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SELENOGLUCOSINOLATES IN NATURE: FACT OR MYTH?

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IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Stanleya pinnata, Lepidium sativum; Armoracia lapathifolia*; Brassicaceae; biosynthesis; glucosinolates; selenoglucosinolates.

Abstract—The glucosinolates in *Stanleya pinnata* have been identified. 3-Butenyl- and 2-hydroxy-3-butenylglucosinolate are the major constituents, yielding 3-butenyl isothiocyanate and 5-vinyl-2-oxazolidinethione on enzymatic hydrolysis. The selenium analogues of these were synthesized. In the selenium-tolerant species *S. pinnata*, grown in water-culture in high selenium concentrations (100 ppm), trace amounts of 3-butenylselenoglucosinolate were shown to be present. This compound could not be detected in plants raised in lower, more normal selenium concentrations. No selenoglucosinolate was found in *Lepidium sativum* (garden cress). A previous report of the presence of selenium in the sinigrin fraction of *Armoracia lapathifolia* (horseradish) could not be substantiated.

INTRODUCTION

Nearly one hundred glucosinolates with the generic formula **1** have been isolated from higher plants. Their natural distribution, chemical structure, biosynthesis, catabolism, biological significance, and economic importance have been repeatedly reviewed [1, 2 and references therein]. The established parallelism in metabolic pathways of sulphur and selenium, reflected, *inter alia*, in the occurrence in higher plants of several selenium isologues of sulphur amino acids [3], prompted the present search for selenoglucosinolates (**2**)* as natural products. In 1974 Stewart *et al.* [5] reported that sinigrin (**1a**), isolated from horseradish plants to which $Na_2{}^{75}SeO_4$ had been administered, contained selenium. On this basis the authors suggested that 'selenosinigrin' had been synthesized in the plant. No other studies of the natural occurrence of selenoglucosinolates seem to be on record.

Prior to our search for naturally occurring selenoglucosinolates, we needed information about their stability, chromatographic behaviour and spectroscopic properties. Hence, two characteristic representatives of this previously unknown class of compounds were synthesized and the required data collected [6]. Thus prepared we set out to look for selenoglucosinolates in glucosinolate-producing plants raised on high-selenium substrates or offered ^{75}Se in the form of sodium selenite or selenate.

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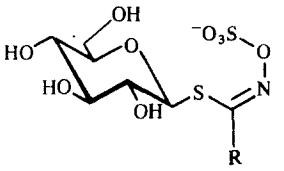
*The term 'selenoglucosinolate' is here introduced as a semi-systematic name for molecular species possessing the general structure (**2**). It is coined by analogy to internationally recommended names such as selenocysteine, selenoglutathione etc. [4]. i.e. by adding the prefix 'seleno' to the name of a naturally occurring sulphur compound with an accepted trivial name, here 'glucosinolate'.

RESULTS AND DISCUSSION

Glucosinolates (**1**) are, as far as we know, ubiquitously present in members of the large family Brassicaceae. Among its *ca* 350 genera, one, the North American *Stanleya*, stands out as a collection of six to eight species, reputed as selenium indicators and accumulators, highly tolerant of selenium [7]. With this in mind we reasoned that *Stanleya pinnata* (Pursh) Britton might be a suitable taxon in which to look for selenoglucosinolates. Accordingly, we first established the glucosinolate pattern in seeds and vegetative parts of this species, collected in the Mojave desert in the southern part of California.

The glucosinolate fractions, isolated by ion exchange chromatography in the usual way, were subjected to persilylation and GC/CIMS analysis as previously described [8]. Two major (**1b** and **1c**) and three minor glucosinolates (**1d**, **1e** and **1f**) were unequivocally identified. Enzymic hydrolysis, catalysed by a myrosinase preparation, converted the glucosinolates into a mixture of isothiocyanates (**3b**, **3d**, **3e**, **3f**) and 5-vinyl-2-oxazolidinethiones (**4a** and/or **4b**). The former were identified by GC/MS, the latter by TLC comparison with authentic specimens. The optical rotation of a sample of **4**, derived from seed material, revealed its identity as a mixture of the (*R*)-(**4a**) and (*S*)-enantiomer (**4b**), deriving from 2(*S*)- and 2(*R*)-hydroxy-3-butenylglucosinolate, respectively. HPLC-analysis of the corresponding desulphoglucosinolates by a standard method [9] showed that the 2(*R*)-glucosinolate predominates in the vegetative parts, the 2(*S*)-epimer in the seeds. The concomitant occurrence of the two diastereomers within the same species is not without precedent [10]. Quantitative data were obtained by HPLC analysis of the genuine glucosinolates [9] (Table 1). As shown in Table 1, a number of glucosinolates, all previously known from other sources, were found as trace constituents in addition to **1b–1f**.

Assuming that selenoglucosinolates, if at all present in *Stanleya*, would be qualitatively and quantitatively simi-



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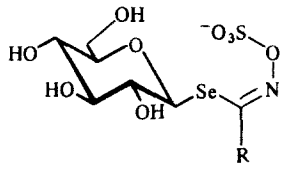
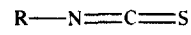


2

1a R = $CH_2CH{:}CH_2$
1b R = $[CH_2]_2CH{:}CH_2$
1c R = $CH_2CH(OH)CH{:}CH_2$
1d R = $[CH_2]_3CH{:}CH_2$
1e R = $CH[Me]_2$
1f R = CH(Me)Et



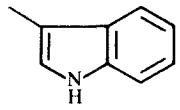
3

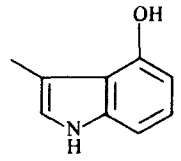
3b R = $[CH_2]_2CH{:}CH_2$
3c R = $CH_2CH(OH)CH{:}CH_2$
3d R = $[CH_2]_3CH{:}CH_2$
3e R = $CH[Me]_2$
3f R = CH(Me)Et

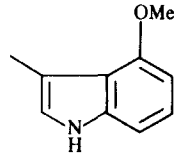
Table 1. Glucosinolates in *S. pinnata*, determined by HPLC analysis

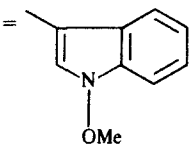
Glucosinolates (**1**)* (side-chain R)	Glucosinolate (μmol/g) Seeds	Freeze-dried, vegetative parts
(*R*)-$CH_2CH(OH)CH{:}CH_2$	15.9	15.9
(*S*)-$CH_2CH(OH)CH{:}CH_2$	28.9	6.0
$CH[Me]_2$ (**1e**)	0.2	0.2
$[CH_2]_2CH{:}CH_2$ (**1b**)	26.8	17.6
CH(Me)Et (**1f**)	1.3	3.7
$[CH_2]_3CH{:}CH_2$ (**1d**)	0.4	0.4
$CH_2CH(Me)Et$	0.2	1.1
$[CH_2]_2Ph$	0.3	tr
$CH_2In(A)$†	0.05	tr
$CH_2In(B)$‡	tr	—
CH_2In (C)§	—	tr
CH_2In (D)‖	0.1	—

*Identified by co-chromatography with authentic specimens.

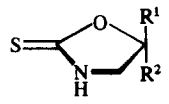
†In(A)= 

‡In(B)= 

§In(C) 

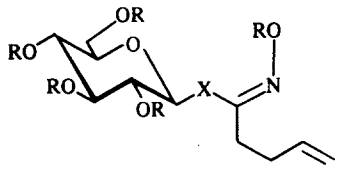
‖In(D)= 

lar to the glucosinolates just described, a need arose for reference samples of desulpho-3-butenylselenoglucosinolate (**5c**), 3-butenyl isoselenocyanate (**6**), and (*R,S*)-5-vinyl-2-oxazolidineselone (**7**). Our previous synthesis [11] of 3-butenylglucosinolate (**1b**) has been improved by generating 4-pentenohydroxamoyl chloride (**9**) from 4-pentenaldoxime (**8**) rather than from 5-nitro-1-pentene. Substituting selenium for sulphur in the glucose



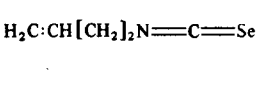
4

4a R^1 = H, R^2 = $CH{:}CH_2$
4b R^1 = $CH{:}CH_2$, R^2 = H



5

5a X = S, R = H
5b X = S, R = Ac
5c X = Se, R = H
5d X = Se, R = Ac



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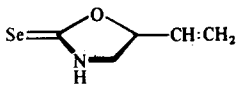

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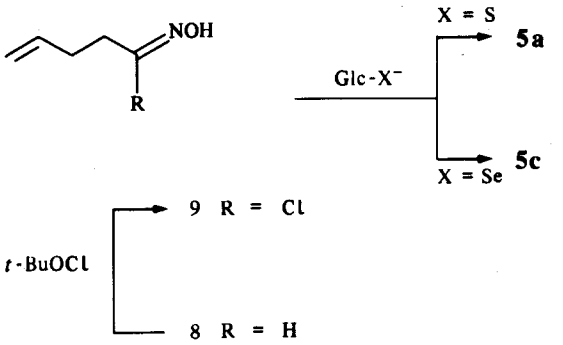

moiety resulted in the desired desulphoselenoglucosinolate (**5c**). In both series the reaction products were purified by chromatography of their pentaacetates, (**5b**) and (**5d**), followed by deacetylation. On HPLC analysis, **5a** and **5c** could be separated to base line, the selenium isologue possessing a slightly higher elution time. The GC/MS method for analysing glucosinolates [8] proved applicable also to persilylated desulphoselenoglucosinolates. Thus, silylated **2** (R = Me) and **5c** on CIMS (*iso*-butane) showed $[M+1]^+$-ions of moderate intensity whereas the spectrum of **2** ($R = PhCH_2$), for unknown reasons, came out much weaker. We decided to focus most of our search for selenoglucosinolates on **3b**, the selenium isologue of the major glucosinolate in *S. pinnata*, and ascertained that its silylated desulpho-derivative (**5c**) on GC had a R_t intermediate between those of the corresponding desulpho-derivatives of **1b** and **1d**. In our previous paper [6], we demonstrated that the plant myrosinase catalysing the hydrolysis of glucosinolates, and hence the formation of isothiocyanates, just as easily hydrolized selenoglucosinolates to isoselenocyanates. For the synthesis of 3-butenyl isoselenocyanate (**6**), a possible hydrolysis product from *S. pinnata*, we adopted the method developed by Henriksen and Ehrbar [12] for the preparation of alkyl isoselenocyanates. The light-sensitive **6** was characterized as *N*-(3-butenyl)-1-piperidinecarboselenoamide formed upon reaction with piperidine. The same procedure was applicable to the synthesis of (±)-5-vinyl-2-oxazolidineselone (**7**) from 1-amino-3-buten-2-ol and to other 2-oxazolidineselones as well (to be published).

Twenty-day-old plants of *S. pinnata*, grown from seedlings in a standard nutrient solution [13], were transferred, in separate experiments, to similar solutions supplemented with 10, 100, and 1000 ppm of selenium in the form of Na_2SeO_3. Whereas plants kept in the exorbitantly high 1000 ppm concentration, showed withering of leaves after four days, no difference in appearance was noted for plants grown for three weeks in nutrients with 100, 10 and 0 ppm of selenium. The glucosinolate fraction from plants exposed to 100 ppm of selenium for three weeks was isolated and subjected to GC/CIMS analysis as before. The glucosinolate profile was virtually identical to that of plants collected in the wild. Only when highly concentrated solutions were employed and the fractions were monitored through a 'window' at *m/z* 693–707 did a minute peak appear in the 100 ppm sample, with a R_t equal to that of an authentic specimen of persilylated desulpho-3-butenylselenoglucosinolate (**5c**), known to produce an $[M+1]^+$-ion at *m/z* 702 (based on ^{80}Se) (Fig. 1). Unfortunately, the quantity of the unknown component was insufficient for a

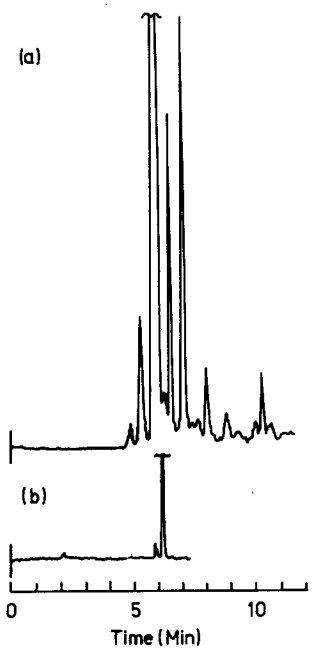

Fig. 1 (a) GC profile, monitored through a MS 'window' at *m/z* 693–707, of persilylated desulphoglucosinolates from *S. pinnata* plants, grown at a selenium concentration of 100 ppm; (b) GC of synthetic, persilylated desulpho-3-butenylselenoglucosinolate (**5c**).

useful mass spectrum to be recorded. Hence, recourse was taken to a GC/EIMS study of the isothiocyanate fraction arising from myrosinase-catalysed hydrolysis of the glucosinolates in *S. pinnata* plants grown in 100 ppm of selenium. At maximum amplification and monitoring through a 'window' at *m/z* 157–162 a GC peak appeared, with a R_t identical to that of synthetic 3-butenyl isoselenocyanate (**6**). Its mass spectrum, as observed in the oscilloscope, exhibited an isotope pattern that was nearly identical to that of authentic **6** in the molecular ion region; a slight deviation from the theoretical selenium isotope pattern occurred in the print-out of the spectrum (Fig. 2). It was concluded that the ratio of 3-butenyl isothiocyanate (**3b**) to 3-butenyl isoselenocyanate (**6**) exceeded 2000:1, a remarkable fact in view of the nutrient concentrations employed: 80 ppm of sulphur, and 100 ppm of selenium. The analysis was repeated with identical results. Plants, collected in the wild, were devoid of the selenium-containing component in their isothiocyanate fraction. Based on the detection limit for **6** it can be estimated that the ratio of **6** to the analogous isothiocyanate (**3b**), and therefore of **2** ($R = [CH_2]_2CH{:}CH_2$) to **1b**, whence they derive, does not exceed 1:50 000.

Additional information was sought by incorporation studies with $Na_2{}^{75}SeO_3$. Excised leaves of one-year-old plants of *S. pinnata* were allowed to absorb a nutrient solution containing a total of 5.6×10^6 Bq of $Na_2{}^{75}SeO_3$ at a concentration of 5 ppm of selenium. After 72 hr, the glucosinolate fraction was isolated and part of it chromatographed on paper, followed by radioautography. No significant activity associated with the glucosinolates could be detected. Another part of the glucosinolates was subjected to enzymatic hydrolysis. The resulting mixture of isothiocyanates was separated from the alkali-soluble 2-oxazolidinethiones. The former were converted into thioureas upon reaction with ammonia. Paper chromatography of both the thiourea mixture and the oxazolidinethiones along with authentic specimens, followed by radioautography and dilution control in the case of the latter, provided no indication of radiolabel in the enzymatic hydrolysis products and hence in the glucosinolate fraction.

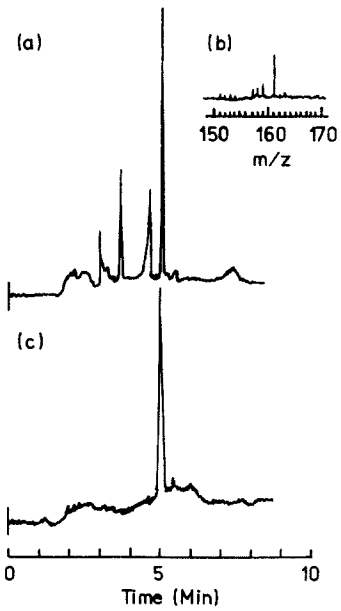

Fig. 2 (a) GC profile, monitored through a MS window at *m/z* 157–162, of the isothiocyanate fraction resulting from enzymatic hydrolysis of the glucosinolates in *S. pinnata* plants, grown at a selenium concentration of 100 ppm. (b) EIMS of the major peak in the profile shown in (a). (c) GC of synthetic 3-butenyl isoselenocyanate (**6**).

The non-incorporation of selenium into selenoglucosinolates in *S. pinnata*, grown at low or moderate selenium concentrations, made us suspect that the observed failure might be related to the known ability of selenium-accumulating taxa to direct selenium away from the protein selenoamino acids (selenocysteine and selenomethionine) and into non-protein selenoamino acids [14]. Since cysteine is believed to be the source of the thioglucosidic sulphur atom in the biosynthesis of glucosinolates, we decided to study the incorporation of $Na_2{}^{75}SeO_3$ in a non-accumulating species of the Brassicaceae. For this purpose we selected *Lepidium sativum* (garden cress), a traditional source of benzylglucosinolate. The administration of radiolabel and the analyses were performed as described for *S. pinnata*. No significant incorporation was observed.

In view of these results, we decided to repeat the experiment of Stewart *et al.* [5] who, in two experiments, reported incorporation in *Armoracia lapathifolia* (horseradish) of $Na_2{}^{75}SeO_4$ into the sinigrin (**1a**) fraction to the extent of 1.7 and 5.4%. Closely following their directions, except for using a 30-fold increase in the activity of the radioprecursor, we found no significant incorporation (< 0.009%) of radioactivity into or near the sinigrin area on paper chromatograms. We have no explanation to offer for the discrepancy other than a possible, unnoticed contamination of the sinigrin, isolated by the Canadian authors, with the radioactive precursor ($^{75}SeO_4^{2-}$) or, possibly, its reduction product ($^{75}SeO_3^{2-}$).

In summary, selenium-containing glucosinolates do not seem to be congeners of glucosinolates in higher plants; a finding in line with a recent dictum: 'a unique biochemistry exists for selenium; this element can no longer be considered merely in terms of its equivalence to sulphur' [14]. An apparent exception seems to be the selenium-accumulating *S. pinnata*. Even when selenoglucosinolates were not detected in this species when grown at low or moderate selenium concentrations, evidence has been presented above for the production of trace amounts of the selenoglucosinolate derived from (**5c**) in plants kept at a selenium concentration as high as 100 ppm. One may speculate that a high selenium uptake under these conditions may force open a narrow passage in a biosynthetic pathway normally inaccessible to selenium substrates. Once again, we are faced with the difficult question of defining a 'natural product'.

EXPERIMENTAL

Plant material. Seeds and aerial parts of *Stanleya pinnata* were collected in the summer of 1986 from an area near Garlock, northeastern Kern County, California by L. Maynard Moe. A voucher specimen is on deposit in the Botanical Museum of the University of Copenhagen.

Methods and materials. Mps: uncorr; TLC: Merck $60F_{254}$ silica gel sheets; PC: Whatman paper no. 1 unless otherwise indicated. Detection of glucosinolates, selenoglucosinolates, 2-

oxazolidinethiones and 2-oxazolidineselones was with $AgNO_3$/aq. NH_3 and heating; thioureas and selenoureas were made visible on spraying with Grote's reagent [15]. The myrosinase soln was prepared according to the lit. [16]. HPLC analyses of glucosinolates and desulphoglucosinolates were carried out according to published standard methods [9]. The ^{75}Se activity was measured using a NaI-well-crystal (background *ca* 600 cpm). In dilution experiments measurements were made at the 13.6 keV emission peak.

Isolation and analysis of compounds. Ground, air-dried *S. pinnata* plants (leaves, stems, inflorescenses) (100 g) were thoroughly extracted with 90% MeOH. The extract was freed of solvent, washed with Et_2O, dissolved in H_2O (1 l), and passed through an Amberlite IR-45 ion-exchange column (25 × 3 cm) in the Cl^--form. Elution was performed by first passing 0.1 M K_2SO_4 through the column until most Cl^- had been displaced, followed by 0.1 M KOH until the breakthrough of base. The last eluate was concd *in vacuo* to near dryness. The glucosinolate fraction was extracted from inorganic salts by boiling 80% EtOH. Evaporation left a hygroscopic solid consisting essentially of a mixture of glucosinolates; GC/CIMS-analysis of the mixture, according to our published method [8], showed the presence of **1e**, **1f**, **1b**, **1d**, and **1c**, in the order of increasing R_t, with **1b** as the predominant species. On PC (*n*-BuOH–pyridine–H_2O, 6:4:3), the glucosinolate pattern of a seed extract did not deviate from that of the aerial parts. Ground, air-dried parts of *S. pinnata* (100 g) were blended with hot CCl_4 (250 ml × 2). The dry plant powder was suspended in Na–Pi buffer (pH 6.5) with 0.0025 M ascorbic acid (0.5 l), and myrosinase-soln (10 ml) was added. After 4 hr at 20°, the mixture was steam-distilled; saturation of the distillate with salt, Et_2O extraction, and careful concn of the Et_2O extract gave a residue that on GC/EIMS, by our previously described technique [17], disclosed the presence of **3e**, **3f**, **3b**, and **3d**, with **3b** greatly dominating. Extraction with hot 90% MeOH of CCl_4-defatted seeds of *S. pinnata* (6 g) gave a residue which was subjected to myrosinase hydrolysis as just described. Extraction with CH_2Cl_2 gave a yellow oil (100 mg) which, after flash chromatography on silica gel in EtOAc–hexane (1:1), afforded an oily specimen of 5-vinyl-2-oxazolidinethione (24 mg), $[\alpha]_D^{20} + 13°$ (MeOH; *c* 2.1) (lit. [18] $[\alpha]_D^{20} - 72.8°$ and $+70.5°$ for the two enantiomers, later shown [19] to represent the (*S*)–(**4b**) and (*R*)-enantiomer (**4a**), respectively. On seeding with (±)-(4), the racemate crystallized from the oily mixture, mp 60–62° (lit [18] mp 64–65°).

Synthesis of reference compounds. Desulpho-3-butenylselenoglucosinolate (**5c**). The oily 4-pentenaldoxime (**8**, a *ca* 1:1 mixture of *Z*- and *E*-isomers) (150 mg), prepared from 4-pentenal by a standard technique [20], was dissolved in MeOH (5 ml) and cooled to −78°. *t*-Butyl hypochlorite (0.18 ml) was added and the bright blue soln was allowed to come to 0° when the colour disappeared as **9** was formed. $NaBH_4$ was added in small portions at 0° to an Ar-covered, stirred soln of the yellow di(1-β-D-glucopyranosyl) diselenide (120 mg) [21] in H_2O (3 ml) until colourless. The above soln of **9** was slowly injected into the glucoselenide-soln and stirring continued for 30 min. The acidic soln was neutralized with 5% $NaHCO_3$ and taken to dryness; the residue was redissolved in H_2O, the soln was repeatedly extracted with Et_2O, and the aq. soln concd to a syrup which was extracted with hot EtOH. Filtration and evapn gave a residue which was purified by flash chromatography on silica gel (CH_2Cl_2–EtOH, 5:1). The oily product (**5c**) was acetylated (Ac_2O–pyridine) and subjected to flash chromatography on silica gel (EtOAc–hexane, 1:1). The fastest moving and major component proved to be the pentaacetate (**5d**) (94 mg, 34%). Mp 136–137° (from EtOAc–Et_2O–hexane). ^1H NMR (90 MHz, $CDCl_3$); δ 2.02, 2.04, 2.06 (12H, *s*, Ac), 2.17 (3H, *s*, NOAc), 2.30–3.00 (4H, *m*, CH_2CH_2), 3.57–3.87 (1H, *m*, H-5), 4.02–4.28 (2H, *m*, H-6α and H-6β), 4.89–5.43 (6H, *m*, HC = C<u>H</u>$_2$, H-1, H-2, H-3, H-4), 5.62–6.18 (1H, *m*, <u>H</u>C=CH$_2$). Deacetylation of **5d** with NH_3–MeOH afforded the homogeneous desulphoselenoglucosinolate (**5c**) as a hygroscopic syrup. CIMS (*iso*-butane) of TMSi-derivative [8], *m/z* (based on ^{80}Se) (rel. int.): 702 $[M+1]^+$ (2.3), 686 $[M-15]^+$ (0.1), 612 $[M-89]^+$ (0.1), 596 $[M-15-90]^+$ (0.1), 361 (100), 252 $[HSe(:NHOTMSi)C_4H_7]^+$ (2.3), 250 $[Se(:NOTMSi)C_4H_7]^+$ (2.5), 170 $[TMSiON:CC_4H_7]^+$ (2.5), 82 $[HN:CC_4H_7]^+$ (31).

3-Butenyl isoselenocyanate (**6**). 3-Butenylamine (0.5 g) was added to a stirred soln of $HgCl_2$ (1.9 g) in MeCN (28 ml). The colourless ppt. was filtered off and resuspended in CH_2Cl_2 (30 ml). CSe_2(0.45 ml) was added with a syringe to the Ar-covered suspension, followed by Et_3N (1.95 ml). The mixture turned black after a few sec and was stirred for 20 min. HgSe was filtered off through a pad of Celite, and the filtrate was concd to *ca* 100 ml, washed with brine, dried, and freed of solvent. A hexane soln of the residue (15 ml) was filtered through a pad of silica gel (1 × 3 cm). Removal of the solvent left the isoselenocyanate **6** as an orange liquid (0.8 g, 70%). ^1H NMR (90 MHz, $CDCl_3$); δ 2.48 (2H, *dt*, *J* = 6.5 Hz, *J* = 6.5 Hz, H-2), 3.67 (2H, *t*, *J* = 6.5 Hz, H-1), 5.06–5.36 (2H, *m*, H-4), 5.54–6.07 (1H, *m*, H-3). EIMS, 70 eV, *m/z* (rel. int.): 161 $[M^+$, based on $^{80}Se]$ (5), 80 (19), 55 (25), 54 (85), 53 (26), 41 (100), 39 (51). For further characterization the isoselenocyanate (**6**) (445 mg), dissolved in CH_2Cl_2 (8 ml), was treated with piperidine (0.31 ml). Removal of the solvent after 1 hr and purification of the product on a silica gel column (2 × 8 cm) (EtOAc–hexane, 1:2) afforded a light-sensitive, homogeneous product of *N*-(3-butenyl)-1-piperidinecarboselenoamide, mp 56–57.5° (from EtOAc–hexane) (Found: C, 49.04; H, 7.47; N 11.32. $C_{10}H_{18}N_2Se$ requires: C, 48.98; H 7.40; N 11.42).

(±)-*5-Vinyl-2-oxazolidineselone* (**7**). (±)-1-Amino-3-buten-2-ol (440 mg) in MeCN (5 ml) was treated with $HgCl_2$ (1.56 g) and CSe_2 (0.3 ml) exactly as described above for the synthesis of **6**. Chromatographic purification afforded **7** as colourless, light-sensitive prisms (217 mg, 25%), mp 80–82° (from Et_2O). (Found: C, 34.36; H, 4.16; N, 7.81. C_5H_7NOSe requires: C, 34.11; H, 4.01; N, 7.95). ^1H NMR (90 MHz, $CDCl_3$); δ 3.52 (1H, *dd*, *J* 8 Hz, *J* 10 Hz, H-4a), 3,92 (1H, *dd*, *J* 10 Hz, *J* 9 Hz, H-4b), 5.14–5.67 (3H, *m*, H-5, HC:C<u>H</u>$_2$, 5.73–6.22 (1H, *m*, <u>H</u>C:CH$_2$), 8.65 (1H, broad *s*, NH).

Stanleya pinnata *plants in high-selenium nutrient solutions.* Seeds of *S. pinnata* were germinated and the seedlings grown as previously described by one of us [13]. The 3–4 cm high plants were kept in normal strength nutrient soln for 6 days, after which they were replaced into fresh solns containing various concentrations of selenium as Na_2SeO_3, $5H_2O$: 1000, 100, 10 and 1 ppm. The solns were adjusted to pH 6.5 with 1 M HCl. After four days the plants subjected to the 1000 ppm conc. of selenium had dried out, whereas no difference in appearance was noted for the other plants. Twelve plants, grown for an additional 21 days at 100 ppm of selenium, with change of nutrient soln every week, were excised at the base of the stems, the roots were discarded and the aerial parts freeze-dried to give 49 g of dry wt. The glucosinolate fraction (275 mg) was isolated as described above. The GC/CIMS analysis of the latter (Fig. 1) was made by using a fused silica megabore column, 30 m × 0.54 mm, 1.5 μ film thickness, preconditioned by repeated injections of the silylating reagent prior to the GC/MS analysis, He at 5 ml/min, temp. programmed 220–280° at 6°/min, injector temp. 200°, transfer lines 250°, sample size 0.5 μl, and isobutane at 4×10^{-6} torr as the reactant gas; silylations were performed as previously reported [8]. An especially concentrated sample was prepared by treating the glucosinolate fraction (10 mg) with BSTFA (20 μl)

and TMCS (3 μl) in pyridine (50 μl), followed by heating at 120° for 15 min. Another part of the glucosinolate fraction (*ca* 150 mg) was dissolved in a Na–Pi buffer (pH 6.5) with 0.0025 M ascorbic acid. A myrosinase soln (0.8 ml) was added; the soln was stirred in the dark for 2 hr, extracted with Et_2O (3 ml × 2), and the extract conc. to 20–30 μl in an Ar stream. GC/EIMS analysis of the isothiocyanate fraction (Fig. 2) was performed on the same column as discussed above: He at 5 ml/min, isothermal 100°, injector temp. 150°, transfer lines 200°, ion source 220°, ionizing potential 70 eV and sample size 1 μl.

Incorporation studies with ^{75}Se-*selenite.* From a one-year-old plant of *S. pinnata* 16 leaves were excised at the base of the petioles and immediately transferred to four conical tubes, each containing an aq. soln of $Na_2{}^{75}SeO_3$ (4 ml, 1.4×10^6Bq, 25 μg of selenium) and a two-fold strength of nutrient soln (1 ml), adjusted to pH 6.5. After 36 hr, the solns had been totally absorbed and half-normal strength of nutrient solns (4 ml) were added. At the end of 72 hr, the healthy looking leaves were removed from the solns and freeze-dried (2.9 g dry wt). The glucosinolate fraction was isolated and the major part subjected to myrosinase hydrolysis in the dark as described above, followed by extraction with CH_2Cl_2 (5 ml), H_2O (5 ml) and 0.1 M KOH (5 ml × 2). The combined aq. extracts were neutralized with 1 M HCl and extracted with CH_2Cl_2 (5 ml × 2); the extract was washed with H_2O, dried, evapd to dryness (2 mg) (total activity 7200 cpm) and subjected to descending PC (C_6H_6–heptane–H_2O, 9:2:9, upper layer) in the dark. PC analysis of synthetic specimens of (±)-5-vinyl-2-oxazolidinethione (**4**) and (±)-5-vinyl-2-oxazolidineselone (**7**) under the same conditions showed them to possess R_f-values of 0.44 and 0.30, respectively. The PC was exposed to an X-ray film for one week but showed no significant incorporation in this area. Furthermore, synthetic **7** (30 mg) was added to the 2 mg-residue and three consecutive recrystallizations from Et_2O were performed giving 23 mg (360 cpm), 20 mg (228 cpm), and 12 mg (168 cpm) of **7**, to be compared with a background of 150 cpm. The CH_2Cl_2-extract from the enzymic hydrolysis was washed with H_2O, dried and treated with NH_3-saturated MeOH in the dark for 1 hr. Evapn afforded a thiourea fraction (8 mg) with a total activity of 2.2×10^5 cpm. Upon ascending PC(H_2O-saturated $CHCl_3$) and radioautography, no detectable activity was noted in the area of synthetic *N*-3-butenylselenourea (R_f 0.50) but rather in an unknown constituent with a considerably higher R_f-value. Radioautography of a PC (*n*-BuOH–pyridine–H_2O, 6:4:3) of the glucosinolate fraction before enzymatic hydrolysis showed weak activities in two spots with R_f-values slightly lower than those of **1b** and **1c**, ruling out their identities as the corresponding selenoglucosinolates, inasmuch as it was shown that in the same solvent system, methyl- and benzyl-glucosinolate possess R_f-values indistinguishable from those of the corresponding, synthetic selenium isologues.

Seeds of *Lepidium sativum* were germinated on wet filter paper, transferred to 1/5-fold strength of nutrient soln, and after 7 days, kept in full-strength soln for an additional 14 days. 12 leaves were excised from the plants and immediately divided between 3 conical flasks, each containing an aq. soln of $Na_2{}^{75}SeO_3$ (4 ml) (9.9×10^5Ba/ml) and a two-fold strength of nutrient soln (1 ml) adjusted to pH 6.5. After 72 hr the yellow leaves had absorbed half of the soln (a total of *ca* 1.5×10^6Bq). The leaves were freeze-dried (dry wt 2.8 g) and the glucosinolate fraction was isolated as described above. PC and radioautography showed no increase over the background activity at the expected site of benzylselenoglucosinolate. Likewise, enzymatic hydrolysis, followed by conversion of the isothiocyanate(s) into the corresponding thiourea(s) with piperidine showed no increase in activity over the background at the site of synthetic *N*-benzyl-1-piperidinecarboselenoamide [6].

Incorporation studies with $Na_2{}^{75}SeO_4$ *in horseradish.* The procedure of Steward *et al.* [5] was strictly followed. 6 leaves from 1-month-old horseradish plants were transferred to normal strength nutrient soln (17.5 ml), containing 35 ppm of selenium as Na_2SeO_4, to which an aq soln (2.5 ml) of $Na_2{}^{75}SeO_4$ (3.7×10^6Bq) was added, so that the total concentration of selenium was at 31 ppm. The soln was absorbed within 18 hr and additional nutrient soln with inactive Na_2SeO_4 was added. After 48 hr, the healthy-looking leaves were freeze-dried (dry wt 1.4 g) and extracted with hot 90% MeOH; the glucosinolate fraction was isolated by passing the extract, first through an Amberlite IR-120 ion exchange resin (H^+ form), and then through a Dowex-2 × 8 column (Cl^--form) which was then rinsed with H_2O, until Cl^--free, and the glucosinolate fraction expelled from the column with 0.1 M potassium salicylate. The fractions containing **1a** were located by PC analysis, acidified to pH with 0.5 M H_2SO_4, extracted with Et_2O to remove salicylic acid, neutralized with 0.1 M KOH, and lyophilized. Extraction of the residue with hot MeOH, evapn, and renewed extraction with boiling 90% EtOH, gave, after evapn, a yellow syrup (16 mg) which was chromatographed on 4 strips of Whatman no. 3 paper in *n*-PrOH–EtOAc–H_2O (7:1:1). The strips were cut into sections, these were placed in vials and scanned. The sections, covering an R_f-range of 0.28–0.47, inside which **1a** and, undoubtedly, also its selenium isologue resided, did not exhibit activity higher than that of the neighbouring sections (i.e 1500–2400 cpm). The sections, corresponding to R_f-values of 0.0–0.15, showed considerably higher activity ($14\text{-}50 \times 10^3$ cpm). The product(s) responsible are likely to be SeO_3^{2-} and/or SeO_4^{2-}.

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