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Tetrahedron Letters 49 (2008) 292–295
Tetrahedron Letters

Thio-functionalised glucosinolates: unexpected transformation of desulfoglucoraphenin

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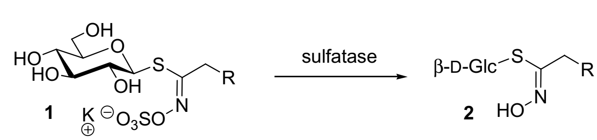
Received 26 July 2007; revised 5 November 2007; accepted 9 November 2007
Available online 17 November 2007

Abstract—Enzymatic desulfation of stable glucoraphenin affords desulfoglucoraphenin, which unexpectedly undergoes further transformations into cyclic nitrone-type derivatives.

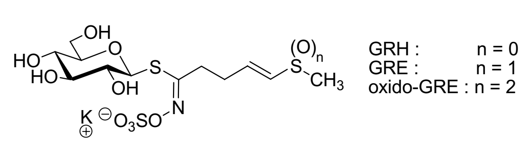
All plants of the Brassicale order contain glucosinolates (GLs) **1**, thiosaccharidic secondary metabolites which display a remarkable structural homogeneity: a hydrophilic β-D-glucopyrano framework bearing an O-sulfated anomeric (*Z*)-thiohydroximate moiety connected to a fairly hydrophobic aglycon side chain.

In the over 120 known GLs, the aglycon chain is the sole structural variant, in which diversified aliphatic, arylaliphatic or heterocyclic arrangements can be found.[1] It is also noteworthy that more than one-third of the known aliphatic aglycons contain an additional terminal thiofunction.[2] Among these thio-functionalised GLs, glucoraphanin (GRA, 4-methylsulfinylbutyl GL)—present in broccoli—is one of the most popular: its enzymatic hydrolysis produces sulforaphane (4-methylsulfinylbutyl isothiocyanate) which has been shown to exert chemopreventive effects against some cancers.[3] In other respects, glucoraphasatin (GRH, 4-methylsulfanyl-3-butenyl GL) and glucoraphenin (GRE, 4-methylsulfinyl-3-butenyl GL) (Scheme 2) have attracted much attention in recent years as bio-relevant redox couple in some neutraceutical applications.[4]

Analysis of GLs in Brassicaceae vegetables is currently based on their enzymatic O-desulfation and HPLC of desulfoglucosinolates **2** (DS-GLs) according to the EU official method ISO-9167-1 (Scheme 1).[5,6] This method was applied in combination with NMR spectrometry[7] to evaluate the purity of GRE extracted from *Raphanus sativus* seeds according to Ref. 4. The desulfoglucoraphenin (DS–GRE) produced through standard desulfation of GRE showed some abnormality: whereas GRE displays a stability similar to that of most GLs, its desulfated derivative is not stable and undergoes progressive degradation in water to produce three more polar major compounds P1–P3, as evidenced by chromatographic study (Fig. 1). From diode array UV apex spectra obtained for peaks P1 and P2–P3, the λ_{max} measured—257 and 259 nm, respectively—were indicative of a dramatic functional transformation of DS–GRE, for which λ_{max} was 226 nm.



Scheme 1. Glucosinolates (GLs) and desulfoglucosinolates (DS-GLs).



Scheme 2. Vinylthiofunctionalised glucosinolates.

Keywords: Glucosinolates; Desulfoglucosinolates; Vinylthio functions; Thioimidate N-oxides.

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doi:10.1016/j.tetlet.2007.11.059

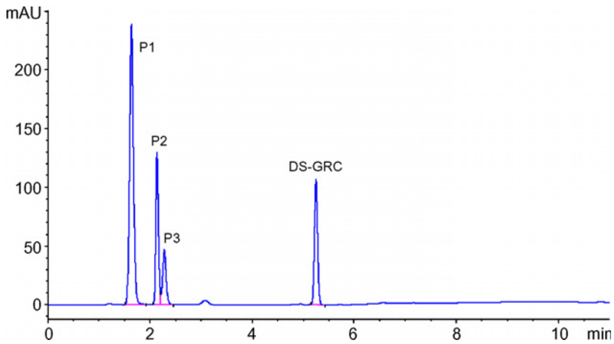


Figure 1. C-18 reversed phase HPLC analysis[8] of the mixture obtained through partial degradation of DS–GRE in water.

Careful preparation and isolation of pure DS–GRE was therefore effected according to a protocol previously described for DS–GRH[4a] using freeze-drying recovery and DS–GRE was characterised by NMR spectrometry—both in deuterium oxide[9] and in DMSO-d_6.[10,11] Compound P1 was isolated by C-18 reversed phase preparative chromatography (HR 16/10 column and Pharmacia FPLC equipment) after the total transformation of DS–GRE (water, 40 °C, 24 h). To preclude concomitant formation of P1, compounds P2–P3 were prepared from DS–GRE in modified conditions (methanol, 40°C, 24 h).[12]

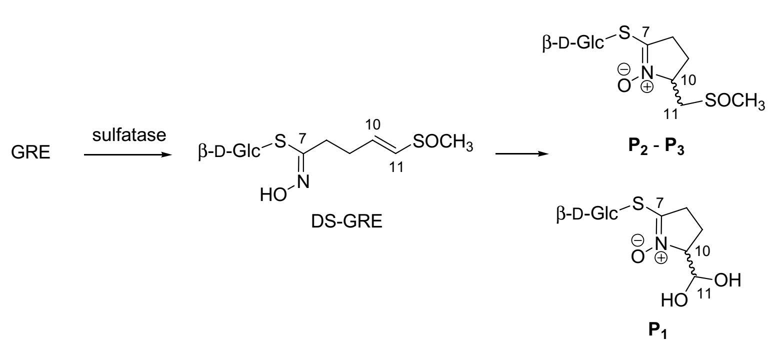
Low and high resolution mass spectrometry analyses[13] of the degradation products led to the following informations:

– P1 is a mixture of diastereoisomers $C_{11}H_{17}NO_7S$.
– P2–P3 is a mixture of diastereoisomers $C_{12}H_{21}NO_7S_2$ isomeric to DS–GRE.

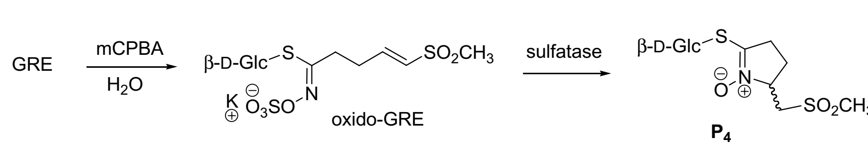
The NMR study[14] of the latter mixture did not show notable changes in the D-gluco moiety but in contrast revealed a dramatic modification of the aglycon part, in which positions C-7, C-9, C-10 and C-11 are especially involved. The missing NMR tags of the vinyl system of DS–GRE were indicative of a likely ring closure, based on a concerted equivalent of the Michael addition reaction, analogous to the 1,3-azaprotio cyclotransfer pathway previously investigated by Grigg and colleagues.[15]

Physical data supported our hypothesis correctly. Carbon-NMR showed a ca. 11 ppm shielding of C-7, while a strong bathochromic effect (λ_{max} from 226 to 259 nm) was observed, in agreement with the transformation of the thiohydroximate into a thioimidate N-oxide—a seldom encountered nitrone-type function.[16] Other NMR features for sites C-9, C-10 and C-11 were relevant to the structure established for P2–P3 (Scheme 3).

To our knowledge, the above transformation is unprecedented: outside of the field of glucosinolates indeed, the thiohydroximate group remains a moderately considered and poorly studied function[17] and therefore its oxime-like nucleophilic behaviour noted in the above transformation called for confirmation. We thus decided to extend the reaction to another glucosinolate in which the sulfoxide group would be replaced by a more powerful EWG. Although mentioned in the literature,[1] the vinyl sulfone counterpart of GRE ('oxido-GRE') was not isolated, but only detected in radish (*R. sativus*) in the form of the derived isothiocyanate.[18] Thus, oxido-GRE had to be synthesised by MCPBA oxidation of natural GRE (Scheme 4).[19] Submitting in turn this sulfone to the standard desulfation conditions (sulfatase, pH 5.6 acetate buffer, 30 °C, 24 h)[6] resulted in progressive transformation of the transient DS-GL into compound P4, showing only peak in the chromatogram with $\lambda_{max} = 260$ nm (UV apex spectra), consistent with a thioimidate N-oxide structure (Scheme 4).[16] Compound P4 was isolated by C-18 reversed phase preparative chromatography (HR 16/10 column and Pharmacia FPLC equipment)[12] and NMR data were relevant



Scheme 3. Spontaneous transformation of DS–GRE.



Scheme 4. *m*CPBA oxidation to oxido-GRE and desulfation–spontaneous cyclisation to P4.

to a cyclic thioimidate N-oxide bearing a sulfone appendage.[20]

In the case of both GRE and oxido-GRE, it could thus be demonstrated that through enzymatic removal of the deactivating sulfate group, the nucleophilic character of the thiohydroximate nitrogen was released and cyclisation involving the EWG-activated end of the aglycon was allowed, resulting in a thioimidate N-oxide.

Structural identification of fraction P1 led to unexpected results: when compared to DS–GRE and P2–P3, the molecular formula $C_{11}H_{17}NO_7S$ was indicative of a formal loss of methanethiol but the UV spectrum ($\lambda_{max} = 257$ nm) remained consistent with a thioimidate N-oxide structure.[16] NMR spectra[21] again did not show notable changes in the D-gluco moiety but revealed functional modification at positions C-10 and C-11: finally, all physical data led to assign to P1 the structure of an aldehyde in its hydrated form (Scheme 3). Considering that no such compound could ever be detected during the sulfatase-induced transformation of oxido-GRE, it may be hypothesized that aldehyde P1 could result from an in situ Pummerer-type rearrangement of sulfoxides P2–P3: this however remains to be investigated.

In summary, we have disclosed a new and unique chemical transformation of GRE during the enzymatic desulfation process:[22] as exemplified by the case of oxido-GRE, such intramolecular cyclisation could likely be extended to other GLs bearing a well-suited EWG in the aglycon chain. More generally, the above results open future prospects for investigating the ambident nucleophilic character of thioxydroximates, as compared with oximes and other hydroximino functional groups: this study is currently under way in our laboratory.

Acknowledgments

The authors wish to thank Dr. Bruno Perly (CEA Saclay) and Julie Schleiss for assistance.

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7. *Selected NMR data (D_2O) for GRE*: ^{1}H δ 6.63 (m, 2H, H-10 & H-11), 5.09 (d, 1H, $J_{1,2} = 9.7$ Hz, H-1), 3.93 (dd, 1H, $J_{6a,6b} = 12.4$ Hz, $J_{6a,5} = 1.5$ Hz, H-6a), 3.74 (dd, 1H, $J_{6b,5} = 5.5$ Hz, H-6b), 3.61 (m, 2H, H-3 & H-5), 3.49 (m, 2H, H-2 & H-4), 2.98 (t, 2H, $J_{8,9} = 7.1$ Hz, H-8), 2.77 (s, 3H, H-12), 2.76 (m, 2H, H-9); ^{13}C δ 162.8 (C-7), 141.7 (C-10), 133.4 (C-11), 82.1 (C-1), 80.5 (C-5), 77.5 (C-3), 72.4 (C2), 69.6 (C-4), 61.1 (C-6), 39.2 (C-12), 30.9 (C-8), 29.1 (C-9).
8. Chromatographic conditions reported in Barillari, J.; Gueyrard, D.; Rollin, P.; Iori, R. *Fitoterapia* **2001**, *72*, 760–764, peaks were monitored at 254 nm.
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10. *Selected NMR data (DMSO-d_6) for DS–GRE*: ^{1}H δ 11.06 (s, 1H, NOH), 6.64 (br d, 1H, $J_{vic} = 15.2$ Hz, H-11), 6.34 (dt, $J_{9,10} = 6.3$ Hz, H-10), 5.38 (d, 1H, $J_{vic} = 6.3$ Hz, OH), 5.13 (d, 1H, $J_{vic} = 4.7$ Hz, OH), 5.02 (d, 1H, $J_{vic} = 5.0$ Hz, OH), 4.74 (d, 1H, $J_{1,2} = 9.5$ Hz, H-1), 4.63 (t, 1H, $J_{vic} = 5.5$ Hz, OH-6), 3.69 & 3.40 (2 dd, 2H, $J_{6a,6b} = 11.5$ Hz, $J_{6a,5} = 5.0$ Hz, $J_{6b,5} = 5.8$ Hz, H-6a & H-6b), 3.22 (m, 2H, H-3 & H-5), 3.08 (m, 2H, H-2 & H-4), 2.75 (m, 2H, H-8), 2.54 (s, 3H, H-12), 2.53 (m, H-9); ^{13}C δ 150.7 (C-7), 136.5, 135.3 (C-10, C-11), 81.6 (C-1), 81.1 (C-5), 78.2 (C-3), 73.0 (C-2), 69.9 (C-4), 61.1 (C-6), 40.2 (C-12), 30.1 (C-8), 28.5 (C-9).
11. In DMSO-d_6 solution, a strong shielding effect (^{1}H and ^{13}C) was observed notably on the C-10 vinylic β-site of DS–GRE (and GRE), for which the normal positioning compared to the α-site C-11 was inverted. Such observation was recently reported for vinyl ketones: Lien, J.-C.; Chen, S.-C.; Huang, L.-J.; Kuo, S.-C. *J. Chin. Chem. Soc.* **2004**, *51*, 847–852.
12. Solid P1, P2–P3 and P4 samples were obtained by freeze-drying of water solutions.
13. *ZabSpecTOF Micromass instrument using positive ESI and LSIMS injection modes*: P1 pseudo-molecular ion $[M+H]^+ m/z$ 308.0793 (calcd for $C_{11}H_{18}NO_7S$ 308.0804); P2–P3 pseudo-molecular ion $[M+H]^+ m/z$ 356.0837 (calcd for $C_{12}H_{22}NO_7S_2$ 356.0838).
14. *Selected NMR data (D_2O) for the P2–P3 mixture (two C-10 diastereoisomers)*: ^{1}H δ 5.05 (d, 1H, $J_{1,2} = 9.0$ Hz, H-1), 4.63, 4.59 (2m, 1H, H-10$_M$ & H-10$_m$), 3.91 (br d, 1H, $J_{6a,6b} = 12.5$ Hz, H-6a), 3.72 (dd, 1H, $J_{6b,5} = 5.6$ Hz, H-6b), 3.52–3.62 (m, 2H, H-3 & H-5), 3.43–3.52 (m, 2H, H-2 & H-4), 3.19–3.26 (m, 2H, H-8 & H-11), 2.83, 2.79 (2s, 3H, H-12$_m$ & 12$_M$), 2.63–2.73 (m, 1H, H-9a), 2.21–2.39 (m, 1H, H-9b). ^{13}C δ 154.0, 153.3 (C-7$_m$ & C-7$_M$), 84.6 (C-1), 82.6 (C-10), 79.3 (C-5), 74.3 (C-3), 71.4 (C-2), 69.2, 68.5 (C-4$_m$ & C-4$_M$), 63.0 (C-6), 58.0, 56.9 (C-11$_M$ & C-11$_m$), 40.0, 39.2 (C-12$_M$ & C-12$_m$), 31.3 (C-8), 26.5, 25.4 (C-9$_M$ & C-9$_m$).
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19. Further purification was carried out by gel filtration chromatography (column XK16/60 Sephadex G-10 and Farmacia FPLC equipment) to give oxido-GRE in the form of a white amorphous solid, $[\alpha]_D$ −27 (*c* 1.1, H_2O). Selected NMR data (D_2O): ^{1}H δ 7.03 (td, 1H, $J_{10,11} = 15.2$ Hz, $J_{10,9} = 6.7$ Hz, H-10), 6.75 (d, 1H, $J_{10,11} = 15.2$ Hz, H-11), 5.09 (d, 1H, $J_{1,2} = 9.6$ Hz, H-1), 3.94 (br d, 1H, $J_{gem} = 12.4$ Hz, H-6a), 3.74 (dd, 1H, $J_{6b,5} = 5.6$, $J_{gem} = 12.4$ Hz, H-6b), 3.50 (m, 2H, H-2 & H-4), 3.59 (m, 2H, H-3 & H-5), 3.14 (s, 3H, H-12), 3.02 (t, 2H, $J_{vic} = 6.7$ Hz, H-8), 2.80 (q, 2H, $J_{vic} = 6.7$ Hz, H-9). ^{13}C δ 162.5 (C-7), 148.1 (C-10), 129.8 (C-11), 82.2 (C-1), 80.7 (C-5), 77.6 (C-3), 72.5 (C-2), 69.8 (C-4), 61.3 (C-6), 42.5 (C-12), 30.6 (C-8), 28.5 (C-9).
20. *Selected NMR data (D_2O) for P4 (major C-10 diastereoisomer)*: 1H δ 5.06 (m, 1H, H-1), 4.70 (m, 1H, H-10), 4.02 (dd, 1H, $J_{gem} = 14.4$ Hz, $J_{vic} = 2.4$ Hz, H-11a), 3.93 (br d, 1H, $J_{6a,6b} = 12.3$ Hz, H-6a), 3.72 (dd, 1H, $J_{6b,5} = 5.3$ Hz, H-6b), 3.45–3.65 (m, 5H, H-2, H-3, H-4, H-5, H-11b), 3.17–3.27 (m, 2H, H-8), 3.24 (s, 3H, H-12), 2.73 & 2.43 (2m, 2H, H-9). ^{13}C δ 157.5 (C-7), 84.7 (C-1), 82.2 (C-10), 79.0 (C-5), 74.1 (C-3), 71.2 (C-2), 68.3 (C-4), 62.7 (C-6), 56.7 (C-11), 43.8 (C-12), 32.1 (C-8), 26.3 (C-9).
21. *Selected NMR data (D_2O) for P1 (major C-10 diastereoisomer)*: 1H δ 5.54 (br s, 1H, H-11), 5.04 (d, 1H, $J_{1,2} = 9.1$ Hz, H-1), 4.24 (m, 1H, $J_{vic} = 6.4$ Hz, H-10), 3.92 (br d, 1H, $J_{gem} = 12.5$ Hz, H-6a), 3.73 (br dd, 1H, $J_{6b,5} = 5.4$ Hz, H-6b), 3.48–3.65 (m, 4H, H-2, H-3, H-4, H-5), 3.16 (br t, 2H, $J_{vic} = 7.2$ Hz, H-8), 2.39 (m, 2H, H-9). ^{13}C NMR: δ 156.0 (C-7), 87.5 (C-11), 82.8 (C-1), 80.4 (C-5), 77.2 (C-3), 74.6 (C-10), 72.3 (C-2), 69.3 (C-4), 60.8 (C-6), 30.9 (C-8), 17.7 (C-9).
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