# Selenoglucosinolates: Synthesis and Enzymatic Hydrolysis

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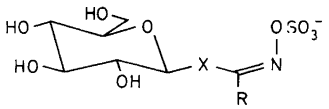
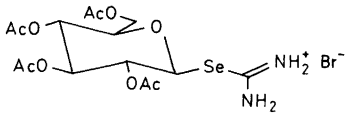
The first selenoglucosinolates have been synthesized and characterized. The synthetic selenium analogues of benzyl- and methylglucosinolate both undergo a facile, ascorbate-activated hydrolysis, catalyzed by an enzyme preparation ('myrosin') traditionally used for hydrolyzing glucosinolates. Benzyl and methyl isoselenocyanates are identified and characterized as products of the enzymatic hydrolysis.

The glucosinolates (*1*), today encompassing about a hundred naturally occurring anions, constitute a strikingly uniform collection of secondary metabolites, discontinuously distributed within the class of dicotyledons. Their chemical character and natural distribution are matters of considerable biological, technical and economic interest.[1] Intrigued by the structural similarity of the few known, naturally occurring selenoorganic compounds (amino acids) and their sulfur counterparts, we have synthesized the first selenoglucosinolates (*2*) with the purpose of studying their properties, including their behaviour towards glucosinolate-hydrolyzing enzymes.

Our synthesis follows an established path to glucosinolates (*1*),[2] with minor, yet important modifications (see Scheme 1). Tetra-*O*-acetyl-l-seleno-β-D-glucopyranose anion (*4*), generated in methanol from the isoselenurium salt (*3*)[3] with slightly less than two equiv. of sodium methoxide, reacted instantaneously with phenylacetohydroximinoyl chloride (*5a*),[2b] probably *via* the nitrile oxide, to give the crystalline *Se*-[(2,3,4,6-tetra-*O*-acetyl)-β-D-glucopyranosyl]phenylacetoselenohydroximate (*6a*). Conversion, by sulfonation, into potassium benzylselenoglucosinolate tetraacetate (*7a*) and thence into benzylselenoglucosinolate (*2a*), isolated as the crystalline tetramethylammonium salt, proceeded unexceptionally. Repetition of the sequence, starting from *3* and the unstable acetohydroximinoyl chloride (*5b*),[2a] led, without isolation of *6b*, to the crystalline potassium methylselenoglucosinolate tetraacetate (*7b*) which, on deacetylation, afforded methylselenoglucosinolate (*2b*), isolated as the crystalline potassium salt. The IR spectra (in KBr) of the selenoglucosinolates [(*2a*; $Me_4N^+$-salt) and (*2b*; $K^+$-salt)] were virtually identical with those of the corresponding salts of the analogous glucosinolates, (*1a*) and (*1b*). Similarly, only minor deviations were noted on comparing the $^1H$- and $^{13}C$-NMR spectra of the two



(1): X = S (2): X = Se
(1a): R = $PhCH_2$ (2a): R = $PhCH_2$
(1b): R = Me (2b): R = Me



(3)

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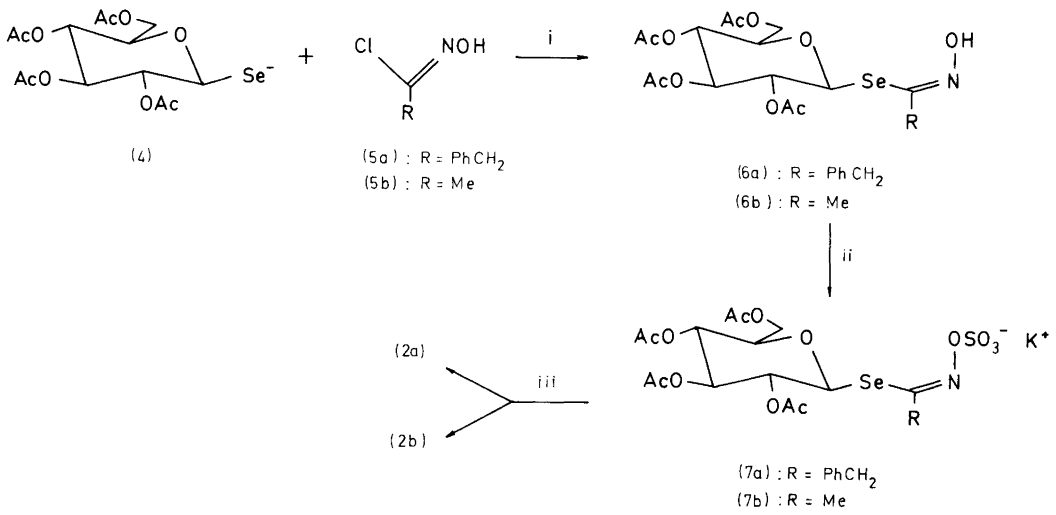



*Scheme 1.* Reagents and conditions: (i) MeOH, 0 °C, argon atmosphere; (ii) $SO_3$: pyridine complex, pyridine, 17 h, 20 °C; (iii) $NH_3$ in MeOH, 0 °C.

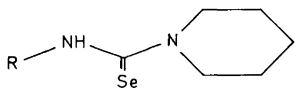
selenoglucosinolates with those of the corresponding glucosinolates (Tables 1 and 2). Hence, the (*Z*)-configuration, generally accepted for the glucosinolates, almost certainly obtains also for the selenoglucosinolates.

Enzymatically induced hydrolysis of the *S*-glucose linkage, followed by degradation of the aglucone to, *inter alia*, isothiocyanates, RNCS, is a characteristic property of glucosinolates (*1*). We now report that selenoglucosinolates (*2*) can be handled analogously and expeditiously with the same, non-purified enzyme preparation ('myrosin') that catalyzes the hydrolysis of glucosinolates.[4] Thus, benzylselenoglucosinolate (*2a*), subjected to the usual hydrolysis conditions (see Experimental), rapidly produces benzyl isoselenocyanate which is converted, upon reaction with piperidine, into *N*-benzyl-l-piperidi-

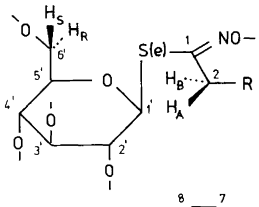
*Table 1.* $^{13}C$ Chemical shift values (ppm relative to $Me_4Si$) for glucosinolates, selenoglucosinolates and synthetic intermediates.

| Compd | Carbon atoms[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 1′ | 2′ | 3′ | 4′ | 5′ | 6′ |
| *6a*[b,c] | 150.7 | 39.8 | 135.9 | 129.0 | 128.2 | 127.4 | ← | | 68.1–75.7 | | → | 62.3 |
| *7a*[d,e] | 155.5 | ~43 | 140.1 | 132.5 | 132.4 | 130.8 | ← | | 71.7–79.4 | | → | 65.8 |
| *2a*[f,g] | 163.4 | 40.7 | 136.6 | 130.5 | 129.6 | 128.8 | 82.2 | 73.6 | 78.4 | 70.1 | 80.8 | 61.7 |
| *1a*[f,g,h] | 163.9 | 39.5 | 136.4 | 130.5 | 129.4 | 128.9 | 82.6 | 73.1 | 78.3 | 70.1 | 81.1 | 61.6 |
| *7b*[d,i] | 154.3 | 22.9 | | | | | ← | | 72.1–79.4 | | → | 66.1 |
| *2b*[f,j] | 163.0 | 20.8 | | | | | 82.4 | 73.6 | 78.5 | 70.6 | 80.9 | 62.1 |
| *1b*[f,j] | 163.9 | 19.4 | | | | | 83.0 | 73.1 | 78.4 | 70.5 | 81.2 | 62.0 |

[a]Numbering as indicated in (*9*). [b]In chloroform-*d*. [c]Acetyl groups: δ 20.6–20.7 ($4CH_3$), 169.4–170.7 (CO). [d]In dimethyl sulfoxide-$d_6$. [e]Acetyl groups: δ 24.2–24.4 ($4CH_3$), 173.2–173.9 (CO). [f]In water-$d_2$. [g]Tetramethylammonium cation: δ 56.5 ($4CH_3$). [h]Spectrum previously reported (Ref. 5). [i]Acetyl groups: δ 23.0–24.4 ($4CH_3$), 173.3–173.5 (CO). [j]Potassium salt.



(8a) : R = $PhCH_2$
(8b) : R = Me



(9) R = H, or 

necarboselenoamide (*8a*), identical with an independently synthesized sample.[7] Similarly, methylselenoglucosinolate (*2b*) affords the somewhat unstable methyl isoselenocyanate,[7,8] identified by GLC-comparison with an authentic sample as well as by conversion into *N*-methyl-l-piperidinecarboselenoamide (*8b*), identical with an authentic sample.[7]

The hydrolysis of glucosinolates, catalyzed by enzyme(s) extracted from seeds of white mustard (*Sinapis alba*), is known to be accelerated dramatically by small amounts of ascorbic acid.[9,10] Working with such an enzyme preparation[4] we have noted a similar enhancement in the rate of hydrolysis of the selenoglucosinolates (*2a*) and (*2b*). In the presence of only enzyme *or* extraneous ascorbic acid, the enzymatic hydrolysis is extremely slow or totally absent, respectively. Limited specificity of the enzyme(s) or the presence in the crude enzyme preparation of ascorbate-activated protein(s) specific for selenoglucosinolates are possibilities between which we cannot distinguish at present.

## Experimental

Melting points are uncorrected. $^{13}C$- (at 125 MHz) and $^{1}H$- (at 500 MHz) NMR spectra were measured on a Bruker HX500 instrument.

*Phenylacetohydroximinoyl chloride* (5a). The method of Liu *et al.*[11] for the preparation of benzohydroximinoyl chlorides was adopted. To a stirred solution of phenylacetaldoxime (5 g, 37 mmol) in *N,N*-dimethylformamide (35 ml) was added one-fifth of 4.95 g (37 mmol) of *N*-chlorosuccinimide (NCS). The temperature (internal thermometer) was regulated by intermittent

*Table 2.* $^{1}H$ NMR Data [chemical shifts, δ-scale (*J* in Hertz)] for glucosinolates, selenoglucosinolates and synthetic intermediates.

| Compd | Atom[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | H-2 | (H-3)–(H-8) | H-1′ | H-2′ | H-3′ | H-4′ | H-5′ | $H_S$-6′ | $H_R$-6′ |
| *6a*[b,c] | 3.98 and 4.04[d] | 7.35 (m) | ← | | | 3.5–5.1 | | | → |
| *7a*[e,c] | 4.07 and 4.15[d] | 7.45 (m) | 5.49[f] | 4.98[g] | 5.35[h] | 5.03[i] | 3.99 (m) | 3.88[i,l] | 4.14[k,l] |
| *2a*[m,n] | 4.19 (s) | 7.43 (m) | 4.90[o] | 3.40 (m) | 3.30 (m) | 3.40 (m) | 3.23 (m) | 3.71[i,l] | 3.66[k,l] |
| *1a*[m,n,p] | 4.16 (s) | 7.43 (m) | 4.72 (m) | 3.34 (m) | 3.34 (m) | 3.40 (m) | 3.28 (m) | 3.69[j,l] | 3.64[k,l] |
| *7b*[e,c,q] | 2.40 (s) | | 5.82[f] | ← | | 4.2–5.5 | | | → |
| *2b*[m,q] | 2.47 (s) | | 5.32 (m) | 3.56 (m) | 3.56 (m) | 3.48 (m) | 3.56 (m) | 3.92[r,l] | 3.71[s,l] |
| *1b*[m,q] | 2.43 (s) | | 5.10[f] | 3.49 (m) | 3.59 (m) | 3.49 (m) | 3.59 (m) | 3.93[r] | 3.73[s] |

[a]Numbering as in formula (*9*). [b]In chloroform-*d*. [c]Acetyl groups: δ 2.05–2.10 ($4CH_3$). [d]Doublets, $^{2}J_{AB}$ *(−16.5).* [e]In dimethyl sulfoxide-$d_6$. [f]Doublet (10). [g]Double doublet [(10) and (9)]. [h]Triplet (9). [i]Triplet (10). [j]Double doublet, $^{2}J_{H(S),H(R)}$ (−12.5), $^{3}J_{H(S),H-5'}$ (3). [k]Double doublet, $^{2}J_{H(R),H(S)}$ (−12.5), $^{3}J_{H(R),H-5'}$ (5). [l]The assignment based on literature data (Ref. 6). [m]In water-$d_2$. [n]Tetramethylammonium cation: δ 3.20 (*s*) ($4CH_3$). [o]Doublet (10). [p]Spectrum previously reported (Ref. 5). [q]Potassium salt. [r]Double doublet, $^{2}J_{H(S),H(R)}$ (−12.5), $^{3}J_{H(S),H-5'}$ (2.5). [s]Double doublet, $^{2}J_{H(R),H(S)}$ (−12.5), $^{3}J_{H(R),H-5'}$ (6).

cooling (dry ice-acetone bath) such that it did not exceed 35 °C during the addition of the remaining NCS in two portions. After cessation of the exothermic reaction, the mixture was poured into ice-water (125 ml), the reaction product extracted into ether (2×75 ml), and the ether extract washed with water (3×35 ml), dried and evaporated to give the chlorinated oxime (5.92 g, 94 %), homogeneous according to NMR spectroscopic analysis. Recrystallization from carbon tetrachloride afforded colourless crystals, m.p. 84–85 °C (lit.[2h] m.p. 89–91 °C).

*Se-[(2,3,4,6-tetra-O-acetyl)-β-D-glucopyranosyl] phenylacetoselenohydroximate* (6a). A solution (2.90 ml, 5.05 mmol) of sodium methoxide in methanol (1.74 M) was slowly injected into an argon-protected solution of the isoselenurium bromide (*3*)[3] (1.4 g, 2.62 mmol), cooled to 0 °C. After stirring for 5 min, a solution of phenylacetohydroximinoyl chloride (*5a*) (0.58 g, 3.41 mmol) in methanol (14 ml) was added dropwise, causing the separation of colourless crystals. Methanol (5 ml) was added and the mixture was stirred for 30 min at 22 °C, cooled to 0 °C, and filtered to give the desired product (0.93 g) which was homogeneous by TLC (hexane:ethyl acetate, 1:1). Additional material was secured from the filtrate by chloroform extraction, followed by a passage through silica gel (1.5×5 cm) in hexane:ethyl acetate (1:1). Evaporation and recrystallization from the same solvent mixture afforded an additional crop of homogeneous *6a* (0.34 g). Total yield (based on *3*) 1.27 g (89 %). M.p. 171–174°; $[\alpha]_D^{20}$ −33.6° (*c* 0.9, $CHCl_3$); NMR data: see Tables 1 and 2. Anal. $C_{22}H_{27}NO_{10}Se$: C, H, N.

*Potassium benzylselenoglucosinolate tetraacetate* (7a). To a solution of *6a* (1.0 g, 1.83 mmol) in dry pyridine (35 ml), was added sulfur trioxide-pyridine complex[12] (2.08 g, 13.0 mmol). After stirring for 17 h at room temp., an additional portion (0.2 g, 1.2 mmol) of the complex was added and stirring was continued for 2 h. A solution of potassium carbonate (1.98 g, 14.3 mmol) in water (40 ml) was added slowly. The aqueous phase was extracted with ether (2×90 ml) and lyophilized. The residue was extracted repeatedly with boiling ethanol. On cooling, the alcohol solution deposited colourless needles (0.52 g), homogeneous by TLC (in water-saturated butanol). An additional crop (0.28 g) was obtained on evaporation of the filtrate and recrystallization from ethanol containing a few drops of water. Total yield of *7a* 0.8 g (64 %). $[\alpha]_D^{20}$ −27.2° (*c* 1.1, $H_2O$); NMR data: see Tables 1 and 2. Anal. $C_{22}H_{26}NO_{13}SSeK$: C, H, N, S.

*Tetramethylammonium benzylselenoglucosinolate* (2a). A solution of the tetraacetate (*7a*) (400 mg) was dissolved in ammonia-saturated methanol (20 ml). After 2 h at 20 °C, deacetylation was complete according to TLC analysis (propanol:ethyl acetate:water, 7:1:2). The solution was taken to dryness *in vacuo*, the residue was redissolved in water (5 ml) and the solution passed through Amberlite IR-120 ion-exchange resin (1.5×15 cm) in the tetramethylammonium form. The column was rinsed with water (20 ml) and the combined effluents were lyophilized. The residue was dissolved in a small volume of methanol, a 1.5-fold volume of ethanol was added, and the solution was evaporated *in vacuo* until turbidity appeared. Cooling and scratching resulted in the crystallization of colourless needles (0.27 g, 85 %) of *2a*. M.p. 187–190° (decompn.); $[\alpha]_D^{20}$ −40.9° (*c* 1.5, $H_2O$); NMR data: see Tables 1 and 2. Anal. $C_{18}H_{30}N_2O_9SSe$: C, H, N, S.

*Potassium methylselenoglucosinolate tetraacetate* (7b). *t*-Butyl hypochlorite (0.84 ml, 7.4 mmol) was added to a stirred solution of acetaldoxime (0.46 g, 7.9 mmol) in methanol (20 ml) at −60 °C (chloroform-dry ice) to give a blue solution, which was allowed to warm to 0 °C. After stirring for 0.5 h at 0 °C, argon was passed through the light-blue solution.

A methanolic solution of sodium methoxide (1.74 M; 2.90 ml, 5.0 mmol) was slowly injected into a stirred, argon-covered solution of the isoselenurium bromide (*3*) (1.4 g, 2.6 mmol) in methanol (14 ml), kept at 0 °C. After stirring for 5 min, the above chlorooxime solution was slowly injected and stirring was continued for another 0.5 h, after which the acidic reaction mixture was neutralized with sodium hydroxide (2 M) and taken to dryness *in vacuo*. Water (30 ml) and chloroform (30 ml) were added. The organic phase was dried and evaporated to dryness and the residue redissolved in pyridine (25 ml). Sulfur trioxide-pyridine complex[12] (1.63 g, 10.3 mmol) was added and the solution was kept at 20 °C for 4 h. A solution of potassium carbonate (1.85 g, 13.4 mmol) in water (40 ml) was then slowly

added at 0 °C. Extraction with ether (3×20 ml) removed glucopyranosyldiselenide octaacetate, formed as a by-product, from the aqueous phase which was then freeze-dried. The residue was extracted with boiling 95 % ethanol which deposited crystalline *7b* (0.81 g) on cooling, homogeneous by TLC analysis (water-saturated butanol). From the mother liquor an additional crop (0.13 g) was obtained. Total yield (based on *3*) 0.94 g (61 %). $[\alpha]_D^{20}$ −36.7° (*c* 1.6, $H_2O$); NMR data: see Tables 1 and 2. Anal. $C_{16}H_{22}NO_{13}SSeK$: C, H, N.

*Potassium methylselenoglucosinolate* (2b). The tetraacetate *7b* (0.6 g, 1.02 mmol) was subjected to deacetylation with methanolic ammonia as described above for *7a* to give crystalline *2b* (0.38 g, 88 %), separating from ethanol as colourless crystals. $[\alpha]_D^{20}$ −40.8° (*c* 1.8, $H_2O$); NMR data: see Tables 1 and 2. Anal. $C_8H_{14}NO_9SSeK$: C, H, N.

*Enzymatic hydrolysis.* Tetramethylammonium benzylselenoglucosinolate (*2a*) (0.10 g) was dissolved in a phosphate buffer (6 ml, pH 6.5). Ascorbic acid (10 mg) and a myrosinase solution[4] (0.5 ml) were added, and the solution was kept in the dark at 20 °C for 2 h. The now cloudy mixture was extracted with ether (2×10 ml), piperidine (0.4 ml) was added and the mixture was stirred in the dark for 20 min. The ether phase was washed with water (3×10 ml), dried and evaporated. Recrystallization of the residue from ethyl acetate-hexane afforded *N*-benzyl-1-piperidinecarboselenoamide (*8a*) (33 mg, 63 %) as colourless, light-sensitive stout prisms, m.p. 97–99 °C, alone and in admixture with an authentic sample.[7]

Potassium methylselenoglucosinolate (*2b*) was subjected to enzymatic hydrolysis in a similar fashion, but with a shorter hydrolysis time (0.5 h) owing to the instability of methyl isoselenocyanate in an aqueous environment, even in the dark. An aliquot of the ether solution was subjected to GLC (OV-101 column, $N_2$, 5 °C $min^{-1}$), showing a single peak with a retention time identical to that of an authentic sample of methyl isoselenocyanate.[7] The remainder of the ether solution was treated with piperidine to give *N*-methyl-1-piperidinecarboselenoamide (*8b*). Colourless needles (from ethyl acetate-hexane), m.p. 136–138 °C, alone or in admixture with an authentic sample.[7]

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