Updated Glucosinolate Profile of *Dithyrea wislizenii*

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Fruit extracts of *Dithyrea wislizenii* were analyzed for desulfoglucosinolates and intact glucosinolates using HPLC-APCI-MS and HPLC-ESI-MS, respectively. 2-Propenylglucosinolate (sinigrin) was shown to be present in the extracts. 6-Methylsulfanylhexyl- (glucolesquerellin **9**), 6-methylsulfinylhexyl- (glucohesperin **10**), 7-methylsulfanylheptyl- (**11**), and 5-methylsulfanylpentylglucosinolate (glucoberteroin **12**) were isolated from the extracts and characterized by NMR and MS data. 7-Methoxyglucobrassicin was not detected in *D. wislizenii* extracts.

Glucosinolates (GLs) are natural products derived from amino acids that are found in the plants of the Brassicaceae family.[1] There is convincing epidemiological evidence that shows that consumption of vegetables from the Brassicaceae family is associated with a decreased risk of cancer, in particular gastrointestinal and respiratory tract cancers.[2] GLs are hydrolyzed to several products such as isothiocyanates, thiocyanates, and nitriles by several biochemical and chemical processes, including the activity of the plant enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.147), during food preparation, cooking, or chewing.[3] Indole-3-carbinol, a degradation product of 3-indolylmethylGL (glucobrassicin **1**) (Figure 1), and 4-methylsulfinybutyl isothiocyanate, a degradation product of 4-methylsulfinylbutylGL (glucoraphanin), are believed to induce beneficial effects on human health through different mechanisms.[2]

Dithyrea wislizenii Engelm. (Brassicaceae) (syn. *Dimorphocarpa wislizenii* (Engelm.) Rollins) is an endemic annual plant found in Nevada, Colorado, Utah, Arizona, New Mexico, and Texas. This species grows in open, sandy soil in dry grasslands and deserts. This grayish, hairy plant is either branched or unbranched, has pinnately lobed leaves and white flowers in dense, thick racemes, and is commonly referred to as "spectacle pod".[4] Many Native American tribes used this plant as food for animals,[5] as a drug to reduce swelling and to treat delirium and wounds, and in miscellaneous ceremonies.[6]

In previous phytochemical studies, the fatty acid content of the seed oil of *D. wislizenii* was investigated.[7,8] In addition, a GC analysis of the hydrolysis products of the compounds extracted from the seeds showed the presence of 5-methylsulfanylpentyl-, 6-methylsulfanylhexyl-, 7-methylsulfanylheptyl-, 5-methylsulfinylpentyl-, and 6-methylsulfinylhexyl isothiocyanates (Table 1), which suggests that the seeds contained the corresponding GLs.[9] Furthermore, dithyreanitrile (7-methoxy-α,α-bis(methylsulfanyl)-1*H*-indole-3-acetonitrile), an indole alkaloid with two sulfur atoms and a nitrile attached to the same carbon, was isolated from the seeds of *D. wislizenii*. This compound was shown to inhibit the feeding of the fall army worm (*Spodoptera frugiperda*) and the European corn borer (*Ostrinia nubilalis*) larvae.[10]

Presently, seven indole GLs have been indexed: glucobrassicin (GBS) (**1**), neoglucobrassicin (**2**), sulfoglucobrassicin (**3**), 1-acetyl-glucobrassicin (**4**), 4-hydroxyglucobrassicin (**5**), 4-methoxyglucobrassicin (**6**), and 1,4-dimethoxyglucobrassicin (**7**) (Figure 1).[1,11]

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Figure 1. Natural indole GLs and 7-methoxyglucobrassicin (**8**).
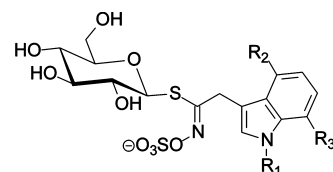
	R₁	R₂	R₃
Glucobrassicin (GBS) (**1**)	H	H	H
Neoglucobrassicin (**2**)	CH₃O	H	H
Sulfoglucobrassicin (**3**)	OSO₃⁻	H	H
1-AcetylGBS (**4**)	CH₃C=O	H	H
4-HydroxyGBS (**5**)	H	HO	H
4-MethoxyGBS (**6**)	H	CH₃O	H
1,4-DimethoxyGBS (**7**)	CH₃O	CH₃O	H
7-MethoxyGBS (**8**)	H	H	CH₃O

As part of our continued interest in the chemistry of the Brassicaceae, and particularly indole GLs, we sought to determine whether 7-methoxyglucobrassicin (**8**) was present in the fruit of *D. wislizenii* as a possible GL biogenetic precursor of dithyreanitrile. The HPLC-APCI-MS analysis of desulfoglucosinolates (DS-GLs), resulting from the removal of the sulfate group of the GLs by sulfatase-catalyzed hydrolysis, was performed. In addition, a systematic investigation of the GL content in *D. wislizenii* fruits, using a well-established method for intact GLs, involving HPLC-ESI-MS coupled with a photodiode-array detector, was initiated, and the intact GLs were isolated and characterized using spectroscopic techniques.

Results and Discussion

The GLs were extracted from *D. wislizenii* fruit according to the ISO 9167-1 method.[12] This method, specific for GLs, allows detection of the DS-GLs resulting from sulfatase-catalyzed hydrolysis and removes any undesired secondary metabolites. In Figure 2, the chromatogram of the crude extract analyzed by HPLC-APCI-MS coupled to a photodiode-array detector shows the

Table 1. Isothiocyanates Characterized from *D. wislizenii* and Their Precursor GLs


GLs R side-chain	Sulfanyl (X=S)	Sulfinyl (X=SO)
$CH_3(X)CH_2CH_2CH_2CH_2CH_2$— (7 6 5 4 3 2)	Glucoberteroin (**12**)	Glucoalyssin (**13**)
$CH_3(X)CH_2CH_2CH_2CH_2CH_2CH_2$— (8 7 6 5 4 3 2)	Glucolesquerellin (**9**)	Glucohesperin (**10**)
$CH_3(X)CH_2CH_2CH_2CH_2CH_2CH_2CH_2$— (9 8 7 6 5 4 3 2)	7-Methylsulfanylheptyl GL (**11**)	-

presence of two major peaks at $t_R = 23.9$ and 36.6 min and three minor peaks at $t_R = 9.2$, 31.6, and 40.1 min. The minor peak at $t_R = 9.2$ min was found to be sinigrin by comparison with a DS-sinigrin standard. It is the first time that spectacle pod fruit is shown to produce this alkenyl GL. Except for sinigrin, the UV spectra and retention time of the other peaks of the chromatogram did not match any of the data for the DS-GLs in our standard library. Nevertheless, the UV spectra (Figure 3) suggest that they are methylsulfinylalkyl- and methylsulfanylalkyl GLs, respectively.[13] These findings are consistent with the GC analysis of the hydrolysis products of the compounds extracted from the seeds of *D. wislizenii* by Daxenbichler et al.,[9] which showed the presence of 5-methylsulfanylpentyl-, 6-methylsulfanylhexyl-, 7-methylsulfanylheptyl-, 5-methylsulfinylpentyl-, and 6-methylsulfinylhexyl isothiocyanates, suggesting that the seeds contain the corresponding GLs. Indole GLs were not detected using this analytical method. Under our experimental conditions, the DS-GLs were detected in the negative mode. No peak with a mass of 397 amu, corresponding to [M − H]⁻, and with a UV spectrum indicating an indole moiety consistent with DS-7-methoxyGBS, was detected. We have been unable to detect the hypothetical 7-methoxyGBS in our extract either because it does not exist in the plant or because it is present in only a very small amount. Therefore, in order to rule out the latter hypothesis

and to elucidate the structure of the four compounds detected, we decided to switch to a different analytical approach.

The crude extract obtained from a large-scale extraction of *D. wislizenii* fruit, with boiling MeOH (see Experimental Section), was analyzed using an HPLC-ESI-MS coupled to a photodiode-array detector, employing an ion-pairing HPLC method for intact GLs.[14] This enabled us to obtain UV and MS spectra for each intact GL detected in the chromatogram. The clues to detect **8** in this plant extract were to search for a compound displaying an indole-type UV spectrum and a mass spectrum indicating a mass of 477 amu. The HPLC chromatogram displayed a profile similar to the one obtained by DS-GLs analysis. Sinigrin was identified by comparing its t_R, UV, and mass spectra with those of a standard in our HPLC library, thus confirming what we had observed in our previous analysis of DS-GLs.

The major peak in the chromatogram, with a mass of 448 amu, displayed a UV spectrum characteristic of a nonaromatic compound, indicating that it was not **8**. Because this major compound did not match any of the spectra in our HPLC library of standards, we isolated it and elucidated its structure using spectroscopic methods. The UV spectrum of **9** showed a λ_{max} at 225 nm. From the high-resolution mass spectrum, the molecular formula $C_{14}H_{26}NO_9S_3$ was established. FTIR data showed absorption bands at 3431 (OH), 2922
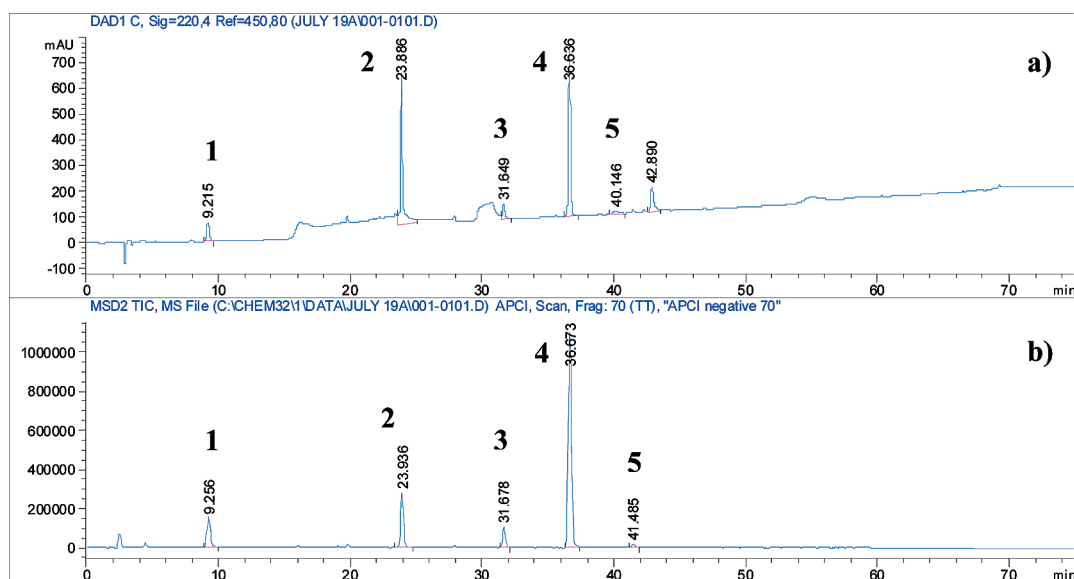
Figure 2. (a) HPLC chromatogram of DS-GLs in spectacle pod fruits at 220 nm. (b) TIC chromatogram APCI⁻. (**1**) DS-sinigrin; (**2**) DS-glucohesperin; (**3**) DS-glucoberteroin; (**4**) DS-glucolesquerellin; (**5**) DS-7-methylsulfanylheptylGL.

a)　　　　　　　　　　　　　　**b)**
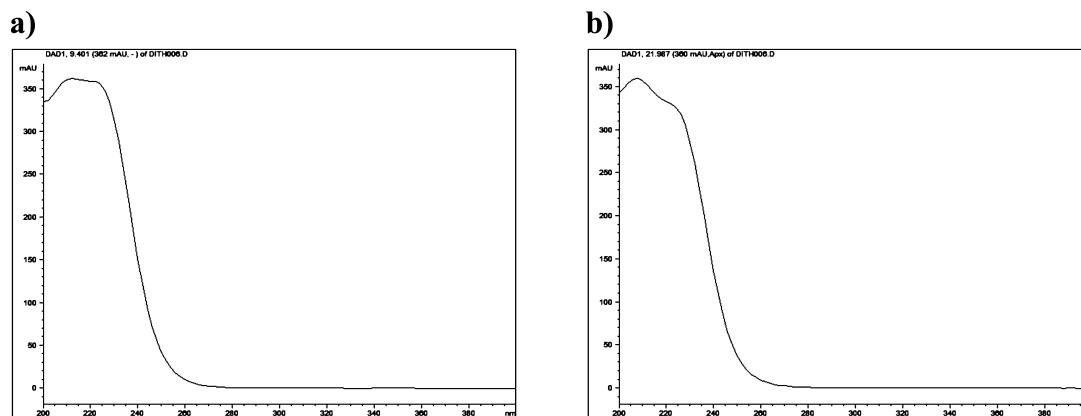
Figure 3. (a) UV spectrum of methylsulfinylalkyl DS-GL **2** of Figure 2; (b) UV spectrum of methylsulfanylalkyl DS-GLs **3** and **4** of Figure 2.

Table 2. NMR Data (δ in ppm; J in Hz) of Compounds **10**−**12** (in D_2O)

	9		10		11		12	
position	δ_C, mult.	$\delta_H{}^a$ (J in Hz)	δ_C, mult.	$\delta_H{}^b$ (J in Hz)	δ_C, mult.	$\delta_H{}^b$ (J in Hz)	δ_C, mult.	$\delta_H{}^b$ (J in Hz)
Glucose moiety								
1′	81.8, CH	5.00, d (9.9)	81.8, CH	5.05, d (9.8)	81.8, CH	5.03, d (9.9)	81.8, CH	4.94, d (9.4)
2′	71.8, CH	3.45, m	71.9, CH	3.49, dd (4.0, 9.1)	71.9, CH	3.48, m	71.9, CH	3.40, m
3′	77.0, CH	3.55, m	77.1, CH	3.59, t (9.1)	77.1, CH	3.57, t (8.9)	77.1, CH	3.49, m
4′	69.0, CH	3.45, m	69.1, CH	3.49, dd (4.0, 9.1)	69.1, CH	3.48, m	69.1, CH	3.40, m
5′	80.1, CH	3.55, m	80.2, CH	3.59, t (9.1)	80.1, CH	3.57, t (8.9)	80.1, CH	3.49, m
6′	60.5, CH_2	H-6′A 3.88, dd (1.8, 12.6) H-6′B 3.70, dd (5.7, 12.6)	60.7, CH_2	H-6′A 3.92, dd (1.8, 12.6) H-6′B 3.75, dd (5.6, 12.6)	60.6, CH_2	H-6′A 3.91, d (12.7) H-6′B 3.74, dd (5.7, 12.7)	60.6, CH_2	H-6′A 3.82, dd (1.9, 13.0) H-6′B 3.64, dd (5.6, 12.0)
Aliphatic moiety								
1	164.7, qC		164.4, qC		164.8, qC		164.6, qC	
2	32.0, CH_2	2.70, t (7.2)	31.9, CH_2	2.76, t (7.3)	32.1, CH_2	2.72, t (7.8)	32.0, CH_2	2.65, t (7.0)
3	26.7, CH_2	1.71, t (7.2)	26.4, CH_2	1.78, m	26.8, CH_2	1.73, m	26.4, CH_2	1.67, m
4	27.5, CH_2	1.41, m	27.3, CH_2	1.53, m	27.7, CH_2	1.41, m	27.3, CH_2	1.41, m
5	27.6, CH_2	1.41, m	27.4, CH_2	1.53, m	27.8, CH_2	1.41, m	27.9, CH_2	1.58, m
6	28.0, CH_2	1.61, t (7.3)	21.6, CH_2	1.78, m	27.9, CH_2	1.41, m	33.1, CH_2	2.49, t (7.3)
7	33.1, CH_2	2.54, t (7.3)	52.5, CH_2	2.93, t (7.4)	28.1, CH_2	1.62, m	14.1, CH_3	2.03, s
8	14.1, CH_3	2.09, s	36.4, CH_3	2.72, s	33.3, CH_2	2.57, t (7.3)		
9					14.1, CH_3	2.11, s		

^{a} 600 MHz. ^{b} 500 MHz.

(CH), 1655 (CN), 1061 (C−O), 881 (sulfonic acid), and 637 (C−S) cm^{-1}. The ^{1}H NMR spectrum exhibited the presence of six pairs of protons due to the CH_2 groups of an aliphatic chain, a signal due to a methylsulfanyl group (δ_H 2.09 and δ_C 14.1), and signals attributable to one glucose unit (Table 2).[15−17] The doublet of the anomeric proton, H-1′, at 5.00 ppm, has a coupling constant of 9.9 Hz, which reveals a β-linked glucopyranosyl moiety.[15−19] The assignment of carbons and protons, achieved by HMBC and HMQC 2D shift correlations, led to identification of the compound as 6-methylsulfanylhexylGL, also known as glucolesquerellin (**9**). The second major peak, with a molecular mass of 464 amu, showed similar NMR and UV spectroscopic properties to those of compound **9**. The difference in the masses of these two compounds is 16 amu, which corresponds to one oxygen atom. This compound was isolated and characterized using spectroscopic methods (^{1}H, ^{13}C NMR, HMBC, and HMQC). The ^{1}H and ^{13}C NMR data showed the presence of a glucopyranosyl unit similar to that of **9**, a hexamethylenic chain, and a methyl singlet (δ_H 2.72 ppm and δ_C 36.4 ppm), confirming the presence of a methylsulfinyl group (Table 2).[17−19] The molecular formula of this compound was confirmed to be $C_{14}H_{26}NO_{10}S_3$ by HR-ESI-MS. These spectroscopic data allowed us to establish that the compound was 6-methylsulfinyl-hexylGL or glucohesperin (**10**).

In the course of our isolation, we focused on the two minor compounds. These two metabolites were isolated and characterized using spectroscopic methods. The molecular formula of the first minor compound was determined to be $C_{15}H_{28}NO_9S_3$ by HR-ESI-MS. The mass spectrum gave a mass of 462 amu. The NMR spectra of this compound (D_2O) displayed a glucose unit similar to that of **9** and **10**, a heptamethylenic chain, and a signal due to a methylsulfanyl group (δ_H 2.11 and δ_C 14.1) (Table 2).[18,19] Analysis of the HMBC and HMQC data led to its identification as 7-methylsulfanylheptyl glucosinolate (**11**). The molecular formula of the second minor compound was determined to be $C_{13}H_{24}NO_9S_3$ by HR-ESI-MS. The mass spectrum gave a mass of 434 amu The NMR spectra of this compound (D_2O) displayed a glucopyranosyl unit similar to that of **9**, **10**, and **11**, a pentamethylenic chain, and a signal due to a methylsulfanyl group (δ_H 2.03 and δ_C 14.1) (Table 2).[18,19] Analysis of the HMBC and HMQC data led to its identification as 5-methylsulfanylpentylGL, also known as glucoberteroin (**12**).

We are reporting for the first time the spectroscopic data of the intact GLs **9**, **10**, **11**, and **12** isolated from *D. wislizenii* fruit. These findings are in agreement with the detection of 5-methylsulfanyl-pentyl-, 6-methylsulfanylhexyl-, 6-methylsulfinylhexyl-, and 7-methylsulfanylheptyl isothiocyanate by Daxenbichler et al.[9] In addition, we are reporting for the first time the DS-GLs profile for *D. wislizenii* (Figure 3 and Table 3).

In summary, we have performed experiments to probe for the presence of 7-methoxyglucobrassicin and to examine the comprehensive glucosinolate profile in *D. wislizenii* fruit using the HPLC-APCI-MS method for analysis of DS-GLs. We were able to

Table 3. Assignment of the Peaks in Figure 2 Originating from the Analysis of a Crude Extract of *D. wislizenii* Fruit

peak	retention time (min)	[M − H]⁻ (m/z)	[M + HCOO]⁻ (m/z)	UV λ$_{max}$ (nm)	corresponding GLs
1	9.2	278	324	222	sinigrin
2	23.9	384	430	224	**10**
3	31.6	354	400	225	**12**
4	36.6	368	414	224	**9**
5	40.1	382	428	224	**11**

demonstrate for the first time the presence of sinigrin and to detect two major and two minor DS-GLs. Moreover, we have determined the profile of intact GLs using an HPLC-ESI-MS method and isolated each intact GL. During this investigation, we have been able to isolate and identify 6-methylsulfanylhexylGL (**9**), 6-methylsulfinylhexylGL (**10**), 7-methylsulfanylheptylGL (**11**), and 5-methylsulfanylpentylGL (**12**) in *D. wislizenii* fruit. The spectroscopic data of **9**−**12** are reported for the first time. Despite the fact that Daxenbichler et al. reported the detection of 5-methylsulfinylpentyl isothiocyanate in *D. wislizenii* fruit, which would indicate that 5-methylsulfinylpentylGL (**13**) (also called glucoalyssin) is present in the plant, we were not able to detect this GL in our extract.[9] This may be due to environmental factors. Regardless of the method used, analysis of intact GLs or analysis of DS-GLs, we were not able to detect 7-methoxyGBS. Furthermore, we did not detect dithyreanitrile probably because it seems to be a minor compound (10⁻³% yield).[10] Because of its structure, dithyreanitrile could have a common biosynthesis pathway with indoleGLs. The structure of dithyreanitrile being close to that of arvelexin (4-methoxyindole-3-acetonitrile), a phytoalexin from the crucifer stinkweed (*Thlaspi arvense*) and that of caulilexin C (*N*-methoxyindole-3-acetonitrile), a phytoalexin from cauliflower,[20] it would therefore be interesting to determine whether this alkaloid is a phytoalexin in *D. wislizenii*.

Epidemiological studies have shown that *Brassica* vegetable consumption reduces significantly cancer risk.[21] Recently, the redox properties of 4-methylthio-3-butenylGL have been investigated.[22] Therefore, it would also be of great interest to test the potential of **9**−**12** for their health benefits.

Experimental Section

General Experimental Procedures. All solvents were ACS grade and used as such, except for CHCl₃, which was redistilled. All deuterated solvents were purchased from Cambridge Isotopes Laboratories, Inc. (Andover, MA). Formic acid was purchased from BDH (Toronto, ON). HPLC-grade MeOH and Et₃N (reagent grade) were purchased from Fisher Scientific (Whitby, ON). Sinigrin was purchased from Sigma-Aldrich (Oakville, ON). HPLC-grade H₂O was generated in the laboratory through a Nanopure Diamond Ultrapure water system by Barnstead (Dubuque, IA). Kieselgel 60 F₂₅₄ analytical TLC aluminum sheets were purchased from EM Science (Gibbstown, NJ); compounds were visualized under UV light and by dipping the plates in sulfuric acid thymol solution containing 1% (w/v) thymol, 10% (v/v) H₂SO₄, and 95% EtOH, followed by heating. Flash column chromatography (FCC) was carried out using 70−230 mesh 60 Å normal-phase silica gel (Aldrich). Sephadex LH-20 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). C-18 silica gel cartridges (Mega Bond Elut Flash, 10 g sorbent mass, 60 mL volume) were obtained from Varian, Inc. (Mississauga, ON). Preparative cellulose TLC glass plates (0.10 mm thickness, no fluorescent indicator) are from Fisher Scientific (Whitby, ON). UV spectra were determined on a Biochrom Ultrospec 2100 pro UV/visible spectrophotometer (Cambridge, England). Infrared spectra were recorded with a Perkin-Elmer Paragon 1000PC IR spectrometer (Waltham, MA) or a Bruker optics Tensor 27 FTIR instrument (Boston, MA) using KBr (VWR, Mississauga, ON) disks. NMR spectra were recorded on a Bruker Avance spectrometer at 600 MHz (¹H) and 150 MHz (¹³C) at the Saskatchewan Structural Science Centre (Saskatoon, Canada) or on a Bruker DRX 500 spectrometer at 500 MHz (¹H) and 125 MHz (¹³C) at Laurentian University (Sudbury, Canada); δ values were referenced to D₂O at 4.80 ppm. HR-ESI-MS measurements were recorded using an API Qstar

XL mass spectrometer at the Saskatchewan Structural Science Centre (Saskatoon, Canada).

Plant Material. Dry spectacle pod fruit was obtained from the company Plants of the South West (Santa Fe, NM). A voucher specimen is deposited at the Department of Biology Herbarium at Laurentian University (Sudbury, Canada).

HPLC-APCI-MS Analysis of Desulfoglucosinolates. The GL extraction was carried out according to the ISO9167-1 method.[12] The use of sinigrin as an internal standard was avoided due to its presence in the sample. Around 400 mg of fruits was successively extracted in 2 × 17 mL of warm EtOH−H₂O (70:30 v/v) using a U-Turrax homogenizer. The extractions were performed at 75 °C to quickly deactivate the endogenous myrosinase so intact compounds could be extracted. The *D. wislizenii* concentrated extract (1 mL) was loaded onto a minicolumn filled with 0.6 mL of DEAE-Sephadex A-25 resin (Amersham Biosciences), conditioned with 25 mM acetate buffer (pH 5.6). After washing with 3 mL of buffer, 100 μL (0.5 U/mL) of prepurified sulfatase was loaded onto the minicolumn and left overnight at room temperature. The DS-GLs were then eluted with 3 mL of distilled H₂O and finally injected into an LC-MS. Analysis was performed by injecting a 20 μL aliquot of the solution of crude extract into an Agilent Technologies HP 1100 (New Castle, DE) high-performance liquid chromatograph equipped with a quaternary pump, automatic injector, diode-array detector (wavelength range 190−600 nm) degasser, and a Hypersil ODS column (5 μm, 4.6 × 200 mm). The two mobile phase solvents, MeOH and H₂O, were prepared with 0.15% Et₃N and 0.18% HCO₂H, added as ion-pairing reagents. Both solutions were filtered using 0.45 μm nylon membranes. The initial mobile phase was 100% HPLC-grade H₂O. At 10 min, the mobile phase was switched to a linear gradient of 100% H₂O to 100% MeOH over 60 min.[14] After each run, the initial mobile phase conditions were set and the system was allowed to equilibrate. The flow rate was kept constant at 1 mL/min. The column temperature was held at room temperature. The HPLC is interfaced to an Agilent model 6120 mass spectrometer (Toronto, ON) with a Chemstation LC-MSD B.03.01 data system. The standard APCI source was operated with a capillary voltage of −4.5 and 3 kV and temperature of 325 °C. The corona needle was 4 μA (positive) and 7 μA (negative), respectively. The system was operated in the negative and positive ion modes. Nitrogen was used as nebulizing and drying gas at a flow rate of 5 L/min (60 psig). The mass spectrometer was programmed to perform full scans between m/z 100 and 1000.

HPLC-ESI-MS Analysis of Intact Glucosinolates. Spectacle pod extract (50 mg) was dissolved in 2 mL of MeOH and filtered through a plug of cotton prior to analysis by HPLC. A 20 μL aliquot of the solution of crude extract was injected into the Agilent Technologies HP 1100 (New Castle, DE) HPLC, described above, using the same column, the same mobile phase, and the same chromatographic conditions used to analyze the DS-GLs. The electrospray interface was a standard ES source operating with a capillary voltage of 4 kV and temperature of 350 °C. The system was operated in the negative and positive ion electrospray modes. Nitrogen was used as nebulizing and drying gas at a flow rate of 10 L/min (35 psig). The mass spectrometer was programmed to perform full scans between m/z 100 and 1000.

Extraction and Isolation. Spectacle pod fruits (347 g) were frozen in liquid N₂, ground in a mortar, and immediately extracted three times with boiling MeOH (1.4 L for the first extraction, 1.05 L for the other extractions) for 30 min. The methanolic solution was filtered and concentrated to dryness (33 g). This extract was dissolved in H₂O (200 mL) and submitted to liquid−liquid extractions with solvents of increasing polarity, i.e., CHCl₃, EtOAc, and BuOH. Each organic layer was concentrated (CHCl₃ fraction 15.2 g, EtOAc fraction 1.2 g, and BuOH fraction 6.1 g), and the aqueous layer was freeze-dried (11.7 g). The BuOH fraction (3.6 g) was separated by FCC (normal phase, gradient CHCl₃−MeOH, 8:2 and 7:3 v/v, 25 mL fractions). Fractions 36 to 125, obtained from the FCC, were combined (622 mg) and fractionated on a Sephadex LH-20 column (H₂O−MeOH, 100:0 and 0:100 v/v, 5 mL fractions). Fractions 4 to 6, obtained from the Sephadex column, were combined (400 mg) and submitted to solid-phase separation (C18 cartridge, H₂O−MeOH, 100:0, 50:50 and 0:100 v/v, 3 mL fraction). Fractions 6 and 7, obtained from the solid-phase separation (C18 cartridge), eluted with H₂O, were combined (27.9 mg), submitted to cellulose preparative TLC (n-BuOH−EtOH−H₂O, 4:1:3, upper phase, 2 successive migrations), and yielded compound **12** (2

mg). Fractions 8 to 50, obtained from the solid-phase separation (C18 cartridge), mentioned above and eluted with H_2O, yielded compound **9** (230 mg). Fractions 53 to 64, obtained from the same solid-phase separation (C18 cartridge), eluted with H_2O–MeOH (50:50, v/v), were combined (39 mg) and submitted to another solid-phase separation (C18 cartridge, H_2O–MeOH, 100:0, 95:5, 90:10, 85:15, 80:20, 50:50, and 0:100 v/v, 3 mL fraction from fractions 1 to 31 and 10 mL fraction from fractions 32 to 80). Fractions 46 to 63, obtained from this separation, eluted with H_2O–MeOH (85:15, v/v), yielded compound **11** (7 mg).

Fractions 126 to 177, obtained from the FCC (normal phase, gradient $CHCl_3$–MeOH, 8:2, 7:3 v/v, 25 mL fractions), mentioned above, were combined (611 mg) and fractionated on a Sephadex LH-20 column (H_2O–MeOH, 100:0 and 0:100 v/v, 5 mL fractions). Fraction 3 (219 mg), obtained from this Sephadex column, was submitted to solid-phase separation (C18 cartridge, H_2O–MeOH, 100:0 and 0:100 v/v, 3 mL fraction). Fractions 10 to 36, obtained from a C18 column, eluted with H_2O–MeOH (100:0), yielded compound **10** (37 mg).

6-Methylsulfanylhexylglucosinolate (9): white, amorphous powder; HPLC, t_R = 31.6 min; UV λ_{max}^{MeOH} nm (log ϵ) 225 (2.8); IR (KBr) ν_{max} 3431, 2922, 2853, 1686, 1655, 1273, 1061, 881, 799, 637, 587 cm^{-1}; ^{1}H NMR (D_2O, 600 MHz) and ^{13}C NMR (D_2O, 150 MHz) data, see Table 2; HR-ESI$^-$-MS *m/z* measured 448.0762 [M]$^-$ (calcd for $C_{14}H_{26}NO_9S_3$, 448.0769).

6-Methylsulfinylhexylglucosinolate (10): white, amorphous powder; HPLC, t_R = 22.3 min; UV λ_{max}^{MeOH} nm (log ϵ) 224 (2.8); IR (KBr) ν_{max} 3380, 2935, 2839, 1621, 1454, 1402, 1274, 1061, 882, 803, 623, 578 cm^{-1}; ^{1}H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; HR-ESI$^-$-MS *m/z* measured 464.0708 [M]$^-$ (calcd for $C_{14}H_{26}NO_{10}S_3$, 464.0719).

7-Methylsulfanylheptylglucosinolate (11): white, amorphous powder; HPLC, t_R = 35.6 min; UV λ_{max}^{MeOH} nm (log ϵ) 224 (3.7); IR (KBr) ν_{max} 3406, 2925, 2854, 1733, 1624, 1457, 1427, 1272, 1060, 878, 802, 646 cm^{-1}; ^{1}H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; HR-ESI$^-$-MS *m/z* measured 462.0934 [M]$^-$ (calcd for $C_{15}H_{28}NO_9S_3$, 462.0926).

5-Methylsulfanylpentylglucosinolate (12): white, amorphous powder; HPLC, t_R = 27.7 min; UV λ_{max}^{MeOH} nm (log ϵ) 222 (3.7); IR (KBr) ν_{max} 3417, 2924, 2853, 1739, 1630, 1463, 1380, 1258, 1118, 1065, 800, 663 cm^{-1}; ^{1}H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; HR-ESI$^-$-MS *m/z* measured 434.0608 [M]$^-$ (calcd for $C_{13}H_{24}NO_9S_3$, 434.0613).

Supporting Information Available: HPLC chromatograms, ^{1}H NMR and ^{13}C NMR spectra of compounds **9**–**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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