Complex *C*-Glycosyl Flavonoid Phytoalexins from *Cucumis sativus*

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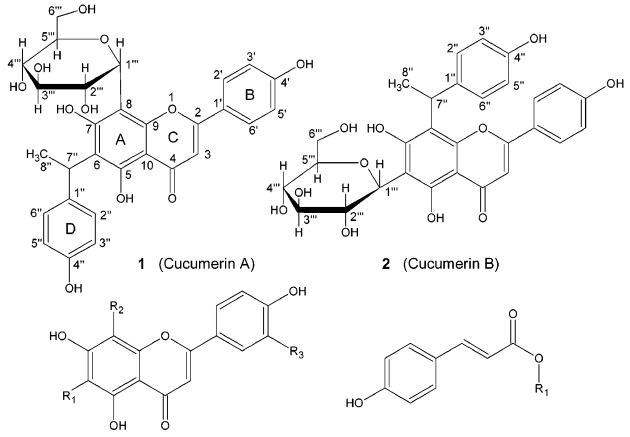
Extraction of cucumber leaf tissue expressing induced resistance against powdery mildew fungi revealed the presence of two new major *C*-glycosyl flavonoid products: vitexin-6-(4-hydroxy-1-ethylbenzene) (cucumerin A, **1**) and isovitexin-8-(4-hydroxy-1-ethylbenzene) (cucumerin B, **2**). In addition, the known *C*-glycosyl flavonoids apigenin-8-*C*-*β*-D-glucopyranoside (vitexin, **3**), apigenin-6-*C*-*β*-D-glucopyranoside (isovitexin, **4**), luteolin-8-*C*-*β*-D-glucopyranoside (orientin, **5**), and luteolin-6-*C*-*β*-D-glucopyranoside (isoorientin, **6**), as well as 4-hydroxycinnamic acid (*p*-coumaric acid, **7**) and its methyl ester (*p*-came, **8**), were found in higher quantities within resistant plants. The structures of **1**−**8** were elucidated using spectroscopic methods and unambiguously confirmed for **3**−**8** using co-chromatography experiments with authentic standards. On the basis of the results of this study and the reported biological activities of *C*-glycosyl flavonoids, these compounds would play a vital role in the defense strategy of this species by acting as phytoalexins.

Powdery mildew fungi are so widespread and ever present among crop plants and ornamentals that the total losses in plant growth and crop yield they cause each year on all crops probably surpass the losses caused by any other single type of plant disease.[1] Cucumber, *Cucumis sativus L.* (Cucurbitaceae), a species particularly affected by powdery mildew, has been recently shown to react to the presence of the fungus *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*, Schlechtend: Fr., Pollacci) by producing phenolic compounds acting as phytoalexins.[2−4] However, despite previous phytochemical studies,[2−9] compounds implicated in the induced resistance phenomenon of this species are unknown largely due to the complexities inherent to such natural products, which are rapidly synthesized in minute quantities at precise cellular locations following inoculation.[10−12] Accordingly, the goal of this investigation was to characterize induced phenolic compound(s) produced by powdery mildew-resistant cucumber plants to better understand the mechanisms of induced resistance for this species.

Extraction of powdery mildew-resistant leaf tissue yielded two new major *C*-glycosyl flavonoids (**1**, **2**) and six known compounds including four *C*-glycosyl flavonoids (**3**−**8**). We report herein the structure elucidation of two new *C*-glycosyl flavonoids and the biological evaluation of compounds **1**−**8** isolated from the leaves of resistant plants.

Approximately 18 mg of compound **1** and 20 mg of compound **2** were isolated from 2.6 kg (fresh weight) of elicited, powdery mildew-resistant cucumber leaves as optically active, yellowish amorphous solids (yields ca. 0.0007% and 0.0008%, respectively). Both **1** and **2** had nearly identical UV absorptions typical of flavonoids with maxima at 272 and 335 nm. The UV spectra of both **1** and **2** differed from the standard $C_6-C_3-C_6$ flavonoid skeleton in that a hyperchromic shift was observed for the absorbance at 272 nm and seemed to be unique to the $C_6-C_2-C_6-C_3-C_6$ cucumerin skeleton (Figure 1).[13,14]



1 (Cucumerin A) **2** (Cucumerin B)

3 R_1 = H, R_2 = *C*-B-D-Glc, R_3 = H (Vitexin)
4 R_1 = *C*-B-D-Glc, R_2 = H, R_3 = H (Isovitexin)
5 R_1 = H, R_2 = *C*-B-D-Glc, R_3 = OH (Orientin)
6 R_1 = *C*-B-D-Glc, R_2 = H, R_3 = OH (Isoorientin)
7 R_1 = H (*p*-Coumaric acid)
8 R_1 = CH_3 (*p*-Came)

^{1}H NMR analysis of the aglucone moieties revealed resonances at *δ* 7.39 (2H, d, 8.8 Hz), 6.78 (2H, d, 8.6 Hz) for **1** and at *δ* 7.39 (2H, d, 8.8 Hz), 6.79 (2H, d, 8.7 Hz) for **2**, corresponding to their *para*-substituted flavonoid B-rings (Table 1).[14] Furthermore, **1** and **2** showed resonances at *δ* 6.51 (1H, s) and 6.53 (1H, s), respectively, which are typical of flavonoid position 3 vinyl protons.[13] Additional aromatic signals were observed at *δ* 7.13 (2H, d, 8.3 Hz), 6.69 (2H, d, 8.6 Hz) for **1** and at *δ* 7.13 (2H, d, 8.5 Hz), 6.69 (2H, d, 8.7 Hz) for **2**, corresponding to 4-hydroxy-1-ethylphenyl protons (D-ring). TOCSY and COSY spectra for **1** and **2** were very similar, and both showed a strong correlation between the 8″ methyl protons at *δ* 1.71 (3H, d, 7.3 Hz) and 1.72 (3H, d, 7.2 Hz) and the 7″ proton resonances at *δ* 4.72 (1H, q, 7.1 Hz) and 4.70 (1H, q, 7.1 Hz), respectively. In addition, TOCSY and COSY correlations were observed for the 1‴ anomeric proton of **1** and **2** at *δ* 4.72 (1H, d, 9.7 Hz) and 4.71 (1H, d, 9.6 Hz) and other sugar multiplets resonating at around *δ* 3.4, as well as among B-ring and D-ring protons. The structure elucidation of **1** and **2** was

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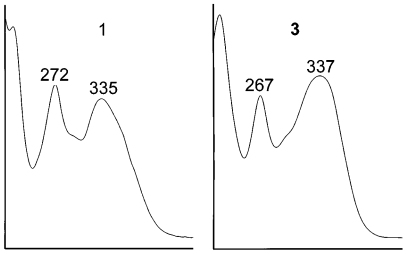

Figure 1. UV spectra of cucumerin A (**1**) and vitexin (**3**) recorded in MeOH at 254 nm with UV maxima indicated (nm).

Table 1. ^{1}H NMR Data (δ, ppm) for Compounds **1** and **2** (in DMSO-d_6)

H	**1**	**2**
3′	6.51 (1H, s)	6.53 (1H, s)
2′	7.39 (1H, d, 8.8 Hz)	7.39 (1H, d, 8.8 Hz)
3′	6.78 (1H, d, 8.6 Hz)	6.79 (1H, d, 8.7 Hz)
5′	6.78 (1H, d, 8.6 Hz)	6.79 (1H, d, 8.7 Hz)
6′	7.39 (1H, d, 8.8 Hz)	7.39 (1H, d, 8.8 Hz)
2″	7.13 (1H, d, 8.3 Hz)	7.13 (1H, d, 8.5 Hz)
3″	6.69 (1H, d, 8.6 Hz)	6.69 (1H, d, 8.7 Hz)
5″	6.69 (1H, d, 8.6 Hz)	6.69 (1H, d, 8.7 Hz)
6″	7.13 (1H, d, 8.3 Hz)	7.13 (1H, d, 8.5 Hz)
7″	4.72 (1H, q, 7.1 Hz)	4.70 (1H, q, 7.1 Hz)
8″	1.71 (3H, d, 7.3 Hz)	1.72 (3H, d, 7.2 Hz)
C-Glc		
1‴	4.72 (1H, d, 9.7 Hz)	4.71 (1H, d, 9.6 Hz)
2‴	4.07 (1H, m)	4.07 (1H, m)
3‴	3.43 (1H, m)	3.43 (1H, m)
4‴	3.39 (1H, m)	3.39 (1H, m)
5‴	3.34 (1H, m)	3.34 (1H, m)
6‴	3.70 (1H, d, 11.8 Hz)	3.70 (1H, d, 11.8 Hz)
	3.92 (1H, d, 11.8 Hz)	3.92 (1H, d, 11.8 Hz)

at first complicated by the overlapping cross-peaks originating from the 1‴ anomeric doublet and the broad 7-methine quadruplet in COSY and TOCSY correlation maps. A careful examination of HMBC spectra revealed the occurrence of two separately correlated sets of resonances; the ones emerging from the anomeric glucose center and the rest of the sugar unit, and the ones between the 7″ and 8″ centers of the hydroxyethylbenzene system. NOESY spectroscopy of **1** and **2** showed strong correlations for 7″ and 8″ protons; 7″, 2″, and 6″ protons; 8″, 2″, and 6″ protons; 1‴ and glucosyl moiety protons, and among B-ring and among D-ring aromatic protons.

^{13}C NMR, DEPT-135, and DEPT-90 spectra showed 29 carbon resonances for **1**, 19 of which were identical to vitexin,[13,15] and 29 carbon resonances for **2**, 16 of which were identical to isovitexin,[13,16] and 26 were identical to **1** with resonances for carbons 6, 8, and 1″ being distinct (Table 2). ^{1}H and ^{13}C NMR analyses of both flavonoids suggested the presence of only one glucopyranose moiety, whose anomeric configuration was determined to be β on the basis of the large coupling constant values of the anomeric protons at ca. δ 4.7, i.e., 9.7 Hz for **1** and 9.6 Hz for **2**.[13,17] The glycosidic linkage for both **1** and **2** was determined to be C−C on the basis of the relatively upfield anomeric carbon resonances at δ 75.3 for **1** and δ 75.3 for **2**. By contrast, the anomeric carbons of *O*-glycosides normally resonate at ca. δ 100.[13,17] Moreover, the glycosidic linkage of **1** and **2** resisted acid hydrolysis (2 N HCl, 2 h, 100 °C, reflux) and enzymatic digestion (β-glucosidase, 48 h, 37 °C), supporting a C−C glycosidic bond for these compounds.[13,17] The substitution patterns of the A-ring in **1** and **2**, 6-(4-hydroxy-1-ethylbenzene) and 8-(4-hydroxy-1-ethylbenzene), including their glycosidation positions, 6-*C*-glucosyl and 8-*C*-glucosyl, respectively, were unambiguously determined using three-bond HMBC correlations, which are shown for **2** in Figure 2. Of particular importance, the ^{1}H and ^{13}C resonances for vitexin's unsubstituted aromatic 6-methine group at δ 6.27 (1H, s) and 98.9[13,15] were absent for **1** and isovitexin's aromatic 8-methine resonances at δ 6.56 (1H, s) and 95.3[13,16] were absent for **2**, corroborating the substitution of these compounds at those positions.

Table 2. ^{13}C NMR Data (δ, ppm) for Compounds **1** and **2** (in DMSO-d_6)[a]

C	**1**	**2**	C	**1**	**2**
2	167.3	166.8	1″	148.1	136.7
3	103.4	103.6	2″	128.3	128.4
4	182.8	183.1	3″	115.6	115.7
5	158.5	158.4	4″	154.9	154.9
6	115.1	108.7	5″	115.6	115.7
7	164.5	164.7	6″	128.3	128.4
8	108.6	112.4	7″	33.8	32.3
9	155.9	156.2	8″	18.9	19.3
10	104.1	104.3	*C*-Glc		
1	122.1	122.1	1‴	75.3	75.3
2	129.2	129.3	2‴	72.7	72.8
3	116.7	116.7	3‴	78.8	78.7
4	162.0	162.0	4‴	70.1	69.9
5	116.7	116.7	5‴	82.0	82.0
6	129.2	129.3	6‴	60.7	60.7

[a] The assignments were based upon DEPT, COSY, TOCSY, HMQC, and HMBC experiments.

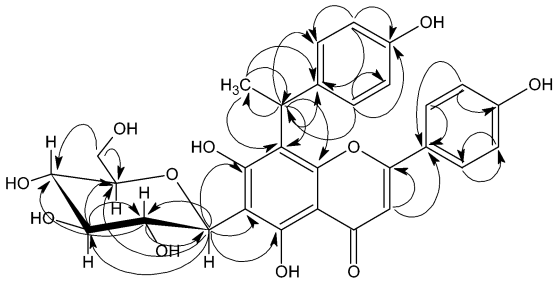

Figure 2. HMBC correlations of **2** measured in DMSO-d_6.

The molecular masses of **1** and **2** were determined to be 552.5 using MALDI-TOF, which was later corroborated with ESI/TOF-LCMS. For the ESI/TOF-LCMS analysis of **2**, a clear fragment ion was observed at *m*/*z* 534.4 (M − H_2O), confirming the ion at 552.5 as M^+.[18] On the basis of the above data, the structures of **1** and **2** were determined to be vitexin-6-(4-hydroxy-1-ethylbenzene) and isovitexin-8-(4-hydroxy-1-ethylbenzene), two new *C*-glycosyl flavonoids having the molecular formula $C_{29}H_{28}O_{11}$ and named cucumerin A and cucumerin B, respectively.

Cucumerin A (**1**) and cucumerin B (**2**) are regioisomers and are thus analogous to vitexin (**3**) and isovitexin (**4**), or orientin (**5**) and isoorientin (**6**). An isomeric relationship between cucumerins A and B was supported by the spectroscopic (UV, NMR, MS) and chromatographic (TLC R_f and HPLC elution time) similarities shared by both compounds. Such regioisomers can be produced through a Wessely−Moser rearrangement, which is a known isomerization for *C*-glycosyl flavonoids having an unsubstituted 5-OH group involving opening of the flavone heterocycle and cyclodehydration of the intermediary β-diketone.[13] This isomerization of *C*-glycosyl flavonoids complicates the interpretation of NMR spectra, since often their frequency of rotation is close to the NMR frequency resulting in double and broadened signals.[19] Despite the recent development of new corrective NMR techniques for *C*-glycosyl flavonoids, ^{1}H NMR and ^{13}C NMR results are comparatively very limited for this rare class of flavonoid,[19] making their identification challenging.

Little is known about cucumerin biosynthesis, but it may resemble the pathway proposed in 1992 by Asai et al. for production of calomelanols, which are structurally related 6-*C*- and 8-*C*-substituted $C_6-C_3-C_6-C_3-C_6$ flavonoid lactones.[20] Biosynthesis of the cucumerin flavonoid skeleton may involve a calomelanol-like $C_6-C_3-C_6-C_3-C_6$ precursor formed through a Michael-type addition of apigenin at position 6 or 8 onto *p*-coumaric acid's unsaturated carbon-3 center, followed by rearomatization of the apigenin A-ring and decarboxylation of the *p*-coumaric acid residue. This synthetic route would require two *p*-coumaric acid residues for every cucumerin molecule formed and would explain the induction of high levels of *p*-coumaric acid and its derivative, *p*-came, observed within the leaves of powdery mildew-resistant plants.

Six known compounds were found in higher concentrations within the leaves of elicited, powdery mildew-resistant cucumber plants and identified as apigenin-8-*C*-*β*-D-glucopyranoside (vitexin, **3**),[13,15] apigenin-6-*C*-*β*-D-glucopyranoside (isovitexin, **4**),[13,16] luteolin-8-*C*-*β*-D-glucopyranoside (orientin, **5**),[13] luteolin-6-*C*-*β*-D-glucopyranoside (isoorientin, **6**),[13] 4-hydroxycinnamic acid (*p*-coumaric acid, **7**),[3] and 4-hydroxycinnamic acid methyl ester (*p*-came, **8**),[2] by comparison of their UV spectral data and unambiguous authentication by HPLC and TLC co-chromatography experiments using appropriate standards. The presence of compounds **3**−**8** within cucumber leaves, including demonstration of **7** and **8** as phytoalexins within this species, has been previously reported.[2,3,21−23] However, this is the first report linking production of compounds **3**−**6** in cucumber with a biological role.

Compared to other classes of flavonoids, little is available in the literature about *C*-glycosyl flavonoids; however, vitexin (**3**) and isoorientin (**6**) were shown to be efficient *β*-glucosidase and pectinase inhibitors.[24] In addition, a *C*-glycosyl flavonoid produced by insect-resistant maize plants was demonstrated to have very high affinity for proteins and antinutritive properties imparting resistance to host plants by disrupting the digestion of insect pests.[25] More recently, fungitoxicity of certain *C*-glycosyl flavonoids against important horticultural fungal pathogens such as *Colletotrichum musae*, *Verticillium albo-artrum*, and *Phytophthora parasitica* has been demonstrated.[24,26] For cucumber, the induction and rapid accumulation of *C*-glycosyl flavonoids **1**−**6** within the leaves of powdery mildew-resistant plants following inoculation, taken together with their near absence within susceptible control plants observed during this study, strongly suggest a role for these compounds in this species as phytoalexins. Furthermore, the timing of production and subsequent accumulation to high concentrations correlating with a substantial reduction in the level of powdery mildew infection was particularly striking for compounds **1**−**4** and is characteristic of phytoalexins.[10] In light of the reported biological activities for *C*-glycosyl flavonoid compounds, it is reasonable to infer that **1**−**6** may adversely affect the proper functioning of fungal proteins, thereby conferring resistance to cucumber plants against powdery mildew.

Experimental Section

General Experimental Procedures. Authentic standards for **3**−**6** were purchased from Indofine Chemical Company (Hillsborough, NJ) and from Aldrich (Sheboygan, WI) for **7** and **8**. *β*-Glucosidase (from almonds, 500 units G-0395, lot # 105H4016) was purchased from Sigma-Aldrich. Melting points were measured using a Gallenkamp capillary melting point apparatus and were uncorrected. Optical rotations were determined with a Jasco DIP-360 digital polarimeter. UV spectra were recorded on a Hewlett-Packard 8453 spectrometer and a Waters 996 photodiode array detector. All NMR spectra were recorded using a Bruker DMX-600 NMR spectrometer (using standard pulse programs and acquisition parameters for 2D spectra). Mass spectra were recorded using a PerSeptive Biosystems (Framingham, MA) Voyager MALDI-TOF instrument operating in a positive-ion linear mode with a nitrogen laser (387 nm) at an accelerating voltage of 3.5 kV, and the matrix used was α-cyano-4-hydroxycinnamic acid (10 mg mL^{-1}) in H_2O−trifluoroacetic acid−acetonitrile (4:1:5). Samples were diluted 10-fold and then mixed with the matrix solution in a ratio of 1:12. Masses were confirmed using a LECO LCMS-ESI ChromaTOF Jaguar instrument (St. Joseph, MI) with an Agilent 1100 series HPLC (Palo Alto, CA) in MeOH (acidified with 0.1% formic acid) with a negative ionizing voltage of −30 kV using direct infusion (10 μL/min). HPLC was performed using a Waters 717 autosampler, 996 photodiode array detector, and 600 controller equipped with either a Waters 8 × 20 reversed-phase C18 column or a Waters RCM 25 × 10 reversed-phase C18 column for final purification of compounds. Desalting of extracts was achieved using Sep-Pak reversed-phase C18 cartridges purchased from Waters Corporation. Semipreparative flash column chromatography was performed using a jacketed Chromaflex column (2.5 cm i.d. × 60 cm) from Kontes. Reversed-phase C18 silica gel was purchased from Silicycle (Québec city, Qc). TLC was performed on Si gel 60 F_{254} using EtOAc−formic acid−HOAc−H_2O (100:11:11:27, solvent A).

Plant Material. Cucumber plants (*Cucumis* sativus L., cv. Corona) were grown from seed in climate-controlled growth chambers (24 °C day/20 °C night, 16 h photoperiod, 145 μE m^{-2} s^{-1}) (Conviron, Winnipeg, Manitoba). Two treatments were created: (1) plants infected with the powdery mildew *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*, Schlechtend: Fr., Pollacci) rendered resistant with a Milsana Bioprotectant treatment (KHH BioSci Inc., Raleigh, NC, 0.5%, sprayed once a week until runoff), a reported elicitor of phytoalexin production,[2] and (2) the control, which consisted of infected, nonelicited susceptible plants. Leaf tissue from each treatment was then carefully harvested, freeze-dried, and stored in the dark at −80 °C until further analysis.

Extraction and Isolation. Freeze-dried leaf tissue from both resistant and control plants was extracted with 80% MeOH (80 mL/g of dry mass plant material) for 48 h on a rotary shaker (100 rpm). Extracts were filtered using a Büchner apparatus to remove particulate matter, then rotoevaporated at 38 °C until only water remained. Pigments, lipids, free phenolics, and other unwanted nonpolar compounds were eliminated by partitioning with Et_2O (5 × 30 mL). Extracts were then hydrolyzed by adding an equal volume of 4 N HCl to each extract and heating for 90 min at 100 °C under reflux using an oil bath as heat source. Extracts were partitioned with Et_2O (3 × 30 mL) and EtOAc (3 × 30 mL) to recover aglycones. Both organic fractions were then combined, rotoevaporated to dryness, and resuspended in MeOH. Methanolic extracts containing aglycones were then desalted using Sep-Pak reversed-phase C18 cartridges by rinsing with 10 mL of H_2O prior to elution with 10 mL of MeOH. Comparison of extracts from both treatments using HPLC revealed induction of **1**−**8** within resistant plant extracts. Semipreparative flash column chromatography with reversed-phase Si gel (600 g) eluted in sequence with 200 mL of H_2O, 200 mL of H_2O/MeOH (4:1, 1:1, 1:4), and 200 mL of MeOH (all solvents were acidified with 2.5% glacial acetic acid to suppress the ionization of compounds) was used to fractionate crude, aglycone-containing methanolic extracts prepared from resistant leaf tissue only (flow rate 6 mL min^{-1}). The H_2O−MeOH (1:4) fraction yielded **1**−**6**, and **7** and **8** were afforded from the 100% MeOH fraction. Final purification of **1** (18 mg) and **2** (20 mg) was achieved using semipreparative HPLC. The detailed extraction and purification protocol used to characterize **1**−**8** is described in a previous paper.[27]

Cucumerin A (1): yellowish amorphous solid; mp 81−82 °C; $[\alpha]^{22}_D$ −224.3° (*c* 0.01, DMSO); UV (MeOH) λ_{max} (log ϵ) 272 (5.08), 335 (5.17) nm; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2); MALDI-TOF *m*/*z* 575.5 $[M + Na]^+$ (90), 552.5 $[M]^+$ (63), 539.2 (24), 522.9 (36), 515.2 (39), 491.2 (83), 481.6 (22), 463.4 90 (26), 431.4 (24), 421.3 (38), 413.4 (78), 393.5 (53), 385.2 (28), 360.6 (25), 353.3 (61), 352.3 (100), 332.5 (89), 318.8 (28), 305.5 (97), 296.5 (33), 279.0 (47), 261.3 (93) (calcd for $C_{29}H_{28}O_{11}Na$, 575.5); ESI/TOF-LCMS *m*/*z* 552.5 $[M]^-$ (31), 413.3 (8), 285.3 (4), 255.1 (4), 214.2 (7), 213.1 (15), 212.1 (100), 210.1 (17), 193.0 (8), 171.1 (11), 157.1 (17), 127.1 (30), 125.1 (5) (calcd for $C_{29}H_{28}O_{11}$, 552.5); R_f 0.91 (Si gel, solvent A).

Cucumerin B (2): yellowish amorphous solid; mp 88−89 °C; $[\alpha]^{22}_D$ −181.1° (*c* 0.01, DMSO); UV (MeOH) λ_{max} (log ϵ) 272 (4.95), 335 (5.04) nm; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2); MALDI-TOF *m*/*z* 575.5 $[M + Na]^+$ (47), 552.5 $[M]^+$ (100), 537.5 (29), 534.4 (19), 522.7 (28), 513.5 (49), 507.6 (34), 505.5 (22), 491.2 (37), 477.5 (16), 469.5 (27), 457.6 (22), 445.4 (19) (calcd for $C_{29}H_{28}O_{11}Na$, 575.5); ESI/TOF-LCMS *m*/*z* 552.5 $[M]^-$ (3), 533.4 (5), 473.2 (7), 461.2 (13), 457.3 (16), 431.2 (97), 389.5 (28), 367.2 (100), 337.4 (84), 327.3 (12), 295.3 (16), 249.1 (8), 186.8 (6), 175.2 (8) (calcd for $C_{29}H_{28}O_{11}$ 552.5); R_f 0.91 (Si gel, solvent A).

Compounds 3−8. These were identified by comparison of their spectral data with literature values[2,3,13,15,16] and unambiguously confirmed using HPLC and TLC co-chromatography experiments with authentic standards.

Acid Hydrolysis and Enzymatic Digestion of 1 and 2. Compounds **1** and **2** (5 mg each) were subjected to acid hydrolysis (2 N HCL, 2 h, 100 °C refluxed over an oil bath). Concurrently, 5 mg of **1** and **2** was digested with β-glucosidase (48 h, 37 °C, buffer: 500 mL of H_2O/0.1 M NaOCl/40 mM $CaCl_2$, pH adjusted to 5.0 with HOAc). HPLC and ESI/TOF-LCMS analysis of acid hydrolysis and enzymatic digestion products revealed that the native structures of **1** and **2** remained unaltered, indicating a *C*-glycoside linkage rather than an *O*-glycoside linkage for these compounds.

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References and Notes

(1) Agrios, G. N. *Plant Pathology*; Academic Press: New York, 1997; p 295.
(2) Daayf, F.; Bel-Rhlid, R.; Bélanger, R. R. *J. Chem. Ecol.* **1997**, *23*, 1517−1526.
(3) Daayf, F.; Ongena, M.; Boulanger, R.; El Hadrami, I.; Bélanger, R. R. *J. Chem. Ecol.* **2000**, *26*, 1579−1593.
(4) Fawe, A.; Abou-Zaid, M.; Menzies, J. G.; Bélanger, R. R. *Phytopathology* **1998**, *88*, 396−401.
(5) Bar-Nun, N.; Mayer, A. M. *Phytochemistry* **1990**, *29*, 787−791
(6) Dean, R. A.; Kuć, J. *Physiol. Mol. Plant Pathol.* **1987**, *31*, 69−81.
(7) Hammerschmidt, R.; Kuć, J. *Physiol. Plant Pathol.* **1982**, *20*, 61−71.
(8) Robertson, B.; Svalheim, Ø. *Physiol. Plant.* **1990**, *79*, 512−518.
(9) Siegrist, J.; Jeblick, W.; Kauss, H. *Plant Physiol.* **1994**, *105*, 1365−1374.
(10) Hammerschmidt, R. *Annu. Rev. Phytopathol.* **1999**, *37*, 285−306.
(11) Snyder, B. A.; Nicholson, R. L. *Science* **1990**, *248*, 1637−1639.
(12) Nicholson, R. L.; Wood, K. V. *Physiol. Mol. Plant Pathol.* **2001**, *59*, 63−69.
(13) Harborne, J. B.; Mabry, T. J. *The Flavonoids: Advances in Research*; Chapman and Hall: New York, 1982.
(14) Harborne, J. B. *Comparative Biochemistry of the Flavonoids*; Academic Press: New York, 1967.
(15) Nawwar, M. A. M.; El-Mousallamy, A. M. D.; Barakat, H. H.; Buddrus, J.; Linscheid, M. *Phytochemistry* **1989**, *28*, 3201−3206.
(16) Ramarathnam, N.; Osawa, T.; Namiki, M.; Kawakishi, S. *J. Agric. Food Chem.* **1989**, *37*, 316−319.
(17) Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307−3330.
(18) Silverstein, R. M.; Webster, F. X. *Spectrometric Identification of Organic Compounds*; John Wiley & Sons: New York, 1998; p 9.
(19) Nørbæk, R.; Brandt, K.; Kondo, T. *J. Agric. Food Chem.* **2000**, *48*, 1703−1707.
(20) Asai, F.; Iinuma, M.; Tanaka, T.; Takenaka, M.; Mizuno, M. *Phytochemistry* **1992**, *31*, 2487−2490.
(21) Linard, A.; Paris, R. R. *Plant Med. Phytother.* **1977**, *11*, 270−274.
(22) Krauze-Baranowska, M.; Cisowski, W. *Biochem. Syst. Ecol.* **2001**, *29*, 321−324.
(23) Fawe, A. Étude du mode d'action du silicium dans l'accroissement de résistance du concombre au blanc poudreux: intervention de composés à activité antifongique et identification. Ph.D. Thesis, Université Laval, Québec, Qc, 1997; pp 59−72.
(24) Ravisé, A.; Chopin, J. *Phytopath. Z.* **1981**, *100*, 257−269.
(25) Wiseman, B. R.; Carpenter, J. E. *J. Econ. Entomol.* **1995**, *88*, 1037−1043.
(26) Harborne, J. B.; Baxter, H. *The Handbook of Natural Flavonoids*; John Wiley & Sons: England, 1999; Vol. 1.
(27) McNally, D. J.; Wurms, K.; Labbé, C.; Bélanger, R. R. *Can. J. Plant Pathol.* **2002**, *24*, 429−436.

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