Constituents of the Underground Parts of *Glehnia littoralis*

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From the underground parts of *Glehnia littoralis* FR. SCHMIDT ex MIQUEL (Umbelliferae), 26 compounds, including two new lignan glycosides [glehlinosides A (1) and B (2)], a new neolignan glycoside [glehlinoside C (3)], and a new phenylpropanoid glycoside [4-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]-3-methoxypropiophenone (4)], were obtained and their structures were determined by analysis of their spectral data. The 1,1-diphenyl-2-picrylhydrazyl radical-scavenging assay disclosed quercetin (8), isoquercetin (9), rutin (10), chlorogenic acid (11), and caffeic acid (24) as the major antioxidative constituents in this crude drug.

Key words traditional Chinese medicine; *Glehnia littoralis*; lignan glycoside; neolignan glycoside; phenylpropanoid glycoside; 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity

Glehnia littoralis (G.) FR. SCHMIDT ex MIQUEL (Umbelliferae) is a perennial herb growing on the sandy beaches of eastern Asia.[1)] The dried roots and rhizomes of this plant are used in traditional Chinese medicine as a tonic, antiphlogistic, and mucolytic for the treatment of respiratory and gastrointestinal disorders,[1)] while they are used in Japan as diaphoretic, antipyretic, and analgestic medicine.[2)] They are also a major component in anti-aging and health promotion prescriptions.[3)] Previous investigations resulted in the isolation of coumarins,[2)] coumarin glycosides,[4)] and polyacetylenes[5)] from constituents of the underground parts of *G. littoralis*.

Recently, free radicals have been implicated in many age-related diseases including cerebral ischemia, Parkinson's disease, Alzheimer's disease, and cancer.[6)] Natural antioxidants can scavenge free radicals and prevent the human body from aging by reducing oxidative stress. Thus we examined the antioxidative constituents of *G. littoralis* and isolated two new lignan glycosides, called glehlinosides A (**1**) and B (**2**), a new neolignan glycoside, called glehlinoside C (**3**), and a new phenylpropanoid glycoside (**4**), together with 22 known compounds (**5**—**26**) (Fig. 1). This paper reports their isolation, structure elucidation, and scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals.

The underground parts of *G. littoralis* were extracted with ethanol, and the extract was suspended in water, defatted with petroleum ether, and then successively partitioned with EtOAc and BuOH. The EtOAc fraction was chromatographed on a silica gel column to give eight known compounds (**19**—**26**), while the BuOH fraction was separated by a combination of Sephadex LH-20 column chromatography and preparative TLC to afford four new compounds (**1**—**4**) and 14 known compounds (**5**—**18**). The known compounds **6**—**26** were identified by means of comparison with published data or with authentic samples as: citrusin A (**6**),[7)] (−)-*seco*isolariciresinol (**7**),[8)] quercetin (**8**),[9)] isoquercetin (**9**),[9)] rutin (**10**),[9)] chlorogenic acid (**11**),[10)] 4″-hydroxyimperatorin 4″-*O*-β-D-glucopyranoside (**12**),[4)] bergaptol-*O*-β-D-glucopyranoside (**13**),[11)] marmesinin (**14**),[4)] (3′*R*)-hydroxymarmesin 4′-*O*-β-D-glucopyranoside (**15**),[4)] osthenol-7-*O*-β-D-gentiobioside (**16**),[2)] uridine (**17**),[12)] adenosine (**18**),[13)] isoimperatorin (**19**),[2)] psoralen (**20**),[2)] scopoletin (**21**),[2)] xanthotoxol (**22**),[2)] ferulic acid (**23**),[14)] caffeic acid (**24**),[14)] vanillic acid (**25**),[15)] and 3-methoxy-4-β-D-glucopyranosyloxypropiophenone (**26**)[16)] (Fig. 1). Among these, compounds **6**—**11**, **13**, **17**, **18**, and **23**—**26** were first identified in *G. littoralis*.

Compound **5** was obtained as colorless amorphous solid, $[\alpha]_D$ −182.1° (MeOH). Its molecular formula was determined to be $C_{26}H_{36}O_{11}$ by positive-ion high-resolution (HR)-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra, analyzed by ^{1}H–^{1}H shift correlation spectroscopy (COSY), heteronuclear mutiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) spectra, suggested the presence of a secoisolariciresinol and a β-glucose moiety, which was confirmed by acid hydrolysis of **5** to (−)-*seco*isolariciresinol (**7**)[8)] and glucose. The glucose was determined to be the D-configuration by gas chromatographic (GC) analysis of a chiral derivative of the acid hydrolysate.[17)] The HMBC spectrum revealed the correlation between the anomeric proton and C-4 or C-4′. Because the aglycon (−)-*seco*isolariciresinol has a symmetry axis between C-8 and C-8′, two aryl groups are equivalent. Thus the β-D-glucose was concluded to be located at C-4; *i.e.*, **5** is (−)-*seco*isolariciresinol 4-*O*-β-D-glucopyranoside. Previously, this compound was identified in *Urtica dioica* (Urticaceae) by GC-MS analysis,[18)] but to our knowledge this is the first report of the isolation of this compound.

Glehlinoside A (**1**) was isolated as a colorless amorphous solid, $[\alpha]_D$ −158.3° (MeOH). Positive-ion HR-FAB-MS showed the quasimolecular ion at *m*/*z* 675.2641, consistent with the molecular formula $C_{34}H_{42}O_{14}$, while the UV spectrum of **1** indicated the presence of aromatic rings (λ_{max} 275 nm). The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of (−)-*seco*isolariciresinol 4-*O*-β-D-glucopyranoside (**5**), but they were characterized by the presence of additional signals due to a 1,3,4-trisubstituted benzoyl group and a methoxyl group (Table 1). The benzoyl group was determined to be a vanilloyl group and to be located at C-6″ of the glucose based on the HMBC correlations depicted in Fig. 2. Thus glehlinoside A was concluded to be (−)-*seco*isolariciresinol 4-*O*-β-D-(6-*O*-vanilloyl)glucopyranoside (**1**), which was confirmed by the alkaline hydrolysis of **1** to **5** and vanillic acid (**25**).

Glehlinoside B (**2**) was obtained as colorless amorphous solid, $[\alpha]_D$ −54.5° (MeOH), of which the molecular formula

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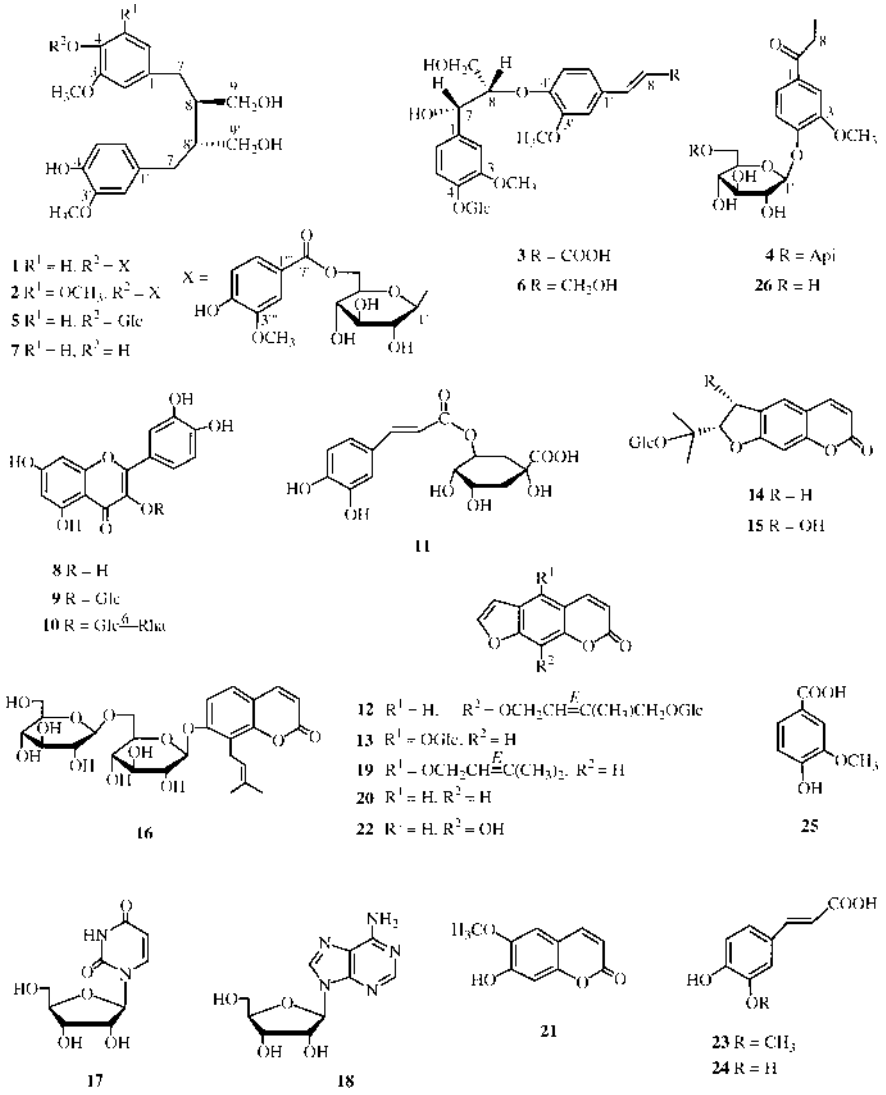

Fig. 1. Structures of Isolated Compounds

Glc: β-D-glucopyranosyl, Api: β-D-apiofuranosyl, Rha: β-D-rhamnopyranosyl.

was established to be $C_{35}H_{44}O_{15}$ by HR-FAB-MS. The UV spectrum of **2** closely resembled that of **1**. Both the ^{1}H- and ^{13}C-NMR spectra of **2** were also similar to those of **1**, but they were characterized by the presence of signals due to a 1,3,4,5-tetrasubstituted benzene ring and an additional methoxyl group instead of the 1,3,4-trisubstituted benzene ring (Table 1). Detailed analysis of the COSY, HMQC, and HMBC spectra of **2** indicated that **2** is a 5-methoxyl derivative of **1**, while the absolute configuration was assigned as 8*R*,8′*R*, based on the same negative sign of the specific rotation as that of **1**. Thus glehlinoside B was determined to have the structural formula **2**.

Glehlinoside C (**3**) was obtained as an off-white amorphous solid, $[\alpha]_D$ −34.3° (MeOH), and its molecular formula, $C_{26}H_{32}O_{13}$, was obtained by HR-FAB-MS. The ^{1}H-NMR spectrum of **3** displayed the signals of two 1,3,4-trisubstituted benzene rings, two oxygenated methines, an oxygenated methylene, and a *trans*-olefine, together with a β-glucose and two methoxyl groups (Table 1). On the other hand, its ^{13}C-NMR spectrum showed the signals of 26 carbons, including a carboxyl carbon, an olefine, and a β-glucopyranosyl group (Table 1). The presence of D-glucose was confirmed by acid hydrolysis followed by GC analysis of the acid hydrolysate. The COSY, HMQC, and HMBC spectra suggested that **3** has a carboxylic acid instead of the allyl alcohol in citrusin A (**6**),[7] a known compound isolated from the same extract. The location of the glucose was determined to be at C-4 from the HMBC correlation between H-1″ of the glucose moiety and C-4 (Fig. 2). The relative stereochemistry between C-7 and C-8 was concluded to be the same as **6**, *i.e.*, *erythro*, based on the small coupling constant (J=5.1 Hz),[19] From these data, glehlinoside C was determined to have the structural formula **3**.

Compound **4** was obtained as an off-white amorphous solid, $[\alpha]_D$ −61.3° (MeOH), and its molecular formula was determined to be $C_{21}H_{30}O_{12}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **4** were similar to those of 3-methoxy-4-β-D-glucopyranosyloxypropiophenone (**26**),[16] a known compound isolated from the same extract, indicating the presence of a 1,3,4-trisubstituted benzene ring, a methyl, a methylene, a methoxyl, and a β-glucopyranosyl group, but they were characterized by the presence of signals due to an additional apiofuranosyl group. Mild acid hydrolysis of **4** furnished **26** and apiose. The location of the apiofuranosyl group was de-

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1**, **2**, **3**, and **5**[a)]

Position	**1**[b)]		**2**[b)]		**5**[b)]		**3**[c)]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		137.2		139.3		137.1		135.8
2	6.63 d (2.0)	114.2	6.29 s	107.7	6.66 d (2.0)	114.0	7.07 d (2.0)	111.8
3		150.3		153.9		150.2		148.3
4		145.8		133.9		145.8		145.7
5	6.89 d (8.3)	117.4		153.9	6.99 d (8.0)	117.7	7.00 d (8.3)	114.6
6	6.34 dd (8.3, 2.0)	122.6	6.29 s	107.7	6.63 dd (8.0, 2.0)	122.6	6.90 dd (8.3, 2.0)	119.2
7	2.53 m	36.1	2.56 m	36.9	2.58 dd (13.8, 7.8)	36.0	4.76 d (5.1)	71.4
	2.65 m		2.68 m		2.70 dd (13.8, 7.0)			
8	1.85 m[d)]	44.0	1.89 m[f)]	44.4	1.89 m[j)]	44.0[k)]	4.45 m	83.3
9	3.58 d (5.4)[e)]	62.0	3.58 d (5.0)[g)]	62.0[h)]	3.58 d (4.7)	61.7[l)]	3.62 d (4.4)	60.0
3-OMe	3.72 s	56.3	3.68 s	56.8	3.76 s	56.1	3.80 s	55.6[m)]
5-OMe			3.68 s	56.8				
1′		133.7		133.6		133.4		127.0
2′	6.48 d (2.0)	113.2	6.59 d (2.0)	113.5	6.57 d (2.0)	113.2	7.19 d (2.0)	111.0
3′		148.7		148.6		148.5		149.6
4′		145.4		145.4		145.2		150.1
5′	6.64 d (8.0)	115.7	6.64 d (8.0)	115.8	6.65 d (8.0)	115.6	7.03 d (8.3)	114.7
6′	6.51 dd (8.0, 2.0)	122.7	6.52 dd (8.0, 2.0)	122.6	6.52 dd (8.0, 2.0)	122.4	7.12 dd (8.3, 2.0)	122.1
7′	2.50 m	36.1	2.56 m	36.1	2.54 dd (13.8, 7.8)	36.0	7.47 d (15.8)	143.9
	2.62 m		2.68 m		2.66 dd (13.8, 7.0)			
8′	1.82 m[d)]	44.0	1.87 m[f)]	43.9	1.86 m[j)]	43.8[k)]	6.35 d (15.8)	116.6
9′	3.56 d (5.4)[e)]	62.0	3.56 d (5.0)[g)]	61.7[h)]	3.58 d (4.7)	61.6[l)]		167.7
3′-OMe	3.66 s	56.5	3.76 s	56.3[i)]	3.74 s	56.4	3.80 s	55.5[m)]
1″	4.82 d (7.8)	102.7	4.74 d (7.6)	105.3	4.79 d (7.3)	102.9	4.83 d (7.0)	100.3
2″	3.51 m	74.8	3.50 m	75.5	3.46 m	74.6	3.28 m	73.1
3″	3.51 m	77.7	3.43 m	77.7	3.47 m	77.4	3.32 m	76.9[n)]
4″	3.43 t (9.0)	72.0	3.43 m	71.9	3.44 m	71.1	3.19 t (9.0)	69.6
5″	3.75 m	75.5	3.50 m	75.7	3.38 m	77.8	3.32 m	76.8[n)]
6″	4.40 dd (11.9, 7.0)	65.0	4.39 dd (11.7, 6.5)	64.9	3.68 dd (12.2, 6.2)	62.3	3.67 dd (12.0, 2.0)	60.8
	4.64 dd (11.9, 2.2)		4.51 dd (11.7, 2.0)		3.87 dd (12.2, 2.0)		3.47 dd (12.0, 5.6)	
1‴		122.4		122.3				
2‴	7.52 d (2.0)	113.8	7.39 d (2.0)	113.7				
3‴		148.7		148.6				
4‴		152.9		152.9				
5‴	6.85 d (8.3)	116.0	6.79 d (8.8)	115.8				
6‴	7.57 dd (8.3, 2.0)	125.3	7.44 dd (8.8, 2.0)	125.2				
7‴		167.8		167.8				
3‴-OMe	3.84 s	56.5	3.84 s	56.4[i)]				

a) *J* values (in Hz) in parentheses. *b*, *c*) Measured in MeOH-d_4 and dimethyl sulfoxide (DMSO)-d_6, respectively. *d*—*o*) Assignments may be interchanged.

termined to be at C-6′ of the glucose moiety, based on the glycosylation shift of C-6′ (**4**, δ 68.7; **26**, δ 62.4) and C-5′ (**4**, δ 77.1; **26**, δ 77.8) of the glucose.[20)] Kitagawa *et al*. confirmed the β-configuration of the anomeric proton of the apiofuranosyl group in apioglycyrrhizin with Klyne's rule.[21)] The difference in the molecular rotation between **4** and **26** was negative (−109°), *i.e*., the same as methyl β-D-apiofuranoside; molecular rotations of methyl α-D- and β-D-apiofuranosides are +190° and −156°, respectively.[21)] Thus **4** was concluded to be 4-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]-3-methoxypropiophenone.

The DPPH radical-scavenging activities of the above compounds **1**—**26**, together with the well-known potent antioxidant ascorbic acid were investigated (Table 2). Five compounds with a catechol group (**8**—**11**, **24**) showed potent activities (EC_{50}, 5.0—11.9 μM) comparable to that of ascorbic acid (EC_{50}, 9.1 μM), while among the lignan derivatives, only compounds **1** (EC_{50}, 28.0 μM) and **7** (EC_{50}, 21.3 μM) exhibited mild activities.

Experimental

Optical rotations were determined in MeOH solutions on a JASCO DIP-140 digital polarimeter at 25 °C. UV spectra were obtained with a Shimadzu UV-160A spectrophotometer. NMR spectra were recorded on a JEOL JNM-GX400 spectrometer with tetramethylsilane (TMS) as internal standard, and FAB-MS measurements were performed on a JEOL JMS-700T spectrometer using glycerol as a matrix. GC analysis was performed on a Shimadzu GC-14AH system. Column chromatography was performed on silica gel (Fuji Silysia, BW-820MH) or macroreticular absorption resin D101 (Tianjin Insecticide Manufacture, China) or Sephadex LH-20 (Pharmacia, Sweden). Analytical and preparative TLC was carried out on precoated Merck Kieselgel 60F_{254} (0.25 or 0.5 mm) or RP-18F_{254} plates (0.25 mm).

Plant Material Dried underground parts of *G. littoralis* were collected in Laiyang, Shandong Province, China, in May 1999. The plant material was identified by Professor Weichun Wu of Shenyang Phamaceutical University, Shenyang, China. A voucher specimen is preserved in the Department of Traditional Chinese Medicine, Shenyang Pharmaceutical University.

Isolation and Identification The dried underground parts (3.0 kg) of *G. littoralis* were extracted with ethanol (5 l, reflux, 2 h, ×3), and the ethanol extract was concentrated under reduced pressure. The residue (219 g) was suspended in water, defatted with petroleum ether, and then successively partitioned with EtOAc (2.5 l) and BuOH (2.5 l). The EtOAc fraction (35 g) was chromatographed over silica gel [$CHCl_3$→$CHCl_3$–MeOH (95 : 5, 90 : 10)→$CHCl_3$–MeOH–H_2O (80 : 20 : 2, 70 : 30 : 2)→MeOH] to give six fractions (frs. 1—6). Fraction 1 (4.6 g) was further subjected to repeated sil-

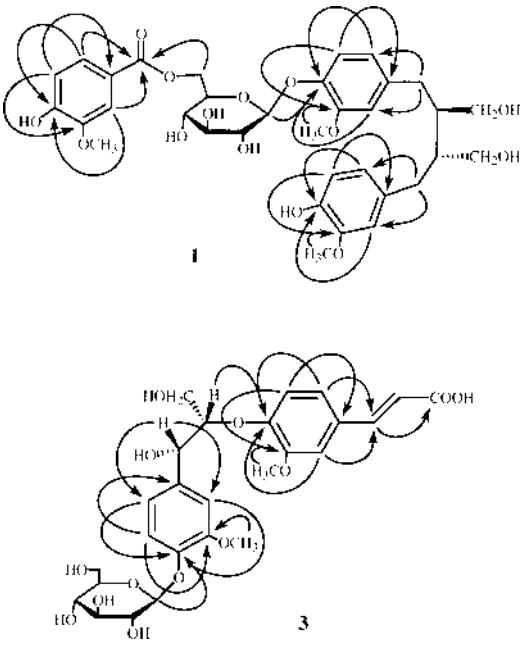

Fig. 2. Significant Correlations Observed in the HMBC Spectra of Glehlinosides A (**1**) and C (**3**)

Table 2. DPPH Radical-Scavenging Activities of Compounds **1**—**26**

Compounds	EC_{50} (in μg/ml)
1	18.9 (28.0)[a]
2	>50.0 (37)[b]
3	>50 (18)[b]
4	>50 (14)[b]
5	21.0 (40.0)[a]
6	>50 (23)[b]
7	7.7 (21.3)[a]
8	1.5 (5.0)[a]
9	3.1 (6.7)[a]
10	5.0 (8.2)[a]
11	4.2 (11.9)[a]
12	>50 (42)[b]
13	>50 (19)[b]
14	>25 (24)[b]
15	>50 (24)[b]
16	16.3 (29.4)[a]
17	>200 (2)[b]
18	>50 (4)[b]
19	34.6 (171)[a]
20	>50 (41)[b]
21	>33 (34)[b]
22	25.7 (192)[a]
23	3.8 (19.6)[a]
24	1.4 (7.8)[a]
25	>50 (43)[b]
26	>50 (18)[b]
Ascorbic acid	1.6 (9.1)[a]

a) EC_{50} in μM. *b*) Inhibitory ratio (%) at the indicated concentration.

ica gel column chromatography with $CHCl_3$–MeOH (97 : 3) to give **19** (20.6 mg) and **20** (9.0 mg). Similarly, repeated silica gel column chromatography of fraction 2 (4.8 g) with $CHCl_3$–MeOH (95 : 5) afforded **21** (18.6 mg), **22** (3.3 mg), **23** (15.3 mg), **24** (11.4 mg), and **25** (9.8 mg). Fraction 4 (6.9 g) was rechromatographed with $CHCl_3$–MeOH–H_2O (80 : 20 : 2) to give **26** (14.6 mg).

The BuOH extract (47 g) was subjected to a macroreticular resin D101 column and eluted with a stepwise gradient of H_2O, H_2O–EtOH (1 : 4 and 1 : 1), and EtOH to give four fractions (frs. 1—4). Fraction 2 (4.9 g) was subjected to Sephadex LH-20 column chromatography with a H_2O–MeOH gradient system to give four fractions (frs. 2-1—2-4). Fraction 2-1 (1.3 g) was rechromatographed with H_2O to give five subfractions. Subfractions 1 (50 mg) and 2 (46 mg) were subjected to preparative TLC with $CHCl_3$–MeOH–H_2O (80 : 20 : 2) to give **12** (8.2 mg), **13** (3.4 mg), and **17** (6.1 mg); or **14** (6.5 mg) and **18** (5.9 mg), respectively. Fraction 2-2 (3.0 g) was also subjected to Sephadex LH-20 column chromatography with H_2O to give four subfractions. Subfractions 1 (50 mg) and 3 (40 mg) were subjected to preparative TLC with $CHCl_3$–MeOH–H_2O (70 : 30 : 2) to afford **6** (7.2 mg) and **11** (7.0 mg); or **3** (3.5 mg), respectively.

Fraction 3 (7.2 g) was subjected to Sephadex LH-20 column chromatography with a H_2O–MeOH gradient system to give four fractions (frs. 3-1—3-4). Fraction 3-1 (1.3 g) was rechromatographed with H_2O to give five subfractions. Subfractions 1 (50 mg) and 2 (66 mg) were seperated by preparative TLC with $CHCl_3$–MeOH–H_2O (80 : 20 : 2) to give **7** (6.5 mg) and **15** (4.1 mg); or **5** (13.0 mg) and **8** (11.0 mg), respectively. Fraction 3-2 (1.0 g) was again subjected to Sephadex LH-20 column chromatography to give five subfractions. Subfractions 1 (41 mg) and 2 (50 mg) were subjected to preparative TLC with $CHCl_3$–MeOH–H_2O (75 : 25 : 2) to give **1** (13.5 mg), and **2** (2.3 mg), respectively, while preparative TLC of subfraction 3 (73 mg) with $CHCl_3$–MeOH–H_2O (80 : 20 : 2) afforded **4** (9.6 mg) and **16** (12.0 mg). Fraction 3-3 (1.6 g) was subjected to repeated Sephadex LH-20 column chromatography with MeOH–H_2O (1 : 5) to give **9** (5.3 mg) and **10** (4.6 mg).

Glehlinoside A (**1**): Colorless amorphous solid, $[\alpha]_D$ −158.3° (c=0.08, MeOH). UV λ_{max} (MeOH) nm: 230, 275. FAB-MS m/z: 697 $(M+Na)^+$, 675 $(M+H)^+$. HR-FAB-MS m/z: 675.2642 (Calcd for $C_{34}H_{43}O_{14}$ 675.2641). ^{1}H- and ^{13}C-NMR: see Table 1.

Glehlinoside B (**2**): Colorless amorphous solid, $[\alpha]_D$ −54.5° (c=0.107, MeOH). UV λ_{max} (MeOH) nm: 230, 275. FAB-MS m/z: 727 $(M+Na)^+$, 705 $(M+H)^+$. HR-FAB-MS m/z: 727.2570 (Calcd for $C_{35}H_{44}O_{15}Na$ 727.2569). ^{1}H- and ^{13}C-NMR: see Table 1.

Glehlinoside C (**3**): Off-white amorphous solid, $[\alpha]_D$ −34.3° (c=0.087, MeOH). UV λ_{max} (MeOH) nm: 230, 275. FAB-MS m/z: 575 $(M+Na)^+$. HR-FAB-MS m/z: 575.1743 (Calcd for $C_{26}H_{32}O_{13}Na$ 575.1740). ^{1}H- and ^{13}C-NMR: see Table 1.

4-[β-D-Apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]-3-methoxypropiophenone (**4**): Colorless amorphous solid, $[\alpha]_D$ −61.3° (c=0.180, MeOH). FAB-MS m/z: 474 $(M+Na)^+$. HR-FAB-MS m/z: 497.1631 (Calcd for $C_{21}H_{30}O_{12}Na$ 497.1635). ^{1}H-NMR (MeOH-d_4) δ: 1.17 (3H, t, J=7.1 Hz, H_3-9), 3.02 (2H, q, J=7.1 Hz, H_2-8), 3.35 (1H, m, H-4′), 3.45 (1H, t, J=9.0 Hz, H-3′), 3.53 (1H, dd, J=9.0, 7.6 Hz, H-2′), 3.56 (2H, s, H_2-5″), 3.60 (1H, m, H-5′), 3.62 (1H, dd, J=11.5, 6.0 Hz, H-6′), 3.72 (1H, d, J=9.8 Hz, H-4″), 3.90 (1H, d, J=2.4 Hz, H-2″), 3.93 (3H, s, 3-OCH_3), 3.97 (1H, d, J=9.8 Hz, H-4″), 4.00 (1H, dd, J=11.5, 2.0 Hz, H-6′), 4.94 (1H, d, J=2.4 Hz, H-1″), 4.96 (1H, d, J=7.6 Hz, H-1′), 7.21 (1H, d, J=8.6 Hz, H-5), 7.57 (1H, d, J=2.0 Hz, H-2), 7.66 (1H, dd, J=8.6, 2.0 Hz, H-6). ^{13}C-NMR (MeOH-d_4) δ: 8.8 (q, C-9), 32.3 (t, C-8), 56.6 (q, 3-OMe), 65.4 (t, C-5″), 68.7 (t, C-6′), 71.5 (d, C-4′), 74.6 (d, C-2′), 74.9 (t, C-4″), 77.1 (d, C-5′), 77.8, 77.9 (each d, C-3′, C-2″), 80.4 (s, C-3″), 101.9 (d, C-1′), 110.9 (d, C-1″), 112.3 (d, C-2), 116.4 (d, C-5), 123.9 (d, C-6), 132.7 (s, C-1), 150.6 (s, C-3), 152.1 (s, C-4), 201.9 (s, C-7).

(−)-*Seco*isolariciresinol 4-*O*-β-D-Glucopyranoside (**5**): Colorless amorphous solid, $[\alpha]_D$ −182.1° (c=0.05, MeOH). UV λ_{max} (MeOH) nm: 230, 270. FAB-MS m/z: 547 $(M+Na)^+$, 525 $(M+H)^+$. HR-FAB-MS m/z: 547.2195 (calcd. for $C_{26}H_{36}O_{11}Na$ 547.2199). ^{1}H- and ^{13}C-NMR: see Table 1.

Alkaline Hydrolysis of Glehlinoside A (1) Glehlinoside A (**1**, 4.5 mg) was dissolved in 0.2 N KOH (2.0 ml) and stirred overnight at room temperature. After neutralization with acetic acid, the reaction mixture was extracted with EtOAc. The extract was concentrated under reduced pressure to give vanillic acid (**25**, 0.8 mg), while the water layer was concentrated under reduced pressure to afford (−)-*seco*isolariciresinol 4-*O*-β-D-glucopyranoside (**5**, 2.5 mg).

Sugar Analysis of Compounds 3 and 5 Compound **3** (2.3 mg) or **5** (3.0 mg) was hydrolyzed with 2 N H_2SO_4 at 75 °C for 3 h. The reaction mixture was passed through Amberlite IRA67 (OH^- form) column, and eluate was concentrated to dryness *in vacuo*. To the residue, 0.1 M L-cysteine methyl ester hydrochloride in pyridine (0.3 ml) was added. The mixture was heated at 60 °C for 2 h. Then trimethylsilylimidazole (0.1 ml) was added and the mixture was heated at 60 °C for 1.5 h. The reaction mixture was partitioned between hexane and water (0.2 ml each), and the hexane layer was analyzed by GC (column, Shimadzu CBJ17-S30-025, 0.32×30 m; column temperature, 230 °C; detector temperature, 270 °C; injection temperature, 270 °C). Standard D- and L-glucose gave one peak at t_R 5.92 and 6.25 min,

respectively.

Acid Hydrolysis of Compound 4 Compound **4** (3.7 mg) was stirred in 0.1 N H_2SO_4 at 60 °C for 30 min, and the reaction mixture was treated as described above to give 3-methoxy-4-β-D-glucopyranosyloxypropiophenone (**26**, 1.6 mg) and apiose (0.7 mg).

DPPH Radical-Scavenging Activities DPPH radical-scavenging activity was measured according to the procedure described by Hatano *et al*.,[22] as reported previously.[23] The sample dissolved in EtOH or in water (500 μl) was mixed with an equal volume of DPPH solution (60 μM). The resulting solution was thoroughly mixed by vortexing and absorbance was measured at 520 nm after 30 min. The scavenging activity was determined by comparing the absorbance with that of the blank (100%) containing only DPPH and solvent. Measurement was done in triplicate with at least three different concentrations, and for compounds showing inhibition higher than 50%, EC_{50} values were caluculated from the data.

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