonylethyl-cysteine (I) undergo fission of the thioether group on treatment with silver salts at pH 7·8—8. Cysteine is formed in both cases. With (I), phenyl vinyl sulphone has also been identified.

s.-2′-Phenylsulphonylethylthioacetic acid (VI) also undergoes fission under similar conditions, but *o*-2-phenylsulphonylethylthiobenzoic acid (V) does not.

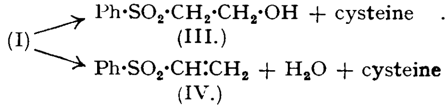
Divinyl sulphone, first prepared by (the late) E. Walker (Ministry of Supply Report, 1926; *J.*, 1931, 1914), is a very reactive substance, and Alexander and McCombie (*J.*, 1931, 1913) have shown that compounds containing hydroxy-, mercapto-, or amino-groups react with it very readily, a hydrogen atom adding to the α-carbon atom and the rest of the molecule to the β-carbon atom. Reaction with a mercapto-group is particularly rapid, and, when divinyl sulphone reacts with a substance such as cysteine, that contains both mercapto- and amino-groups, combination occurs predominantly with the former. This reactivity extends to those compounds, like *phenyl vinyl sulphone* (IV), that only contain one vinyl group.

It has now been shown that the reaction between thiols and compounds containing the group

$\cdot SO_2\cdot CH{:}CH_2$ is, under conditions approximating to the biological, reversible. Peters and Wakelin (Ministry of Supply Report, No. 39, 1941; *Biochem. J.*, 1947, **41**, 555) have found that the thioether linkage in the *product* (II) of reaction of cysteine with divinyl sulphone and in that (I) from cysteine and phenyl vinyl sulphone can be split by treatment, at slightly alkaline pH, with silver salts, since the cysteine fragment from (II) can be isolated as its oxidation product,

$$\underset{\text{(I.)}}{\mathrm{Ph{\cdot}SO_2{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H}} \qquad \underset{\text{(II.)}}{\mathrm{SO_2[CH_2{\cdot}CH_2{\cdot}S{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H]_2}}$$

cystine. The sulphone fragment from (II) does not readily lend itself to isolation and characterisation; so in order to simplify the problem, attention was turned to a compound such as (I), which only contains one cysteinyl group and which also contains a readily identified sulphone fragment. On fission, (I) should give cysteine (or its oxidation product, cystine) and either phenyl 2-hydroxyethyl sulphone (III) or phenyl vinyl sulphone (IV):



$$\text{(I)} \longrightarrow \underset{\text{(III.)}}{\mathrm{Ph{\cdot}SO_2{\cdot}CH_2{\cdot}CH_2{\cdot}OH}} + \text{cysteine} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad (a)$$

$$\text{(I)} \longrightarrow \underset{\text{(IV.)}}{\mathrm{Ph{\cdot}SO_2{\cdot}CH{:}CH_2}} + \mathrm{H_2O} + \text{cysteine} \quad . \; . \; . \; . \; . \; . \; . \quad (b)$$

For identification of the sulphone fragment, derivatives of (III) and (IV) have been prepared. (III) can be characterised as its p-*nitrobenzoate*. (IV) is itself a crystalline solid but can be further characterised as its p-*tolylthio*-derivative by treatment with *p*-thiocresol.

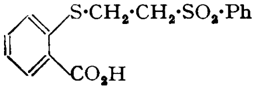
(I) was prepared from thiophenol as follows:

$$\mathrm{Ph{\cdot}SH} \xrightarrow[\mathrm{NaOH}]{\mathrm{Cl{\cdot}[CH_2]_2{\cdot}OH}} \underset{\text{(VII.)}}{\mathrm{Ph{\cdot}S{\cdot}CH_2{\cdot}CH_2{\cdot}OH}} \xrightarrow{\mathrm{SOCl_2}} \underset{\text{(VIII.)}}{\mathrm{Ph{\cdot}S{\cdot}CH_2{\cdot}CH_2Cl}} \xrightarrow{\mathrm{H_2O_2}} \underset{\text{(IX.)}}{\mathrm{Ph{\cdot}SO_2{\cdot}CH_2{\cdot}CH_2Cl}}$$

$$\xrightarrow{\mathrm{NEt_3}} \text{(IV)} \xrightarrow[\mathrm{NaHCO_3}]{\text{Cysteine}} \text{(I)}$$

On treatment with silver salts at the appropriate pH, (I) breaks down according to scheme (*b*), the cysteine appearing as cystine and the sulphone fragment as (IV).

In an attempt to obtain a more rigid proof of the course of the reaction, the condensation product of (IV) with *o*-mercaptobenzoic acid, namely o-2-*phenylsulphonylethylthiobenzoic acid* (V), was prepared. It was thought that, if this compound could be split on "silver treatment," the thio-fragment would appear either as *o*-mercaptobenzoic acid or as its oxidation product, the corresponding disulphide; both these two compounds are crystalline solids of definite melting point. Unfortunately, (V) did not respond to "silver treatment." This is in all probability due to the fact that fission is preceded by the formation of a silver complex at the thioether atom. (V) is less likely to form such a complex than is a purely alkyl sulphide. That fission is not due to the presence of an amino-group was demonstrated by preparing 2-*phenylsulphonylethylthioacetic acid* (VI) and showing that this compound undergoes fission in a similar manner to (I) and (II), since it gives the nitroprusside reaction on "silver treatment."



(V.)

$$\underset{\text{(VI.)}}{\mathrm{Ph{\cdot}SO_2{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}CH_2{\cdot}CO_2H}}$$

EXPERIMENTAL.

Phenyl 2-*hydroxyethyl sulphide* (VII) was prepared from ethylene chlorohydrin (41 g., 33 c.c.) and a solution of thiophenol (55 g.) in 20% aqueous sodium hydroxide (120 c.c.), the reaction being completed by heating on a steam-bath for 1 hour. After cooling, the mixture was extracted with chloroform, the chloroform layer separated, and the solvent distilled off. The residue was distilled and a fraction collected at 135°/8 mm. (141°/11 mm.) (Nenitzescu and Scarlatescu, *Ber.*, 1935, *B*, **68**, 587, give b. p. 119—120°/4 mm.) (yield, 61·5 g., 80%); the product had d_{15}^{24} 1·1145, n_D^{22} 1·5870, and was characterised as p-*nitrobenzoate*, m. p. 57—58° (from methanol) (Found: C, 59·6; H, 4·2. $C_{15}H_{13}O_4NS$ requires C, 59·4; H, 4·3%).

Phenyl 2-*chloroethyl sulphide* (VIII), prepared from thionyl chloride (45 c.c.) and (VII) (55 g.) in dry chloroform (150 c.c.), boiled at 107°/7 mm. (123°/14 mm.) (yield, 56 g.) and had $d_{15}^{23\cdot5}$ 1·1820 (Dawson, *J. Amer. Chem. Soc.*, 1933, **55**, 2070, gives b. p. 88—89°/0·637 mm., *d* 1·1769) (Found: Cl, 20·85. Calc. for C_8H_9ClS: Cl, 20·5%). Although not so active as mustard gas, this substance is strongly vesicant, and care should be exercised in handling it; contaminated apparatus may be rendered harmless by treatment with concentrated nitric acid.

Phenyl 2-chloroethyl sulphone (IX) was prepared by oxidising (VIII) (8·5 g.) with 30% hydrogen peroxide (15 c.c.) in glacial acetic acid (35 c.c.), the reaction being completed by boiling the mixture gently under reflux for 0·5 hour. The product was isolated by pouring the mixture into water and purified by crystallising from ethanol (yield, pure, 8 g., 78·5%); m. p. 55° (Kretov and Toropova, *J. Gen. Chem. U.S.S.R.*, 1937, **7**, 2009, give m. p. 52°) (Found : Cl, 17·6. Calc. for $C_8H_9O_2ClS$: Cl, 17·3%).

Phenyl 2-hydroxyethyl sulphone (III) was prepared by oxidising (VII) with a slight excess of 30% hydrogen peroxide in acetic acid. It did not solidify but distilled without decomposition at 177°/2 mm. and had n_D^{11} 1·5573. It was characterised as its p-*nitrobenzoate*, m. p. 151—152° (from acetic acid) (Found : C, 53·4; H, 3·9. $C_{15}H_{13}O_6NS$ requires C, 53·7; H, 3·9%). The same ester was obtained by the oxidation of the *p*-nitrobenzoate of (VII) with hydrogen peroxide in acetic acid.

Phenyl vinyl sulphone (IV) was prepared by treating (IX) (10 g.) in benzene (100 c.c.) with triethylamine (5 g., 7 c.c.) and setting the mixture aside overnight. After the triethylamine hydrochloride had been removed, the benzene was distilled off and the residue treated with light petroleum (b. p. 40—60°). The product solidified and was recrystallised from benzene–light petroleum (b. p. 40—60°). The yield of pure product melting at 68·5° was 6·5 g. (79%) (Found : C, 57·1; H, 5·0. $C_8H_8O_2S$ requires C, 57·1; H, 4·8%).

p-*Tolyl 2-Phenylsulphonylethyl Sulphide*.—(*a*) Thio-*p*-cresol (1·5 g.) and (IV) (2 g.) were warmed with a trace of triethylamine. Heat was evolved and, on cooling, the *sulphide* solidified. It was crystallised from ethanol and melted at 95—96° (Found : C, 61·3; H, 5·2. $C_{15}H_{16}O_2S_2$ requires C, 61·6; H, 5·5%).

(*b*) Thio-*p*-cresol (3 g.), dissolved in a solution of sodium (0·8 g.) in ethanol (30 c.c.), was treated with (IX) (5 g.), and the mixture heated on a steam-bath for 1 hour. The mixture was filtered hot; when cooled, the filtrate deposited crystals of the sulphide, which after recrystallisation from ethanol melted at 95—96°, not altered on admixture with the product from (*a*).

S-2-*Phenylsulphonylethylcysteine* (I).—Cysteine hydrochloride (1·5 g.) was added to (IV) (1·7 g.) in 5% aqueous sodium hydrogen carbonate (25 c.c.) at 40°. The mixture was heated on a steam-bath for 2 hours and then cooled, and any unchanged (IV) extracted with chloroform. The *cysteine* derivative (2·5 g., 86·5%) separated when the solution was kept. It was recrystallised from water and melted with decomposition at 200—203° (Found : C, 45·3; H, 5·2. $C_{11}H_{15}O_4NS_2$ requires C, 45·6; H, 5·2%).

o-2-*Phenylsulphonylethylthiobenzoic Acid* (V).—*o*-Mercaptobenzoic acid (3·7 g.), (IV) (4 g.), benzene (30 c.c.), and a trace of triethylamine were heated under reflux for 0·5 hour. When it had cooled, the benzene solution was extracted with aqueous sodium carbonate, and the latter separated and acidified with hydrochloric acid. The separated *acid* was filtered off and crystallised from ethanol; m. p. 203° (Found : C, 56·3; H, 4·7. $C_{15}H_{14}O_4S_2$ requires C, 56·7; H, 4·7%).

2-*Phenylsulphonylethylthioacetic Acid* (VI).—Equimolar parts of (IV) and 90% thioglycollic acid were heated on a steam-bath for 4 hours with a trace of triethylamine. The product was treated with an aqueous suspension of barium carbonate and filtered. The filtrate, on acidification with hydrochloric acid, deposited an oily *acid* which rapidly solidified. It melted at 84° after crystallisation from benzene (Found : C, 46·3; H, 4·75. $C_{10}H_{12}O_4S_2$ requires C, 46·45; H, 4·5%).

Divinyl Sulphone.—2 : 2′-Dichlorodiethyl sulphone (100 g.; m. p. 57°; cf. Helfrich and Reid, *J. Amer. Chem. Soc.*, 1920, **42**, 1208), precipitated calcium carbonate (55 g.) and water (500 c.c.) were heated in a three-necked flask equipped with a sealed stirrer, a thermometer (bulb in liquid), and a reflux condenser, and set in a water-bath. After the mixture had been heated at 85—90° for about 4 hours, the reaction was complete. Some polymeric material was filtered off through glass wool, and the filtrate extracted with benzene in a continuous extractor for 2 hours. The extract was dried (anhydrous $MgSO_4$), and the benzene distilled off. The residue was distilled, a fraction being collected at 123—125°/18 mm. (98—100°/7 mm.) (Alexander and McCombie, *loc. cit.*, give b. p. 120—121°/18 mm.; their method is less convenient than ours for large quantities. Cf. also Levin, *J. pr. Chem.*, 1930, **127**, 77). The yield was 47—53 g. (79—85%). When cooled in " dry ice " and acetone, the sulphone froze at −16°.

The crude sulphone, obtained by oxidising an appropriate amount of mustard gas with hydrogen peroxide (2·5 moles) in acetic acid, may be used with practically no diminution in yield. Sodium hydrogen carbonate may be employed in place of calcium carbonate but, in this case the reaction temperature must be kept between 40° and 45° during the hydrolysis and any free alkali at the end of the reaction neutralised with hydrochloric acid before benzene extraction. The material may be stabilised by the addition of triethylamine (0·05%) and storage in a cool, dark place.

Di-[2-(2′-*amino*-2′-*carboxyethylthio*)*ethyl*] *Sulphone* (II).—A solution of divinyl sulphone in excess of 5% aqueous sodium hydrogen carbonate at 38° was treated with two molar equivalents of cysteine hydrochloride. Precipitation of the *biscysteine* derivative started almost at once and was complete in about 5 minutes. It was filtered off and crystallised from water (in which it was rather sparingly soluble) in minute, hair-like needles, melting with decomposition at 251—252° (Found: C, 33·2; H, 5·7. $C_{10}H_{20}O_6N_2S_3$ requires C, 33·3; H, 5·6%).

Divinyl sulphone can be conveniently determined by precipitation as the above compound. An aqueous solution containing about 0·15 g. of the sulphone is treated with 50 c.c. of 3% sodium hydrogen carbonate solution, and cysteine hydrochloride (not less than 0·4 g. per 0·15 g. of sulphone) in a little water is added. After 15 minutes the precipitate is collected on a weighed Gooch crucible, washed with a little ice-water, dried at 100°, and weighed. 0·234 Mg. is added to this weight for each c.c. of filtrate plus washings. 1 G. of condensation product corresponds to 118/360 g. of divinyl sulphone.

Fission of (II) *on Silver Treatment*.—500 Mg. of (II) were treated with 10 c.c. of 10% silver nitrate and adjusted to pH 3·0 with 4N-hydrochloric acid. After the mixture had been centrifuged, the filtrate still contained unchanged (II) [strong nitroprusside reaction on addition of silver nitrate (2 c.c.) and subsequently sodium hydroxide]. After the mixture had been re-adjusted to pH 3·0, the combined precipitates were washed with dilute hydrochloric acid and extracted with a solution of sodium iodide (2 g.) with the addition of hydrochloric acid to pH 3·0. Nitroprusside reactions on the centrifugate indicated the liberation of cysteine. The combined cysteine-containing extracts were evaporated to dryness *in vacuo* with the addition of acid to pH 3·0. Aqueous ammonia (*d* 0·880; 0·4 c.c.) was added and, after shaking, the whole was allowed to oxidise at room temperature for 24 hours. The remaining ammonia was removed *in vacuo*, and the residue dissolved in dilute hydrochloric acid, the small amount of insoluble material being removed by micro-filtration. The cystine was precipitated in crude form by adjusting the pH to 4·0 in successive fractions. The first fractions required redissolution in 0·1N-hydrochloric acid with slight warming and further filtration before typical hexagonal plates of cystine (26 mg.) could be obtained. Fission was not complete, as *ca.* 300 mg. of unchanged (II) were recovered from the filtrate from the silver precipitate.

Isolation of (IV) *from the Silver Treatment of* (I).—(I) (200 mg.) was treated as described for (II). The aqueous filtrate at pH 3·0 from the silver–cysteine complex after alkali treatment was evaporated to dryness *in vacuo* at $<50°$, traces of sodium hydroxide being added during the concentration to prevent a fall in pH. The residue was extracted with chloroform, and the latter removed *in vacuo*, leaving an oil that slowly crystallised. On re-extraction and centrifugation, 62 mg. of crystals, m. p. 66·5° [67° on admixture with an authentic specimen of (IV)], were obtained.

The identity of the sulphone fragment was confirmed by converting 45 mg. of the recovered compound into its *p*-tolylthio-derivative by warming with *p*-thiocresol (37 mg.) and a trace of triethylamine. The product, after crystallisation from light petroleum and then from ethanol, melted at 95°, not altered on admixture with an authentic specimen.

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DEPARTMENT OF BIOCHEMISTRY, OXFORD.
CHEMICAL DEFENCE EXPERIMENTAL ESTABLISHMENT,
PORTON, WILTS.

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