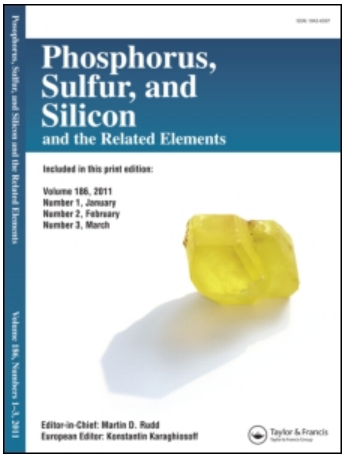

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COMPARATIVE REACTIVITIES OF SELENOSULFATES AND SELENENYLTHIOSULFATES WITH THIOLS

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COMPARATIVE REACTIVITIES OF SELENOSULFATES AND SELENENYLTHIOSULFATES WITH THIOLS

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Nitroaryl selenosulfates (**1**) and selenenylthiosulfates (**2**) reacted instantaneously with a thiol, *N*-succinylcysteamine, in water at pH 4.6 to give an insoluble selenenyl sulfide and diselenide. The selenenyl sulfide was the major product from the 2-nitro compounds whereas the diselenide was the major (almost exclusive) product from the 4-nitro compounds. However when the 4-nitro compounds reacted with *N*-succinylcysteamine in dimethylformamide the selenenyl sulfide was the major and the diselenide the minor product indicating the formation of selenenyl sulfide preceded that of diselenide. Also in the dimethylformamide reactions a short-lived, light-sensitive, purple intermediate was formed which was identified as 4-nitroselenophenol.

It was impossible to compare reactivities of selenosulfates and selenenylthiosulfates in water since all reacted immediately to form insoluble products. However, when formation of the purple complex of 4-nitroselenophenol in dimethylformamide was used as an indicator, Se-(4-nitrophenyl) selenenylthiosulfate reacted significantly faster with *N*-succinylcysteamine than did Se-(4-nitrophenyl) selenosulfate thus correlating with previously observed rates of inactivation of the enzyme papain by these compounds.

Alkyl selenosulfates $RSeSO_3^-$ have been shown to react instantly with simple thiols,[1] to be potent inhibitors of thiol-dependent enzymes[2] and to have promise as antifungal reagents[3]. They react with the thiol cysteine (CySH) to form RSeSCy, some of which reacts further to form RSeSeR and $CySSO_3^-$ in a sequence of reactions believed to be as follows[1]

$$RSeSO_3^- + CyS^- \rightarrow RSeSCy + SO_3^{2-} \quad \text{(i)}$$

$$RSeSCy + SO_3^{2-} \rightarrow RSe^- + CySSO_3^- \quad \text{(ii)}$$

$$RSe^- + RSeSO_3^- \rightarrow RSeSeR + SO_3^{2-} \quad \text{(iii)}$$

$$RSe^- + RSeSCy \rightarrow RSeSeR + CyS^- \quad \text{(iv)}$$

The aim of the current work was to compare the reactivities of some aryl selenosulfates $ArSeSO_3^-$ (**1**) and their analogous selenenylthiosulfates $ArSeS_2O_3^-$ (**2**) with thiols.

In aqueous solution at pH 4.6 compounds **1** and **2** reacted immediately with the thiol, *N*-succinylcysteamine (RSH) to form the insoluble ArSeSR and ArSeSeAr with ArSeSR being the major product in all cases except those of the 4-nitrophenyl compounds (Table I). Thus the ArSeSR : ArSeSeAr ratio appeared to depend on the position of the nitro group.

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TABLE I

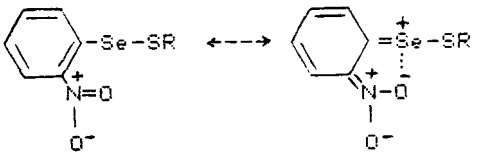
Products from reaction of $RSeSO_3K/ArSeSO_3K/ArSeS_2O_3K$ with RSH in pH 4.6 acetate buffer at room temperature

Reactants	Products: $(RSe—)_2$ or $(ArSe—)_2$ MP °C	Yield, %	RSeSR or ArSeSR MP °C	Yield, %	Analysis (C, H, N,: Calcd/Found)
$PhCH_2SeSO_3K$	90–91 Lit.[13] 92–93	15	114–116	66	45.09, 4.95, 4.04/ 45.29, 5.00, 4.03
$4\text{-}NO_2C_6H_4CH_2SeSO_3K$	105–106 Lit.[13] 107.5	31	80–82	54	39.90, 4.12, 7.16/ 39.76, 4.20, 6.96
$PhSeS_2O_3K$	59–60 Lit.[17] 61–62	19	100–101	68	43.38, 4.55, 4.21/ 43.35, 4.61, 4.21
$2\text{-}NO_2C_6H_4SeS_2O_3K$	212–214 Lit.[18] 214	32	113–114	57	38.20, 3.74, 7.42/ 38.26, 3.80, 7.14
$2\text{-}NO_2C_6H_4SeSO_3K$		35		48	
$4\text{-}NO_2C_6H_4SeS_2O_3K$	181–182 Lit.[19] 180–182	77*	110–112	23*	38.20, 3.74, 7.42/ 38.01, 3.70, 7.17
		87#		13#	
$4\text{-}NO_2C_6H_4SeSO_3K$		82*		17*	
		84#		6#	

* 5°C, not room temperature.
# Determined spectrophotometrically.

Previous studies on the reactions of Se-(4-nitrobenzyl) selenosulfate with CySH have shown that pH also plays an important role in the RSeSCy : RSeSeR ratio.[1] For example, RSeSCy was the major product at pH 5 while RSeSeR was the major (only) product at pH 7. The reason for the difference in product ratio was believed to be the greater ease of reaction (ii) at pH 7 since the sulfite is a stronger nucleophile than bisulfite.

In the present work, ease of reaction (ii) almost certainly determines the product ratio also. In the case of Se-(4-nitrophenyl) selenosulfate **1a**, although bisulfite is a relatively weak nucleophile at pH 4.6, conjugation of selenium with the nitroaromatic ring would tend to weaken the Se—S bond of ArSeSR while the driving force of reaction (ii) would be the resonance stabilization of 4-nitroselenophenol ArSeH making it a weaker S-base and therefore a better leaving group than bisulfite or RSH. In the case of Se-(2-nitrophenyl) selenosulfate **1b**, although conjugation of selenium with the nitroaromatic ring undoubtedly occurs, back donation of charge from the nitro group to the selenium atom[4] probably ensures that the Se—S bond is not as weak as in the case of the 4-nitrophenyl compounds.



The proximity of the negatively charged nitro group to the Se—S bond may also have the effect of repelling an attacking nucleophile.

It is believed that **1** and **2** follow the same mechanism of reaction as do $RSeSO_3^-$ although the compound S-thiosulfo-*N*-succinylcysteamine ($RSS_2O_3^-$) has not been

positively identified and attempts to make it as a reference material by a published method[5] have been unsuccessful. However a water-soluble compound with a slightly higher R_f (TLC on silica gel: BuOH : AcOH : H_2O; 4 : 1 : 1) than S-sulfo-*N*-succinyl-cysteamine $RSSO_3^-$ (0.53 cf 0.44) was observed in the reaction mixture.

As mentioned previously, the aim of this work was to compare the reactivities of **1** and **2** with thiols. Since all compounds reacted instantaneously with RSH in aqueous solution to form precipitates at pH 4.6 it was not possible to detect reactivity differences in water. Therefore another solvent was sought in which all reactants and products were soluble and which would offer the possibility of spectrophotometric determination of reaction rates. Dimethylformamide (DMF) fulfilled these criteria. The 4-nitrophenyl compounds **1a** and **2a** were compared since, to a first approximation, they formed only one selenium product at room temperature in water, bis-(4-nitrophenyl) diselenide and it was believed that this would simplify the comparison.

However, on reaction with RSH in DMF, instead of the yellow diselenide being formed, a purple colour (λ_m 530 nm) appeared almost immediately (the rate and duration of appearance varying with concentration of RSH). The purple compound also appeared to be light-sensitive. When the reactions were studied in a spectrophotometer the color was stable for several minutes (Figure 1) but, on removal of the cuvette from the spectrophotometer, the color disappeared rapidly. It was felt that this purple compound could be either 4-nitroselenophenol ArSeH formed as in equation (ii) or 4-nitrobenzeneselenenic acid ArSeOH produced by hydrolysis of

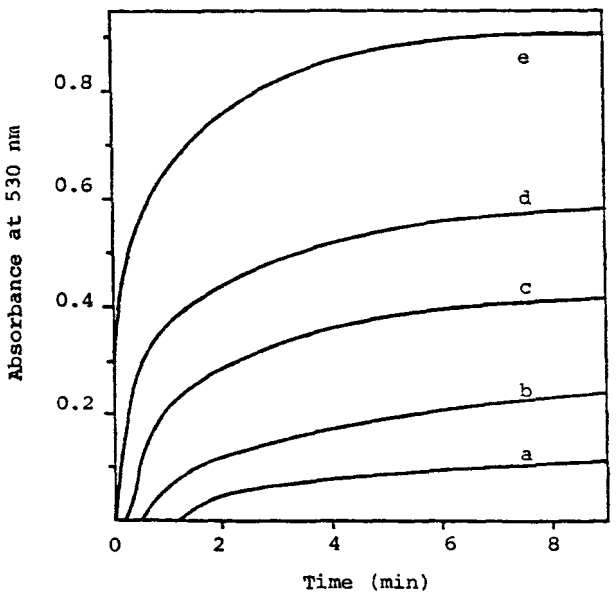


FIGURE 1 Effect of concentration of *N*-succinylcysteamine (RSH) on the rate of formation of 4-nitroselenophenol (ArSeH) from Se-(4-nitrophenyl) selenosulfate **1a** and Se-(4-nitrophenyl) selenenylthiosulfate **2a** in DMF. Molar ratios of reactants: (a) **1a**: RSH—1 : 1; (b) **2a**: RSH—1 : 1; (c) **1a**: RSH—1 : 5; (d) **2a**: RSH—1 : 5; (e) **2a**: RSH—1 : 10 (for details see Experimental).

S-(4-nitrobenzeneseleno)-*N*-succinylcysteamine ArSeSR[6]

$$\text{i.e.} \quad \text{ArSeSR} + \text{HOH} \rightarrow \text{ArSeOH} + \text{RSH} \qquad \text{(v)}$$

Although the formation of ArSeOH was considered less likely, there was sufficient water in the DMF (0.5%) for the reaction to occur and it was felt that the dipolar aprotic DMF might stabilise the species. Neither 4-nitroselenophenol nor 4-nitrobenzeneselenenic acid has even been isolated in the solid state since both are too unstable.[8,9] However 4-nitroselenophenol is known in solution and has been reported to have λ_m 18,800 cm^{-1}, (= 531 nm) in DMF.[9]

Since both S-(4-nitrobenzeneseleno)-*N*-succinylcysteamine ArSeSR and bis-(4-nitrophenyl) diselenide ArSeSeAr on reaction with RSH in DMF gave the purple compound, this was good evidence for its being 4-nitroselenophenol

$$\text{e.g.} \quad \text{ArSeSR} + \text{RSH} \rightarrow \text{ArSeH} + \text{RSSR} \qquad \text{(vi)}$$

$$\text{ArSeSeAr} + \text{RSH} \rightarrow \text{ArSeH} + \text{ArSeSR} \qquad \text{(vii)}$$

Further evidence of its identity was obtained by methylation of the purple reaction mixture from which methyl 4-nitrophenyl selenide was obtained as a major product.

By contrast with the aqueous reactions of **1a** and **2a**, in which ArSeSeAr was the major product, in DMF ArSeSR was the major product and ArSeSeAr the minor product. The reason for this may be due to the ability of sulfite and thiosulfate to complex with DMF, thus reducing their nucleophilicity in reaction (ii), and also the fact that none of the products was insoluble in DMF thus removing a possible driving force of the reaction.

The products of the DMF reactions, the fact that significantly more ArSeSR was formed in the aqueous reactions at 5°C than at room temperature (Table I) and the intermediacy of ArSeH are all additional evidence that the course of the reaction follows equations (i)–(iv) at least in the case of **1** and probably also in the case of **2**.

This being so, it was felt that a comparison of the rates of formation of ArSeH from **1a** and **2a** would be a valid comparison of their relative reactivities.

Figure 1 shows that, at both concentrations of RSH **2a** reacted more rapidly than **1a** thus correlating directly with their rates of inactivation of the thiol-dependent enzyme papain.[11] Since sulfite is rated a stronger S-base than thiosulfate,[10] it would be expected that reaction (ii) would be faster for **1a** than for **2a**. This suggests that the overall rate of formation of ArSeH must be controlled by reaction (i) and that the difference in reactivity of **1a** and **2a** is due to the superior leaving potential of thiosulfate.

EXPERIMENTAL

Melting points were determined on a Leitz hot stage microscope apparatus and are uncorrected. UV spectra were measured on a Cary 17 spectrophotometer using 1 cm quartz cells. IR spectra were recorded on a Hitachi EPI-G grating spectrophotometer or on a Perkin-Elmer 580B spectrophotometer. ^1H NMR spectra were recorded using a JEOL JNM-FX 100 Fourier Transform spectrometer and chemical shifts (δ) are expressed in parts per million downfield from internal tetramethylsilane. Electron impact mass spectra were recorded on an AEI-MS 12 mass spectrometer at an ionization potential of 70 eV. Chemical ionization mass spectra were obtained on a GEC-AEI MS 902 spectrometer. GC-MS spectra were obtained on a Shimadzu GC-6A gas chromatograph connected through an all glass line with a straight split to an AEI-MS 12 mass spectrometer using a S.C.O.T. OV-101 (30 m × 0.5 mm) column. GLC separations were performed at 160°C on an OV 1 (3% on Chromosorb W) column using a Tracor 560 gas

chromatograph with flame ionization detector and nitrogen carrier gas. Microanalyses were performed on a Perkin-Elmer 240 instrument. TLC was carried out on silica gel (60 F_{254}) plates (Merck, Germany).

Materials. Diselenides,[7,13] potassium Se-(4-nitrobenzyl) selenosulfate,[13] potassium Se-benzyl selenosulfate,[13] potassium Se-(2-nitrophenyl) selenosulfate **1b**,[12,14] potassium Se-(2-nitrophenyl) selenenylthiosulfate **2b**,[12,14] *N*-succinylcysteamine[15] and potassium S-sulfo-*N*-succinylcysteamine[16] were prepared to satisfactory purity by established methods. Potassium Se-(4-nitrophenyl) selenosulfate **1a**, and potassium Se-(4-nitrophenyl) selenenylthiosulfate **2a** were prepared to satisfactory purity by a modification of a known method[14] as follows:

To a stirred, cooled (5°C) solution of 4-nitrobenzeneselenenyl bromide (2.65 g, 9.4 mmol) (from bis-(4-nitrophenyl) diselenide (2 g, 5 mmol) and bromine (0.8 g, 5 mmol)) in dry chloroform (40 ml) was added a cold (5°C) solution of potassium sulfite (1.58 g, 10 mmol) or potassium thiosulfate (1.9 g, 10 mmol) in water (30 ml) all at once. After 30 min the separated crystals were filtered off, washed with chloroform (3 × 15 ml) and extracted with acetone (4 × 10 ml). The residue from the freeze-dried aqueous layer was washed with chloroform (3 × 15 ml) and then extracted with acetone (2 × 10 ml). The acetone extracts were combined and the solvent removed *in vacuo*. **1a** and **2a** were recrystallised from acetone/chloroform to give creamy crystals.

Methods

Reaction of $RSeSO_3K$, **1**, *and* **2** *with N-succinylcysteamine (RSH).* All reactions were carried out at pH 4.6 and room temperature for 30 minutes except those of 4-nitrophenyl salts which were also carried out at 5°C for 20 minutes. Results appear in Table I. A typical protocol follows.

Potassium Se-benzyl selenosulfate with RSH. To a stirred solution of potassium Se-benzyl selenosulfate (249 mg, 0.86 mmol) in pH 4.6 acetate buffer (18 ml, 0.1*M*) was added RSH (152 mg, 0.86 mmol) in pH 4.6 acetate buffer (5 ml, 0.1*M*) at room temperature. Precipitation of S-(benzylseleno)-*N*-succinylcysteamine occurred immediately and after 30 min it was removed by centrifugation, washed successively with water (5 × 10 ml) and light petroleum (40–60°C; 5 × 10 ml) and recrystallised from chloroform/light petroleum (40–60°C) to give white needles mp 114–116°C (196 mg, 66%). The supernatant, after standing for 2 h at room temperature, was extracted with chloroform (3 × 8 ml), the chloroform extracts combined with the light petroleum wash of the precipitate, dried (Na_2SO_4) and the solvent removed *in vacuo* to give dibenzyl diselenide (22 mg, 15%; mp 90–91°C (Lit.[13] mp 92–93°C)).

The supernatant was examined by TLC (silica gel/BuOH : AcOH : H_2O—4 : 1 : 1; location iodine vapour) and revealed the presence of S-sulfo-*N*-succinylcysteamine (R_f 0.34, same as authentic material). S-(benzylseleno)-*N*-succinylcysteamine was identified by: IR(KBr): 693,757 (phenyl); 1555, 1650, 3300 (—CONH); 1692 (—COOH) cm^{-1}; ^1H NMR ($CDCl_3$): 2.58 (m, 6 H, —CO—CH_2—CH_2—COOH, —S—CH_2—); 3.38 (apparent q, 2 H, J = 6.1 Hz, —CH_2—NH—); 4.09 (s, 2 H, Ph—CH_2—Se); 5.97 (broad s, 1 H, —*NH*—CO—); 7.3 (broad s, 5 H, aromatic) ppm. Anal. Calcd. for $C_{13}H_{17}NO_3SSe$: C, 45.09; H, 4.95; N, 4.04. Found: C, 45.29; H, 5.00; N, 4.03.

1a/2a *with RSH. Spectrophotometric determination of products.* To a stirred solution of **1a** or **2a** (5 μmol) in pH 4.6 acetate buffer (15 ml, 0.1*M*) was added a solution of RSH (0.9 mg, 5 μmol) in pH 4.6 acetate buffer (5 ml, 0.1*M*) at room temperature. After 30 min, the reaction mixture, without removal of precipitate, was extracted with chloroform (3 × 25 ml), the combined extracts washed with water (2 × 30 ml), dried (Na_2SO_4) and diluted to 100 ml with chloroform. Concentrations of ArSeSR and ArSeSeAr in the chloroform solution were assayed by measuring absorbance at 270 nm and 340 nm and substituting the values in the following simultaneous equations

$$A' = \varepsilon_1' c_1 + \varepsilon_2' c_2 \quad \text{at 270 nm}$$

$$A'' = \varepsilon_1'' c_1 + \varepsilon_2'' c_2 \quad \text{at 340 nm}$$

A' and A'' are the measured absorbances of the chloroform solution at 270 and 340 nm respectively. ε_1'(7,784) and ε_1''(13,960) are the molar absorbances (1 m^{-1} cm^{-1}) of ArSeSeAr while ε_2'(3,462) and ε_2''(10,162) are those of ArSeSR. c_1 and c_2 are the concentrations (mol/l) of ArSeSeAr and ArSeSR respectively.

1a/2a *with RSH in DMF. Identification of purple intermediate.* To a solution of **1a** or **2a** (0.1 mmol) in DMF (6 ml) was added RSH (18 mg, 0.1 mmol) in DMF (2 ml). A purple colour developed immediately (λ_m 530 nm). Silica gel (1 g) was added followed by a cold ethereal solution of diazomethane, which caused the purple colour to disappear instantaneously, and the reaction mixture was left for 3 h at 0°C

and then overnight at room temperature in a closed vessel. The excess ether and diazomethane were removed *in vacuo* and the reaction mixture submitted to GC-MS analysis.

The major product (m/z (ei) 217; ci (isobutane) 218) was identified as methyl 4-nitrophenyl selenide and hence the purple compound from which it was derived was 4-nitroselenophenol (ArSeH).

1a/2a *with RSH in DMF. Rates of reaction.* To a solution of **1a** or **2a** (0.3 μmol) in DMF (2.8 ml), thermally equilibrated in a spectrophotometer cell at 20°C for 10 min was added a solution of RSH (0.3–3.0 μmol) in DMF (0.2 ml). Reaction velocity was measured by increase in absorbance at 530 nm of ArSeH.

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