THE SYNTHESIS OF TRITIUM LABELLED DIALKENYL SULPHIDES STRUCTURALLY RELATED TO SULPHUR CROSSLINKS IN VULCANIZED NATURAL RUBBER.

G.Ayrey *, D.Barnard ** and T.H.Houseman ***
* Isotope Unit, Queen Elizabeth College, London University, London, W8 7AH, U.K.
** Natural Rubber Producers'Research Association, Welwyn Garden City, U.K.
*** Tobacco Research Council Laboratories, Harrogate HG3 1 PY, U.K.

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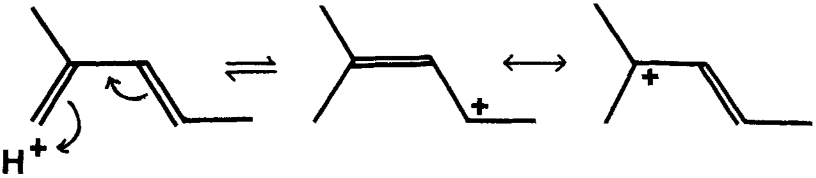
SUMMARY

Di-(1,3-dimethylbut-2-enyl-$\left\{4-{}^{3}H_{1}\right\}$*mono-, di-and trisulphides have been synthesised via the common intermediate S-(1,3-dimethylbut-2-enyl-*$\left\{4-{}^{3}H_{1}\right\}$ *thiouronium bromide (I). Protonation of a mixture of 2-methylpenta-1,3- and -2,4-dienes during formation of (I) was shown to be accompanied by a large kinetic isotope effect such that protonation was 8-10 times more probable than tritonation.*

INTRODUCTION

Recent studies[1] of the oxidative behaviour of sulphur crosslinks in vulcanized natural rubber (NR) necessitated synthesis of a series of radiolabelled dialkenyl sulphides structurally related to the sulphur crosslinks. The A_1-sulphides (where A_1 = 1,3-dimethylbut-2-enyl) were chosen since earlier studies[2] using the model olefin 2-methylpent-2-ene have indicated the presence of a significant proportion of this type of structure in sulphur vulcanizates of NR.

The general synthesis of these allylically unsaturated sulphides has been discussed in the literature[3-5] along with mechanistic considerations of their formation, and the synthetic route is outlined in Figure 1. Steps common to the synthesis of all three sulphides include formation of S-(1,3-dimethylbut-2-enyl) thiouronium bromide (I) from the mixture of 2-methylpenta-1,3- and 2,4-dienes which in turn is obtained by dehydration of 2-methylpentan-2,4-diol. Saville[4] has suggested that polar addition to the diene of hydrobromic acid and thiourea proceeds via an intermediate allylic carbonium ion formed by initial protonation of the diene.



This is followed by nucleophilic addition of thiourea at the sterically less-hindered carbon atom to give the thiouronium compound (I). The protonation step offers an easy means of introducing a radiolabel into the molecule since tritiated water added to the hydrobromic acid/ thiourea mixture exchanges rapidly with all the labile hydrogen atoms in the system. Thus, in the absence of a kinetic isotope effect, tritium competes equally with hydrogen for reaction with the diene. The actual radioactivity incorporated into the molecule in this way is dependent on the specific activity of the tritiated water used and the relative proportions of labelled and unlabelled exchangeable hydrogen atoms present in the system. The amino-hydrogen atoms of thiourea also become labelled by exchange but the radioactivity resulting from this is lost either during subsequent washing with an excess of water or during the next stage of the synthesis.

Alkaline hydrolysis[4] of I yields A_1-thiol (V), which is either oxidised to $A_1S_2A_1$ using alkaline hydrogen peroxide[6] or added to the diene in the presence of an acid catalyst to form A_1SA_1[4]. The route to $A_1S_3A_1$[5] (IV) involves nucleophilic substitution of the thiouronium cation[7] by thiosulphate to form the Bunté salt (II), half of which is then converted to the perthio-anion (III) using sodium sulphide. A_1-trisulphide is finally obtained by nucleophilic attack of III on the remainder of the Bunté salt.

This paper describes the synthesis, by the above route, of tritium labelled dialkenyl mono-, di- and tri-sulphides. During these preparations, the step involving protonation of 2-methylpenta-1,3-diene was shown to be accompanied by a large hydrogen isotope effect. Crystalline $A_1S_3A_1$ was obtained for the first time by crystallisation of the oily product from a methanolic solution at low temperature.

EXPERIMENTAL

S-(1,3-Dimethylbut-2-enyl-[4-$^3H_1$]) thiouronium bromide (I).

The diene mixture (32.8g; 0.4 mole), obtained by slow dehydration of 2-methylpentan-1,4-diol[4], was added to a solution of recrystallised thiourea (33.4g; 0.44 mole) in 48% aqueous hydrobromic acid (60 ml) and tritiated water (0.40 ml; 110.3 mCi/ml) at 10°C. The mixture solidified exothermically after 20 min of vigorous shaking and cooling in an ice-water bath. After another 30 min the cake was shaken into a slurry with cold 20% v.v. aqueous hydrobromic acid (40 ml). The crude A_1-thiouronium bromide was filtered off, sucked fairly dry (2 hr), and then re-suspended in acetone (40 ml) before being filtered again and dried <u>in vacuo</u>. The dried thiouronium salt (68.6g; 0.29 mole) had a melting point of 134.0°C (lit. value[4] = 133.5 - 134.3°C) and a specific activity of 28.9 mCi/mole. The yield was 92% based on the percentage of 1,3-diene in the mixture. (Found: C, 35.3; H, 6.5. Calc. for $C_7H_5N_2S$ Br: C, 35.2; H, 6.3%).

Three such syntheses of A_1-thiouronium bromide were separately performed for use in the reactions described below.

S-(1,3-Dimethylbut-2-enyl-[4-$^3H_1$]) thiol. (A_1-Thiol).

Finely powdered, tritium-labelled A_1-thiouronium bromide (40g; 0.167 mole, 28.9 mCi/mole) was added in portions to a cold, stirred solution of sodium hydroxide (10g; 0.25 mole) and potassium cyanide (ca. 0.1g) in water (50 ml) under nitrogen. The temperature of the solution was raised gradually to boiling after 3 hr and the crude thiol distilled directly into a separating funnel at 95°C/745 mm. The thiol (22 ml) was separated, washed twice with water (2 x 30 ml), dried (magnesium sulphate), and fractionated in a small unit still fitted with Vigreux column and nitrogen bleed. A main fraction (9.58g) was collected at 132.0°C/747 mm. (lit. value[4] = 133.7°C/747 mm.) and found to have a specific activity of 0.616 (± 0.003) mCi/mole. The material was shown to be ca. 99.7% pure by gas chromatography using a glass column packed with 7% w.w. dinonyl phthalate on Chromosorb G operating at 65°C (Found: C, 62.1; H, 10.4; S, 27.5. Calc. for $C_6H_{12}S$: C, 62.05; H, 10.35; S, 27.6%).

A second sample of A_1-thiol, prepared by the above route from an alternative batch of A_1-thiouronium bromide had a specific activity of 0.801 (± 0.005) mCi/mole.

Di-(1,3-dimethylbut-2-enyl-[4-$^3H_1$]) sulphide. (A_1SA_1).

Perchloric acid (0.06 ml, 78%) was added to the radioactive A_1-thiol (6.3g; 0.054 mole, 0.801 mCi/mole) with stirring under nitrogen at 10°C. The mixture of 2-methylpenta-1,3- and -2,4-dienes (3.7g; 0.046 mole) was then added from a dropping funnel over a period of 2 hr. The cooling source was removed and stirring continued for 1 hr before adding water (12 ml), extracting with ether (2 x 10 ml) and washing the extracts with a saturated aqueous solution of sodium hydrogen carbonate. The ethereal extracts were then dried (magnesium sulphate) and solvent removed by rotary evaporation. Crude A_1SA_1 (6.48g) was recrystallised twice at -40°C and once at -10°C from a 1:3 w.v. methanolic solution. The white crystalline solid was washed well with methanol at -40°C, dried _in vacuo_ (0.72g, m.p. 17.3 - 17.5°C) and stored in the dark at -10°C. It had specific activity 0.803 (± 0.014) mCi/mole and was shown to be >99.8% pure by gas chromatography using a glass column packed with 5% w.w. polyethylene glycol on celite, operating at 118°C. The radiochemical purity was 100 ± 0.3% by reverse isotope dilution analysis.

Di-(1,3-dimethylbut-2-enyl-[4-$^3H_1$]) disulphide. ($A_1S_2A_1$).

To a cooled, stirred solution of sodium hydroxide (15.6g; 0.39 mole) in water (65 ml) was added radioactive A_1SH (7.5g; 0.065 mole, 0.616 mCi/mole), the temperature being maintained at ca. 10°C. After slow addition of 30% w.v. hydrogen peroxide (4.4g; 0.039 mole) the mixture was stirred for a further 45 min at room temperature and extracted with petroleum spirit (2 x 250 ml, b.p. 30 - 40°C). The extract was washed with saturated sodium thiosulphate solution (2 x 200 ml) and dried over magnesium sulphate. Solvent was removed by rotary evaporation and the crude $A_1S_2A_1$ (6.2g) fractionated under high vacuum in a unit still fitted with Vigreux column. A main fraction (4.0g) was collected at 70.0°C/0.01 mm. The product had a specific activity of 1.29 (± 0.011) mCi/mole. It was examined by thin layer chromatography on silica gel G using n-heptane as solvent and 3% w.v. sodium azide in N/10 iodine as locating spray. A single spot, R_f = 0.702, contained >99.6% of the total radioactivity recovered from the developed thin layer plate. A weight percent purity of 100.4 was indicated by gas chromatography using di-t-butyl phenol as standard on a glass column packed with XF - 1105, a cyano silicone, on Chromosorb G operating at 118°C.

Di-(1,3-dimethylbut-2-enyl-[4-$^3H_1$]) trisulphide. ($A_1S_3A_1$).

Tritium-labelled A_1-thiouronium bromide (34.3g; 0.143 mole) from a third preparation as previously described was added to a stirred solution of sodium thiosulphate pentahydrate (54.5g; 0.22 mole) in 1:2 methanol : water (114 ml) and the mixture heated at 70°C for 15 min. The solution of Bunté salt was extracted with chloroform (2 x 60 ml) and with redistilled petroleum ether (2 x 60 ml; b.p. 30 - 40°C) and the pH then adjusted to *ca.* 7 using sodium bicarbonate. The solution was extracted a further twice with petroleum ether (2 x 60 ml; b.p. 30 - 40°C), transferred to a dimpled flask and then vigorously stirred under a layer of petroleum ether (300 ml; b.p. 30 - 40°C). Sodium sulphide nonahydrate (17.3g; 0.072 mole) in water (60 ml) was added slowly over a period of 1.5 hr; the organic phase was separated, washed with water (3 x 100 ml) and dried over magnesium sulphate. Solvent was removed by rotary evaporation and the pale yellow oil (7.57g) recrystallised three times at -10°C from a 1:15 w.v. methanolic solution. The white crystalline solid (2.29g) melted at 33.5 - 34.0°C and had a specific activity 1.50 (± 0.01) mCi/mole. Gas chromatography gave only one peak (column conditions as for $A_1S_2A_1$) and reverse isotope dilution analysis indicated a radiochemical purity of 100 ± 0.2% (Found: C, 54.9; H, 8.5; S, 36.6. Calc. for $C_{12}H_{22}S_3$: C, 54.9; H, 8.45; S, 36.6%).

Radiochemical Assay

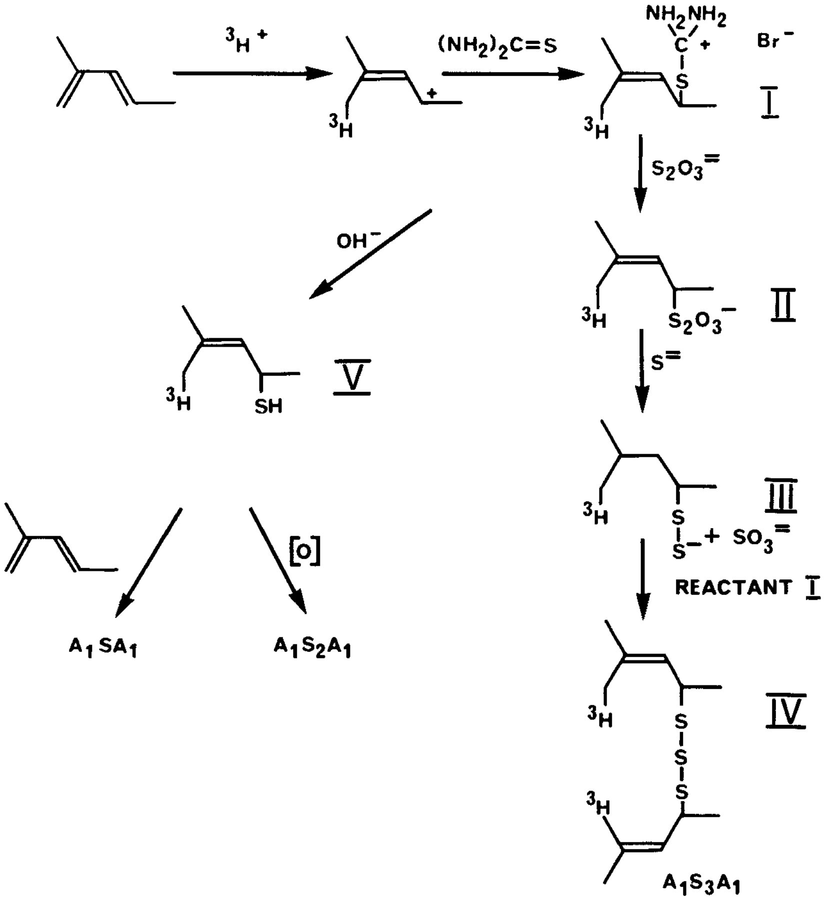
Sample radioactivity was measured using a Tracerlab Coru/matic 25 liquid scintillation spectrometer with optimised gain and window settings. Samples to be assayed were dissolved in a toluene solution of butyl-PBD (15 ml, 0.6% w.v.) in standard screw cap counting vials. The external standard method of quench correction was used and all samples were counted to at least 1% accuracy.

RESULTS AND DISCUSSION

The reaction scheme shown in figure 1 indicates that the initial protonation step in the presence of tritiated water should result in the incorporation of one labelled hydrogen atom (on carbon 4) and that the resultant monosulphide should contain one labelled atom per molecule while the di- and trisulphide should contain 2. Consideration of the specific activity of the tritiated water and its dilution by exchangeable hydrogens in the total reaction mixture enables calculation of the expected specific activity for the sulphides. Experimentally, it was found that the mono-, di- and trisulphides prepared as described in the experimental section contained only 13, 11 and 10% respectively of the expected amount of tritium (Table I). This unexpected "loss" of activity was investigated further using accurately known quantities of reagents (and hence labelled and unlabelled hydrogen atoms) in the following experiments:-

(i) A_1-thiouronium bromide prepared on a 0.1 mole scale

Figure I SYNTHESIS OF TRITIUM LABELLED A_1− SULPHIDES.



and isolated immediately after formation, without further washing with dilute hydrobromic acid, was dried to constant specific activity and found to contain 4.13 rather than the expected 5.0 labelled atoms per molecule. A_1-trisulphide prepared from this material contained 0.18 labelled atoms per molecule (0.09 atoms per A_1 structure). This compared well with the figure of 0.20 obtained in the earlier trisulphide synthesis (Table I).

(ii) A sample of the A_1-thiouronium bromide prepared in (i) above was repeatedly recrystallised from water to a constant specific activity which corresponded to only 0.13 labelled atoms per molecule. This treatment clearly removed the 4 labile tritium atoms associated with the thiouronium group.

(iii) A_1-thiouronium bromide, prepared from labelled thiourea containing exactly 4.0 labelled atoms per molecule (introduced by prolonged exchange with tritiated water), was found to contain 4.16 "diluted" labelled atoms. The net specific activity per gram-atom of tritium was calculated on the assumption that under the conditions used there was complete equilibration between the tritium atoms of labelled thiourea and the unlabelled hydrogen atoms of hydrobromic acid. By comparison with the results of earlier experiments this appeared to be a valid assumption. $A_1S_3A_1$ synthesised from this A_1-thiouronium bromide had a specific activity equivalent to 0.26 labelled atoms per molecule (0.13 labelled atoms per A_1 structure).

From these observations it was concluded that tritium introduced into the A_1 moiety during initial protonation of

TABLE 1. Comparison of Calculated and Observed Specific Activities of A_1-Sulphides

Sulphide:	A_1SA_1	$A_1S_2A_1$	$A_1S_3A_1$
Specific activity of tritiated water used in synthesis (mCi/mole):	2.04	1.98	2.02
Total quantity of exchangeable hydrogen in the system (g atoms):	18.629	18.501	18.629
Calculated specific activity of sulphides assuming complete exchange (mCi/mole):	6.08	5.95	7.57
Observed specific activity of sulphides (mCi/mole):	0.803	1.29	1.50
Number of labelled hydrogen atoms per molecule of sulphide:	0.13*	0.22	0.20

* In A_1SA_1, only <u>one</u> A_1-structure is labelled by virtue of the synthetic route used

the diene was not partially removed by subsequent reaction, but instead this step was subject to a large isotope effect, the *apparent* overall rate of protonation being 8 - 10 times faster than that of tritonation.

Similar hydrogen isotope effects have been observed in the hydration of isobutene[8], the first step of which involves protonation of the olefin in the presence of an acid catalyst. It has been shown[9] that in most reactions of this type, formation of a carbon-hydrogen bond is favoured relative to formation of carbon-deuterium or carbon-tritium bonds. For hydration of isobutene the molar activity of the resulting *tert*-butyl alcohol, after washing to remove labile hydroxylic tritium, was 6.9 times *smaller* than the activity of tritium in the reaction medium (per gram-atom exchangeable hydrogen). It was concluded[8] from application of the Gross-Butler theory[10] governing isotopic fractionation in an isotopically mixed solvent that the large isotope effects derived from measurements of relative isotopic abundances in the final product were due not to a kinetic isotope effect but largely to a thermodynamic isotope effect.

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