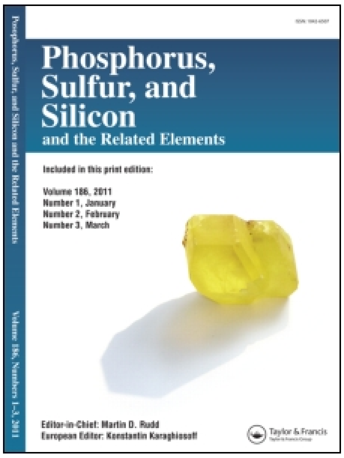

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# COMPARATIVE REACTIVITIES OF ALKYL SELENOSULFATES (SELENO-BUNTE SALTS) AND ALKYL THIOSULFATES (BUNTE SALTS) WITH CYSTEINE

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# COMPARATIVE REACTIVITIES OF ALKYL SELENOSULFATES (SELENO-BUNTE SALTS) AND ALKYL THIOSULFATES (BUNTE SALTS) WITH CYSTEINE

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The comparative reactivities towards cysteine (CySH) of potassium benzyl selenosulfate, potassium *p*-nitrobenzyl selenosulfate and their thiosulfate isologues have been examined in neutral and slightly acidic media. The selenosulfates ($RSeSO_3^-$) reacted more rapidly and gave better yields of products than the thiosulfates ($RSSO_3^-$) at pH 5 and pH 7 and in each series *p*-nitrobenzyl derivatives reacted more completely than the benzyl. The products from both types of compound were the cysteine derivatives RX–SCy and $CyS{-}SO_3^-$ together with the symmetrical diselenides/disulfides RXXR (X = S or Se). In each class of compound the *p*-nitrobenzyl derivatives produced a greater percentage of RXXR, at the expense of RX–SCy. The nature and distribution of products indicated that several competing reactions were proceeding simultaneously. An investigation of mechanistic possibilities by way of model systems suggested that, in the case of the selenosulfates, the initial products were RSe–SCy plus $SO_3^{2-}$ which reacted further to form $RSe^-$ plus $CyS{-}SO_3^-$. The reaction of $RSe^-$ with $RSeSO_3^-$ and RSeSCy produced RSeSeR. By contrast the reaction of $RSSO_3^-$ with CySH may yield RS–SCy plus $SO_3^{2-}$ together with $RS^-$ plus $CyS{-}SO_3^-$ directly, both sets of compounds are then capable of further reaction.

Although $RS^-$ may react with $RSSO_3^-$ to form RSSR, its tendency to react with RSSCy is far less than that of $RSe^-$ to react with RSeSCy.

Although both sulfur and selenium are biologically indispensible, selenium is required at a far lower level.[1] This has led to investigations of comparative reactivities of analogous sulfur and selenium compounds from which it can be generally stated that selenium compounds are more reactive than their sulfur counterparts.[2–6]

As further evidence of this greater reactivity, potassium *p*-nitrobenzyl selenosulfate and potassium benzyl selenosulfate have recently been shown to possess antimicrobial activity[7] and to be very potent inhibitors of the enzyme glutathione reductase.[8] By contrast their thiosulfate isologues had no antimicrobial activity and no inhibitory activity.

As glutathione reductase is known to contain a number of thiol groups, including a potential dithiol at the active site,[9,10] and alkyl thiosulfates are known to react readily with thiols,[11] it seemed possible that the inhibitory activity of the selenosulfates might be due to differences in reactivity with this group.

The aim of the present work was to test this hypothesis by comparing the reactivities of selenosulfates ($RSeSO_3^-$) and their thiosulfate isologues ($RSSO_3^-$) with a model thiol (cysteine, CySH) in neutral and slightly acid media.

## RESULTS AND DISCUSSION

The major products of the reactions of $RXSO_3^-$ with CySH were the derivatives (RX–SCy) together with symmetrical disulfides/diselenides (RXXR) and S-sulfocysteine ($CyS{-}SO_3^-$). The yields of RX–SCy and RXXR, together with the rates of reaction (time for precipitation of RX–SCy) are included in Table I.

In view of changes in rate of reaction and yield and distribution of products with change in R and X, it became apparent that the reactions were only broadly described by the expression

$$RX{-}SO_3^- + CySH \rightarrow RX{-}SCy + RXXR + CyS{-}SO_3^- \tag{i}$$

TABLE 1
Products and % yield from reaction $RX{-}SO_3^- + CySH$

| Reactant $RX{-}SO_3^-$ | pH 5 RXXR | pH 5 RX–SCy | pH 5 Time for precipitation (min) | pH 7 RXXR | pH 7 RXSCy | pH 7 Time for precipitation (sec) |
|---|---|---|---|---|---|---|
| $C_6H_5CH_2{-}S{-}$ | 2 | 42 | 9 | 4 | 68 | 20 |
| $p\text{-}NO_2C_6H_4CH_2{-}S{-}$ | 6 | 52 | 10 | 26 | 54 | 20 |
| $C_6H_5CH_2{-}Se{-}$ | 7 | 76 | *i** | 22 | 69 | *i** |
| $p\text{-}NO_2C_6H_4CH_2{-}Se{-}$ | 20 | 69 | *i** | 94 | 0 | *i** |

*i** = instantaneous.

### *Influence of pH*

Both rate of reaction and distribution of products were subject to pH control. In the arbitrarily selected 30 minute period for most reactions, the selenosulfates reacted significantly faster and to a greater extent (judged from the sum of RXXR and RX–SCy) than the thiosulfates, particularly at pH 5. The degree of reaction of thiosulfates, extended to 60 minutes at pH 5, increased markedly with change in pH, that of the selenosulfates less so.

Formation of RXXR increased with pH in both selenosulfate and thiosulfate reactions. By contrast formation of RX–SCy increased with pH in the thiosulfate series but decreased with the selenosulfates (from 68% to 0% for *p*-nitrobenzyl selenosulfate). However formation of $CySSO_3^-$ (followed semi-quantitatively by TLC) was greater in selenosulfate than in thiosulfate reactions and increased with pH and reaction time.

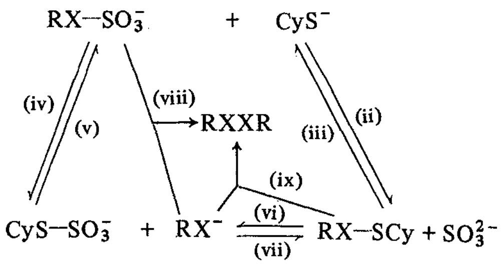
### *Substituent influence*

In both classes of compound the effect of the *p*-nitro group was to increase the extent of reaction at both pH 5 and 7 and to increase the formation of RXXR at the expense of RX–SCy at pH 7.

### *Product distribution*

The products may be grouped into two classes: (a) cysteine derivatives (RX–SCy) and $CyS{-}SO_3^-$) and (b) disulfides and diselenides (RXXR); The complexity of reaction mixtures indicates that several competing reactions may occur simultaneously and formation of both classes of product may now be considered separately.

(a) Cysteine derivatives. Formation of RX–SCy and $CyS{-}SO_3^-$ is possible by the following mechanistic routes



Since the initial reaction leads to either RX–SCy (ii) or $CyS{-}SO_3^-$ (iv) the formation of disulfides and diselenides means that secondary reactions of the type (vi) and (vii) must occur. The extent to which these reactions occur has been tested by model experiments.

(*i*) *Reaction of RXSCy with* $SO_3^{2-}$ The products of the reaction of RX–SCy with $SO_3^{2-}$ are included in Table II and reveal different distributions. In spite of the fact that RX–SCy was completely insoluble in water at pH 7, in each case partial decomposition to $CyS^-$ and $CyS{-}SO_3^-$ occurred. The extent of decomposition was greater for the selenocysteines (complete for *p*-nitrobenzyl selenocysteine ($NO_2C_6H_4CH_2$-

TABLE 2
TLC of reaction products from $RX{-}SCy + SO_3^{2-}$ at pH 7

| Reactant RX–SCy | Products[a] RXXR | Products[a] $RX{-}SO_3^-$ | Products[a] CySH | Products[a] $CyS{-}SO_3^-$ |
|---|---|---|---|---|
| $C_6H_5CH_2{-}S{-}$ | – | – | *t** | *t** |
| $p\text{-}NO_2C_6H_4CH_2{-}S{-}$ | + | ++ | ++ | ++ |
| $C_6H_5CH_2{-}Se{-}$ | + | – | + | + |
| $p\text{-}NO_2C_6H_4CH_2{-}Se{-}$ | +++ | + | +++ | +++ |

[a] located as described–yields assessed semiquantitatively by comparison of spot size.

*t** = trace.

Se–SCy) than for the thiocysteines and greater for the *p*-nitrobenzyl than for the benzyl compounds. Formation of RXXR, greatest for $NO_2C_6H_4CH_2Se$–SCy, was detected in all reactions except that of S-(benzylthio) cysteine ($PhCH_2S$–SCy). RX–$SO_3^-$ was obtained from both *p*-nitrobenzyl derivatives but not from either benzyl derivative. These results indicate the differences in reactivity of the selenium and sulfur compounds, and the substituent influence on product distribution.

Displacement reactions from organosulfur compounds have been interpreted on the basis of relative basicity of the displaced species.[12–14] Thus it has been shown that in nucleophilic attack on unsymmetrical disulfides the least S-basic thiol is displaced as illustrated in the reaction

$$ArS{-}SCH_2CH_3 + CN^- \rightarrow ArS^- + CN{-}S{-}CH_2CH_3 \qquad (x)$$

where Ar = 2,4-dinitrophenyl

Notwithstanding the theoretical expectation of reaction (iii), reaction (vi) must become involved to an extent conditioned by the fate of $RX^-$ (as in the complete disappearance of $NO_2C_6H_4CH_2Se$–SCy to form $(NO_2C_6H_4CH_2Se)_2$). Again because selenols ($RSe^-$) are stronger S- bases than thiols ($RS^-$) it might be predicted that RS–SCy would be more reactive than RSe–SCy and that the *p*-nitrobenzyl derivatives would be the most reactive in each case. However RSeSCy was more reactive than RS–SCy, but the *p*-nitrobenzyl derivatives displayed greater reactivity than the benzyl derivatives in each case. This situation, reflecting the greater ease of displacement of $RSe^-$, may be attributable to a weaker Se–S bond in RSe–SCy compared to the S–S bond in RS–SCy, (as suggested by extrapolation of values for the S–S and Se–Se bonds[15]) combined with greater reactivity of the displaced $RSe^-$ tending to promote reaction (vi), confirmed as mentioned, by the greater yield of diselenide than of disulfide for both benzyl and *p*-nitrobenzyl compounds.

*(ii) Reaction of $RX^-$ with CyS–$SO_3^-$* The products of the reaction of $RX^-$ with CyS–$SO_3^-$, also a heterogeneous reaction due to the water insolubility of RXH, are included in Table III. Again imbalance in the distribution and yields of products are shown. Contrasting with significant amounts of RX–SCy formed from RSH, only traces were formed from RSeH and were only visible in the first 10 minutes of the reactions. $RXSO_3^-$ was only formed from *p*-nitrobenzyl thiol ($NO_2C_6H_4CH_2SH$).

As in the previous reaction RXXR was formed in greater yield from RSeH than from RSH and, in each series, the influence of the nitro group is to be noted with more formed from the *p*-nitrobenzyl than benzyl derivatives. CySH was formed to a much greater extent in RSeH than in RSH reactions and was reduced to only traces with benzyl thiol.

TABLE 3

TLC of reaction products from RXH + $CySSO_3^-$ at pH 7

| Reactant RXH | Products[a] RXXR | RX–SCy | RX–$SO_3^-$ | CySH |
|---|---|---|---|---|
| $C_6H_5CH_2$–S– | + | + | – | *t** |
| *p*-$NO_2C_6H_4CH_2$–S– | ++ | ++ | ++ | + |
| $C_6H_5CH_2$–Se– | ++ | *t** | – | +++ |
| *p*-$NO_2C_6H_4CH_2$–Se– | +++ | *t** | – | ++ |

[a] located as described–yields assessed semiquantitatively by comparison of spot size.

*t** = trace.

A major product from the reaction of $RX^-$ with CyS–$SO_3^-$ should be RX–SCy directly (reaction vii), or indirectly through reaction (v) since this leads to RX–$SO_3^-$ and $CyS^-$ succeeded by rapid interaction to form RXSCy. Yet significant amounts of RXSCy were only formed from RSH. Formation of RXSCy seems more favored from RSH than from RSeH due to the greater stability of the S–S than the Se–S bond.[15]

Superimposed on this reaction is the possibility that reaction of RX–SCy, as formed, with $RX^-$ will lead to RXXR + $CyS^-$ in accordance with the displacement reactivity discussed above.

Semi-quantitative TLC experiments carried out on aliquots at intervals over a 30 minute period indicate that reaction (vii) (or reactions (v) + (ii)) is influenced by subsequent reaction of RXSCy with $RX^-$, where clearly the reactivity of the selenium derivatives is highly favoured. This is confirmed by several factors–the yield of RXXR, the lesser residue of RX–SCy and the greater yield of CySH–all of which fit the sequence:

$$RX^- + CyS{-}SO_3^- \longrightarrow RX{-}SCy + SO_3^{2-} \qquad (xi)$$

$$RX^- + RXSCy \longrightarrow RXXR + CyS^- \qquad (xii)$$

Thus although two routes are possible for the formation of RX–SCy and CyS–$SO_3^-$, route A (reactions (ii) + (vi)) and route B (reactions (iv) + (vii)), it seems likely that the former is predominant, particularly for the selenosulfate.

If route B were predominant, RX–SCy would be expected to increase during the reaction, whereas if route A were predominant CyS–$SO_3^-$ should increase.

Aliquots taken at intervals during reactions at pH 7 showed that CyS–$SO_3^-$ increased significantly with time whereas RX–SCy did not appear to alter.

Again if route B were predominant, a good yield of RX–SCy would be expected from the reaction of $RX^-$ (except $p$-$NO_2C_6H_4CH_2Se^-$) with CyS–$SO_3^-$. However, neither selenol produced more than traces of RX–SCy, again favouring route A.

The reaction of $p$-$NO_2C_6H_4CH_2SeSO_3^-$ with $CyS^-$ at pH 7 instantly gave a white precipitate changing to yellow within 5 minutes, suggesting the immediate formation of RX–SCy and its subsequent decomposition to RXXR. This is considered verified by the complete destruction of $p$-$NO_2C_6H_4CH_2Se$–SCy by $SO_3^{2-}$ at pH 7 (xiii).

$$2\,p\text{-}NO_2C_6H_4CH_2SeSCy + SO_3^{2-} \longrightarrow (p\text{-}NO_2C_6H_4CH_2Se)_2 + CyS{-}SO_3^- + CyS^- \qquad \text{(xiii)}$$

(b) Disulfides/diselenides (RXXR). Formation of disulfides and diselenides remains for discussion with two possible sources to be considered. Route A offers the possibility that $RX^-$, as formed, may participate in two further competing reactions (viii) and (ix)–involving starting material $RXSO_3^-$ or the product RX–SCy respectively. Each leads to RXXR, formation of which would provide a driving force for reaction. Although the possibility of formation by oxidation of $RX^-$ is recognized, nevertheless it seems that greater emphasis must be given to the reactions quoted.

Continuing the dissection to individual steps therefore the reactions of $RX^-$ with $R'XSO_3^-$ and R′X–SCy, leading theoretically to unsymmetrical products RXXR′, were separately investigated.

*(i) Reaction of $RX^-$ with $R'XSO_3^-$* The products of the reaction are included in Table IV. Two features distinguish the participation of RSeH–the greater extent of reaction and appearance of rearranged symmetrical derivatives RSeSeR and R′SeSeR′–from that of RSH–appearance of RSSR′ with only traces of rearranged products. In fact the yield of RSSR′ was only equal to RSeSeR′ which itself represented only about one third of the total product mixture.

*(ii) Reaction of $RX^-$ with R′XSCy* The products of this reaction are included in Table V. Both reactants $RX^-$ and R′X–SCy were insoluble in water giving a completely heterogeneous reaction. There was a marked difference between the abilities of RSeH and RSH to undergo reaction. RSeH reacted quickly and extensively with the selenocysteines (R′SeSCy) to give CySH in good yields together with three diselenides RSeSeR′, RSeSeR and R′SeSeR′, whereas RSH in the same time period hardly reacted with thiocysteines (R′SSCy), giving only traces of CySH and disulfides.

Two factors probably combine to ensure the much greater reaction of the selenols–the relative weakness of the Se–S bond of RSe–SCy compared to the S–S bond of RS–SCy and the greater nucleophilicity of $RSe^-$ compared to $RS^-$.

In summary, it is proposed that the products of reaction (i), arise predominantly from reactions (ii), (vi), (viii) and (ix), with reactions (iv) and (vii) making a contribution to the thiosulfate reactions but little, if any, to the selenosulfate reactions. The greater reactivity of selenosulfates over that of thiosulfates is due to the ability of $RSe^-$, produced in reaction (vi), to undergo further reaction, particularly with RSeSCy. In both series greater reactivity is induced by the $p$-nitro group.

TABLE 4
TLC of reaction products from RXH + R′X–$SO_3^-$ at pH 7

| Reactants | | Products[a] | | |
|---|---|---|---|---|
| RXH | R′X–$SO_3^-$ | RXXR | RXXR′ | R′XXR′ |
| $C_6H_5CH_2$–S– | $p$-$NO_2C_6H_4CH_2$–S– | $t$* | ++ | $t$* |
| $p$-$NO_2C_6H_4CH_2$–S– | $C_6H_5CH_2$–S– | + | ++ | $t$* |
| $C_6H_5CH_2$–Se– | $p$-$NO_2C_6H_4CH_2$–Se– | + | ++ | ++ |
| $p$-$NO_2C_6H_4CH_2$–Se– | $C_6H_5CH_2$–Se– | ++ | ++ | ++ |

[a] located as described–yields assessed semiquantitatively by comparison of spot size.
$t$* = trace.

TABLE 5
TLC of reaction products from RXH + R'X–SCy at pH 7

| Reactants | | Products[a] | | | |
|---|---|---|---|---|---|
| RXH | R'X–SCy | RXXR | RXXR' | R'XXR' | CySH |
| $C_6H_5CH_2$–S– | $p$-$NO_2C_6H_4CH_2$–S– | $t$* | + | + | $t$* |
| $p$-$NO_2C_6H_4CH_2$–S– | $C_6H_5CH_2$–S– | + | – | – | $t$* |
| $C_6H_5CH_2$–Se– | $p$-$NO_2C_6H_4CH_2$–Se– | ++ | ++ | ++ | +++ |
| $p$-$NO_2C_6H_4CH_2$–Se– | $C_6H_5CH_2$–Se– | ++ | ++ | ++ | +++ |

[a] located as described–yields assessed semiquantitatively by comparison of spot size.
$t$* = trace.

## EXPERIMENTAL

### *Materials*

Cysteine hydrochloride (1), benzyl thiol (2) and potassium sulfite (3) were commercial preparations.

Potassium benzyl thiosulfate (4),[16,17] potassium $p$-nitrobenzyl thiosulfate (5),[16,17] potassium benzyl selenosulfate (6),[17] potassium $p$-nitrobenzyl selenosulfate (7),[17] dibenzyl disulfide (8),[16,17] di-$p$-nitrobenzyl disulfide (9),[16,17] dibenzyl diselenide (10),[17] di-$p$-nitrobenzyl diselenide (11),[17] potassium S-sulfocysteine (12),[18] and $p$-nitrobenzyl thiol (13)[19] were prepared to satisfactory purity by standard methods.

### *Benzyl p-nitrobenzyl disulfide (14)*

To a vigorously stirred solution of potassium $p$-nitrobenzyl thiosulfate (1.44 g, 5 mmol), and strontium nitrate (1.42 g, 5 mmol to remove sulfite), in water (10 ml) was added 2 (0.62 g, 5 mmol), for convenience in ether (5 ml), and the mixture heated for 30 minutes at 60°, during which time the ether was removed. After a further 1.5 h at room temperature the mixture was extracted with ether (3 x 30 ml), and the combined extracts filtered, washed with water (2 x 20 ml), dried ($Na_2SO_4$) and the solvent removed under nitrogen.

The resulting yellow oil contained 8, 9 and 14. 14 was separated from 8 and 9 by repeated preparative TLC on silica gel (1 mm thickness) with light petroleum (bp 40/60°) ethyl acetate (4 : 1) as developing solvent. 14 crystallized from 95% ethanol as buff, needle-like crystals (yield 0.28 g, 19%) mp 51–52°, gave a single spot ($R_F$ 0.50) on TLC (system 2) and satisfactory elemental analysis.

### *Benzyl p-nitrobenzyl diselenide (15)*

Preparation of 15 was attempted by the above method but it was found impossible to obtain 15 free from 10 and 11. A band of 15, isolated from a TLC plate, always disproportionated to 10 and 11 on re-chromatography. Nevertheless it was assumed that the band with $R_F$ 0.50, midway between 10 and 11, was 15.

### *Benzyl selenol (16) and p-nitrobenzyl selenol (17)*

These materials were prepared immediately before use by hypophosphorous acid reduction of the appropriate diselenide.[20] A typical protocol follows.

To a solution of 10 (0.21 g, 0.63 mmol) in 95% ethanol (10 ml) was added 60% hypophosphorous acid (5 ml) and the mixture refluxed under nitrogen until colorless (approx. 15 minutes). Water (10 ml) was added and 16 extracted with ether (2 x 10 ml). The combined extracts were washed with deoxygenated water (2 x 10 ml), dried ($Na_2SO_4$), and diluted to 25 ml with more ether.

The selenol content of the ether extract was assayed iodometrically and the appropriate amount used in each of the reactions described below. In a similar manner ethereal solutions of 2, 13 and 17 were assayed iodometrically immediately before use.

### *Iodometric Assay of RXH (2, 13, 16, 17)*

Conditions for the assay of RSH and RSeH were chosen to ensure that oxidation of 16 did not proceed beyond 10 (the most labile RXXR). The aim of the assay was to maintain an approximate stoichiometry of 1 : 1 in RXH reactions and for this purpose the assay was sufficiently accurate.

The protocol was as follows: To carbon tetrachloride (15 ml) was added an aliquot of the ether solution of RXH (4–5 ml). Ether was removed by warming to about 40°, (2–3 minutes) and to the cooled solution (ice bath) was added 0.004 M iodine (20 ml). After standing at 0° for several minutes (2' for RSeH; 3' for RSH) excess iodine was titrated with 0.008 M sodium thiosulfate solution.

### *Thin Layer Chromatography*

The major components for all reaction mixtures were separated on silica gel (60 $F_{254}$, Merck) using $n$-butanol/acetic acid/water, (4 : 1 : 1) as developing solvent (system 1).

The following range of $R_F$ values, which includes all sulfur and selenium derivatives, covers each class of compound: RXXR/0.89; RX–$SO_3^-$/0.60–0.65; RX–SCy/0.48–0.52; CySH/0.25; $CySSO_3^-$/0.14; $(CyS)_2$/0.05.

RXXR' was separated from RXXR and R'XXR' on silica gel (60 $F_{254}$, Merck) using light petroleum (bp 40–60°)/ethyl acetate, 4 : 1, as developing solvent (system 2). $R_F$ values (all members of class) were: RXXR/0.62–0.67; RXXR'/0.50; R'XXR'/0.24–0.27; (where R = benzyl; R' = $p$-nitrobenzyl).

Products were visualized under uv light (RXXR, RX–$SO_3^-$, RX–SCy), by ninhydrin reaction (RX–SCy, CySH, CyS–$SO_3^-$, $(CyS)_2$), Folin-Ciocalteu reagent (RXH, CySH), Nakamura-Tamura (NT) fuchsin reagent (RX–$SO_3^-$ and CyS–$SO_3^-$).[21]

### *Reactions of CySH with RX–SO$_3^-$* (1 + (4–7))

All these reactions were allowed to proceed at room temperature for 30 minutes except those at pH 5 where the time was increased to 60 minutes. Major products of the reactions are shown in Table 1.

### *1 + 4 (pH 7)*

To 4 (87 mg, 0.35 mmol) in pH 7 phosphate buffer (5 ml) was added 1 (55 mg, 0.35 mmol) in water (2 ml, adjusted to pH 7). Precipitation soon began (Table 1) and after standing for 30 minutes, the reaction mixture, without filtration, was extracted with ether (4 x 2 ml), the ether extracts combined and solvent removed.

The reaction mixture was then centrifuged, supernatant decanted, the precipitate washed with water (4 x 5 ml), ethanol (2 x 4 ml) and ether (2 x 4 ml), dried *in vacuo* and weighed. Aliquots of the ether extract, and of the precipitate (dissolved in M HCl) together with aliquots of a duplicate, smaller scale reaction mixture taken at intervals (0, 2, 5, 10, 15 and 30 minutes) were chromatographed using system 1.

The products of the reaction were 8 (1.6 mg, 4%–ether extract), S-(benzylthio) cysteine ((18), 59 mg, 68%–precipitate) and 12.

8 was identified by mp 68.5–70° (Lit.[17] 71°) and 12 by its $R_F$ 0.14, system 1, (same as authentic material), ninhydrin reaction (α-amino group) and NT fuchsin reagent (thiosulfate group). 18 was identified by elemental analysis, $R_F$ 0.52 (system 1) located under uv light, ninhydrin reaction (α-amino group), solubility in acid and alkali with degradation in latter and spectroscopic properties: ir (KBr) 692,753 (benzyl); 1498 (NH$_3^+$); 1414, 1587 (CO$_2^-$) cm$^{-1}$; nmr (DSS ref. in 0.1 M $H_2SO_4$ in $D_2O$); δ 2.84 (d, 2, J = 6 Hz β-methylene); 3.99 (s, 2, benzyl methylene); 4.19 (t, 1, J = 5.8 Hz, α-methyne); 7.42 (s, 5, benzyl) ppm.

### *1 + 5 (pH 7)*

In a similar manner 5 (101 mg, 0.35 mmol) in pH 7 phosphate buffer (5 ml) was reacted with 1 (55 mg, 0.35 mmol) in water (2 ml, adjusted to pH 7). Products of reaction were 9 (15.5 mg, 26%), S-(*p*-nitrobenzylthio) cysteine ((19), 54 mg 54%) and 12. 9 was identified by mp 123–125° (Lit.[17] 126.5°) and 12 as previously. 19 was identified by elemental analysis, $R_F$ 0.48 (system 1) located under uv light, ninhydrin reaction (α-amino group) solubility in acid and alkali with degradation in latter and spectroscopic properties: ir (KBr) 710 (benzyl); 1565 (NH$_3^+$); 1345, 1525 ($NO_2$); 1400, 1600 (CO$_2^-$) cm$^{-1}$; nmr (DSS ref. in 0.1 M $H_2SO_4$ in $D_2O$); δ 2.86 (d, 2, *J* = 6Hz β-methylene); 4.11 (s, 2-benzyl methylene); 4.29 (t, 1, *J* = 5.6Hz–α-methyne); 7.64 (d, 2, *J* = 8.7 Hz–benzyl $H_2$); 8.21 (d, 2, *J* = 8.7 Hz–benzyl $H_3$) ppm.

### *1 + 6 (pH 7)*

In a similar manner 6 (88 mg, 0.31 mmol) in pH 7 phosphate buffer (5 ml) was reacted with 1 (47 mg, 0.30 mmol) in water (2 ml, adjusted to pH 7). Products of reaction were 10 (11.3 mg, 22%), S-(benzylseleno) cysteine ((20) 60.7 mg 69%) and 12. 10 was identified by mp 89–90° (Lit.[17] 92–93°) and 12 as previously. 20 was identified by elemental analysis, $R_F$ 0.52 (system 1) located under uv light, ninhydrin reaction (α-amino group), solubility in acid and alkali with degradation in latter and spectroscopic properties: ir (KBr) 692,756 (benzyl); 1490 (NH$_3^+$); 1412, 1585 (CO$_2^-$) cm$^{-1}$; nmr (DSS ref. in 0.1 M $H_2SO_4$ in $D_2O$); δ 2.92 (d, 2, *J* = 6Hz-β-methylene); 4.16 (t, 1, *J* = 6Hz-α-methyne); 4.21 (s, 2-benzyl methylene); 7.41 (s, 5-benzyl) ppm.

### *1 + 7 (pH 5)*

In a similar manner 7 (70 mg, 0.21 mmol) in pH 5 phthalate buffer (3.5 ml) was reacted with 1 (33 mg, 0.21 mmol) in water (2 ml, adjusted to pH 5). Products of reaction were 11 (9.1 mg, 20%), S-(*p*-nitrobenzylseleno) cysteine ((21), 48 mg, 69%) and 12. 11 was identified by mp 105–107.5° (Lit.[17] 107.5°) and 12 as previously. 21 was identified by elemental analysis, $R_F$ 0.49 (system 1) located under uv light, ninhydrin reaction (α-amino group), solubility in acid and alkali with degradation in latter and spectroscopic properties: ir (KBr) 696 (benzyl); 1558 NH$_3^+$); 1345, 1520 ($NO_2$); 1400, 1600 (CO$_2^-$) cm$^{-1}$; nmr (DSS ref. in 0.1 M $H_2SO_4$ in $D_2O$; δ 2.88 (d, 2, *J* = 6.8 Hz-β-methylene); 4.21 (t, 1, *J* = 4.5 Hz-α-methyne); 4.28 (s, 2, benzyl methylene); 7.61 (d, 2, *J* = 8.3 Hz benzyl $H_2$); 8.21 (d, 2, *J* = 8.3 Hz benzyl $H_3$) ppm.

### *Reactions of SO$_3^{2-}$ with RX–SCy (3+(18–21))*

Results for each of these reactions performed at room temperature, as described below, appear in Table II.

### *3 + 20*

To solid 20 (9.6 mg, 0.03 mmol) was added 3 (5 mg, 0.03 mmol) in pH 7 phosphate buffer (1 ml) and the reaction allowed to proceed under nitrogen for 30 minutes. Duplicate aliquots (10 μl) of the reaction mixture (taken after 0, 2, 5, 10, 15 and 30 minutes) were separately chromatographed (System 1). Products were visualized and identified in uv light, by the ninhydrin reaction (Plate 1), and Folin-Ciocalteu reagent (Plate 2).

### *Reactions of CyS–SO$_3^-$ with RXH (12+(2, 13, 16, 17))*

Results for each of these reactions performed at room temperature, as described below, appear in Table III.

### *12 + 16*

To a stirred solution of 12 (12 mg, 0.05 mmol) in pH 7 phosphate buffer (1 ml) under nitrogen was added 16 (0.05 mmol) in ether (1–3 ml) removed in the nitrogen stream within 5 minutes. Duplicate aliquots (10 μl) of the reaction mixture (after 0, 2, 5, 10, 15 and 30 minutes) were separately chromatographed (system 1). Products were visualized and identified under uv light, by the ninhydrin reaction (Plate 1), and by reaction with the Folin-Ciocalteu reagent (Plate 2).

Presence of 16 in the reaction mixture was verified by taking an additional aliquot (10 μl) at each time interval and reacting with 7 (100 μl in pH 7 buffer) to give 15 identified by TLC (System 2).

### *Reactions of RXH (2, 13, 16, 17) with R′X–SO$_3^-$ (4–7)*

Results for each of these reactions performed at room temperature, as described below, appear in Table IV.

### *16 + 7*

To a stirred solution of 7 (14.5 mg, 0.05 mmol) in pH 7 phosphate buffer (1 ml) under nitrogen was added 16 (0.05 mmol) in ether (1-3 ml), removed within 2 minutes in the nitrogen stream.

Aliquots (10 μl) of the reaction mixture (after 0, 2, 5, 10, 15 and 30 minutes) were chromatographed (system 2) and products located under uv light.

### *Reactions of RXH (2, 13, 16, 17) with R'X–SCy (18-21)*

Results for each of these reactions, performed at room temperature, as described below, appear in Table V.

### *16 + 21*

To a stirred mixture of 21 (16.9 mg, 0.05 mmol) in pH 7 phosphate buffer (1 ml) under nitrogen was added 16 (0.05 mmol) in ether (1-3 ml), removed as described above.

Duplicate aliquots (10 μl) of the reaction mixture (after 0, 2, 5, 10, 15 and 30 minutes) were separately chromatographed (System 1). Products were visualized and identified in uv light, by the ninhydrin reaction (Plate 1) and by reaction with the Folin-Ciocalteu reagent (Plate 2).

Presence of 16 in the reaction mixture was verified using an additional aliquot as described above.

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