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Synthesis of phenyl-^{3}H-*sec*-butyl-^{35}S$_{1}$-disulphide

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SUMMARY

*Phenyl-^{3}H-*sec*-butyl disulphide was synthesized via Wilzbach irradiated phenyl benzenethiolsulphonate and phenyl-*sec*-butyl-^{35}S$_{1}$-disulphide was synthesized via elemental sulphur-35. The two disulphides were mixed to give phenyl-^{3}H-*sec*-butyl-^{35}S$_{1}$-disulphide which was assayed by liquid scintillation counting.*

INTRODUCTION

Studies of the radiolytic degradation of polyisobutene have previously indicated the important role played by certain additives (1). A continuation of this work required a labelled radical acceptor, such that yields of polymer radicals could be determined using techniques similar to those described in an earlier study of cold mastication of rubber (2). A suitable radical acceptor was found to be phenyl-*sec*-butyldisulphide and because of its unsymmetrical nature it was decided to label the two halves of the molecule separately. The results of the subsequent polyisobutene degradation studies have already been published (3, 4).

Various routes for the preparation of unsymmetrical disulphides have been described including reactions of alkyl sulphenyl thiocyanates with thiols (5, 6), of organic sodium thiosulphates (Bunte salts) with thiolate ions (7, 8), of organic sulphenyl chorides with thiols (9, 10, 11), and of organic thiolsulphonates with thiolate ions (12, 13). The latter route was chosen in the present work because of its experimental convenience and the fact that yields were expected to be high (14).

Tritium labelled phenyl benzene-thiolsulphonate was prepared by Wilzbach irradiation of the unlabelled material and the labelled substrate reacted with

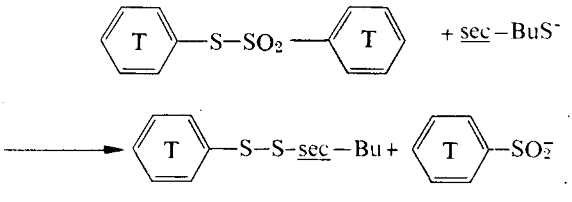

FIG. 1

sec-butane thiol in sodium ethoxide solution to give phenyl-^{3}H-*sec*-butyl disulphide (reaction 1).

The tritium irradiation was carried out with seven curies of tritium gas — a commercial service offered by the Radiochemical Centre, Amersham. Approximately 75 millicuries of labelled material were obtained after purification. The labelling was clearly not uniform as is shown by the fact that appreciably more than half of the activity of the thiolsulphonate appeared in the disulphide after purification ($\sim$ 58% assuming a quantitative reaction).

sec-Butane-^{35}S-thiol was prepared by the reaction of *sec*-butylmagnesium bromide with elemental sulphur-35, using techniques described previously (2). The yield was poor (35%) and not as good as in trial experiments (50-60%). Undoubtedly, better yields could be obtained if more time was devoted to exploring the various reaction variables. However, in the present case the thiol was immediately converted to phenyl-*sec*-butyl-$^{35}S_1$-disulphide by reaction with inactive phenyl benzenethiolsulphonate (reaction 2).

$$\textit{sec}\text{-BuMgBr} + {}^{35}\text{S} \longrightarrow \textit{sec}\text{-Bu}^{35}\text{SH}.$$
$$\textit{sec}\text{-Bu}^{35}\text{S}^- + \text{PhSO}_2\text{Ph} \longrightarrow \textit{sec}\text{-Bu}^{35}\text{S-SPh} + \text{PhSO}_2^- \qquad [2]$$

The overall radiochemical yield of purified disulphide was 20%.

The two disulphides were assayed by liquid scintillation counting and then mixed, further diluted and purified to give phenyl-^{3}H-*sec*-butyl-$^{35}S_1$-disulphide having specific activities of 34.0 μC/mM for tritium and 6.67 μC/mM for sulphur-35. The chemical purity was $>$ 99.9% and the radiochemical purity was about 95 ± 5%.

EXPERIMENTAL

1. *Phenyl benzenethiolsulphonate.*

Phenyl benzenethiolsulphonate was prepared by the method of Douglass and Farah (12) from diphenyl disulphide. After several recrystallisations from ethanol/petroleum ether (b.p. 40-60 °C), a pure product m.p. (uncorr.) 43-44 °C mixed m.p. 43-44 °C (Lit. value 37.5-38.5 °C (14)). Found: S, 25.5% ; $C_{10}H_{10}S_2O_2$ requires S, 25.6%.

2. *Di-*sec*-Butyl disulphide, phenyl-*sec*-butyl disulphide,* and *diphenyl disulphide*

A mixture of these three disulphides was conveniently prepared by oxidising a mixture of thiophenol and *sec*-butane thiol with iodine in alkaline solution. The disulphides were separated by fractional distillation through an 18″ Vigreux column. Di-*sec*-butyl disulphide had b.p. 34 °C/0.1 mm ; phenyl-*sec*-butyl disulphide had b.p. 72-75 °C/0.1 mm ; (Found : C, 60.5; H, 7.1; S, 32.2% ; $C_{10}H_{14}S_2$ requires : C, 60.5 ; H, 7.1 ; S,32.4%) ; diphenyl disulphide remained as a residue in the flask and after two recrystallisations from ethanol/water had m.p. 59-60 °C. All three disulphides were easily resolved on a 4′ gas-liquid chromatography (G.L.C.) column (Pye Argon Chromatograph) consisting of 0.2% Apiezon L on glass beads (80 mesh) at 150 °C. With an argon flow rate of 70 ml/min the retention times were 3 min, 5 min, and 26 min, respectively for dibutyl disulphide, phenyl-*sec*-butyl disulphide and diphenyl disulphide. On a similar column consisting of 10% Apiezon L on celite (100-120 mesh), and under the same conditions, dibutyl disulphide and phenyl-*sec*-butyl disulphide had retention times of 5 minutes and 25 minutes respectively. Less than 0.05% of any disulphide in the presence of the others was readily detectable by this G.L.C. analysis.

3. *Phenyl benzenethiolsulphonate-^{3}H.*

Phenyl benzenethiolsulphonate (0.5 g) was irradiated with 7 curies of tritium gas at the Radiochemical Centre, Amersham. The product was dissolved in benzene (25 ml) and a small potrion diluted and assayed to give a total value for combined tritium of 297 millicuries. A second portion (5 ml) was added to pure inactive phenyl benzenethiolsulphonate (1.0 g) and the mixture chromatographed on a column of silica gel (35 g) (14,15). The material recovered from the column was mixed with further inactive thiolsulphonate (1.0 g) and then recrystallised three times from ethanol/petroleum ether (b.p. 40-60 °C) to yield colourless crystals of phenyl benzenethiolsulphonate-^{3}H (1.2 g; 8.2 mC). Thus, only 71.5 millicuries of the original activity received were present in the desired component.

4. *Phenyl-^{3}H-*sec*-butyl disulphide.*

The reaction was carried out in the same type of vessel as was used for preparation of ^{35}S-thiols (2), and stirring was achieved by a rapid stream of nitrogen bubbles. Phenyl benzenethiolsulphonate-^{3}H (0.626 g; 2.50 mM; 4.18 mC) was dissolved in ethanol (5 ml) and *sec*-butane thiol (0.227 g; 2.52 mM) in 1.056 N potassium ethoxide in ethanol (2.35 ml) added dropwise. After the addition pure carrier phenyl-*sec*-butyl disulphide (0.5 g) was added, ethanol was removed *in vacuo* at room temperature and the residue purified by passage through a silica gel column in carbon tetrachloride solution to yield a yellow oil (0.98 g; 2.48 mC). G. L. C. analysis showed this to be essentially pure phenyl-*sec*-butyl disulphide with a trace (~ 0.15%) of diphenyl disulphide.

5. sec-*Butane thiol-^{35}S.*

sec-Butyl magnesium bromide was prepared in ether solution and the concentration checked by treatment of 5 ml portions in 25 ml distilled water with excess standard hydrochloric acid (0.5 N) followed by back titration with standard sodium hydroxide (0.1 N) using bromophenol green as indicator. Standardised Grignard reagent (15 ml; 3.33 mM) was placed in the apparatus described previously (2) and sulphur-35 (0.066 g; 2.06 m Atom; 1.65 mC) in anhydrous benzene (5 ml) was slowly added at 0 °C. The mixture was refluxed for one hour, decomposed by the addition of 15% aqueous hydrochloric acid (1 ml) and water (4 ml), and the thiol extracted into ether in an atmosphere of nitrogen (2). In the same extraction apparatus the ether was washed with water and then the thiol extracted into 10% aqueous sodium hydroxide solution (3,1,1, and 1 ml). The sodium hydroxide extracts were again acidified and the thiol extracted into petroleum ether (b.p. 30-40 °C). The total extracts which were slightly less than 5 ml were transferred to a 5 ml volumetric flask containing *n*-octane (0.2 ml) and made up to the mark with petroleum ether. Portions of this solution (~ 0.5 µl) were then examined by G.L.C. on a 4′ column consisting of 25% dinonyl phthalate on celite (100-200 mesh) at 100 °C, with an argon flow rate of 65 ml/min. Under these conditions the thiol had a retention time of 3.1 minutes and the internal standard, *n*-octane, a retention time of 5.6 minutes. From the ratio of peak heights, *n*-octane : *sec*-butane thiol, and by reference to a previously prepared calibration curve, the chemical yield of *sec*-butane thiol was calculated to be 0.064 g (0.71 mM ; 35% yield).

6. *Phenyl-*sec*-butyl-$^{35}S_1$-disulphide.*

sec-Butane thiol-^{35}S (0.71 mM) in petroleum ether was mixed with 1.056 N potassium ethoxide in ethanol (0.65 ml) and additional ethanol (4 ml) and added dropwise to phenyl benzenethiolsulphonate (0.7 g; 2.8 mM) in ethanol as described in section 4 above. The product was diluted and purified as before to give a pale yellow oil (0.63 g; 0.32 mC; 20% radiochemical yield). G.L.C. analysis revealed the presence of traces of both dibutyl disulphide (~ 1%) and diphenyl disulphide (~ 2%).

7. *Phenyl-^{3}H-*sec*-butyl-$^{35}S_1$-disulphide.*

Phenyl-^{3}H-*sec*-butyl disulphide (0.4033 g ; 1.02 mC) and phenyl-*sec*-butyl-$^{35}S_1$-disulphide (0.3984 g; 0.202 mC) were mixed with pure inactive phenyl-*sec*-butyl disulphide (4.959 g) and then fractionally distilled through a 6″ semi micro Vigreux column to give : Fraction [1]. b.p. 68-72 °C/0.15 mm ; 1.277 g, consisting of 0.3% di-*sec*-butyl disulphide and 99.7% phenyl-*sec*-butyl disulphide (by G.L.C. analysis) ; Fraction [2]. b.p. 72 °C/0.15 mm ; 3.040 g pure phenyl-*sec*-butyl disulphide (ie. $<$ 0.05% of either di-*sec*-butyl or diphenyl disulphide); Fraction [3]. b.p. 72 °C/0.15 mm ; 0.181 g; pure phenyl-*sec*-butyl disulphide ; Fraction [4]. the residue consisting of 99.3% phenyl-*sec*-butyl disulphide and 0.7% diphenyl disulphide.

8. *Radiochemical Assay.*

All samples were counted in an Ekco liquid scintillation counter (type 664 A) operating at − 10 °C and coupled to an Ekco pulse height analyser/

scaler (type 610 A). The phosphor consisted of PPO (0.3%) and POPOP (0.03%) in redistilled 'Analar' toluene. The single labelled compounds were counted under optimum conditions for the particular isotope and corrections applied for background, quenching, and decay. Quench corrections were made by addition of internal standard to every counting pot : 0.1 ml Toluene-^{3}H for tritium samples and 0.1 ml Toluene-^{14}C for the sulphur-35 samples; by means of an 'Agla' micrometer syringe.

In the case of doubly labelled samples, sulphur-35 was counted under conditions such that tritium made a negligible contribution to the background and then tritium and sulphur-35 were counted together in a narrow « tritium channel ». This is essentially the screening method of Okita *et al.* (16) except that in our case high voltage was maintained constant and the discriminator bias varied, since there was only one channel of pulse height analysis available and it was necessary to re-adjust the controls for each sample. With the equipment described, conditions were : amplifier gain × 1000; high voltage 1050 volts; channel conditions (*i*) : — lower discriminator 5 volts, upper discriminator 10 volts; channel conditions (*ii*) : — lower discriminator 35 volts, no upper discriminator. These settings gave for (*i*), background = 8.5 counts/sec.; tritium efficiency 10% ; sulphur-35 efficiency 4% ; and for (*ii*) background = 0.7 counts/sec ; tritium efficiency, 0.1% ; sulphur-35 efficiency 70%.

Specific activities for the various fractions are given in the table where it can be seen that the radiochemical purity of fraction [2] is of the order of 95% when compared with the values calculated from the specific activities of the singly labelled components before dilution and mixing. The precision of the assay technique was assessed when 22 separate assays of fraction [2] gave maximum deviations from the mean of ± 4.2% for sulphur-35 and ± 5.1% for tritium ; the corresponding standard deviations ($\sqrt{\Sigma\delta^2/n-1}$) were 1.9% and 2.5% respectively.

TABLE

Fraction	Specific Activities	
	Sulphur-35 (c/sec./mM) × 10^{-5}	Tritium (c/sec./mM) × 10^{-6}
[1]	2.71	1.14
[2]	2.47	1.26
[3]	2.49	1.22
[4]	2.44	1.27
Calculated*	2.57	1.30

* Calculated from the values obtained when the single labelled disulphides were assayed separately and using the necessary dilution factor.

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