A New Sinapoyl Derivative of Isovitexin 6″-*O*-*β*-D-Glucopyranoside from *Alliaria petiolata*

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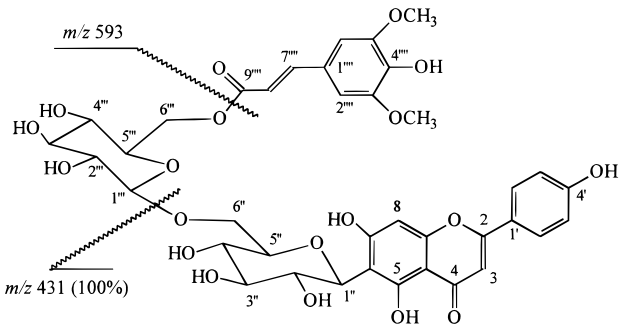
The new compound 6‴-*O*-sinapoylisovitexin 6″-*O*-*β*-D-glucopyranoside (**1**) was isolated from the foliage of *Alliaria petiolata*, and identified by spectral studies.

The foliage of *Alliaria petiolata* Cavara & Grande (Cruciferae) is a rich source of glycosides of flavonoids, especially those based on apigenin[1] and hydroxycinnamic acid derivatives.[2] Previously, we have identified isovitexin 6″-*O*-*β*-D-glucopyranoside from the foliage of *A. petiolata*.[1] A considerable variation in flavonoid content and composition was observed in plants from different sources. Plants from the East Ithaca and Michigan Hill (Tompkins County, NY) areas contained an apigenin derivative that was absent in most other populations. Interestingly, when seedlings from the East Ithaca population were grown under greenhouse conditions, a significantly higher production of this compound was observed. Since this apigenin derivative appeared to be a new compound, we became interested in its identification and biological activity. Here, we report the identification of this compound as a sinapoyl derivative of isovitexin 6″-*O*-*β*-D-glucopyranoside (**1**). This compound was accompanied by another flavonoid as a minor constituent (less than 10%) that appeared to be an isomer of **1**.



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An ethanol extract of *A. petiolata* leaves was diluted with an equal amount of water to precipitate chlorophylls, lipids, and other insoluble material. The mixture was centrifuged, and clear supernatant was evaporated to near dryness and reconstituted in water. This aqueous solution was separated by preparative HPLC into several fractions. The fraction that eluted as a single peak at 23 min under the chromatographic conditions used contained compound **1** as the major constituent. Negative-ion ESIMS of this compound showed a signal for an $(M - 1)^-$ at *m*/*z* 799. The UV spectrum showed absorptions at λ_{max} 272 and 331 nm, suggesting an apigenin chromophore.[3] ^{1}H NMR data showed a pattern typical of a flavonoid glycoside, acylated with a derivative of hydroxycinnamic acid. However, the signals for α,*β*-unsaturated protons of the acylated moiety and the anomeric protons of sugars were accompanied by low-intensity peaks that were similar in pattern but slightly different in shift values than those of the major compound. This observation indicated the presence of a minor component in the sample. From the integration of peak areas, the minor constituent was estimated to be less than 10% of the major compound. However, we were unable to resolve it from **1** by HPLC. Apparently, the associated minor compound is a structural isomer of compound **1**, since the ESIMS showed only a single composite quasimolecular ion peak at *m*/*z* 799.

Final structural assignments for the major compound **1** were made on the basis of 1D and 2D NMR investigations [^{1}H NMR, ^{13}C NMR, DQCOSY, and gradient-enhanced heteronuclear multiple quantum coherence (geHMQC)] and ESIMS analysis of the mixture. The ^{1}H NMR spectrum of the major compound showed the presence of 10 protons in the aromatic and alkene regions. The doublets at *δ* 7.82 and 6.85 (each 2H, $J = 8.2$ Hz, which correlated to carbon signals at 128.4 and 115.8 ppm, respectively) and the two singlets at *δ* 6.84 and 6.78, which correlated to carbon signals at 102.8 (C-3) and 93.5 (C-8) ppm, respectively, suggested that the apigenin moiety in compound **1** is substituted at C-6. The presence of a sinapoyl group was indicated by the occurrence of an ester carbonyl signal at 167.2 ppm in the ^{13}C NMR spectrum and the two singlets at *δ* 6.73 (2H) and 3.56 (6H, OCH_3-2) and two doublets at *δ* 6.52 and 7.52 (each 1H, $J = 16.4$ Hz) in its ^{1}H NMR spectrum. Further confirmation was provided by the fragment ion observed in negative-ion ESIMS at *m*/*z* 593 ($[M]^-$ − sinapoyl group). A sugar anomeric proton signal at *δ* 4.68 (1H, $J = 7$ Hz) that correlated to the carbon signal at 72.6 ppm suggested that this sugar is attached as a *C*-*β*-glycoside to the C-6 carbon of the apigenin moiety. The second sugar anomeric proton signal observed at *δ* 5.06 ($J = 7$ Hz), which correlated to the carbon signal at 100.7 ppm, indicated the second sugar to be an *O*-*β*-glycoside. Downfield shifts of the methylene carbon signals of both sugars (69.3 and 6.31 ppm) suggested that both OH groups at the sugar C-6 positions are substituted. In addition, the two protons attached to C-6‴ of the *O*-sugar appeared at *δ* 4.62 and 4.18. Since C-6‴ proton signals are known to show a downfield shift by about 0.5−1.6 ppm,[4,5] compared to shifts of protons attached to the C-6 position of an unsubstituted sugar, which generally appear in the range *δ* 3.18−3.50, we concluded that in **1** the *O*-glycoside moiety is acylated at the C-6‴ position. Additional support for

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the presence of a sinapoyl group on the *O*-glycoside was provided by significant fragment ions that appeared at *m/z* 431 (100, $[M]^- - 369$) and *m/z* 367 in negative-ion ESIMS.

The hydrolysis of compound **1** produced a sugar that was identified as glucose on the basis of the comparison of the R_f value of the product by paper chromatography with that of an authentic sample and by TMS derivatization and comparison of retention time with that of the TMS derivative of glucose by GC−MS. Furthermore, the organic layer obtained from the hydrolysis of **1**, when compared by TLC with the organic layer of hydrolysis product of isovitexin 6″-*O*-*β*-D-glucopyranoside, showed spots of comparable R_f values. These results indicated that compound **1** is a derivative of isovitexin 6″-*O*-*β*-D-glucopyranoside.

Finally, on the basis of all the evidence available, it was concluded that compound **1** is 6‴-*O*-sinapoylisovitexin 6″-*O*-*β*-D-glucopyranoside. Many of the ^{1}H NMR and ^{13}C NMR data of compound **1** were congruent with corresponding data of isovitexin 6″-O-*β*-D-glucoside.[1]

The minor isomer showed two doublets at δ 5.11 and 4.64 (J = 7−8 Hz) for the anomeric sugar protons and two doublets at δ 7.45 and 6.52 (J = 15.2 Hz), which integrated for less than 10% of the signal for the major compound. Other signals could not be easily distinguished, suggesting that probably the minor compound is an isomer of compound **1** that is substituted differently. *C*-Glycosides are known to rearrange in the presence of acidic protons; therefore, the impurity may well be an artifact formed by a rearrangement during the isolation process.[6]

Previously, we reported that isovitexin 6″-*O*-*β*-D-glucopyranoside from this plant acts as a feeding deterrent to fourth-instar larvae of *Pieris napi oleracea* Harris.[1] Since compound **1** does not exhibit similar activity (Haribal, unpublished results), it appears that a free primary hydroxy group at the *O*-glucosyl moiety of isovitexin 6″-*O*-*β*-D-glucopyranoside is essential for its antifeedant activity.

Experimental Section

General Experimental Procedures. GC−MS analyses were conducted using a capillary column (DB-5, 30 m × 0.23 mm) installed in a HP5890 GC linked to a Hewlett-Packard 5970 mass selective detector. UV spectra were recorded on a Perkin-Elmer Lambda 5 UV/vis spectrophotometer. All ^{1}H NMR spectra were recorded at 399.99 MHz and ^{13}C NMR at 25.59 MHz in DMSO-d_6. 2D NMR spectra were recorded on a 499.99 MHz Varian instrument using standard software. Negative-ion ESI mass spectra were recorded on a Micromass (Manchester, U.K.) Quattro 1 instrument, and the samples were introduced by a direct infusion method (capillary voltage 2.7 KV, cone voltage 47 V, source temp 80 °C). HPLC was performed on a Varian Vista 5500 (Limerick, Ireland) instrument, attached to an HP 1040A diode array detector (Hewlett-Packard) monitored at 218 and 254 nm.

Plant Material. Samples of *A. petiolata* were collected from Eastern Heights around Ithaca in Tompkins County, NY, in November 1996 and were grown in a greenhouse under controlled conditions (16:8 L/D photoperiod and RH of 65%).

Extraction and Isolation. Young rosette leaves (50 g) of *A. petiolata* from the greenhouse were extracted into 200 mL of 95% boiling EtOH. The resulting mixture was homogenized in a blender, and the insoluble plant material was removed by filtration. The extract was concentrated to 50 mL, diluted with an equal amount of water, and vortexed. The insoluble material was centrifuged, and the supernatant was evaporated to dryness, redissolved in water, and diluted to 50 mL. Compound **1** was collected as the fraction that eluted as a single peak at 23 min by HPLC on a reversed-phase column (C_{18} Bondex 10, 5 μm; 300 × 7.0 mm, Phenomenex, Torrance, CA) using a water−acetonitrile gradient at a flow rate of 2 mL/min (0−5 min 0% acetonitrile; 25% acetonitrile at 40 min; 100% acetonitrile at 50 min).

Table 1. ^{13}C and ^{1}H NMR Data of Compound 1 in DMSO-d_6

position	δ^{13C}	δ^1H
2	164.1	
3	102.8	6.84
4	182.0	
5	156.3	
6	106.0	
7	162.2	
8	93.5	6.78
9	161.2	
10	104.8	
1′	120.7	
2′	128.4	7.82 (1H, d, J = 8.2 Hz)
3′	115.8	6.85 (1H, d, J = 8.2 Hz)
4′	159.3	
5′	115.8	6.85 (1H, d, J = 8.2 Hz)
6′	128.4	7.82 (1H, d, J = 8.2 Hz)
1″	72.6	4.68 (1H, d, J = 7 Hz)
2″	69.8	4.75
3″	78.5	3.93
4″	73.6	3.60
5″	70.7	3.10 or 3.80
6″	69.3	3.35, 3.85
1‴	100.7	5.06 (1H, d, J = 7 Hz)
2‴	73.6	3.10
3‴	75.5	3.18
4‴	72.6	3.30
5‴	73.6	3.84
6‴	63.1	4.62, 4.18
sinapoyl		
1⁗	120.7	
2⁗	106.0	6.73
3⁗	147.7	
4⁗	138.2	
5⁗	147.7	
6⁗	106.0	6.73
7⁗	145.9	7.52 (1H, d, J = 16.4 Hz)
8⁗	114.3	6.52 (1H, d, J = 16.4 Hz)
9⁗	167.2	
OCH_3	55.7	3.56

Compound 1: brownish-yellowish flakes; UV λ_{max} at 272 and 331 nm in MeOH; 389, 360, 315 sh, 307 213 nm, in MeOH + NaOMe; 382 sh, 337.5, 301, 279 nm in MeOH + $AlCl_3$; 382 sh, 337, 301, 279 nm in MeOH + $AlCl_3$ + HCl; 395 sh, 334, 270 nm in MeOH + NaOAc; 331, 270 nm in MeOH + NaOAc + H_3BO_3; ^{1}H NMR (in DMSO-d_6) and ^{13}C NMR (in DMSO-d_6) data, see Table 1; negative-ion ESIMS *m/z* 799 $(M - H)^-$, 593 $(M - 207)^-$, 431 $(M - 369)^-$ (100), 367, 311, 255, 209, 205.

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