319. *Oxidation of Organic Sulphides. Part XI.*[1] *The Synthesis of Specifically Labelled Phenyl* [^{35}S]*Benzenethiolsulphinate and Phenyl Benzenethiol*[^{35}S]*sulphonate.*

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A simple method is described which gives high yields of phenyl [^{35}S]benzenethiolsulphinate, labelled at either of the two possible positions, from [^{35}S]thiophenol. Reaction of these products with 1-chloro-2,4-dinitrobenzene is shown to give 2,4-dinitrophenyl phenyl sulphoxide, which can be used to assess the specificity of the radio-labelling. The synthesis of phenyl benzenethiol[^{35}S]sulphonate from [^{35}S]thiophenol is also described.

Since the identification of the antibacterial principle of garlic (*Allium sativium*) as allyl propene-2-thiolsulphinate,[2] interest in thiolsulphinates has been largely concentrated on their biochemistry, *e.g.*, bacteriostatic powers,[2] enzyme inactivation,[3] and possible anti-tumour properties.[4] More recently the importance of thiolsulphinates as the primary oxidation product of disulphides has been recognised in three separate fields: in photosynthesis, as the oxidised form of 6-thioctic acid; [5] as the unstable intermediates leading to S–S bond scission in the conversion of disulphides into thiolsulphonates; [6] and as retarders of free-radical chain processes such as autoxidation and vinyl addition polymerisation.[7]

For the more detailed study of the S–S bond scission and retardation reactions, examples of a thiolsulphinate specifically labelled at each of the two sulphur atoms were required. Phenyl benzenethiolsulphinate was chosen as a well-characterised and reasonably stable model with the required order of reactivity. Of the various methods of synthesis of thiolsulphinates,[8,9] only the condensation of a thiol and sulphinyl chloride appeared able to give the unsymmetrical labelling required.

$$\mathrm{Ph{\cdot}SH + Ph{\cdot}{}^{35}SO{\cdot}Cl \longrightarrow Ph{\cdot}{}^{35}SO{\cdot}SPh} \quad \text{(I)}$$

$$\mathrm{Ph{\cdot}SOCl + Ph{\cdot}{}^{35}SH \longrightarrow Ph{\cdot}SO{\cdot}{}^{35}SPh} \quad \text{(II)}$$

By a slight adaptation of Douglass and Farah's elegant synthesis of alkanesulphinyl chlorides,[10] thiophenol was converted quantitatively into benzenesulphinyl chloride, on the 10-mmole scale, in a one-step reaction suitable for radioactive materials. Thus [^{35}S]thiophenol could be used as the starting material for both compounds (I) and (II). Condensation of radioactive thiophenol with inactive benzenesulphinyl chloride, and *vice versa*, was carried out in the vessel used for the synthesis of the latter, giving an overall yield of 85% (after recrystallisation) of the appropriately labelled phenyl benzenethiolsulphinate. Elemental and functional-group analyses [11] indicated a chemical purity of 100 ± 1%; the radiochemical purity, by isotope dilution analysis, was 100 ± 2%.

Specificity of Labelling.—To prove that the radioactive label was solely in the intended position, a reaction was required which, by splitting the S–S bond of the thiolsulphinate, would give a product containing specifically either the sulphenyl- or the sulphinyl-sulphur.

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[1] Part X, Barnard, Bateman, Cain, Colclough, and Cunneen, *J.*, 1961, 5339.

[2] Cavallito and Bailey, *J. Amer. Chem. Soc.*, 1944, **66**, 1950.

[3] Willis, *Biochem. J.*, 1956, **63**, 514.

[4] Weisberger and Pensky, *Science*, 1957, **126**, 1112.

[5] Barltrop, Hayes, and Calvin, *J. Amer. Chem. Soc.*, 1954, **76**, 4348.

[6] Barnard and Percy, *Chem. and Ind.*, 1960, 1332.

[7] Barnard, Bateman, Cole, and Cunneen, *Chem. and Ind.*, 1958, 918.

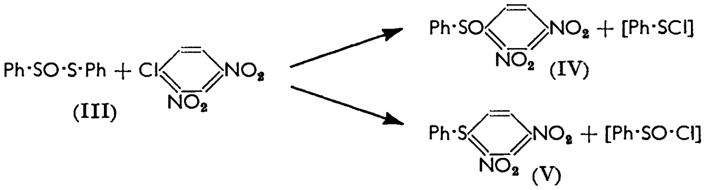
[8] Small, Bailey, and Cavallito, *J. Amer. Chem. Soc.*, 1947, **69**, 1710; Stoll and Seebeck, *Helv. Chim. Acta*, 1949, **32**, 866; Backer and Kloosterziel, *Rec. Trav. chim.*, 1954, **73**, 129; Colclough and Cunneen, *Chem. and Ind.*, 1960, 626.

[9] Vinkler and Klivenyi, *Acta Chim. Acad. Sci. Hung.*, 1957, **11**, 15.

[10] Douglass and Farah, *J. Org. Chem.*, 1958, **23**, 330.

[11] Barnard and Cole, *Analyt. Chim. Acta*, 1959, **20**, 540.

Thioalkylation of a thiol to give disulphide was not suitable since the sulphenic acid concomitantly formed also reacts with the thiol to give the same disulphide.[12] Reaction with 1-chloro-2,4-dinitrobenzene, with which Leandri *et al.*[13] had cleaved aryl thiolsulphonates to aryl 2,4-dinitrophenyl sulphones, gave 2,4-dinitrophenyl phenyl sulphoxide in 50% yield, with a 30% yield of the corresponding sulphide. Since the sulphoxide can only contain the sulphinyl sulphur of the thiolsulphinate from which it is derived, and is readily isolated and purified, its specific activity should provide an accurate measure of the [^{35}S] in that position. The same argument does not apply to the sulphide since disproportionation of the thiolsulphinate, which is quite likely under the reaction conditions, has been shown to lead to diphenyl disulphide,[14] and hence to 2,4-dinitrophenyl phenyl sulphide,[15] whose sulphur is not solely derived from the sulphenyl sulphur of the thiolsulphinate.



The results in Table 1 indicate a slight lack of specificity of labelling which would not be expected from the method of synthesis. The labelling was stable for a long time and was not randomised in acetic acid solution, or by shaking the thiolsulphinate with dilute mineral acid, with which limited contact is made during the synthesis. Therefore, an acid-catalysed rearrangement can be discounted. There is a definite possibility that the apparent unspecificity is an artefact. Thus, (*a*) a small amount of sulphide might be oxidised to sulphoxide, by thiolsulphinate for example; and (*b*) the sulphenyl chloride, which accompanies sulphoxide formation, is known, under alkaline conditions, to give rise to thiolsulphinate[9] which, in turn, would give sulphoxide having the activity of the sulphenyl sulphur of the original thiolsulphinate.

TABLE 1.

The distribution of activity in compounds (I) and (II).

Thiolsulphinate (III)	Specific activity of sulphur in (III) (c./min. × 10^{-5}) *	Specific activity of sulphur in (IV) (c./min. × 10^{-5}) *		Activity in sulphinyl position (%)
		Calc.	Found	
Ph·^{35}SO·S·Ph	4·84	9·66	9·47	98·1
Ph·SO·^{35}S·Ph	4·84	0	0·107	1·1

* Counted as an " infinitely thick " precipitate of benzidine sulphate.

In any case, the percentage of the radioactivity apparently in the wrong position was regarded as acceptable since it was little greater than the counting errors expected in the subsequent applications of the thiolsulphinate.

Cleavage of Thiolsulphinates by 1-*Chloro*-2,4-*dinitrobenzene*.—Leandri *et al.*[13] conclude that, in the reaction of thiolsulphonates with 1-chloro-2,4-dinitrobenzene, the nucleophilicity of the sulphonyl-sulphur is greater than that of the sulphenyl-sulphur. In the case of thiolsulphinates, the formation of sulphoxide as the major product argues that the sulphinyl-sulphur similarly has the greater nucleophilicity, although the sulphide found suggests that the 1-chloro-2,4-dinitrobenzene also attacks the sulphenyl-sulphur. The sulphide could, however, arise from diphenyl disulphide formed either by

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[12] Schöberl and Gräfje, *Annalen*, 1958, **617**, 71.
[13] Leandri and Tundo, *Ann. Chim.* (*Italy*), 1954, **44**, 264.
[14] Barnard, *J.*, 1957, 4675.
[15] Leandri and Tundo, *Ann. Chim.* (*Italy*), 1954, **44**, 330.

disproportionation of the thiolsulphinate or as one of the products of hydrolysis of the postulated sulphenyl chloride.

Since the completion of this work, Vinkler and Klivenyi[16] have reported the reaction of 1-chloro-2,4-dinitrobenzene with phenyl benzene-, *p*-tolyl toluene-*p*-, and *p*-chlorophenyl *p*-chlorobenzene-thiolsulphinate. They claim that, in each case, the product is the appropriate aryl 2,4-dinitrophenyl sulphide, isolated in yields of 72%, 85%, and 96%, respectively. We have repeated the cleavage of phenyl benzenethiolsulphinate under their experimental conditions, which differ from ours in the use of equimolar quantities of reactants in a solvent containing more water. This results in a heterogeneous system and, possibly, a slightly higher reaction temperature. In our hands the sulphoxide was still the major product (yield 45%), and only a small amount of sulphide (16%) could be isolated. The degradation of *p*-chlorophenyl *p*-chlorobenzenethiolsulphinate under the same conditions gave the sulphide (62%) together with sulphone (26%) and sulphoxide (8%). It was found that, in the absence of 1-chloro-2,4-dinitrobenzene, this thiolsulphinate very rapidly disproportionates into disulphide (56%) and thiolsulphonate (33%) under the given experimental conditions, and it appears likely that these products are the source of the observed high yields of sulphide and sulphone when the reagent is present. Under the conditions used in the present work the last thiolsulphinate gave sulphoxide (39%) and smaller amounts of sulphide and sulphone. Vinkler and Klivenyi's suggestion that the electrons of the S–S bond of the thiolsulphinate are acquired solely by the sulphenyl-sulphur in order to receive the 2,4-dinitrophenylium ion must therefore be refuted.

The Synthesis of a Specifically Labelled Phenyl Benzenethiolsulphonate.—[^{35}S]Thiophenol was converted into the sulphinyl chloride and thence, by hydrolysis with the theoretical amount of water, into benzene[^{35}S]sulphinic acid. Condensation of the latter with an equivalent of inactive benzenesulphenyl chloride, according to the method of Douglass and Farah,[17] gave the thiolsulphonate, $\mathrm{Ph{\cdot}{}^{35}SO_2{\cdot}SPh}$, in a yield of 70% after recrystallisation.

The position of the label was confirmed by degradation of the product with equivalent amounts of thiophenol and sodium hydroxide to give quantitative yields of diphenyl disulphide and sodium benzenesulphinate.[11] The specific activity of the disulphide, after recrystallisation to constant activity, indicated that 99% of the total activity had been located at the sulphonyl position (see Table 2).

TABLE 2.

The distribution of radioactivity in $\mathrm{Ph{\cdot}{}^{35}SO_2{\cdot}SPh}$.

Specific activity of the sulphur of the thiolsulphonate (10^3 c./min.)	Specific activity of the sulphur of the derived disulphide (c./min. × 10^{-3})	Activity in sulphonyl position (%)
8·35	0·105	98·7

EXPERIMENTAL

Synthesis of Phenyl Benzene[^{35}S]*thiolsulphinate* (I).—Chlorine was slowly bubbled into a solution of thiophenol (1·0 g.) and acetic acid (0·55 g.) in dry methylene chloride (10 ml.) at 0° until the initial deep orange colour had faded to a pale yellow (*ca.* 1 hr.). The solvent was removed *in vacuo* and the residue dissolved in dry ether (10 ml.). Trial experiments showed that the yield of pure benzenesulphinyl chloride obtained in this way was 99—100%. [^{35}S]-Thiophenol (0·99 g., 0·44 mc/mmole), purchased from The Radiochemical Centre, Amersham, and pyridine (0·71 g.) in dry ether (5 ml.) were added slowly, with stirring, to the solution of sulphinyl chloride at −20°. The ethereal solution of the resulting thiolsulphinate, containing suspended pyridine hydrochloride, was washed successively with water (10 ml.), N-sulphuric acid (10 ml.), and water (2 × 10 ml.), and dried ($\mathrm{CaCl_2}$). After filtration into a pear-shaped flask equipped with a sintered-glass, side-arm filter, the ether was removed *in vacuo* and the

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[16] Vinkler and Klivenyi, *Acta Chim. Acad. Sci. Hung.*, 1960, **22**, 346.

[17] Douglass and Farah, *J. Org. Chem.*, 1958, **24**, 973.

product recrystallised twice by dissolving it in warm chloroform (1 ml.) and then adding light petroleum (30 ml.; b. p. 40—60°). White needles were thus obtained which changed, on equilibration with the mother-liquor, to the more usual yellow prisms. The product (1·72 g., 86%) was dried *in vacuo* for not more than 30 min. in the dark to avoid disproportionation.[14] The purity was checked by the determination of thiolsulphinate content [11] (39·37 mg., equiv. to 16·78 ml. of 0·0199N-thiosulphate; 99·3%) and by a standard microcombustion (Found: S, 27·4. Calc. for $C_{12}H_{10}OS_2$: S, 27·4%). The sulphate ion from the combustion was suitably diluted with inactive potassium sulphate and assayed for radioactivity as an " infinitely thick " layer of benzidine sulphate in conditions and with equipment previously described.[18] The corrected count rate was 4·84 × 10^5 c./min. A sample of the thiolsulphinate (22·914 mg.) was diluted with highly pure, inactive material (1·0197 g.), and the mixture recrystallised twice from chloroform–light petroleum (b. p. 40—60°) and twice from methanol–light petroleum. The count rate of the product, assayed as above and corrected for dilution, was 4·87 × 10^5 c./min.

The absolute specific activity of the original thiolsulphinate was 1·845 × 10^6 c. $sec.^{-1}$ $g.^{-1}$ as determined by scintillation counting, with [^{14}C]toluene of known specific activity as internal standard.

Phenyl benzenethiol[^{35}S]*sulphinate* (II) was prepared in an identical manner, except that [^{35}S]thiophenol (0·99 g.) was used to make the benzenesulphinyl chloride, which was then condensed with unlabelled thiophenol. The product, obtained in 85% yield, had a corrected count rate (as benzidine sulphate) of 4·84 × 10^5 c./min. (Found: S, 27·3%).

Reaction of Thiolsulphinates with 1-Chloro-2,4-dinitrobenzene.—The procedure adopted is typified by the following example. Phenyl benzenethiolsulphinate (0·492 g.) was dissolved in ethanol (3 ml.), and 1-chloro-2,4-dinitrobenzene (0·880 g.) in ethanol (4 ml.) added, followed by sodium hydrogen carbonate (0·360 g.) and water (0·7 ml.). The mixture was refluxed for 55 min. and then concentrated by allowing ethanol (3—4 ml.) to distil off. Benzene (15 ml.) was added, and the solution washed with water (3 × 20 ml.), diluted with light petroleum (15 ml.; b. p. 40—60°), and run through a column of silica gel (15 g.; 100—200 mesh). The column was washed with 1 : 1 benzene–light petroleum (50 ml.), followed by benzene (30 ml.), to obtain eluate A, and then with chloroform (90 ml.; eluate B). The latter, on evaporation, left *2,4-dinitrophenyl phenyl sulphoxide* (0·37 g.), an oil which crystallised from ethanol as yellow crystals (0·31 g.), m. p. 104—107°. The m. p., and mixed m. p., after a further recrystallisation was 106·5—107° (Found: C, 49·2; H, 2·8; S, 11·0. $C_{12}H_8O_5N_2S$ requires C, 49·3; H, 2·8; S, 11·0%).

The authentic sulphoxide, prepared by the reaction of peracetic acid with 2,4-dinitrophenyl phenyl sulphide, existed in two forms, m. p. 106·5—107° and 114—115°. Each form was converted into the other by crystallisation from ethanol in the presence of the appropriate " seed " crystal. Their infrared spectra were identical in solution (CS_2) but differed in the solid state (KBr disc).

When the position of labelling in a thiolsulphinate was to be assessed, the sulphoxide from eluate B was recrystallised to constant specific activity and assayed in the form of benzidine sulphate. The sulphoxide was found to be a powerful quenching agent in scintillation counting.

Evaporation of eluate A left a yellow oil from which 2,4-dinitrophenyl phenyl sulphide of m. p. and mixed m. p. 118—119° was isolated by repeated fractional crystallisation from ethanol. As losses were obviously high, the yield of sulphide was estimated by an isotopic dilution method. Phenyl benzene[^{35}S]thiolsulphinate (I) (0·565 g.; 4·84 × 10^5 c./min. as benzidine sulphate) was treated with 1-chloro-2,4-dinitrobenzene as above. The residue from eluate A was diluted with pure 2,4-dinitrophenyl phenyl sulphide (0·9875 g.), equilibrated in benzene solution, rechromatographed on silica gel, and recrystallised 4 times from ethanol and twice from benzene–light petroleum (b. p. 40—60°) until of constant specific activity (1·54 × 10^5 c./min. as benzidine sulphate). On the assumption that the sulphide would be derived only from the sulphenyl-sulphur of the thiolsulphinate, the yield was calculated to be 0·187 g. (28%).

Synthesis of Phenyl Benzene[^{35}S]*thiolsulphonate.*—[^{35}S]Thiophenol (2·00 g., 7·5 mc/mole), dissolved in methylene chloride (10 ml.) and acetic acid (1·10 g.), was chlorinated to benzenesulphinyl chloride as before. This was dissolved in methylene chloride and treated with water (0·327 g.) to produce benzene[^{35}S]sulphinic acid. Pyridine (2·88 g.) was added and a solution of

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[18] Ayrey. Barnard, and Moore, *J.*, 1953, 3179.

benzenesulphenyl chloride, prepared by treating diphenyl disulphide (4·0 g.) in carbon tetrachloride (20 ml.) with chlorine (0·64 g.) at 0°, was added slowly with stirring at 0°. After the mixture had been washed with water (3 × 20 ml.) and dried ($MgSO_4$), the solvent was removed, and the residue dissolved in carbon tetrachloride (50 ml.) and absorbed on silica gel (70 g.; 100—200 mesh). Elution with carbon tetrachloride (150 ml.) removed the excess of diphenyl disulphide (1·9 g.). The thiolsulphonate was eluted with chloroform (200 ml.) and thrice recrystallised from ethanol–light petroleum (b. p. 60—80°) to give white crystals (3·24 g., 70%) (Found: S, 29·1. Calc. for $C_{12}H_{10}O_2S_2$: S, 29·3%). Its purity as thiolsulphonate [11] was 99·8% and its specific activity (counted as an infinitely thick layer of benzidine sulphate) $8{\cdot}35 \times 10^3$ c./min.

Location of Label in Phenyl Benzene[^{35}S]*thiolsulphonate.*—A sample (0·43 g.) of the thiolsulphonate prepared above was dissolved in acid-free ethanol (25 ml.) and treated with thiophenol (0·193 g.). The solution required 3·63 ml. of 0·486N-sodium hydroxide for neutrality to Bromophenol Blue. Diphenyl disulphide (0·38 g.) was precipitated by the addition of water, chromatographed on silica gel in light petroleum (b. p. 40—60°) solution and recrystallised twice from ethanol to constant specific activity ($1{\cdot}05 \times 10^2$ c./min. as benzidine sulphate).

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