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# THIOPROPANAL *S*-OXIDE, ALK(EN)YL THIOSULPHINATES AND THIOSULPHONATES: SIMULATION OF FLAVOUR COMPONENTS OF *ALLIUM* SPECIES

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**Abstract**—The importance of thiopropanal *S*-oxide and methyl methanethiosulphinate as intermediate compounds in the production of the volatile components of certain *Allium* species has been established. *In vitro*, a reducing agent such as sodium borohydride is an essential component of the system.

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## INTRODUCTION

The classical work of Stoll and Seebeck [1] established the importance of 2-propenyl propenethiosulphinate (allicin) as an intermediate in production of the characteristic flavour of *Allium sativum*. Isolation and synthesis of thiopropanol *S*-oxide [2] was correspondingly important in relation to *A. cepa*. The reactions, structure and biochemistry of thiosulphinates have been reviewed by Isenberg and Grdinic [3]. The present paper describes properties not hitherto reported of these compounds and their use in simulation of the volatile flavour components of certain *Allium* species.

## RESULTS

### *Thiopropanal* S*-oxide*: *properties*

The lachrymator (thiopropanal *S*-oxide) [2] was synthesised by dehydrochlorination of 1-propanesulphinyl chloride [4] in the presence of triethylamine at −20°. UV: $\lambda_{max}^{hexane}$ ($\epsilon$) 254 (5160) nm; the peak was virtually completely eliminated on reaction with L-cysteine reagent. The first-order rate constants of lachrymator decay at 0° and 25°, the partition coefficient between hexane and water and TLC properties of the L-cysteine derivative and the corresponding properties of diluted onion juice were in satisfactory agreement [5].

Mixtures of thiopropanal *S*-oxide (22·8 μmol) and methyl methanethiosulphinate (2·4 μmol) dissolved in water (9 ml) were incubated for 30 min at 40° under the conditions described for sampling of onion headspace volatile components [6]. The above relative proportions were based on amino-acid analyses of *S*-alk(en)yl-L-cysteine sulphoxides by Matikkala and Virtanen [7], see also Schwimmer [8]. The characteristic peaks obtained from fresh onion were absent from the corresponding GLC traces of the simulation mixture. However, on addition of sodium borohydride (34 mg) (900 μmol) after incubation, chromatograms virtually indistinguishable from those of fresh onion slices (10 g) were obtained, (cf. [9], Fig. 1a) and the identity of 10 major peaks was confirmed by GC–MS. In a parallel experiment with thiopropanal *S*-oxide (22·8 μmol) and sodium borohydride (34 mg), the principal components identified were 1-propyl disulphide, propanal, *cis*- and *trans*-1-propenylpropyl disulphide and 1-propyl trisulphide. 3,4-Dimethylthiophene was tentatively identified as a minor component, cf. [10]. A redox system ($E_0'$ + 0·217V) has been identified in fresh onion juice.

### *1-Propyl propanethiosulphinate*

The thiosulphinate was prepared from 1-propyl disulphide by oxidation with perbenzoic acid [11] or with $H_2O_2$ by a method analogous to that of [12]. The pure compound had an odour characteristic of freshly cut onion, bp 42°/0·67 Pa (0·005 mm); UV: $\lambda_{max}^{hexane}$ ($\epsilon$) 262 (1960) nm; IR: $\nu_{max}$ 1085 cm$^{-1}$, characteristic of thiosulphinates [13]. The MS was consistent with that of thiosulphinate, containing traces of the corresponding thiosulphonate. $E_{262}$ was eliminated to 97% completeness by reaction of a hexane solution with L-cysteine reagent pH 6·7 and partially eliminated (21%) by the reagent at pH 4. An excess of the reagent (pH 6·7) gave crystalline *S*-1-propylmercapto-L-cysteine, mp 227°, elementary analysis in agreement with $C_3H_7SSCH_2CN(NH_2)$-COOH. TLC on cellulose gave a single spot $R_f$, 2·14 (cf. cystine 0·11; cysteine 1·00) developed with 2-methylpropan-2-ol—formic acid (90%)–HCl(conc)–water (95:15:18:12) [14] in close agreement with the corresponding component of onion extracts [9]. The partition coefficient, water–hexane (1:2) of the thiosulphinate was 0·28 at 15°.

The compound decomposed in a GLC column (max. temp. 180°) giving a complex mixture containing 1-propyl disulphide, *cis*- and *trans*-1-propenylpropyl disulphide, 1-propyl trisulphide and minor unidentified peaks. The same pattern of peaks was observed when the thiosulphinate was analysed by the headspace method but the total peak area was only 15·7% of that obtained by column injection owing to its low volatility. Equal quantities (1 $\mu$l each) of 1-propyl propanethiosulphinate and methyl methanethiosulphinate introduced into a GLC column (max. temp. 180°) resulted in identification of many of the peaks found in corresponding chromatograms of fresh onion slices (see above). 1-Propanethiol and other peaks associated with decomposition of thiopropanal *S*-oxide, such as propanal and 2-methylpent-2-enal were, however, absent. A corresponding analysis by the headspace method gave a similar pattern of peaks of which the total area was 9·6% of that of the previous experiment.

### *Methyl methanethiosulphinate*

The thiosulphinate was prepared by $H_2O_2$ oxidation of methyl disulphide [cf. 12], bp 35°/9·3 Pa (0·07 mm), yield 75%, elementary analysis was in agreement with $C_2H_6OS_2$. The distilled product had an unpleasant cabbage-like odour. UV: $\lambda_{max}^{hexane}$ ($\epsilon$) 260 (1930) nm. $E_{max}$ was 99% eliminated on reaction with L-cysteine reagents, pH 4 and 6·7. IR: $\nu_{max}$ 1085 cm$^{-1}$; peaks characteristic of thiosulphonates at 1130 and 1320 cm$^{-1}$ were absent. The MS was consistent with that of the thiosulphinate. The partition coefficient, water–hexane (1:2) was *ca* 17 at 15°. On injection into a GLC column (max. temp. 180°), the thiosulphinate gave a complex mixture containing methyl di, tri- and tetra-sulphides and minor unidentified peaks. The properties of mixtures with 1-propyl propanethiosulphinate are described above.

A mixture of methyl methanethiosulphinate (1·3 $\mu$l) and sodium borohydride (34 mg) in water (9 ml) was analysed by the GLC-headspace method. The major peak was methyl disulphide; minor amounts of methanethiol and methyl sulphide were also detected. The chromatogram was closely similar to that obtained from slices of *A. aflatunense* bulb except for the presence of methanol and ethanol in the latter [9].

### 2-*Propenyl* (*allyl*) *propenethiosulphinate*

2-Propenyl disulphide (allyl disulphide) was prepared from 3-bromo-1-propane via the corresponding Bunte salt [15]. The thiosulphinate was prepared by perbenzoic acid oxidation of the disulphide and purified by method A of Small *et al.* [11]. The thiosulphinate had a garlic-like odour. UV: $\lambda_{max}^{hexane}$ ($\epsilon$) 264 (837) nm. $E_{264}$ was eliminated by 67% completeness on reaction with L-cysteine reagent pH 4 and to 88% completeness with the reagent at pH 6·7. IR : $\nu_{max}$ 1085 cm$^{-1}$. The first order reaction constant for thiosulphinate breakdown in hexane solution was determined by loss of $E_{264}$, $k_1$, $1{\cdot}62 \times 10^{-4}$ S$^{-1}$ at 25°. On this basis, the time for 50% loss ($t = \log_e 2/k_1$) was 71·3 min. The corresponding values for an extract of garlic cloves were $k_1$, $2{\cdot}10 \times 10^{-5}$ S$^{-1}$ at 0° and $1{\cdot}50 \times 10^{-4}$ S$^{-1}$ at 25·0° ($t_{50\%}$, 9·2 hr and 77 min, respectively). The partition coefficient, water–hexane (1:2) was 0·91 and the corresponding value for the garlic extract was 0·76 at 18°.

Brodnitz *et al.* [16] showed that synthetic 2-propenyl propenethiosulphinate and the corresponding compound in solvent extracts of garlic cloves exhibited two characteristic features on GLC. Immediate analysis by injection gave two cyclic isomers of MW 144, 2-propenyl disulphide and trisulphide. After storage at 20° for 20 hr, the major products were 2-propenyl disulphide, sulphide and trisulphide with only traces of the above isomers. Our observations on the synthetic thiosulphinate were consistent with these conclusions.

Mixtures of 2-propenyl propenethiosulphinate (0·90 $\mu$l, 5·6 $\mu$mol) and methyl methanethiosulphinate (0·1 $\mu$l, 0·9 $\mu$mol) dissolved in water (9 ml) were incubated for 30 min at 40° and the volatile components sampled by the headspace method. The characteristic peaks obtained from garlic cloves were virtually absent from the corresponding GLC traces. Addition of sodium borohydride (8·5 mg, 225 $\mu$mol) after incubation gave corresponding chromatograms which were essentially indistinguishable from those from crushed garlic cloves (30 g fr. wt). The major peaks (97% of the total area) from the simulation mixture were 2-propenyl mono, di- and tri-sulphides, methyl-2-propenyl mono-, di- and tri-sulphides and methyl disulphide. The relative proportions of alk(en)yl radicals found in garlic cloves were 2-propenyl–methyl–1-propyl, 82:16:2 and the corresponding proportions in the simulation mixture were 74·6:25:0·4.

### *Properties of the corresponding thiosulphonates*

1-Propyl propane- and methyl methane-thiosulphonates were prepared by $H_2O_2$ oxidation of the corresponding disulphides with 2 mol proportions of the oxidising agent [12].

### 1-*Propyl propanethiosulphonate*

Colourless liquid, bp 75–78°/20 Pa (0·15 mm), the odour was similar to that of the corresponding thiosulphinate but less pungent, $n_D^{22}$ 1·5102. (Found C, 40·6; H, 8·1; S, 35·3. $C_6H_{14}O_2S_2$ requires C, 39·5; H, 7·8; S, 35·1%). UV: $\lambda_{max}^{hexane}$ ($\epsilon$) 256 (274) nm. The peak at 256 nm was eliminated to 32% completeness on reaction with L-cysteine reagent at pH 4 and to 85% completeness with the reagent at pH 6·7. IR: $\nu_{max}$ 1130, 1320 cm$^{-1}$ characteristic of thiosulphonates with a minor peak at 1085 cm$^{-1}$. The MS was consistent with theory. On GLC a major peak corresponding to the thiosulphonate emerged after 170 min. Reaction with L-cysteine at pH 6·7 gave crystalline *S*-1-propylmercapto-L-cysteine identical with that described above.

### *Methyl methanethiosulphonate*

Colourless liquid, bp 56°/20 Pa (0·15 mm) with a characteristic odour similar to but less pungent than that of the corresponding thiosulphinate. (Found, C, 19·3; H, 4·9; S, 50·9. $C_2H_6O_2S_2$ requires C, 19·0; H, 4·8; S, 50·8%). The IR and MS were consistent with theory for the thiosulphonate. The compound was readily soluble in water and in ethanol but only slightly soluble (0·03% at 18°) in hexane. UV: $\lambda_{max}$ (hexane, water or ethanol) 238 nm ($\epsilon$ 65 [hexane and ethanol], 8·3 [water]). The peak at 238 nm was eliminated to 95% completeness on reaction with L-cysteine reagent pH 4. The thiosulphonate eluted from the GLC column as a single peak, $R_t$ 109 min.

### 2-*Propenyl propenethiosulphonate*

The thiosulphonate was prepared by perbenzoic acid oxidation of the corresponding disulphide and purified by method A of Small *et al.* [11]. Colourless, undistillable liquid with a less pungent odour than that of the corresponding thiosulphinate. UV: $\lambda_{max}^{hexane}$ (ε) 266 (580) nm. The peak at 266 nm was eliminated to 79% completeness on reaction with L-cysteine reagent at pH 6·7. IR: $\nu_{max}$ 1130, 1318 cm$^{-1}$. On headspace-GLC in the presence of $NaBH_4$, the major peak was 2-propenyl disulphide with a small amount of 2-propenyl sulphide.

### *Reaction with* N-*ethylmaleimide*

The reactivity of the compounds described above towards *N*-ethylmaleimide was determined under the conditions described by Nakata *et al.* [17]. The relative intensities of the colour reactions were expressed in terms of that of 2-propenyl propenethiosulphinate as standard (Table 1). An extract of garlic cloves [17] gave a strongly +ve reaction (100 units as standard on a dry wt basis) whereas a corresponding extract of onion gave a weakly +ve reaction (4·8 units). These observations are interpreted as evidence that the garlic extract contained the thiosulphinate rather than the thiosulphonate.

### *Properties of the synthetic compounds in relation to those of extracts of* Allium *species*

(i) *Onion.* The close correspondence of properties of thiopropanal *S*-oxide with those of onion extracts with respect to the following properties has been demonstrated by Freeman and Whenham [5]: (a) $\lambda_{max}$ and loss of $E_{254}$ on reaction with L-cysteine reagent, (b) rate of decay of $E_{254}$ at 0° and 25°, (c) partition coefficient between water and hexane and (d) TLC. It was concluded that the *S*-oxide was the major component which accounted for the observed optical absorbance of onion extracts.

(ii) *Garlic.* Comparison of the following properties showed that there was a corresponding correlation between extracts of garlic cloves and 2-propenyl propenethiosulphinate: (a) reaction rate of loss of $E_{264}$ and (b) partition coefficient as described above, (c) $\lambda_{max}$ of hexane extracts of garlic cloves was 262 nm, (d) the peak at 262 nm in the extracts was eliminated to 86% completeness on reaction with L-cysteine reagent, pH 4 and 93% completeness with the reagent at pH 6·7, (e) the major spot observed on TLC plates of extracts of garlic cloves by the method described by Lukes [14] ($R_f$ 0·83, $R_{cysteine}$ 1·77, reddish colour) corresponded closely with that obtained from the reaction product of 2-propenyl propenethiosulphinate with L-cysteine.

(iii) Allium aflatunense. The similarities in TLC properties between the L-cysteine derivative of methyl methanethiosulphinate and extracts of certain *Allium* species of which *A. aflatunense* is typical have been demonstrated by Freeman and Whenham [9].

Table 1. Intensity of colour reactions of thiopropanal *S*-oxide, thiosulphinates and thiosulphonates with *N*-ethylmaleimide [17] expressed in relation to 2-propenyl propenethiosulphinate as standard

| Compound | Relative colour intensity | |
|---|---|---|
| Thiopropanal *S*-oxide | 5·5 | |
| | Thiosulphinate | Thiosulphonate |
| Methyl methane | 74 | 0·56 |
| 1-Propyl propane | 71 | 1·14 |
| 2-Propenyl propene | 100 | 20·9 |

### $\lambda_{max}$ *and* ε *of the corresponding disulphides*

Because certain thiosulphinates and thiosulphonates undergo molecular transformations leading to disulphides, it is relevant to record $\lambda_{max}$ and ε values of the disulphides in question. Methyl disulphide had $\lambda_{max}$ 255 nm (ε 343) and 1-propyl disulphide had $\lambda_{max}$ 252 nm (ε 395). 2-Propenyl-disulphide had no peak between 207 and 350 nm, but at 250 nm, ε was 1134.

## DISCUSSION

The evidence presented above supports the conclusion that thiopropanal *S*-oxide, 1-propyl propanethiosulphinate and methyl methanethiosulphinate are intermediate compounds in the production of the volatile components of onion and other *Allium* species. Characteristic components identified by GLC were formed from these intermediate compounds in GLC columns and may be artefacts of this treatment rather than native components of freshly sliced *Allium* tissues. In spite of the unambiguous identification of methyl methane-, propyl methane- and propyl propane-thiosulphonates in extracts of raw onion [10], it was concluded that thiosulphonates are less significant as intermediate compounds than the corresponding thiosulphinates. Properties of corresponding alliinase-precursor (*S*-alk(en)yl-L-cysteine sulphoxide) systems are presented in the following paper [18].

## EXPERIMENTAL

*GLC headspace method* [6]. The FID chromatograph had dual 2 m × 6 mm glass columns containing 8% Carbowax 1540 on DMCS-treated Chromosorb W. The sample was incubated for 30 min in a 500 ml, 3-necked flask at 40°. An aliquot (250 ml) of the headspace gas was displaced by a slow stream of argon and the volatile components condensed in a trap, containing glasswool, cooled in solid $CO_2$. The trap was transferred to the column inlet, heated for 2 min at 180° and the sample flushed out in the carrier gas stream ($N_2$) at 25 ml/min. The column temp. was maintained at 50° for 10 min and then programmed at 2°/min to 130°.

*GC-MS.* GLC separation was carried out in either a 1·5 m × 4 mm glass column packed with Carbowax 1540 as described above or a 15 m SCOT column with Carbowax 1540 as stationary phase. Part of the effluent from the GLC column was passed into a mass spectrometer using a membrane separator mounted in a glass envelope. MS were recorded at a scan rate of 4 s/decade, a source temp. of 200° and an ionisation energy of 70 eV. (We are grateful to Mr. R. Self, Food Research Institute, Norwich, for MS data.).

L-*Cysteine reagents* [14]. L-Cysteine HCl (100 mg) was dissolved in $H_2O$ (7·5 ml) and the pH value of the soln adjusted either to pH 4 with M Na citrate or to pH 6·7 with 1·25 M NaOH. Hexane solns of the compounds (7 ml) were shaken with the cysteine reagents (0·5 ml), respectively, for 1 hr at room temp.

*Simulation of onion essence: preparation of distilled oil.* Thiopropanal *S*-oxide (5·4 g, 0·06 mol), methyl methanethiosulphinate (0·33 g, 3 mmol) and aq. 0·1 M $NaBH_4$ (160 ml) were mixed and the resultant oil–$H_2O$ emulsion was submitted to *n*-pentane extraction:co-distillation in a Likens and Nickerson apparatus [19]. Solvent was cautiously removed at 20°/1·2 × 10$^4$ Pa (90 mm) giving 2·56 g of yellow oil, the properties of which were closely similar to the corresponding product from fresh onion and to commercial onion oil. The presence of 10 major components previously identified in fresh onion [10] or onion oil [20] was established by $R_f$ data and GC-MS

*Redox potential of onion juice.* Onion juice diluted 1:10 with $H_2O$ (pH 5·4) reduced phenolindophenol and phenolindo-2,6-dichlorophenol, i.e. $E'_0$ was *ca* + 0·217 V. $NaBH_4$ soln (0·1 M) gave the same result.

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