Constituents of the Underground Parts of *Glehnia littoralis*

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**From the underground parts of *Glehnia littoralis*** Fr. Schmidt ex Miqael (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-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-3-methoxypropio
differentiation, antioxidant activity, and anti-inflammatory activity. Furthermore, the glycoside was identified by HPLC analysis and confirmed by acid hydrolysis.

**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 Miqael (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, anthphlo
gistic, and mucolytic for the treatment of respiratory and gastrointestinal disorders, 1) while they are used in Japan as diaphoretic, antipyretic, and analgesic medicine. 2) They are known to have antipyretic, antiphlogistic, and mucolytic properties for the treatment of respiratory diseases, and they are also used in Japan to treat respiratory and gastrointestinal disorders. 3) Previous investigations resulted in the isolation of 26 compounds, of which 23 were identified as constituents of the underground parts of *G. littoralis*.

Recently, free radicals have been implicated in many age-related diseases including cerebral ischemia, 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 antioxidant 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: eremophil A (6), 21) (–)-secoisolariciresinol (7), 3) quercetin (8), 9) isorhamnetin (9), 10) rutin (10), 11) chlorogenic acid (11), 12) 4′-hydroxyimperatorin 4′-O-β-D-glucopyranoside (12), 13) bergap
tol-6-O-β-D-glucopyranoside (13), 5) masregenol (14), 6) (3R,6'R)-hydroxymarmesin 4′-O-β-D-glucopyranoside (15), 9) ostholest-7-O-β-D-gentiobioside (16), 17) uridine (17), 12) adenosine (18), 13) isomethylavon (19), 21) psoralen (20), 22) scopoletin (21), 23) xanthotoxin (22), 24) ferulic acid (23), 14) caffeic acid (24), 14) vanillic acid (25), 15) and 3-methoxy-4′-β-D-glucopyra
nosylpropiofiltrone (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, [α] D 0 –182.1° (MeOH). Its molecular formula was determined to be C 26H 34O 11 by positive-ion high-resolution (HR)-FAB-MS. The 1H- and 13C-NMR spectra, analyzed by 1H–1H shift correlation spectroscopy (COSY), heteronuclear multiple 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 (–)-secoisolariciresinol (7) 9) and glucose. The glucose was determined to be the α-configuration by gas chromatographic (GC) analysis of a chiral derivative of the acid hydrolysate. 17) The HMBC spectrum revealed the correlation between the anemic proton and C-4 or C-4′. Because the aglycon (–)-secoisolariciresinol 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 (–)-secoisolariciresinol 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, [α] D 0 –158.3° (MeOH). Positive-ion HR-FAB-MS showed the quasimolecular ion at m/z 675.2641, consistent with the molecular formula C 34H 26O 14, while the UV spectrum of 1 indicated the presence of aromatic rings (λ max 275 nm). The 1H- and 13C-NMR spectra of 1 were similar to those of (–)-secoisolariciresinol 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 vanillyl 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 (–)-secoisolariciresinol 4-O-β-D-(6-O-vanillyl)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, [α] D 0 –54.5° (MeOH), of which the molecular formula...
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, \([\alpha]_D -34.3^\circ\) (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 \(\beta\)-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 \(\beta\)-glucopyranosyl group (Table 1). The presence of \(\alpha\)-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., \(\text{erythro}\), 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, \([\alpha]_D -61.3^\circ\) (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-\(\beta\)-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 \(\beta\)-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. 1H- and 13C-NMR Data for Compounds 1, 2, 3, and 5\(^a\)

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta_H)</th>
<th>(\delta_C)</th>
<th>(\delta_H)</th>
<th>(\delta_C)</th>
<th>(\delta_H)</th>
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<th>(\delta_H)</th>
<th>(\delta_C)</th>
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<td>2</td>
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<td>114.2</td>
<td>6.29 s</td>
<td>107.7</td>
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<td>114.0</td>
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<td>145.8</td>
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<td>6.34 dd (8.3, 2.0)</td>
<td>122.6</td>
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<td>107.7</td>
<td>6.63 dd (8.0, 2.0)</td>
<td>122.6</td>
<td>6.90 dd (8.3, 2.0)</td>
<td>119.2</td>
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<td>2.53 m</td>
<td>36.1</td>
<td>2.56 m</td>
<td>36.9</td>
<td>2.58 dd (13.8, 7.8)</td>
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<td>9</td>
<td>1.85 m(^b)</td>
<td>44.0</td>
<td>1.89 m(^b)</td>
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<td>3-OMe</td>
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<td>62.0(^d)</td>
<td>3.58 d (4.7)</td>
<td>61.7(^b)</td>
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<td>3.80 s</td>
<td>55.6(^n)</td>
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\(^a\) \(J\) 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.

terminated to be at C-6' of the glucose moiety, based on the glycosylation shift of C-6' (4, \(\delta\) 68.7; 26, \(\delta\) 62.4) and C-5' (4, \(\delta\) 77.1; 26, \(\delta\) 77.8) of the glucose.\(^{20}\) Kitagawa \textit{et al.} confirmed the \(\beta\)-configuration of the anomeric proton of the apiofuranosyl group in apioglycyrrhizin with Klyne's rule.\(^{21}\)

The difference in the molecular rotation between \(\text{API}\) and ascorbic acid were investigated (Table 2). Five compounds (frs. 1—6) of the extract was concentrated under reduced pressure. The residue (219 g) was partitioned with EtOAc (2.5 l) and BuOH (2.5 l). The EtOAc fraction (35 g) was used for preparative TLC. TLC was carried out on precoated silica gel 60F254 (0.25 or 0.5 mm) or RP-18F254 plates (0.25 mm).

**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 tetrakis(dimethylamino)benzene (TMS) as internal standard, and FAB-MS measurements were performed on a JEOI 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 60F254 (0.25 or 0.5 mm) or RP-18F254 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 Pharmaceutical 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 collected in Laiyang, Shandong Province, China, in May 1999. The plant material was identified by Professor Weichun Wu of Shenyang Pharmaceutical University, Shenyang, China. A voucher specimen is preserved in the Department of Traditional Chinese Medicine, Shenyang Pharmaceutical University.
Table 2. DPPH Radical-Scavenging Activities of Compounds 1—26

<table>
<thead>
<tr>
<th>Compounds</th>
<th>EC₅₀ (μg/ml)</th>
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| 1         | 18.9 (28.0)² | 76
| 2         | >50.0 (37)²  | 76
| 3         | >50.0 (18)²  | 76
| 4         | >50.0 (14)²  | 76
| 5         | 21.0 (40.0)² | 76
| 6         | >50.0 (23)²  | 76
| 7         | 7.7 (21.3)²  | 76
| 8         | 1.5 (5.0)²   | 76
| 9         | 3.1 (6.7)²   | 76
| 10        | 5.0 (8.2)²   | 76
| 11        | 4.2 (11.9)²  | 76
| 12        | >50.0 (42)²  | 76
| 13        | >50.0 (19)²  | 76
| 14        | >25 (24)²    | 76
| 15        | >25 (24)²    | 76
| 16        | 16.3 (29.4)² | 76
| 17        | >200 (2)²    | 76
| 18        | >50.0 (43)²  | 76
| 19        | 34.6 (171)²  | 76
| 20        | >50.0 (41)²  | 76
| 21        | >33 (34)²    | 76
| 22        | 25.7 (192)²  | 76
| 23        | 3.8 (19.6)²  | 76
| 24        | 1.4 (7.8)²   | 76
| 25        | >50.0 (43)²  | 76
| 26        | >50.0 (18)²  | 76
| Ascorbic acid | 1.6 (9.1)²  | 76

a) EC₅₀ in μg/ml. b) Inhibitory ratio (%) at the indicated concentration.

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

**Acid Hydrolysis of Compound 4** Compound 4 (3.7 mg) was stirred in 0.1 N H2SO4 at 60 °C for 30 min, and the reaction mixture was treated as described above to give 3-methoxy-4-β-D-glucopyranosylsoxypirophenone (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., as reported previously. The sample dissolved in EtOH or in water (50 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%, EC50 values were calculated from the data.

**References**

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