

Two New Phenolic Glycosides from *Curculigo orchoides*Ai-Xue Zuo,* Yong Shen,[†] Zhi-Yong Jiang,[‡] Xue-Mei Zhang,[‡] Jun Zhou,[‡] Jun Lü,[§] and Ji-Jun Chen^{‡*}

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Two new phenolic glycosides were isolated from the rhizomes of *Curculigo orchoides* 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-methoxyphenol-1-*O*-[β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-glucopyranoside (**1**) and 1',3'-dimethoxy-4-hydroxyalangifolioside (**2**).

Key Words : Phenolic glycosides, *Curculigo orchoides*

Introduction

Curculigo orchoides Gaertn., belonged to the Amaryllidaceae family, was widely distributed in China, India, Malaya, Japan and Australia.¹ The rhizomes of *C. orchoides* had been collected as a famous traditional Chinese medicine in the Chinese pharmacopeia. Previous phytochemical investigation on the rhizomes of *C. orchoides* revealed that it contained cycloartane triterpenes,² phenolic glycosides,³ and chlorophenonic glycosides.⁴ Some chemicals from *C. orchoides* have exhibited stimulating immune response,⁵ antioxidative activities.⁶ During the last two years, our group had found some anti-depressant active phenolic compounds⁷ and several new phenolic glycoside dimers,⁸ phenolic glycosides^{9,10} and cycloartane triterpenes¹¹ from *C. orchoides*. As a further phytochemical investigation on this plant, two new phenolic glycosides, named 3-hydroxyl-5-methoxyphenol-1-*O*-[β -D-glucopyranosyl-(13)- β -D-glucopyranoside and 1',3'-dimethoxy-4-hydroxyalangifolioside were isolated from the 70% extract of the rhizomes of *C. orchoides*. 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 $[\alpha]_D^{24.1} -42.42$ (c 0.32, C₅H₅N). Its molecular formula was determined to be C₁₉H₂₈O₁₂ on the basis of negative HR-ESI-MS at m/z 447.1501 [M-H]⁻ (calcd for C₁₉H₂₇O₁₂, 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 H₂SO₄ liberated glucose which was identified by comparing with the authentic sample on Paper Chromatography (PC) [BuOH-EtOAc-H₂O

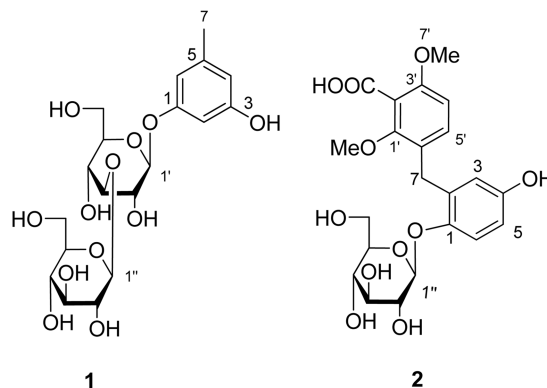
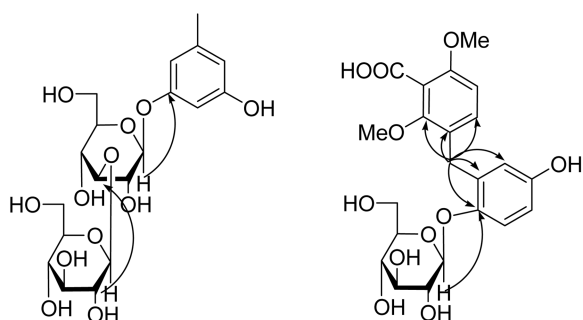


Figure 1. The structures of compounds **1-2**.

4:1:5, upper layer; PhOH-H₂O, 4:1]. In the ¹H-NMR spectrum of compound **1**, three aromatic proton signals corresponding 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 glucoside¹² revealed that compound **1** contained one more glucopyranose unit than orcinol glucoside; In order to determine 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

Table 1. ^1H - and ^{13}C NMR data of compound **1** in $\text{C}_5\text{D}_5\text{N}$ (600/150 MHz δ in ppm, J in Hz)

| No. | Glc-1' | | Glc-1'' | |
|-----|---------------------|---------------------|------------------------|------------------------|
| | δ_{H} | δ_{C} | δ_{H} | δ_{C} |
| 1 | | 160.6 (s) | 5.62 (1H, d, 7.6) | 102.2 (d) |
| 2 | 7.07 (1H, br. s) | 102.6 (d) | 4.34-4.35 (overlapped) | 74.3 (d) |
| 3 | | 160.3 (s) | 4.38-4.40 (overlapped) | 88.7 (d) |
| 4 | 6.80 (1H, br. s) | 109.2 (d) | 4.26-4.28 (1H, m) | 69.9 (d) |
| 5 | | 140.0 (s) | 4.28-4.30 (1H, m) | 79.2 (d) |
| 6 | 6.78 (1H, br. s) | 111.7 (d) | 4.38-4.40 (overlapped) | 62.4 (t) |
| 7 | 2.19 (3H, s) | 22.2 (q) | 4.28-4.30 (1H, m) | 4.34-4.35 (overlapped) |

**Figure 2.** The key HMBC correlations of compounds **1-2**.

elucidated as 3-hydroxyl-5-methylphenol-1- O -[β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-glucopyranoside].

Compound **2**, a white amorphous powder, gave a molecular formula of $\text{C}_{22}\text{H}_{26}\text{O}_{11}$ deduced by HR-EI-MS at m/z 465.1400 ($[\text{M}-\text{H}]^-$; calcd. for $\text{C}_{22}\text{H}_{25}\text{O}_{11}$, 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_2O 4:1:5, upper layer; PhOH- H_2O , 4:1]. The ^1H -NMR demonstrated one typical 1,3,4-trisubstituted aromatic ring signals at δ_{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 δ_{H} 6.65 (1H, d, $J = 8.5$ Hz), 6.97 (1H, d, $J = 8.5$ Hz), one methylene at δ_{H} 3.95 (2H, s), two methoxys at δ_{H} 3.80 (3H, s), 3.77 (3H, s), and a β -configuration anomeric proton at δ_{H} 4.74 (1H, d, $J = 7.3$ Hz); The ^{13}C -NMR of compound **2** displayed 22 carbon signals including one methene at δ_{C} 30.0 (t), one carboxyl at δ_{C} 175.0 (s), two methoxys at δ_{C} 62.2 (q), 56.2 (q), a set of glucopyranosyl moiety at δ_{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,¹³ 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-hydroxyalangifolioside.¹⁴ The main difference between them was that there were two additional methoxyl units in compound **2**. The HMBC correlations from methoxyl signals at δ_{H} 3.80 (3H, s) and 3.77 (3H, s) to δ_{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-hydroxyalangifolioside.

Experimental

General Experimental Procedures. Optical rotations

Table 2. ^1H - and ^{13}C NMR data of compound **2** in CD_3OD (400/100 MHz δ in ppm, J in Hz)

| No. | δ_{C} | δ_{H} | No. | δ_{C} | δ_{H} |
|-----|---------------------|--------------------------|--------|---------------------|---------------------|
| 1 | 150.2 (s) | | 1' | 155.6 (s) | |
| 2 | 133.8 (s) | | 2' | 156.1 (s) | |
| 3 | 117.7 (d) | 6.43 (1H, d, 2.8) | 3' | 127.2 (s) | |
| 4 | 153.6 (s) | | 4' | 107.8 (d) | 6.65 (1H, d, 8.5) |
| 5 | 114.2 (d) | 6.54 (1H, dd, 8.8, 2.8) | 5' | 131.0 (d) | 6.97 (1H, d, 8.5) |
| 6 | 118.4 (d) | 7.01 (1H, d, 8.8) | 6' | 126.3 (s) | |
| 7 | 30.0 (t) | 3.95 (2H, s) | 7' | 175.0 (s) | |
| Glc | | | | | |
| 1'' | 104.1 (d) | 4.74 (1H, d, 7.3) | OMe-1' | 62.2 (q) | 3.80 (3H, s) |
| 2'' | 75.0 (d) | 3.43-3.47 (overlapped) | OMe-2' | 56.2 (q) | 3.77 (3H, s) |
| 3'' | 78.2 (d) | 3.43-3.47 (overlapped) | | | |
| 4'' | 71.4 (d) | 3.37-3.38 (overlapped) | | | |
| 5'' | 78.0 (d) | 3.37-3.38 (overlapped) | | | |
| 6'' | 62.6 (t) | 3.87 (1H, dd, 12.2, 2.2) | | | |
| | | 3.70 (1H, m) | | | |

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 internal standard; chemical shift (δ) were expressed in ppm and coupling constants (J) in Hz. FAB-MS was recorded on VG-Auto-spec-3000 mass spectrometer (Manchester, England); ESI and HR-ESI-MS were taken on a API Qstar-Pulsar-1 mass spectrometer (Applied Biosystems/MDS Sciex, Ontario, Canada). Column chromatography (CC) were performed on silica gel (200-300 mesh, Qingdao Meigao Chemical Co., Ltd., Qingdao, P.R. China), Al_2O_3 (Shanghai Wusi Chemical Reagents Company), D_{101} macroporous resins (Tianjin Pesticide Chemical Company), Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd. USA) and Lichroprep RP-18 (40-63 mm; Merck, Darmstadt, Germany); Fractions were monitored by TLC and visualization by spraying with 10% H_2SO_4 in EtOH followed by heating.

Plant Material. The rhizomes of *Curculigo orchioideis* 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. orchioideis* (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 D_{101} , 200 kg) with gradient elution of H_2O , 10% EtOH- H_2O , 30% EtOH- H_2O , 90% EtOH- H_2O to afford four fractions: (Frs. A-D). The Fr. B (10% EtOH- H_2O eluted, 800 g) was subjected to Al_2O_3 CC (8 kg, 14×50 cm) and subsequently eluted with EtOAc-EtOH- H_2O (9:1:0.1), EtOAc-EtOH- H_2O (8:2:0.2) and EtOAc-EtOH- H_2O (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- H_2O (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 CHCl_3 -MeOH- H_2O (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- H_2O (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, CHCl_3 -MeOH- H_2O 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, CHCl_3 -MeOH 1:1) and further purified by silica gel CC (15 g, 1×15 cm) with the eluent of EtOAc-EtOH- H_2O (8:2:0.2) to yield compound **1** (11 mg).

Compound (1): Colorless crystal (MeOH); $\text{C}_{19}\text{H}_{28}\text{O}_{12}$;

$[\alpha]_{\text{D}}^{24.1} -42.42$ (c 0.32, $\text{C}_5\text{H}_5\text{N}$); UV (MeOH) λ_{max} (log ϵ) 273 (3.30) nm; IR (KBr) ν_{max} 3364, 2862, 1601, 1507, 1458, 1086, 1058, 574 cm^{-1} ; ^1H - and ^{13}C -NMR see Table 1; (–) ESI-MS m/z 447 $[\text{M}-\text{H}]^-$; (–) HR-ESI-MS m/z 447.1501 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{19}\text{H}_{27}\text{O}_{12}$, 447.1502).

Compound (2): Amorphous powder; $\text{C}_{22}\text{H}_{26}\text{O}_{11}$; $[\alpha]_{\text{D}}^{21.4} -48.87$ (c 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 284 (3.70); IR (KBr) ν_{max} 3407, 2921, 1703, 1600, 1495, 1460, 1396, 1213, 1081, 810, 609, 583 cm^{-1} ; ^1H - and ^{13}C -NMR see Table 2; (–) FAB-MS m/z 465 $[\text{M}-\text{H}]^-$, 451 $[\text{M}-\text{Me}]^-$, 413 $[\text{M}-\text{Me}-\text{CO}]^-$, 399 $[\text{M}-\text{Me}-\text{CO}-\text{CH}_2]^-$, 381 $[\text{M}-\text{Me}-\text{CO}-\text{CH}_2-\text{H}_2\text{O}]^-$; (–) HR-ESI-MS m/z 465.1400 $[\text{M}-\text{H}]^-$; calcd. for $\text{C}_{22}\text{H}_{25}\text{O}_{11}$, calcd. for 465.1396).

Acid Hydrolysis. Each of compounds **1-2** (2 mg) was dissolved in MeOH (1.0 mL) and 4 M H_2SO_4 (1.0 mL) solution and hydrolyzed under reflux for 2 h. The hydrolysate was allowed to cool, diluted with 2 mL H_2O , and extracted with 2 mL EtOAc. The aq. layer was neutralized with aq. $\text{Ba}(\text{OH})_2$ and concentrated in *vacuum* to give a residue, in which glucose was identified by comparing with authentic sample on PC [BuOH-EtOAc- H_2O 4:1:5, upper layer, $R_f = 0.45$; PhOH- H_2O , 4:1, $R_f = 0.40$ on PC respectively].

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