Two New Phenolic Glycosides from *Curculigo orchioides*

Ai-Xue Zuo, Yong Shen, Zhi-Yong Jiang, Xue-Mei Zhang, Jun Zhou, Jun Lü, and Ji-Jun Chen

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China. †E-mail: aixuezuo@163.com
Yunnan University of Traditional Chinese Medicine, 1076 Yu-Hua Road, Kunming 650500, China
Yunnan Agricultural University, Kunming 650201, China. ‡E-mail: chenjj@mail.kib.ac.cn
Kunming Jingbiao Biosciences R&D Co. Ltd., Kunming, Yunnan 650000, China

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Two new phenolic glycosides were isolated from the rhizomes of *Curculigo orchioides* Gaertn., based on comprehensive spectroscopic analyses including IR, MS, 1D- and 2D NMR (COSY, HSQC, and HMBC), their structures were elucidated as 3-hydroxyl-5-methylen-1-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (1) and 1',3'-dimethoxy-4-hydroxyalangifolioside (2).

Key Words : Phenolic glycosides, *Curculigo orchioides*

**Introduction**

*Curculigo orchioides* Gaertn., belonged to the Amaryllidaceae family, was widely distributed in China, India, Malaya, Japan and Australia. The rhizomes of *C. orchioides* had been collected as a famous traditional Chinese medicine in the Chinese pharmacopeia. Previous phytochemical investigation on the rhizomes of *C. orchioides* revealed that it contained cycloartane triterpenes, phenolic glycosides, and chlorophenonic glycosides. Some chemicals from *C. orchioides* have exhibited stimulating immune response, antioxidative activities. During the last two years, our group had found some anti-depressant active phenolic compounds and several new phenolic glycoside dimers, phenolic glycosides and cycloartane triterpenes from *C. orchioides*. As a further phytochemical investigation on this plant, two new phenolic glycosides, named 3-hydroxyl-5-methylen-1-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (1) and 1',3'-dimethoxy-4-hydroxyalangifolioside (2) were isolated from the 70% extract of the rhizomes of *C. orchioides*. This paper deals with the isolation, structure elucidation of two new phenolic glycosides based on spectroscopic techniques including MS, IR, 1D- and 2D NMR.

**Results and Discussion**

Compound 1 was obtained as colorless crystals (MeOH) with an optical rotation of [α]$_D^{24}$ = -42.42 (c 0.32, CH$_3$N). Its molecular formula was determined to be C$_{19}$H$_{26}$O$_{12}$ on the basis of negative HR-ESI-MS at m/z 447.1501 [M-H]~
(calcd for C$_{19}$H$_{26}$O$_{12}$, 447.1502); The IR spectrum of compound 1 showed the absorption bands for hydroxyl group (3364 cm$^{-1}$), aromatic ring (1601, 1507, 1458 cm$^{-1}$), and glycosidic linkage (1086 cm$^{-1}$) in the molecule. Hydrolysis of compound 1 with 2 M H$_2$SO$_4$ liberated glucose which was identified by comparing with the authentic sample on Paper Chromatography (PC) [BuOH-EtOAc-H$_2$O 4:1:5, upper layer; PhOH-H$_2$O, 4:1]. In the 1H-NMR spectrum of compound 1, three aromatic proton signals correspond to 1,3,5-trisubstituted aromatic ring at δ$_H$ 7.07 (1H, br. s), 6.80 (1H, br. s), 6.78 (1H, br. s), and one methyl resonance at δ$_H$ 2.19 (3H, s) were observed, together with two anomeric proton signals at δ$_H$ 5.62 (1H, d, J = 7.6 Hz), 5.41 (1H, d, J = 7.9 Hz), suggesting the two glucose moieties in β-configuration. The 13C-NMR spectrum of compound 1 (Table 1) exhibited 19 carbon resonances, involving an aromatic ring: δ$_C$ 160.6 (s), 102.6 (d), 160.3 (s), 109.2 (d), 140.0 (s), 111.7 (d); two glucopyranosyl moieties: δ$_C$ 102.2 (d), 74.3 (d), 88.7 (d), 69.9 (d), 79.2 (d), 62.4 (t) and 106.4 (d), 76.2 (d), 78.8 (d), 72.1 (d), 78.8 (d), 63.0 (t), and one methyl δ$_C$ 22.2 (q); Comparing the NMR data of compound 1 with those of orcinol glucoside$^{12}$ revealed that compound 1 contained one more glucopyranose unit than orcinol glucoside; In order to determined the location of this additional glucopyranose moiety, an HMBC experiment was conducted. As shown in Figure 2, the HMBC correlation between H-1' (δ$_H$ 5.41, 1H, d, J = 7.9 Hz) and C-3' (δ$_C$ 88.7, d) demonstrated the additional glucopyranose was linked at the C-3' of the inner glucopyranose; Therefore, compound 1 was
elucidated as 3-hydroxyl-5-methylenol-1-O-[β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl].

Compound 2, a white amorphous powder, gave a molecular formula of C$_{22}$H$_{26}$O$_{11}$ deduced by HR-ESI-MS at $m/z$ 465.1400 ([M-H]$^{-}$); calcd. for C$_{22}$H$_{26}$O$_{11}$, 465.1396; In the IR spectrum, the absorption bands at 3407 (OH), 1703 (C=O) and 1600, 1495, 1460 (aromatic ring) cm$^{-1}$ were observed. Acidic hydrolysis of compound 2 afforded glucose identified by comparison with the authentic sample on PC [BuOH-EtOAc-H$_2$O 4:1:5, upper layer; PhOH-H$_2$O, 4:1]. The 1H-NMR demonstrated one typical 1,3,4-trisubstituted aromatic ring signals at $\delta_{H}$ 6.43 (1H, d, $J$ = 2.8 Hz), 6.54 (1H, dd, $J$ = 8.8, 2.8 Hz), 7.01 (1H, d, $J$ = 8.8 Hz), one tetra-substituted aromatic ring protons resonances at $\delta_{H}$ 6.65 (1H, d, $J$ = 8.5 Hz), 6.97 (1H, d, $J$ = 8.5 Hz), one methylene at $\delta_{H}$ 3.95 (2H, s), two methoxyl at $\delta_{H}$ 3.80 (3H, s), 3.77 (3H, s), and a β-conformation anomeric proton at $\delta_{H}$ 4.74 (1H, d, $J$ = 7.3 Hz); The 13C-NMR of compound 2 displayed 22 carbon signals including one methene at $\delta_{C}$ 30.0 (t), one carboxyl at $\delta_{C}$ 175.0 (s), two methoxys at $\delta_{C}$ 62.2 (q), 56.2 (q), a set of glucopyranosyl moiety at $\delta_{C}$ 104.1 (d), 75.0 (d), 78.2 (d), 71.4 (d), 78.0 (d), 62.6 (t), matched to those of β-methyl-glucopyranoside, as well as two aromatic rings (Table 2). Detailed analysis of the NMR data of compound 2 indicated that the compound 2 was structurally similar to 4-hydroxyxylangilofloside.$^{14}$ The main difference between them was that there were two additional methoxyl units in compound 2. The HMBC correlations from methoxyl signals at $\delta_{H}$ 3.80 (3H, s) and 3.77 (3H, s) to $\delta_{C}$ C-1′ (s), 155.6 and C-3′ (156.1, s) constructed that the two additional methoxyls were located at C-1′ and C-3′, respectively. Based on the above evidences, compound 2 was characterized as 1′,3′-dimethoxyl-4-hydroxyxylangilofloside.

### Experimental

#### General Experimental Procedures

Optical rotations

### Table 1. 1H- and 13C NMR data of compound 1 in CD$_3$OD (600/150 MHz $\delta$ in ppm, $J$ in Hz)

<table>
<thead>
<tr>
<th>No.</th>
<th>$\delta_{H}$</th>
<th>$\delta_{C}$</th>
<th>Glc-1′</th>
<th>Glc-1″</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160.6 (s)</td>
<td>5.62 (1H, d, 7.6)</td>
<td>102.2 (d)</td>
<td>5.41 (1H, d, 7.9)</td>
</tr>
<tr>
<td>2</td>
<td>102.6 (d)</td>
<td>4.34-4.35 (overlapped)</td>
<td>74.3 (d)</td>
<td>4.12-4.16 (1H, m)</td>
</tr>
<tr>
<td>3</td>
<td>109.0 (s)</td>
<td>4.38-4.40 (overlapped)</td>
<td>88.7 (d)</td>
<td>3.88-4.02 (1H, m)</td>
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<tr>
<td>4</td>
<td>140.0 (s)</td>
<td>4.26-4.28 (1H, m)</td>
<td>69.9 (d)</td>
<td>4.23-4.24 (1H, m)</td>
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<tr>
<td>5</td>
<td>127.2 (s)</td>
<td>4.28-4.30 (1H, m)</td>
<td>79.2 (d)</td>
<td>4.28-4.30 (1H, m)</td>
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<tr>
<td>6</td>
<td>111.7 (d)</td>
<td>4.38-4.40 (overlapped)</td>
<td>62.4 (t)</td>
<td>4.59 (1H, m)</td>
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<tr>
<td>7</td>
<td>2.19 (3H, s)</td>
<td>22.2 (q)</td>
<td>4.34-4.35 (overlapped)</td>
<td>63.0 (t)</td>
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### Table 2. 1H- and 13C NMR data of compound 2 in CD$_3$OD (400/100 MHz $\delta$ in ppm, $J$ in Hz)

<table>
<thead>
<tr>
<th>No.</th>
<th>$\delta_{C}$</th>
<th>$\delta_{H}$</th>
<th>Glc-1′</th>
<th>Glc-1″</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150.2 (s)</td>
<td>155.6 (s)</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>133.8 (s)</td>
<td>156.1 (s)</td>
<td></td>
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<tr>
<td>3</td>
<td>117.7 (d)</td>
<td>127.2 (s)</td>
<td>3′</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>153.6 (s)</td>
<td>107.8 (d)</td>
<td>4′</td>
<td>6.65 (1H, d, 8.5)</td>
</tr>
<tr>
<td>5</td>
<td>114.2 (d)</td>
<td>131.0 (d)</td>
<td>5′</td>
<td>6.97 (1H, d, 8.5)</td>
</tr>
<tr>
<td>6</td>
<td>118.4 (d)</td>
<td>126.3 (s)</td>
<td>6′</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>30.0 (t)</td>
<td>175.0 (s)</td>
<td>7′</td>
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### Figure 2. The key HMBC correlations of compounds 1-2.
were performed on a Horiba SEPA-300 polarimeter (Tokyo, Japan). IR spectra were recorded on a Bio-Rad FTS-135 spectrometer (Richmond, USA) with KBr pellets, ν in cm⁻¹. UV spectra were measured on UV-210A spectrometer (Shimadzu, Japan); NMR spectra were conducted on Bruker AV-400 or DRX-600 spectrometers (Karlsruhe, Germany) with TMS as standard internal; chemical shift (δ) are expressed in ppm and coupling constants (J) in Hz. FAB-MS was recorded on VG-Auto-spec-3000 mass spectrometer (Applied Biosystems/MDS Sciex, Ontario, Canada). Column chromatography (CC) were performed on silica gel (200-300 mesh, Tianjin Pesticide Chemical Company). Acid Hydrolysis. Each of compounds 1-2 (2 mg) was dissolved in MeOH (1.0 mL) and 4 M H₂SO₄ (1.0 mL) solution and hydrolyzed under reflux for 2 h. The hydrolysate was allowed to cool, diluted with 2 mL H₂O, and extracted with 2 mL EtOAc. The aq. layer was neutralized with aq. Ba(OH)₂ and concentrated in vacuum to give a residue, in which glucose was identified by comparing with authentic sample on PC (BuOH-EtOAc-H₂O 4:1:5, upper layer, Rf = 0.45; PhOH-H₂O, 4:1, Rf = 0.40 on PC respectively).

Plant Material. The rhizomes of Curculigo orchioides Gaertn. were collected in Wenshan county, Yunnan Province, P. R. China, in November 2005, and authenticated by Prof. Dr. Li-Gong Lei, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (NO. 20051106) had been deposited in the Group of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered rhizomes of C. orchioides (200 kg) were extracted with 70% EtOH (each 1000 L 2 h) three times under reflux to yield an extract which was combined and concentrated to a small volume (600 L) and submitted to CC (macroporous resin D₁₀₁, 200 kg) with gradient elution of H₂O, 10% EtOH-H₂O, 30% EtOH-H₂O, 90% EtOH-H₂O to afford four fractions: (Frs. A-D). The Fr. B (10% EtOH-H₂O eluted, 800 g) was subjected to Al₂O₃ CC (8 kg, 14 × 50 cm) and subsequently eluted with EtOAc-EtOH-H₂O (9:1:0.1), EtOAc-EtOH-H₂O (8:2:0.2) and EtOAc-EtOH-H₂O (7:3:0.2) to afford sub-fractions B 1-3.

Fr. B 1 (200 g) was subjected to RP-18 CC (1 kg, 6 × 60 cm) eluted with MeOH-H₂O (2:8) to afford fractions B1a-c. Fr. B 1a (3.0 g) was applied to a silica gel CC (100 g, 3.4 × 27 cm) eluted with CHCl₃-MeOH-H₂O (8.5:1.5:0.15) to give four portions. The second portion (1.2 g) was purified on RP-18 CC (120 g, 2.5 × 33 cm) eluted with MeOH-H₂O (3:97) to give a residue, which was purified by Sephadex LH-20 CC (53 g, 2.2 × 62 cm) eluted with MeOH to afford compound 2 (21 mg). The Fr. B 1c (18.0 g) was performed on silica gel CC (250 g, 4 × 50 cm, CHCl₃-MeOH-H₂O 7:3:0.2) to give a residue (1.2 g) which was submitted to Sephadex LH-20 CC (53 g, 2.2 × 62 cm, CHCl₃-MeOH 1:1) and further purified by silica gel CC (15 g, 1 × 15 cm) with the eluent of EtOAc-EtOH-H₂O (8:2:0.2) to yield compound 1 (11 mg).

**Compound (1):** Colorless crystal (MeOH); C₁₀₂H₂₀O₁₂; [α]D²¹ = −42.42 (c 0.32, CH₃CN); UV (MeOH) λmax (log ε) 275 (3.30) nm; IR (KBr) νmax 3364, 2862, 1601, 1507, 1458, 1086, 1058, 574 cm⁻¹; 1H- and 13C-NMR see Table 1; (ESI)-MS m/z 447 [M-H]⁻; (HR-ESI-MS) m/z 447.1501 [M-H]⁻ (calcd for C₁₀₂H₂₀O₁₂, 447.1502).

**Compound (2):** Amorphous powder; C₂₂H₃₀O₁₁; [α]D²¹ = −48.87 (c 0.12, MeOH); UV (MeOH) λmax (log ε) 284 (3.70); IR (KBr) νmax 3407, 2921, 1703, 1495, 1460, 1396, 1213, 810, 609, 583 cm⁻¹; 1H- and 13C-NMR see Table 2; (ESI)-FAB-MS m/z 465 [M-H]⁻, 451 [M-Me], 413 [M-Me-CO], 399 [M-Me-CO-CH₂H₂O]; (HR-ESI-MS) m/z 465.1400 ([M-H]⁻); calcd for C₂₂H₃₀O₁₁, 465.1396.

**References**