

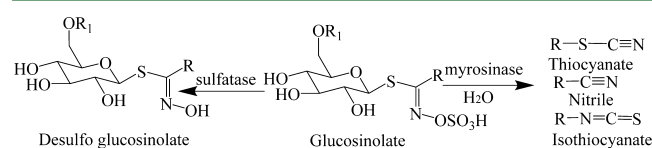
Profiling of Glucosinolates and Flavonoids in *Rorippa indica* (Linn.) Hiern. (Cruciferae) by UHPLC-PDA-ESI/HRMS<sup>n</sup>Long-Ze Lin,<sup>\*,†</sup> Jianghao Sun,<sup>†</sup> Pei Chen,<sup>†</sup> Ren-Wei Zhang,<sup>§</sup> Xiao-E Fan,<sup>§</sup> Lai-Wei Li,<sup>§</sup> and James M. Harnly<sup>†</sup><sup>†</sup>Food Composition and Methods Development Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Building-161, BARC-East, 10300 Baltimore Avenue, Beltsville, Maryland 20705, United States<sup>§</sup>Longjin Pharmaceutical Company, Ltd., Wujiadui News Road, Kunming, Yunnan 650228, China

**ABSTRACT:** An UHPLC-PDA-ESI/HRMS<sup>n</sup> profiling method was used to identify the glucosinolates and flavonoids of *Rorippa indica* (Cruciferae), a wild vegetable and Chinese herb used to treat cough, diarrhea, and rheumatoid arthritis. Thirty-three glucosinolates, more than 40 flavonol glycosides, and 18 other phenolic and common organic compounds were identified. The glucosinolates and polyphenols were separated by UHPLC. High-resolution deprotonated molecules provided high accuracy mass values that were used to determine formulas and provide putative identification of the glucosinolates and flavonoids. The fragments from multistage mass spectrometry were used to elucidate the structures. The concentrations of the main components were based on UV peak areas and molar relative response factors with a single calibration standard. This study found this plant to be a rich source for glucosinolates, containing 24 new glucosinolates, including 14 glucosylated glucosinolates that were previously unidentified.

**KEYWORDS:** glucosinolates, flavonol glycosides, *Rorippa indica* (Cruciferae), Chinese wild vegetable, Chinese herb, UHPLC-PDA-ESI/HRMS<sup>n</sup>, compound identification, main compound quantification

## INTRODUCTION

Glucosinolates (GSs) are sulfur- and nitrogen-containing secondary metabolites found in cruciferous plants. A central carbon is connected to a thioglucose, to a sulfate via a nitrogen atom, and to a side chain (R) (Figure 1). The side chain determines whether the glucosinolate is defined as aliphatic



The identification code, name, and R and R<sub>1</sub> groups of the glucosinolates

GS-1 to GS-3: R=9-(methylsulfonyl)nonyl (straight and branch chain), R<sub>1</sub>=H  
 GS-4: R=7-methylsulfonylheptyl, R<sub>1</sub>=H  
 GS-5: R=8-(methylsulfonyl)octyl, R<sub>1</sub>=H  
 GS-6: R=10-methylsulfonyldecyl, R<sub>1</sub>=H  
 GS-7: R=8-(methylsulfonyl)octyl, R<sub>1</sub>=H  
 GS-8 and GS-9: R=9-(methylsulfonyl)nonyl (straight and branch chain), R<sub>1</sub>=H  
 GS-10: R=10-(methylsulfonyl)decyl, R<sub>1</sub>=H  
 GS-11: R=4-methoxyindol-3-ylmethyl, R<sub>1</sub>=H  
 GS-12: R=hydroxyundecyl, R<sub>1</sub>=H  
 GS-13 to GS-16: R=9-(methylsulfonyl)hydroxynonyl (straight and branch chain), R<sub>1</sub>=H  
 GS-17 and GS-18: R=10-methylsulfonylhydroxydecyl (straight and branch chain), R<sub>1</sub>=H  
 GS-19 to GS-22: R=9-(methylsulfonyl)nonyl (straight and branch chain), R<sub>1</sub>=Glc  
 GS-23: R=9-(methylsulfonyl)nonyl, R<sub>1</sub>=Glc  
 GS-24 to GS-26: R=10-methylsulfonylhydroxydecyl (straight and branch chain), R<sub>1</sub>=Glc  
 GS-27 and GS-28: R=9-(methylsulfonyl)nonyl, R<sub>1</sub>=Glc  
 GS-29: R=10-(methylsulfonyl)decyl, R<sub>1</sub>=Glc  
 GS-30: R=methylthioethylpentyl, R<sub>1</sub>=Glc  
 GS-31: R=7-methylthio-3-oxoheptyl, R<sub>1</sub>=Glc  
 GS-32: R=8-methylthiooctyl, R<sub>1</sub>=Glc; R<sub>1</sub>=H: deoxyglucohirsutin in RS  
 GS-33: R=7-(methylsulfonyl)heptyl, R<sub>1</sub>=Glc

Figure 1. Structures of the glucosinolates of *Rorippa indica*.

(straight- or branched-chain alkyl, alkenyl, and oxo-, hydroxyl forms), aromatic, or indole. Many glucosinolates also contain one or two more sulfur atoms in the side chain to form another sulfur-containing unit, such as methyl-thio- (–S–), methyl-sulfinyl- (–SO–), methyl- or benzyl-sulfonyl- (–SO<sub>2</sub>–), mercapto- (HS–), glucosyl-disulfanyl (–S–S–), and cysteinyl-thio- [HOOC–CH(NH<sub>2</sub>)–CH<sub>2</sub>–S–]. The glucosinolates and their enzyme-hydrolyzed products (thiocyanates, isothiocyanates, and nitriles) have shown important human health benefits, especially with respect to cancer prevention. Thus, many studies have been carried out on the isolation and structural elucidation of glucosinolates and their hydrolyzed products with respect to their biological activities and distribution in the plant kingdom.<sup>1–7</sup>

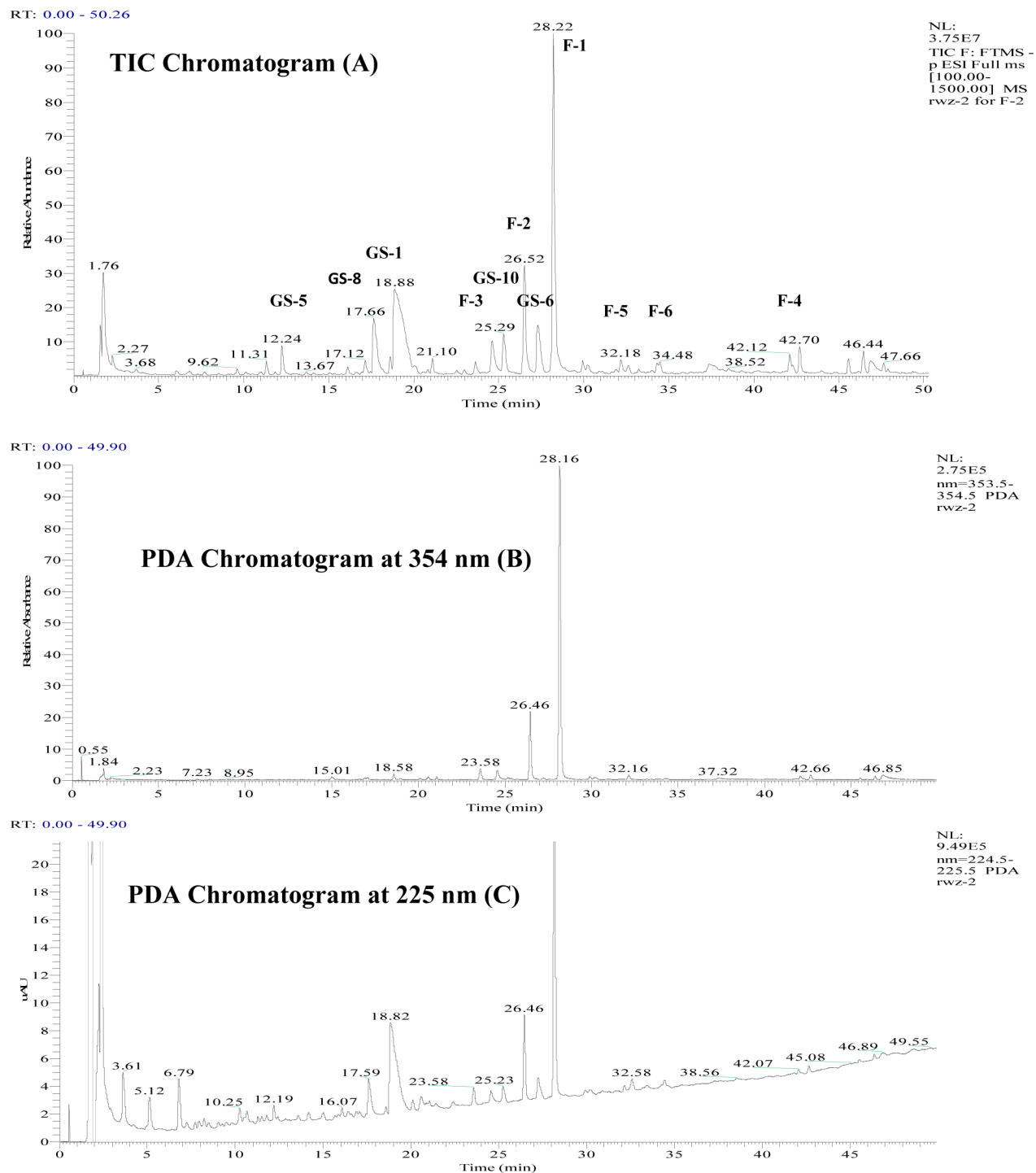
To date, approximately 200 glucosinolates have been reported in plants. An electronic database of the structures, formulas, and accurate masses of 200 known and a further 180 predicted GSs has been established.<sup>8</sup> Desulfoglucosinolates, glucosinolate-related nitriles, and isothiocyanates (Figure 1) formed by the loss of SO<sub>3</sub> or other functional groups through the action of sulfatase and myrosinase have been isolated from plants.<sup>9,10</sup> Glucosinolates, widely distributed in many plants of the Cruciferae family, have also been found in plants from other families although at significantly reduced levels. The glucosinolate profiles of many foods derived from cruciferous plants have been studied qualitatively and quantitatively to

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**Figure 2.** Chromatograms (A, TIC; B, PDA at 354 nm; and C, PDA at 225 nm) of *Rorippa indica* (only the main glucosinolates and flavonoids are labeled).

establish their dietary intake and relationship to health outcomes in biological, epidemiological, and clinical studies.<sup>1–28</sup>

The fragmentation patterns of glucosinolates have been studied using multistage mass spectrometry (MS<sup>n</sup>), primarily in the negative ionization mode. High-performance liquid chromatography and MS<sup>n</sup> with electrospray ionization (HPLC-ESI/MS<sup>n</sup>) has been widely used to identify the intact glucosinolate components of plants.<sup>8,11–18</sup> Most glucosinolates have an ultraviolet (UV) absorbance maximum wavelength

( $\lambda_{\max}$ ) between 219 and 228 nm. They can be quantified directly from the UV absorbance at 225 nm when they are well separated.<sup>8,14,19,20</sup> However, in most cases, quantification has been based on absorbance of the desulfoglucosinolates following sulfatase hydrolysis. Quantification is based on calibration with a single compound and predetermined relative response factors (RRFs).<sup>8,20–26</sup>

*Rorippa indica* (Linn.) Hiern. is a perennial plant of the Cruciferae family that is widely distributed throughout the world. Its fresh spear-shaped leaves and tender seedlings are

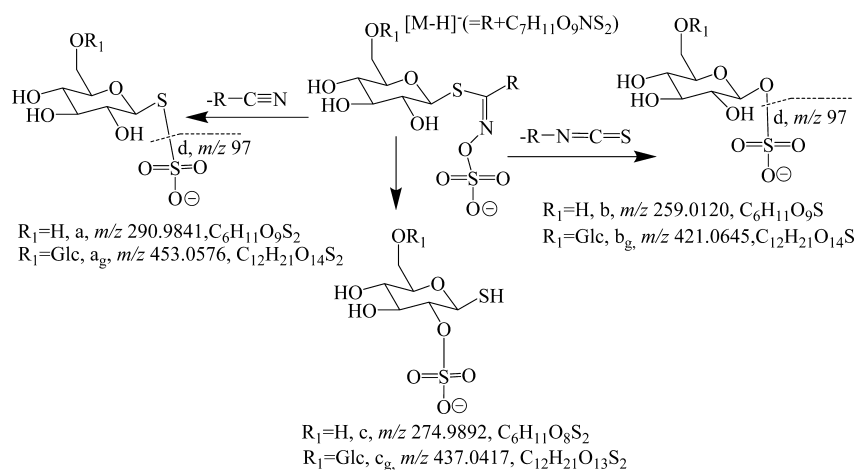


Figure 3. Molecular and typical fragments for the glucosinolates in *Rorippa indica*.

used as a healthy food, and the whole plant is used as an animal feed in China. The dried whole plant material is used as a Chinese traditional herb with purported diuretic, anti-inflammatory, antifever, and anticough properties and to help with blood circulation and rheumatoid arthritis.<sup>27</sup> Some glucosinolates have been reported in *R. indica* and several other *Rorippa* plants.<sup>4,28</sup> Ronifore (9-methylsulfonylnonyl nitrile) and its related amide (rorifamide) were isolated as the anticough components of *Rorippa montana*.<sup>27,29</sup> Fifteen isothiocyanates and desulfoglucosinolates were isolated from the roots of *Rorippa* plants, and several flavonol glycosides, phenolic acids, and derivatives, and other organic compounds have been reported.<sup>9,28–30</sup> This paper presents a comprehensive study of the glucosinolate and polyphenolic components of *R. indica*.

Phenolic compounds are widely distributed in plant-derived foods, whereas the glucosinolates are among the biologically active components of Cruciferous plants. Both types of compounds are known to have many potential benefits to human health. As a part of our project to systematically identify and quantify these compounds, both standardized HPLC-PDA-ESI/MS and UHPLC-PDA-ESI/HRMS<sup>n</sup> methods were developed to identify and quantify the phenolics using their molar relative response factors (MRRFs) for UV absorbance.<sup>31–33</sup> In this study, the UHPLC-PDA-ESI/HRMS<sup>n</sup> method was used to analyze the phenolic and glucosinolate profiles of *R. indica* and led to the identification of 33 glucosinolates, including 24 for the first time, and more than 40 flavonol glycosides. This study found that *R. indica* was a rich plant source for glucosinolates on the basis of its structural variety and total concentration of glucosinolates.<sup>4,5,8</sup>

## MATERIALS AND METHODS

**Standards and Other Chemicals.** Glucocheirolin potassium salt [3-(methylsulfonyl)propyl glucosinolate-K, MW = 439 + 39 Da], rutin 3H<sub>2</sub>O (MW = 610 + 54 Da), quercetin, kaempferol, and isorhamnetin were obtained from Chromadex, Inc. (Irvine, CA, USA). Formic acid, hydrochloric acid (~37%), HPLC grade methanol, and acetonitrile were purchased from VWR International, Inc. (Clarksburg, MD, USA). HPLC grade water was prepared from distilled water using a Milli-Q system (Millipore Lab., Bedford, MA, USA).

**Standard Solutions.** The stock standard solution consisted of dried rutin (1.27 mg)<sup>34</sup> and glucocheirolin-K (1.43 mg) in 10.0 mL of methanol/water (60:40, v/v). The stock solution was prepared at three concentrations (1, 1/2, and 1/4) to provide a range of signals suitable for the quantification of the main glucosinolates and phenolics

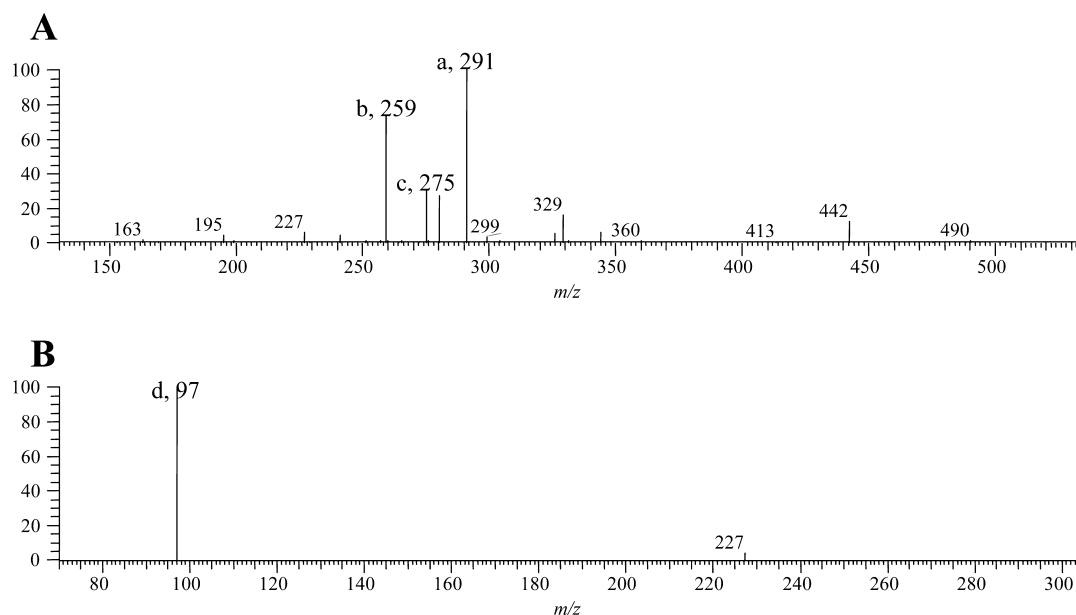
of this sample. Each of the solutions (2  $\mu$ L) was injected in triplicate, and the relative standard deviation (RSD) for each peak was <5.0%. The three standards provided a linear calibration curve for estimating the concentration of the main compounds of the extract.<sup>31,32</sup>

**Plant Materials and Extracts.** The dried *R. indica* plant materials were purchased by the Longjin Pharmaceutical Co., Ltd., from local herb stores in Chongqing, China. The plant materials were ground into powders and passed through 60 mesh sieves prior to extraction. The ground powder (200 mg) was extracted with 5.0 mL of methanol/water (60:40, v/v) using an FS30 ultrasonic sonicator (Fisher Scientific, Pittsburgh, PA, USA) for 60 min at room temperature. The slurry mixture was centrifuged at 2500 rpm for 15 min (IEC Clinical Centrifuge, Damon/IEC Division, Needham, MA, USA), and the supernatant (4.000 mL) was filtered through a 17 mm (0.45  $\mu$ m) PVDF syringe filter (VWR Scientific, Seattle, WA, USA). Two microliters of the extract was injected in triplicate into the HPLC for analysis.<sup>31–33</sup>

**Acidic Hydrolyzed Extracts.** The filtered extracts (0.50 mL) were mixed with concentrated HCl (37%, 0.1 mL) and heated in a capped tube at 85 °C for 2 h. Then, 0.4 mL of methanol was added to the mixture, and the solution was sonicated for 10 min. The solution was refiltered prior to HPLC injection.<sup>31</sup>

**UHPLC-PDA-ESI/HRMS<sup>n</sup> Conditions.** The UHPLC-DAD-ESI/HRMS-MS<sup>n</sup> system used consisted of an LTQ Orbitrap XL mass spectrometer with an Accela 1250 binary Pump, a PAL HTC Accela TMO autosampler, a diode array detector (DAD) (ThermoScientific, San Jose, CA, USA), and a G1316A column compartment (Agilent, Palo Alto, CA, USA). The separation was carried out on an UHPLC column (200 mm  $\times$  2.1 mm i.d., Hypersil Gold AQ-RP-C18, 1.9  $\mu$ m) (ThermoScientific) with an HPLC/UHPLC precolumn filter (Ultra-Shield Analytical Scientific Instruments, Richmond, CA, USA) at a flow rate of 0.3 mL/min. The mobile phase consisted of a combination of A (0.1% formic acid in water, v/v) and B (0.1% formic acid in acetonitrile, v/v). The linear gradient was from 4 to 20% B (v/v) at 40 min, to 35% B at 60 min, to 100% B at 61 min, and held at 100% B to 65 min.<sup>32</sup> The PDA recorded spectra from 200 to 700 nm, and peak intensities at 225 and 354 nm were used for the quantification of the main glucosinolates and flavonoids, respectively.<sup>33</sup>

The MS was operated in the negative ionization mode using the following conditions: sheath gas at 70 (arbitrary units), auxiliary and sweep gas at 15 (arbitrary units), spray voltage at 4.8 kV, capillary temperature at 300 °C, capillary voltage at 15 V, and tube lens at 70 V. The scan range was from  $m/z$  100 to 1500 with a resolution of 15000, FTMS AGC target at 2e5, FT-MS/MS AGC target at 1e5, isolation width of  $m/z$  1.5, and maximum ion injection time of 500 ms. High-accuracy ion mass values were determined for all primary ions (i.e., the deprotonated molecular ions). The most intense ion was selected for the data-dependent scan to provide MS<sup>2</sup>–MS<sup>5</sup> product ions with a normalized collision energy at 35% (CID).<sup>33</sup> The product ions were



**Figure 4.** MS<sup>2</sup> (A) and MS<sup>3</sup> (B) spectra of 9-(methylsulfonyl)nonylglucosinolate (GS-1).

determined with nominal mass accuracy. An additional separate data-dependent scan with high-energy collisional dissociation (HCD) at 75% was also carried out for high-accuracy measurement of some of the MS<sup>n</sup> ions with the resolution of 7500.

## RESULTS AND DISCUSSION

**Identification of Glucosinolates.** Thirty-three glucosinolates were detected in the plant extract. Figure 1 lists the identification codes, putative identification, retention times (recorded on the TIC chromatogram), deprotonated molecule high-resolution mass, formula, error between the experimental and calculated values, and main and important fragments from MS<sup>2</sup>, MS<sup>3</sup>, and MS<sup>4</sup> fragmentations for each peak. The listing is in order of molecular weight. The TIC and PDA (at 225 nm) chromatograms are shown in Figure 2. Thirty-three of the compounds were putatively identified, and nine compounds could not be identified.

The main glucosinolates of this plant consisted of methylsulfonyl straight alkyl chain compounds (CH<sub>3</sub>S(O)<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>) (Figure 1). The most concentrated glucosinolate (GS-1) had TIC and UV peaks with retention times (*t*<sub>R</sub>) at 18.88 and 18.83 min (Figure 2), UV λ<sub>max</sub> at 225 nm, and a [M - H]<sup>-</sup> of 522.1134 Da for C<sub>17</sub>H<sub>32</sub>O<sub>11</sub>NS<sub>3</sub> (calcd 522.1129 Da, with the error of -1.72 ppm) for 9-(methylsulfonyl)nonylglucosinolate (GS-1). The assignment of this compound as 9-(methylsulfonyl)nonyl glucosinolate (GS-1) was based on the fact that the hydrolyzed products, desulfoglucosinolate, isothiocyanate, and nitrile (roffifone), had been previously isolated from this plant.<sup>9,27,28</sup> Furthermore, the authors in China isolated the related nitrile from the same plant material and confirmed the structure by nuclear magnetic resonance.

GS-1 had major MS<sup>2</sup> fragments at *m/z* 291 (100%, [C<sub>6</sub>H<sub>11</sub>O<sub>9</sub>S<sub>2</sub>]<sup>-</sup>), 275 (28%, [C<sub>6</sub>H<sub>11</sub>O<sub>8</sub>S<sub>2</sub>]<sup>-</sup>), and 259 (73%, [C<sub>6</sub>H<sub>11</sub>O<sub>9</sub>S]<sup>-</sup>), ions a, c, and b in Figures 3 and 4A. MS<sup>3</sup> had a major fragment at *m/z* 97 (100%, [HSO<sub>4</sub>]<sup>-</sup>), ion d in Figure 4B. The compositions for ions a, c, and b were confirmed by the experimentally determined masses of 290.9842 (error -2.7 ppm), 274.9890 (error -3.9 ppm), and 259.0117 (error -4.8 ppm, Figure 3), respectively, from the additional data-dependent scan with activation in HCD.

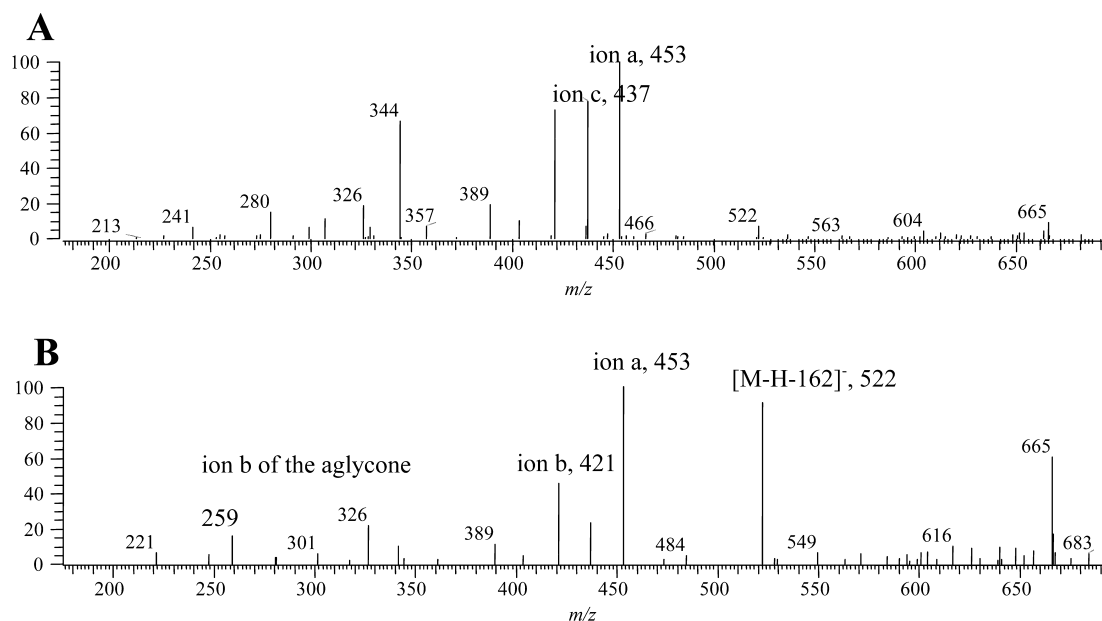
Figure 3 illustrates the fragmentation pathway leading to the four product ions through the loss of RCN and RNCS groups. Some glucosinolates, such as 4-methoxyglucobrassicin, glucobrassicin, 3'-hydroxyglucoisatisin, glucoisatisin, and sinalbin, were reported to present an MS<sup>2</sup> fragment (b, 291 Da) with a relative intensity of 6–61%.<sup>13,14,22</sup> These diagnostic ions fully supported the identification of this compound as a glucosinolate.

Two minor peaks (GS-2 and GS-3, *t*<sub>R</sub> = 10.39 and 21.00 min) had the same high-resolution deprotonated molecular ion masses as GS-1 and similar MS<sup>2</sup> and MS<sup>3</sup> fragments. Thus, they were determined to be isomers of GS-1. Most likely, the R groups are isomeric.

Three glucosinolates, GS4, GS-5, and GS-6, are analogues of GS-1 and identified as 7-methylsulfonylheptyl- (*t*<sub>R</sub> = 6.67 min, [M - H]<sup>-</sup> = 494.0823 Da), 8-(methylsulfonyl)octyl- (*t*<sub>R</sub> = 12.24 min, [M - H]<sup>-</sup> = 508.0978 Da), and 10-methylsulfonyldecylglucosinolates (*t*<sub>R</sub> = 27.25 min, [M - H]<sup>-</sup> = 536.1301 Da), respectively. Their mass differences arise from the numbers of CH<sub>2</sub> units. Their retention times correlate with increasing mass, suggesting that the R groups are straight chains and the increasing number results in increased retention times as the polarity decreases. These data also confirmed the identification of these compounds as glucosinolates.

Four other glucosinolates (GS-7–GS-10) containing methylsulfinyl (CH<sub>3</sub>SO(CH<sub>2</sub>)<sub>n</sub>) were detected. They were putatively identified as 8-(methylsulfinyl)octyl- (*t*<sub>R</sub> = 11.39 min, [M - H]<sup>-</sup> = 492.1031 Da), 9-(methylsulfinyl)nonyl- (*t*<sub>R</sub> = 17.66 and 18.71 min, [M - H]<sup>-</sup> close to 506.1189 Da, two isomers, GS-8 and GS-9), and 10-(methylsulfinyl)decyl glucosinolates (*t*<sub>R</sub> = 25.28 min, [M - H]<sup>-</sup> = 520.1347 Da), respectively. All of them showed the same diagnostic fragments, confirming them as glucosinolates, and their elution time increased with increasing numbers of CH<sub>2</sub> units.

GS-11 (*t*<sub>R</sub> = 11.85 min) contained an indole function and was putatively identified as 4-methoxyindol-3-ylmethylglucosinolate on the basis of its high-resolution mass value of 477.0635 Da. This compound showed the same fragmentation behavior observed above. Eight of the above glucosinolates (GS-1, GS-4,



**Figure 5.** MS<sup>2</sup> spectra of the glucosides (A, GS-19; and B, GS-22) of 9-(methylsulfonyl)nonylglucosinolate.

GS-5, GS-6, GS-7, GS-8, GS-10, and GS-11) were previously reported, and GS-7, GS-8, and GS-11 were found in *Rorippa* plants.<sup>4</sup> Furthermore, the desulfoglucosinolates and/or isothiocyanates of the above glucosinolates (except GS-4) were previously isolated from the roots of this plant.<sup>9</sup> However, compounds GS-2, GS-3, and GS-9 were not known glucosinolates.<sup>4–8</sup>

The remaining 21 peaks were putatively identified as glucosinolates and will be discussed in detail. GS-12 ( $t_R = 33.33$  min,  $[M - H]^- = 488.1622$  Da) had a deprotonated molecular ion of  $C_{18}H_{34}O_{10}NS_2$  (error 0.49 ppm) and showed the classic glucosinolate MS<sup>2</sup> fragments at  $m/z$  259 (100%), 275 (44%), and 291 (33%). After subtraction of ion b ( $m/z$  259,  $C_6H_{11}O_9S$ ) or ion a ( $m/z$  291,  $C_6H_{11}O_9S_2$ ) from the deprotonated molecule (Figure 3), the masses for R-NCS or R-CN were obtained. Each showed a formula for R = HO-(CH<sub>2</sub>)<sub>11</sub>, that is,  $C_{11}H_{23}O$ , the same as that obtained directly from R =  $C_{18}H_{34}O_{10}NS_2 - C_7H_{11}O_9NS_2$  (Figure 3). In this way, the structures for the glucosinolates can be figured out easily. Thus, this compound was putatively identified as hydroxyundecyl glucosinolate. This glucosinolate was not among the predicted 180 new glucosinolates in the electronic database of Clarke because the number of CH<sub>2</sub> groups ( $n$ ) is 11 and 10 was the largest considered in the prediction set.<sup>8</sup>

Two groups of the isomeric alkyl glucosinolates were observed. The first group (GS-13–GS-16) contained four isomers ( $t_R = 5.41, 6.47, 7.74,$  and  $10.55$  min,  $[M - H]^- = 538.1081$  Da). They showed product ions of  $m/z$  291, 275, 259, and 97 and were identified as 9-(methylsulfonyl)-hydroxynonylglucosinolates. They had one more hydroxyl group than that of related 9-(methylsulfonyl)-nonylglucosinolate (GS-1) and its isomers (GS-2 and GS-3). The second group (GS-17 and GS-18) consisted of two isomers ( $t_R = 10.97$  and  $13.34$  min,  $[M - H]^- = 552.1236$  Da). They showed the same diagnostic GS fragments and were putatively identified as 10-methylsulfonylhydroxydecylglucosinolates and to be the hydroxyl derivatives of 10-methylsulfonyldecylglucosinolate (GS-6) and its isomers. The R groups for the isomers can be determined in the same manner as described

previously for GS-12. Among them, GS-13 and GS-17 (with suggested straight chains for the R group) were among the predicted 180 new glucosinolates in the electronic database of Clarke with code numbers 245 and 246, respectively.<sup>8</sup>

Four minor peaks (GS-19–GS-22) had different retention times ( $t_R = 17.68, 16.08, 16.84,$  and  $20.92$  min) but a similar mass ( $[M - H]^- = 684.1658–684.1663$  Da) corresponding to a formula of  $C_{23}H_{42}O_{16}NS_3$  (errors less than  $-2.0$  ppm). GS-19 had major MS<sup>2</sup> fragments at  $m/z$  522 (77%,  $[M - H]^- - 162$ , loss of glucosyl), 453 (100%,  $291 + 162$ , ion a<sub>g</sub>, the “g” means the fragment contained a glucosyl,  $C_6H_{10}O_5$ , Figure 3), 437 (23%,  $275 + 162$ , ion c<sub>g</sub>), 421 (94%,  $259 + 162$ , ion b<sub>g</sub>, Figure 3), and 259 (14%, ion b) (Figures 4 and 5 and Table 1). The detection of ions a, b, and c plus a glucosyl is reasonable, and  $[M - H]^- = 421.0670$  (error of 3.0 ppm) for ion b confirmed its composition as  $C_{12}H_{21}O_{14}S$ . Thus, the data suggest that these compounds are the glucosides of 9-(methylsulfonyl)nonyl glucosinolate (the glucoside of GS-1).

GS-20, GS-21, and GS-22 also showed the typical glucosinolate glucoside fragments at  $m/z$  453, 437, and 421 (Figures 3 and 5 and Table 1). On the basis of both of the high-resolution mass values and the presence of the glucosinolate glucoside characteristic fragments, they were identified as the glucosides of 9-(methylsulfonyl)nonylglucosinolates (the glucosides of GS-1–GS-3). Because there was no hydroxyl function on the R group, this glucosyl was most likely connected to the thioglucoside and most likely at the 6-position as that for a monoacylated glucosinolate.<sup>6,7</sup>

Similarly, four other compounds (GS-23–GS-26) were recognized as the glucosides of glucosinolates on the basis of the fact that they presented the same diagnostic fragments at  $m/z$  453, 437, and 421. GS-23 ( $t_R = 22.55$  min,  $[M - H]^- = 654.1554$  Da,  $C_{22}H_{40}O_{15}NS_3$ , error less than  $-2$  ppm) was putatively identified as a glucoside of 9-(methylsulfonyl)-nonylglucosinolate (the glucoside of GS-8). GS-24–GS-26 ( $t_R = 23.37, 24.29,$  and  $28.18$  min,  $[M - H]^- = 698.1915$  Da,  $C_{24}H_{44}O_{16}NS_3$ , error  $-1.8$  ppm) were putatively identified as the glucosides of 10-methylsulfonyldecylglucosinolates (the glucosides of GS-6 and its isomers).

**Table 1. Compound Code, Putative Identification, Retention Time, High-Resolution Mass of the Deprotonated Molecule, Formula, Error between Calculated and Measured Values, and MS<sup>n</sup> Data of the Glucosinolates in *Rorippa indica***

code	glucosinolate putative identification <sup>a</sup>	$t_R$ (min)	$[M - H]^-$ (Da)	formula	error (ppm)	MS <sup>1</sup> fragments (%)	MS <sup>2</sup> fragments (%)	MS <sup>3</sup> fragments (%)	MS <sup>n</sup> fragments (%)
GS-11	4-methoxyindol-3-ylmethylglucosinolate	11.85	477.0635	C <sub>17</sub> H <sub>21</sub> O <sub>10</sub> N <sub>2</sub> S <sub>2</sub>	-1.697	459 (61), 291 (39), 275 (61), 259 (100), 235 (28)			
GS-12	hydroxyundecylglucosinolate	33.33	488.1624	C <sub>18</sub> H <sub>34</sub> O <sub>10</sub> NS <sub>2</sub>	-1.149	471 (90), 470 (60), 444 (50), 291 (40), 275 (40), 259 (100), 246 (30), 201 (30), 145 (30)	470 (89), 291 (33), 275 (44), 259 (100)		
GS-7	8-(methylsulfinyl)octylglucosinolate (glucoarabtin)	11.39	492.1031	C <sub>16</sub> H <sub>30</sub> O <sub>10</sub> N <sub>2</sub> S <sub>3</sub>	-1.283	428 (100, -64)		275 (36), 259 (100)	
GS-4	7-(methylsulfonyl)heptylglucosinolate	6.67	494.0823	C <sub>15</sub> H <sub>28</sub> O <sub>11</sub> NS <sub>3</sub>	-1.409	476 (21), 291 (100), 275 (32), 259 (79), 252 (21)		275 (46), 259 (100)	241 (12), 139 (100), 97 (44), 89 (8)
GS-8	9-(methylsulfinyl)nonylglucosinolate (glucoarabin)	17.66	506.1188	C <sub>17</sub> H <sub>32</sub> O <sub>10</sub> NS <sub>3</sub>	-0.951	442 (100, -64)			
GS-9	isomer of GS-8	18.71	506.1189	C <sub>17</sub> H <sub>32</sub> O <sub>10</sub> NS <sub>3</sub>	-0.951	442 (100)			
GS-5	8-(methylsulfonyl)octylglucosinolate	12.24	508.0978	C <sub>16</sub> H <sub>30</sub> O <sub>11</sub> NS <sub>3</sub>	-1.665	291 (100), 275 (21), 259 (45),		97 (100)	
GS-10	10-(methylsulfinyl)decylglucosinolate	25.28	520.1347	C <sub>18</sub> H <sub>34</sub> O <sub>10</sub> NS <sub>3</sub>	-0.637	456 (100)		376 (16), 291 (18), 278 (16), 275 (47), 263 (20), 259 (100)	241 (12), 223 (10), 139 (100), 97 (38)
GS-1	9-(methylsulfonyl)nonylglucosinolate	18.88	522.1134	C <sub>17</sub> H <sub>32</sub> O <sub>11</sub> NS <sub>3</sub>	-1.716	442 (12), 329 (16), 291 (100), 280 (27), 275 (30), 259 (73), 227 (6)		97 (100)	
GS-2	isomer of GS-1	21.00	522.1136	C <sub>17</sub> H <sub>32</sub> O <sub>11</sub> NS <sub>3</sub>	-1.333	522 (33), 504 (100), 478 (33), 476 (56), 450 (33), 444 (33), 291 (44), 259 (56)			
GS-3	isomer of GS-1	10.39	522.1138	C <sub>17</sub> H <sub>32</sub> O <sub>11</sub> NS <sub>3</sub>	-0.950	458 (100)		378 (39), 332 (35), 280 (26), 275 (30), 259 (100), 235 (30)	
GS-6	10-(methylsulfonyl)decylglucosinolate	27.25	536.1301	C <sub>18</sub> H <sub>34</sub> O <sub>11</sub> NS <sub>3</sub>	1.125	456 (11), 343 (14), 340 (6), 294 (20), 291 (100), 275 (26), 259 (58)		97 (100)	
GS-13	9-(methylsulfonyl)hydroxynonylglucosinolate	5.41	538.1082	C <sub>17</sub> H <sub>32</sub> O <sub>12</sub> NS <sub>3</sub>	-1.878	520 (53), 494 (24), 458 (24), 291 (100), 275 (41), 259 (59)			
GS-14	isomer of GS-12	6.47	538.1082	C <sub>17</sub> H <sub>32</sub> O <sub>12</sub> NS <sub>3</sub>	-2.594	520 (45), 494 (36), 450 (27), 448 (27), 362 (27), 296 (45), 291 (100), 259 (55)			
GS-15	isomer of GS-12	7.74	538.1060	C <sub>17</sub> H <sub>32</sub> O <sub>12</sub> NS <sub>3</sub>	0.298	296 (16), 291 (100), 275 (24), 259 (44)			
GS-16	isomer of GS-12	10.55	538.1080	C <sub>17</sub> H <sub>32</sub> O <sub>12</sub> NS <sub>3</sub>	-2.250	495 (26), 360 (21), 296 (26), 291 (100), 259 (21)			
GS-17	10-(methylsulfonyl)hydroxydecylglucosinolate	10.97	552.1238	C <sub>18</sub> H <sub>34</sub> O <sub>12</sub> NS <sub>3</sub>	-2.761	508 (17), 310 (11), 291 (100), 275 (20), 259 (37)			
GS-18	isomer of GS-16	13.34	552.1240	C <sub>18</sub> H <sub>34</sub> O <sub>12</sub> NS <sub>3</sub>	-1.559	534 (38), 340 (38), 310 (38), 291 (100)			
GS-30	glucoside of methylthioethylenylpentylglucosinolate	28.07	622.1300	C <sub>21</sub> H <sub>36</sub> O <sub>14</sub> NS <sub>3</sub>	-0.546	326 (100, -296), 299 (22)			
GS-31	glucoside of 7-methylthio-3-oxoheptylglucosinolate	22.16	638.1245	C <sub>21</sub> H <sub>36</sub> O <sub>15</sub> NS <sub>3</sub>	-1.182	620 (7), 522 (20), 326 (100, -312), 299 (22), 280 (33)		494 (14), 442 (11), 423 (7), 407 (25), 391 (100), 264 (7)	
GS-32	glucoside of 8-methylthiooctylglucosinolate	21.15	638.1608	C <sub>22</sub> H <sub>40</sub> O <sub>14</sub> NS <sub>3</sub>	-1.316	574 (100, -64)		423 (11), 407 (37), 391 (100), 191 (16)	
GS-23	glucoside of 9-(methylsulfinyl)heptylglucosinolate	16.51	640.1403	C <sub>21</sub> H <sub>38</sub> O <sub>15</sub> NS <sub>3</sub>	-0.945	623 (62), 550 (62), 391 (100), 390 (38)			
GS-27	glucosides of 9-(methylsulfinyl)nonylglucosinolate	22.55	654.1554	C <sub>22</sub> H <sub>40</sub> O <sub>15</sub> NS <sub>3</sub>	-1.765	423 (88), 407 (42), 391 (100), 373 (12), 359 (21)			
GS-28	isomer of GS-27	18.58	668.1708	C <sub>23</sub> H <sub>42</sub> O <sub>15</sub> NS <sub>3</sub>	-2.102	604 (100)		453 (25), 437 (100), 421 (100), 332 (38), 264 (100)	
GS-29	isomer of GS-27	16.70	668.1712	C <sub>23</sub> H <sub>42</sub> O <sub>15</sub> NS <sub>3</sub>	-1.504	604 (100)		528 (17), 453 (28), 437 (56), 421 (100), 403 (22)	

Table 1. continued

code	glucosinolate putative identification <sup>a</sup>	$t_R$ (min)	$[M - H]^-$ (Da)	formula	error (ppm)	$MS^2$ fragments (%)	$MS^3$ fragments (%)	$MS^4$ fragments (%)
GS-29	glucoside of (R)-10-(methylsulfinyl)decylglucosinolate	22.79	682.1868	$C_{24}H_{44}O_{15}NS_3$	-1.546	618 (100)		
GS-19	glucosides of 9-(methylsulfonyl)nonylglucosinolate	17.68	684.1663	$C_{23}H_{42}O_{14}NS_3$	-1.198	666 (59), 522 (91), 453 (100), 437 (23), 421 (45), 326 (23), 259 (14)		
GS-20	isomer of GS-19	16.08	684.1658	$C_{23}H_{42}O_{14}NS_3$	-1.928	453 (81), 437 (29), 421 (100)		
GS-21	isomer of GS-19	16.84	684.1630	$C_{23}H_{42}O_{14}NS_3$	2.563	643 (21), 453 (100), -231, 437 (42), 421 (96), 389 (42)		
GS-22	isomer of GS-19	20.92	684.1658	$C_{23}H_{42}O_{14}NS_3$	-1.928	453 (100), 437 (83), 421 (72), 389 (18), 344 (66), 326 (18), 307 (11), 280 (15)		
GS-24	glucosides of 10-(methylsulfonyl)decylglucosinolate	23.37	698.1812	$C_{24}H_{44}O_{16}NS_3$	-2.248	681 (31), 680 (23), 639 (23), 453 (100), 437 (31), 421 (23)		
GS-25	isomer of GS-31	24.29	698.1821	$C_{24}H_{44}O_{16}NS_3$	-0.959	680 (10), 662 (12), 453 (95), 437 (25), 421 (100), 389 (8), 343 (10)		
GS-26	isomer of GS-31	28.18	698.1815	$C_{24}H_{44}O_{16}NS_3$	-1.818	680 (100), 652 (45), 626 (27), 537 (27), 528 (27), 453 (64), 437 (36), 421 (64), 358 (64)		

<sup>a</sup>GS, glucosinolate.

One pair of isomers, GS-27 and GS-28 ( $t_R = 16.43$  and  $18.58$  min,  $[M - H]^- = 668.1712$  Da,  $C_{23}H_{42}O_{15}NS_3$ , error  $-1.5$  ppm) were putatively identified as glucosides of 9-(methylsulfinyl)nonylglucosinolate (the glucosides of GS-5 or its isomer). GS-29 ( $t_R = 22.79$  min,  $[M - H]^- = 682.1868$  Da,  $C_{23}H_{44}O_{15}NS_3$ , error  $-1.5$  ppm) was thought to be the glucoside of 10-(methylsulfinyl)decylglucosinolate (the glucosides of GS-10 or its isomer). The base  $MS^2$  fragments ( $m/z$  604 for GS-27 and GS-28 and  $m/z$  618 for GS-29) showed a loss of 64 Da (loss of  $CH_3 - SO - H$ ), indicating that these glucosinolates contained a methylsulfinyl ( $CH_3-SO^-$ ) group in the side chain.<sup>17,18</sup> Both GS-27 and GS-28 had  $MS^3$  spectra containing the diagnostic glucosinolate glucoside fragments at  $m/z$  453 (25 and 28%, respectively), 437 (100 and 56%), and 421 (both 100%) to confirm their identification. Similar fragments were not observed for GS-29. However, the  $MS^2$  signal was very weak.

Four other compounds (GS-30–GS-33) also appeared to be analogues of the glucosides of glucosinolates on the basis of their masses and formulas. GS-30 ( $t_R = 28.07$  min,  $[M - H]^- = 622.1290$  Da,  $C_{21}H_{36}O_{14}NS_3$ , error  $-0.5$  ppm) might be the glucoside of methylthioethenylpentenyl ( $CH_3-S-C=C-(CH_2)_5$ ) or an isomer of methylthioheptenyl. The aglycone with a methylthioethenylpentenyl group is among the 180 predicted new glucosinolates (no. 204) in the electronic database of Clarke.<sup>8</sup>

GS-31 and GS-32 ( $t_R = 22.16$  and  $21.15$  min) have the same nominal masses, but have different high-accuracy masses. GS-31 ( $[M - H]^- = 638.1245$  Da,  $C_{21}H_{36}O_{15}NS_3$ , error  $-1.2$  ppm) was putatively identified as the glucoside of 7-methylthio-3-oxoheptylglucosinolate or 7-methylsulfinylheptenylglucosinolate. GS-32 ( $[M - H]^- = 638.1608$  Da,  $C_{22}H_{40}O_{14}NS_3$ , error  $-1.3$  ppm) might be the glucoside of 8-methylthiooctylglucosinolate.

GS-33 ( $t_R = 16.51$  min,  $[M - H]^- = 640.1403$  Da,  $C_{21}H_{38}O_{15}NS_3$ , error  $-0.9$  ppm) might be the glucoside of 7-(methylsulfinyl)heptyl glucosinolate. Instead of showing the typical glucosinolate glucoside fragment described above, three of them (GS-31–GS-33) had base  $MS^2$  or  $MS^3$  fragments at  $m/z$  391 like GS-23. Consequently, the lack of definitive  $MS^2$  fragments made it impossible to develop a molecular formula for this ion. The putative formula offered in Table 1 was based primarily on the close match of the experimentally measured high-resolution masses with calculated values. The aglycones of these three glucosides were known glucosinolates, but not detected in this plant.

The 14 glucosinolate glucosides mentioned above have not been previously reported in any other plants. They represent a new family of glucosinolates with a glucosyl connected to the thioglucosyl group. All of the observed  $MS^n$  fragments could be related to the structural elements of the glucosinolate skeleton. For examples, ions a, b, c, and d and thioglucosyl ( $m/z$  195), as well as fragments obtained from further losses of oxygen, sulfur, or parts of the glucosyl were observed. However, not enough fragments were obtained to determine the structure of the R groups.<sup>8,11–25</sup> Of course, the R groups for the glucosinolates were not very large (containing fewer than 12 carbon atoms for the aliphatic GSs), but they can be arranged in several different ways to form the isomers. For example, the aliphatic structure can be linear or branched, the oxygen atom can be found in the oxo (keto) or hydroxy form, and the alkenyl double bond can be located at different positions.<sup>3–8</sup> As a result, nuclear

magnetic resonance (NMR) analysis is still the most accurate way to identify GSs structurally.<sup>6,7</sup>

The major glucosinolates of *R. montana* had similar side chains and were well separated, allowing for the measurement of their peak areas at 225 nm (Figure 3). Their concentrations were calculated relative to glucocheirolin using MRRF values of 1.0, on the basis of previous reports that the side chains were similar and did not alter the absorption coefficient of the molecules.<sup>8,14,19,20</sup> The relative peak area ratios for GS-1, GS-8, GS-6, GS-5, and GS-10 were 100.0, 15.5, 9.6, 5.1, and 4.6, respectively. The corresponding concentrations for GS-1, GS-8, GS-6, GS-5, and GS-10 were 0.51, 0.079, 0.049, 0.026, and 0.024% on a dry weight basis, and their total contents was 0.69% or 690 mg/100 g of dried plant materials. GS-1 constituted approximately 71% of the total glucosinolate content.

**Identification of Phenolic and Other Common Compounds.** Table 2 presents the retention times (recorded on the TIC chromatograms), HR masses, and formulas for deprotonated molecules  $[M - H]^-$ , diagnostic  $MS^2$ ,  $MS^3$ ,  $MS^4$ , and  $MS^5$  product ions, and putative identities of 44 flavonol glycoside, 18 phenolic, and other common compounds. The listing is in order of molecular weight. The typical TIC and PDA (at 354 nm) chromatograms are shown in Figure 2. The strategy for identifying phenolic compounds in food samples has been described previously.<sup>31,33</sup> In this study, only the glycosides of flavonols were observed, and most are reported for the first time.

Two tetrahydroxyflavone 3-*O*-rhamnosylhexosides ( $t_R = 28.22$  and  $26.52$  min,  $[M - H]^- = 593.1491$ ) provided the largest peaks (F-1 and F-2 in Figure 2A) in chromatograms of the *R. indica* extract. They had UV band I maximum absorbances at 265 and 346 nm and base  $MS^2$  and  $MS^3$  fragments of  $m/z$  447 (by loss of 146) and  $m/z$  284 (the radical anion species of kaempferol from the loss of  $162 + 1$  rather than the ion at  $m/z$  285), suggesting that they might be kaempferol glycosides containing a rhamnosylhexose. This radical anion had base  $MS^4$  and  $MS^5$  fragments of  $m/z$  255 (100%) and 221 (100%), which suggested that this flavonol was most likely to be kaempferol. This identification was confirmed by the fact that kaempferol was the tetrahydroxyflavone (by direct comparison with kaempferol standards) in the acidic hydrolyzed extract (chromatogram not shown). The close retention times of the flavonol glycoside suggested that they might be kaempferol 3-*O*-rhamnosylglucosides with the rhamnosyl at the 6-position of hexosyl (glucosyl or galactosyl).<sup>33</sup> Their high-resolution mass confirmed their putative identification.

Four other peaks with different retention times ( $t_R = 17.35$ ,  $21.04$ ,  $30.80$ , and  $32.13$  min) had high-resolution masses close to 593.1500 Da, and all had similar fragments, suggesting they were also kaempferol rhamnosylhexosides. However, they were all minor components of the plant and did not provide sufficient UV data or  $MS^n$  fragments to allow identification of a specific isomeric structure. Thus, their position of attachment to the flavone and the type of hexose were not completely identified.

Similarly, flavonoid F-3 ( $t_R = 24.61$  min,  $[M - H]^- = 609.1470$  Da) had a  $MS^2$  fragment at  $m/z$  447 (loss of 162) and an  $MS^3$  fragment at  $m/z$  301 (loss of 146) and was putatively identified as quercetin 3-*O*-hexosylrhamnoside. Its isomers ( $t_R = 23.42$  and  $25.84$  min) had  $MS^2$  fragments at  $m/z$  463 (loss of 146) and  $MS^3$  fragments at  $m/z$  301 (loss of 162), suggesting

they were quercetin 3-rhamnosylhexosides. The isomer with  $t_R = 23.42$  min was further identified as rutin by direct comparison with an authentic standard. Another isomer ( $t_R = 34.50$  min) had a  $MS^2$  fragment at  $m/z$  301, and its sugar connection could not be determined. The  $MS^4$  and  $MS^5$  fragments for  $m/z$  301 were 255 (22–27%), 179 (41–71%), 151 (100%), and 107 (100) and suggested the aglycone was quercetin. This was confirmed by the fact that the pentahydroxyflavone in the acidic hydrolyzed extract was identified as quercetin.

One flavonoid ( $t_R = 30.31$  min,  $[M - H]^- = 623.1611$  Da) had a base  $MS^2$  fragment at  $m/z$  477, a base  $MS^3$  fragment at  $m/z$  314, and  $MS^4$  and  $MS^5$  ions at  $m/z$  285 and 270 to suggest it was isorhamnetin 3-*O*-rhamnosylhexoside. Two rhamnosides and three hexosides of kaempferol were detected. At least one of the rhamnosides and hexosides might have the glycosyl at the 3-position of kaempferol. Another flavonoid ( $t_R = 33.59$  min,  $[M - H]^- = 577.1555$  Da,  $C_{27}H_{29}O_{14}$ ) had a base  $MS^2$  fragment at  $m/z$  431 and a base  $MS^3$  fragment at  $m/z$  285 to suggest it was kaempferol 3-*O*-rhamnosylrhamnoside.

Similarly, six flavonoids conjugated with three monosaccharides were putatively identified. The first two flavonoids ( $t_R = 34.46$  and  $33.19$  min,  $C_{32}H_{37}O_{18}$ ) showed one pentosyl more than that of kaempferol 3-*O*-rhamnosylrhamnoside. The first one (F-6) had a base  $MS^2$  fragment at  $m/z$  563 (loss of 146 Da) and a base  $MS^3$  fragment at  $m/z$  284 (loss of 278 Da, i.e.,  $146 + 132$ ) to indicate it was kaempferol 3-*O*-rhamnosylpentosylrhamnoside. The second one ( $t_R = 33.19$ ) was very similar to F-6 but had a base  $MS^2$  fragment at  $m/z$  447 (loss of 294 Da) for a dirhamnosylpentoside. Another three flavonoids ( $t_R = 34.46$ ,  $33.19$ ,  $24.73$ ,  $26.21$ , and  $27.94$  min,  $C_{33}H_{39}O_{20}$ ) had base  $MS^2$  fragments at  $m/z$  609 Da (loss of 146 Da), a base  $MS^3$  fragment at  $m/z$  285 Da (loss of 324 Da), and a base  $MS^4$  fragment at  $m/z$  151, suggesting them to be isomers of kaempferol 3-*O*-rhamnosyldihexosides. The last flavonoid ( $t_R = 22.95$  min  $[M - H]^- = 771.1971$  Da,  $C_{33}H_{39}O_{21}$ ) had a base  $MS^2$  fragment at  $m/z$  625 (loss of 146 Da), indicating it might be quercetin 3-*O*-rhamnosyldihexoside.

Acylation of the flavonol glycosides was determined by mass difference. An increased mass close to 146.0366, 162.0315, 176.0471, or 206.0576 Da was diagnostic of the presence of a *p*-coumaroyl, caffeoyl, feruloyl, or sinapoyl group. For example, two kaempferol acylglycosides ( $t_R = 37.65$  and  $39.03$  min,  $[M - H]^-$  close to 755.1826 Da,  $C_{36}H_{35}O_{18}$ ) had a base  $MS^2$  fragment at  $m/z$  609 Da (calculated as 609.1460, with error of  $-0.177$  ppm for  $C_{27}H_{29}O_{16}$ , from loss of 146.0366 Da,  $C_9H_6O_2$  for coumaroyl, instead of loss of 146.0576 Da,  $C_6H_{10}O_4$ , rhamnosyl) and base  $MS^{3,4}$  fragments ( $m/z$  447, 285, 257, 241, 229, 151 Da) identifying them as kaempferol 3-*O*-*p*-coumaroyldihexosides. Similarly, three acylated flavonoids ( $t_R = 30.24$ ,  $42.70$ , and  $46.66$  min,  $[M - H]^-$  close to 769.1975 Da,  $C_{37}H_{37}O_{18}$ ) had base  $MS^2$  fragments at  $m/z$  575 (loss of around 194.0576 Da for  $C_{10}H_{10}O_4$ , ferulic acid) suggesting they were kaempferol 3-*O*-feruloylrhamnosylhexosides. In the same way, four acylated glycosides were putatively identified as the 3-*O*-caffeoyldihexoside ( $t_R = 31.67$  min,  $[M - H]^- = 771.1797$  Da), the 3-*O*-sinapoyldihexoside ( $t_R = 41.60$  min,  $[M - H]^- = 831.1974$  Da), the 3-*O*-hexoside-7-*O*-methoxybenzoyldihexoside ( $t_R = 33.57$  min,  $[M - H]^- = 921.2290$  Da), and the 3-*O*-sinapoylhydroxyferuoyldihexoside-7-*O*-hexoside ( $t_R = 39.84$  min,  $[M - H]^- = 1185.2914$  Da) of quercetin, respectively. In the same manner, the remaining ones were identified as two 3-*O*-sinapoylhexoside-7-*O*-rhamnosides ( $t_R = 42.40$  and  $46.26$  min,  $[M - H]^-$  close to 799.2093 Da), the 3-*O*-



**Table 2. Compound Name, Retention Time, High-Resolution Mass of the Deprotonated Molecule, Formula, Error between Calculated and Measured Values, and MS<sup>n</sup> Data of the Flavonol Glycosides and Other Compounds in *Rorippa indica***

compound putative identification (code) <sup>a</sup>	t <sub>R</sub> (min)	[M - H] <sup>-</sup> (Da)	formula	error (ppm)	MS <sup>2</sup> fragments (m/z) (%)	MS <sup>3</sup> fragments (m/z) (%)	MS <sup>4</sup> fragments (m/z) (%)	MS <sup>5</sup> fragments (m/z) (%)
malic acid <sup>b</sup>	2.02	133.0137	C <sub>4</sub> H <sub>5</sub> O <sub>5</sub>	-4.109	115 (100)	71 (100)		
vanillic acid <sup>b</sup>	5.13	167.0349	C <sub>8</sub> H <sub>7</sub> O <sub>4</sub>	-0.491	152 (100), 123 (65), 108 (16)			
citric acid <sup>b</sup>	2.25	191.0193	C <sub>6</sub> H <sub>7</sub> O <sub>7</sub>	-3.276	173 (26), 111 (100)	67 (100)		
<i>trans</i> -ferulic acid <sup>b</sup>	23.48	193.0503	C <sub>10</sub> H <sub>9</sub> O <sub>4</sub>	-1.720	178 (26), 149 (56), 134 (100)	106 (100)		
<i>cis</i> -ferulic acid <sup>b</sup>	25.08	193.0503	C <sub>10</sub> H <sub>9</sub> O <sub>4</sub>	-1.720	178 (26), 149 (56), 134 (100)	106 (100)		
<i>trans</i> -sinapic acid <sup>b</sup>	20.81	223.0241	C <sub>10</sub> H <sub>7</sub> O <sub>6</sub>	-3.189	195 (8), 179 (100)	135 (100), 109 (66)		
<i>cis</i> -sinapic acid <sup>b</sup>	22.00	223.0241	C <sub>10</sub> H <sub>7</sub> O <sub>6</sub>	-3.189	195 (8), 179 (100)	135 (100), 109 (66)		
dihydroxybenzoylpentose <sup>b</sup>	6.90	285.0611	C <sub>13</sub> H <sub>13</sub> O <sub>8</sub>	-1.721	165 (6), 153 (100), 152 (33), 109 (7)	109 (100)		
dihydroxybenzoylpentose <sup>b</sup>	8.10	285.0611	C <sub>13</sub> H <sub>13</sub> O <sub>8</sub>	-1.721	153 (100)	109 (100)		
dihydroxybenzoylpentose <sup>b</sup>	9.23	285.0611	C <sub>13</sub> H <sub>13</sub> O <sub>8</sub>	-1.721	153 (100)	109 (100)		
<i>p</i> -coumaroylmalic acid <sup>b</sup>	20.59	279.0505	C <sub>13</sub> H <sub>11</sub> O <sub>7</sub>	-1.885	163 (100), 133 (70)	93 (100)		
hydroxybenzoylhexose <sup>b</sup>	6.80	299.0767	C <sub>13</sub> H <sub>13</sub> O <sub>8</sub>	-1.808	137 (100)			
hydroxybenzoylhexose <sup>b</sup>	8.44	299.0767	C <sub>13</sub> H <sub>13</sub> O <sub>8</sub>	-1.808	137 (100)			
dihydroxybenzoylhexose <sup>b</sup>	4.27	315.0717	C <sub>13</sub> H <sub>13</sub> O <sub>9</sub>	-1.445	225 (12), 165 (14), 163 (8), 153 (100), 152 (31)			
dihydroxybenzoylhexose <sup>b</sup>	5.62	315.0716	C <sub>13</sub> H <sub>13</sub> O <sub>9</sub>	-1.762	153 (100)			
dihydroxybenzoylhexose <sup>b</sup>	6.33	315.0717	C <sub>13</sub> H <sub>13</sub> O <sub>9</sub>	-1.445	169 (100), 151 (31)			
galloylquinic acids <sup>b</sup>	4.42	343.0665	C <sub>14</sub> H <sub>15</sub> O <sub>10</sub>	-1.661	299 (28), 153 (100)			
km rhamnoside	47.97	431.0982	C <sub>21</sub> H <sub>19</sub> O <sub>10</sub>	-0.394	285 (100), 284 (27)	257 (45), 241 (40), 213 (14), 151 (100)	107 (100), 83 (7)	65 (100), 63 (41)
km rhamnoside	46.87	431.0984	C <sub>21</sub> H <sub>19</sub> O <sub>10</sub>	0.070	285 (100), 284 (30)	257 (39), 241 (35), 213 (13), 151 (100)	107 (100), 83 (7)	65 (100), 63 (41)
sinapoylhexose + formic acid <sup>b</sup>	16.01	431.1919	C <sub>20</sub> H <sub>31</sub> O <sub>11</sub>	-0.859	385 (100)	223 (80), 205 (77), 161 (28), 153 (100)		
km hexoside	32.80	447.0927	C <sub>21</sub> H <sub>19</sub> O <sub>11</sub>	-1.307	327 (22), 301 (49), 300 (11), 285 (100), 284 (93)			
km 3-O-hexoside	30.58	447.0930	C <sub>21</sub> H <sub>19</sub> O <sub>11</sub>	-0.636	327 (16), 285 (57), 284 (100), 255 (11)	255 (100), 227 (10)	255 (24), 227 (88), 211 (100)	
km 3-O-hexoside (F-5) <sup>b</sup>	32.17	447.0931	C <sub>21</sub> H <sub>19</sub> O <sub>11</sub>	-0.413	327 (20), 285 (56), 284 (100), 255 (15)	255 (100), 227 (13)	255 (19), 227 (100), 211 (61)	227 (23), 199 (56), 183 (100)
lactiresinol-4'-glucoside <sup>b</sup>	30.91	521.2023	C <sub>26</sub> H <sub>33</sub> O <sub>11</sub>	-1.026	503 (11), 449 (9), 359 (18), 341 (100), 179 (49)			
km 3-O-dirhamnoside <sup>b</sup>	33.59	577.1555	C <sub>27</sub> H <sub>29</sub> O <sub>14</sub>	-1.349	431 (100), 285 (25)	285 (100), 284 (14)	257 (77), 243 (100), 213 (93)	297 (100)
km rhamnosylhexoside	17.35	593.1505	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub>	-1.169	503 (43), 473 (100), 383 (25), 353 (30)	383 (22), 353 (100)	325 (100), 297 (50)	
km 3-O-hexoside-7-O-rhamnoside	21.04	593.1503	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub>	-1.506	447 (100), 431 (72)	285 (100), 284 (41)	257 (50), 241 (33), 151 (100)	107 (100)
km 3-O-rhamnosylhexoside (F-1) <sup>b</sup>	28.22	593.1491	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub>	-3.529	447 (100)	327 (16), 285 (41), 284 (100), 255 (12)	255 (100)	227 (100), 211 (63)
km 3-O-rhamnosylhexoside (F-2) <sup>b</sup>	26.52	593.1500	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub>	-2.012	447 (100), 431 (41), 285 (16)	327 (22), 285 (20), 284 (100), 255 (15)	255 (100), 227 (11)	255 (13), 227 (100), 211 (57)
km 3-O-rhamnosylhexoside	30.80	593.1504	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub>	-1.337	473 (6), 447 (100), 431 (37), 285 (12)			

Table 2. continued

compound putative identification (code) <sup>a</sup>	$t_R$ (min)	$[M - H]^-$ (Da)	formula	error (ppm)	MS <sup>2</sup> fragments ( $m/z$ ) (%)	MS <sup>3</sup> fragments ( $m/z$ ) (%)	MS <sup>4</sup> fragments ( $m/z$ ) (%)	MS <sup>5</sup> fragments ( $m/z$ ) (%)
km 3-O-rhamnonylhexoside	31.13	593.1504	C <sub>27</sub> H <sub>39</sub> O <sub>15</sub>	-1.337	473 (8), 447 (100), 431 (35), 285 (18)	327 (17), 285 (42), 284 (100), 255 (14)	255 (100)	227 (100), 211 (69)
km 3-O-hexoside-7-O-hexoside	16.92	609.1469	C <sub>27</sub> H <sub>39</sub> O <sub>16</sub>	1.300	489 (14), 447 (100)			
km 3-O-hexoside-7-O-hexoside	18.79	609.1471	C <sub>27</sub> H <sub>39</sub> O <sub>16</sub>	1.629	447 (100)			
qn 3-O-hexosylthamnoside	22.42	609.1470	C <sub>27</sub> H <sub>39</sub> O <sub>16</sub>	1.465	463 (81), 447 (100), 446 (33), 301 (32)			
qn 3-O-rhamnonylhexoside (rutin) <sup>b</sup>	23.42	609.1472	C <sub>27</sub> H <sub>39</sub> O <sub>16</sub>	1.793	463 (100), 447 (23), 301 (12)	301 (100), 300 (26)		
qn 3-O-hexosylthamnoside (F-3) <sup>b</sup>	24.54	609.1470	C <sub>27</sub> H <sub>39</sub> O <sub>16</sub>	1.465	463 (67), 447 (100), 446 (34), 301 (31)	301 (100), 299 (10)	255 (22), 179 (71), 151 (100)	107 (100)
qn 3-O-hexosylthamnoside <sup>b</sup>	25.82	609.1470	C <sub>27</sub> H <sub>39</sub> O <sub>16</sub>	1.465	463 (64), 447 (100), 446 (25), 301 (31), 285 (11)	301 (100)	255 (27), 179 (46), 151 (100)	
qn 3-O-hexosylthamnoside <sup>b</sup>	26.17	609.1470	C <sub>27</sub> H <sub>39</sub> O <sub>16</sub>	-1.467	463 (56), 447 (100), 301 (29)	301 (100)		
is 3-O-rhamnonylhexoside <sup>b</sup>	30.31	623.1611	C <sub>28</sub> H <sub>39</sub> O <sub>17</sub>	-1.056	477 (100), 461 (41), 315 (9)	357 (19), 314 (100), 285 (8), 271 (6)	285 (100), 271 (75), 243 (27)	270 (100)
qn 3-O-hexoside-7-O-hexoside	15.60	625.1398	C <sub>29</sub> H <sub>31</sub> O <sub>16</sub>	-1.956	463 (100), 301 (22)	301 (100), 300 (32)		
km 3-O-acetyl-hexosylthamnoside	31.43	635.1605	C <sub>29</sub> H <sub>31</sub> O <sub>16</sub>	-1.980	489 (57), 431 (100), 430 (12), 285 (73)	413 (35), 285 (39), 284 (100), 255 (17)		
km 3-O-rhamnonylpentasythamnoside (F-6)	34.46	709.1978	C <sub>33</sub> H <sub>37</sub> O <sub>18</sub>	-1.040	563 (100)			
km 3-O-dirhamnonylpentoside	33.19	709.1972	C <sub>32</sub> H <sub>37</sub> O <sub>18</sub>	-1.886	665 (14), 547 (8), 489 (7), 447 (100), 285 (20)			
km 3-O-( <i>p</i> -coumaronylhexosyl) rhamnosside	45.84	739.1877	C <sub>36</sub> H <sub>35</sub> O <sub>17</sub>	-0.369	593 (100), 285 (7)	447 (6), 285 (100)	257 (68), 241 (37), 151 (100)	107 (100), 83 (10)
km 3-O-rhamnonylhexosylhexoside	24.73	755.2031	C <sub>33</sub> H <sub>39</sub> O <sub>20</sub>	-1.479	609 (100)	285 (100)	267 (31), 257 (100), 229 (44), 151 (30)	
km 3-O-rhamnonylhexosylhexoside	26.21	755.2030	C <sub>33</sub> H <sub>39</sub> O <sub>20</sub>	-1.346	609 (100)	285 (100)	267 (42), 257 (100), 239 (29), 229 (56)	
km 3-O-rhamnonylhexosylhexoside	27.94	755.2023	C <sub>33</sub> H <sub>39</sub> O <sub>20</sub>	-2.273	609 (100), 431 (17), 285 (7)	285 (100)	267 (31), 257 (100), 229 (30), 151 (71)	
km 3-O- <i>p</i> -coumaronylhexosylhexoside <sup>b</sup>	37.65	755.1826	C <sub>36</sub> H <sub>35</sub> O <sub>18</sub>	-0.380	609 (100)	447 (16), 323 (49), 285 (100), 221 (6)	257 (100), 241 (42), 229 (45), 151 (74)	
km 3-O- <i>p</i> -coumaronylhexosylhexoside <sup>b</sup>	39.03	755.1814	C <sub>36</sub> H <sub>35</sub> O <sub>18</sub>	-1.969	609 (100)	447 (17), 323 (51), 285 (100)	335 (100)	307 (100), 306 (19), 241 (10)
km 3-O-feruloylhexosylthamnoside <sup>b</sup>	30.24	769.1975	C <sub>37</sub> H <sub>37</sub> O <sub>18</sub>	-1.349	575 (100), 455 (12)	455 (100)		
km 3-O-feruoylthamnosylhexoside (F-4)	42.70	769.1974	C <sub>37</sub> H <sub>37</sub> O <sub>18</sub>	-1.479	623 (100)	323 (100), 299 (66), 285 (68), 284 (15)	263 (46), 221 (100), 179 (82), 177 (36)	
km 3-O-feruloylthamnosylhexoside	46\66	769.1972	C <sub>37</sub> H <sub>37</sub> O <sub>18</sub>	-1.739	653 (7), 623 (100), -146), 285 (7)	447 (6), 299 (24), 285 (100)	257 (77), 241 (43), 213 (23), 151 (100)	
km 3-O-caffeoyldihexoside	31.67	771.1797	C <sub>36</sub> H <sub>35</sub> O <sub>19</sub>	2.461	609 (47), 608 (6), 447 (100), 327 (6), 285 (25)			
qn 3-O-rhamnonyldihexoside <sup>b</sup>	22.95	771.1971	C <sub>33</sub> H <sub>39</sub> O <sub>21</sub>	-2.374	625 (100), 609 (68), 463 (40)	353 (100), 339 (22), 299 (50), 285 (39)	251 (97), 209 (100), 207 (42), 191 (52)	
km 3-O-sinapoylhexoside-7-O-rhamnosside	42.40	799.2093	C <sub>38</sub> H <sub>39</sub> O <sub>19</sub>	0.248	653 (100)			
km 3-O-sinapoylhexoside-7-O-rhamnosside	46.26	799.2080	C <sub>38</sub> H <sub>39</sub> O <sub>19</sub>	-1.379	653 (100), 431 (10), 285 (15)			

Table 2. continued

compound putative identification (code) <sup>a</sup>	$t_R$ (min)	$[M - H]^-$ (Da)	formula	error (ppm)	MS <sup>2</sup> fragments (m/z) (%)	MS <sup>3</sup> fragments (m/z) (%)	MS <sup>4</sup> fragments (m/z) (%)	MS <sup>5</sup> fragments (m/z) (%)
qn 3-O-sinapoyldihexoside	41.60	831.1974	C <sub>38</sub> H <sub>39</sub> O <sub>21</sub>	-1.842	787 (100), 641 (42), 625 (57), 607 (7), 479 (74)			
km 3-O-hydroxybenzoyldihexoside-7-O-dihexoside	31.17	891.2185	C <sub>40</sub> H <sub>43</sub> O <sub>23</sub>	-1.751	729 (96), 567 (100)			
qn3-O-hexoside-7-O-methoxybenzoyldihexoside	33.57	921.2290	C <sub>41</sub> H <sub>45</sub> O <sub>24</sub>	-1.764	593 (25), 463 (100), 457 (37)			
km 3-O-caffeoyldihexoside-7-O-rhamnoside	32.85	917.2340	C <sub>43</sub> H <sub>46</sub> O <sub>23</sub>	-1.865	771 (100), 755 (29), 609 (31)	609 (100)	447 (26), 323 (50), 285 (100), 221 (7)	
km 3-O-feruloyldihexoside-7-O-rhamnoside	34.95	931.2505	C <sub>43</sub> H <sub>47</sub> O <sub>23</sub>	-0.924	785 (100), 623 (10)	623 (100), 447 (47), 285 (7)		
km 3-O-feruloyldihexoside-7-O-rhamnoside	38.95	931.2503	C <sub>43</sub> H <sub>47</sub> O <sub>23</sub>	-1.139	785 (100), 769 (38), 623 (54), 447 (37), 431 (6)	623 (100), -162), 447 (72), 285 (13)		
km 3-O-sinapoyldihexoside-7-O-rhamnoside	34.46	961.2604	C <sub>44</sub> H <sub>49</sub> O <sub>24</sub>	-1.587	815 (100), 799 (12), 653 (18), 447 (10)	653 (100), 447 (45), 285 (8)		
qn 3-O-sinapoyldihydroxyferuloyldihexoside-7-O-hexoside	39.84	1185.2914	C <sub>54</sub> H <sub>57</sub> O <sub>30</sub>	-2.205	1039 (23), 1023 (100), 893 (12), 877 (18)	877 (100), 861 (93), 861 (37), 731 (61)		

<sup>a</sup>is, isorhamnetin; km, kaempferol; qn, quercetin. <sup>b</sup>Identified using in-house database.

hydroxybenzoyldihexoside-7-O-hexoside ( $t_R = 31.17$  min,  $[M - H]^- = 891.2185$  Da), the 3-O-caffeoyldihexoside-7-O-rhamnoside ( $t_R = 32.85$  min,  $[M - H]^- = 917.2340$  Da), the 3-O-feruloyldihexoside-7-O-rhamnosides ( $t_R = 34.95$  and 38.95 min,  $[M - H]^- = 931.2504$  Da), two 3-O-sinapoyldihexoside-7-O-rhamnosides ( $t_R = 34.46$  min,  $[M - H]^- = 961.2604$  Da), and the 3-O-acetylhexosylrhamnoside ( $t_R = 31.43$  min,  $[M - H]^- = 635.1605$  Da) of kaempferol, respectively.

As mentioned in a previous publication, dried rutin (at 354 nm) was used as the quantification standard and the MRRF values of two main flavonoids were used to determine their concentrations.<sup>31</sup> Concentrations of approximately 0.05 and 0.01% (or 50 and 10 mg/100 g of plant material on dry weight basis) were obtained for flavonoids F1 and F2, respectively. The total flavonoid content of this plant is <0.10% because the remaining flavonols were present at very low concentrations and UV peaks were not detectable for absorbance measurements.

In addition to the flavonoids, 1 lignan (lariciresinol 4-O- $\beta$ -D-glucopyranoside) and 18 other common compounds, including the glucosides of hydroxybenzoic and hydroxycinnamic acids and their derivatives (e.g., bound to sugar, quinic acid, and malic acid) and organic acids, were identified by comparison of the chromatographic and MS data with those in our in-house flavonoid database. Most of the common compounds have previously been detected in other plants analyzed in this laboratory.

The results of this study showed that *R. indica* is a rich source of glucosinolates.<sup>1-5,8</sup> These compounds were reasonably separated using the UHPLC conditions described here and identified by their high-accuracy mass measurement and multistage mass fragments. The UV absorbance of the major glucosinolates was used to compute their concentration. In summary, 33 glucosinolates, over 40 flavonoids, and more than 20 other phenolic and organic compounds were identified.

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### Notes

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