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Two New Phenolic Glycosides from *Curculigo orchioides*

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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-methyphenol-1-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (**1**) and 1',3'-dimethoxyl-4-hydroxyalangifolioside (**2**).

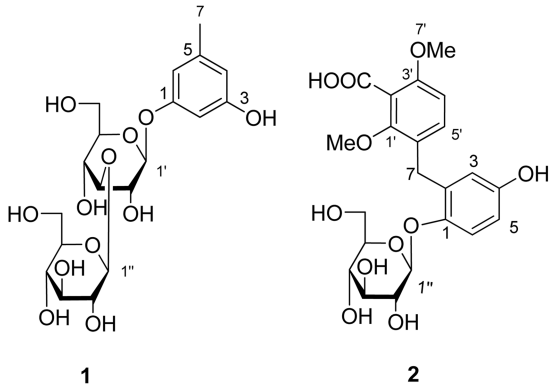
Key Words : Phenolic glycosides, *Curculigo orchioides*

Introduction

Curculigo orchioides Gaertn., belonged to the Amaryllidaceae family, was widely distributed in China, India, Malaya, Japan and Australia.[1] 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,[2] phenolic glycosides,[3] and chlorophenonic glycosides.[4] Some chemicals from *C. orchioides* have exhibited stimulating immune response,[5] antioxidative activities.[6] During the last two years, our group had found some anti-depressant active phenolic compounds[7] and several new phenolic glycoside dimers,[8] phenolic glycosides[9,10] and cycloartane triterpenes[11] from *C. orchioides*. As a further phytochemical investigation on this plant, two new phenolic glycosides, named 3-hydroxyl-5-methyphenol-1-*O*-[β-D-glucopyranosyl-(13)-β-D-glucopyranoside and 1',3-dimethoxyl-4-hydroxyalangifolioside 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 $[\alpha]_D^{24.1}$ −42.42 (*c* 0.32, C_5H_5N). Its molecular fomula was determined to be $C_{19}H_{28}O_{12}$ on the basis of negative HR-ESI-MS at *m/z* 447.1501 $[M-H]^-$ (calcd for $C_{19}H_{27}O_{12}$, 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_2SO_4 liberated glucose which was identified by comparing with the authentic sample on Paper Chromatography (PC) [BuOH-EtOAc-H_2O 4:1:5, upper layer; PhOH-H_2O, 4:1]. In the ^{1}H-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 ^{13}C-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 moeities: 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



1 **2**

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

Table 1. ^{1}H - and ^{13}C NMR data of compound **1** in C_5D_5N (600/150 MHz δ in ppm, *J* in Hz)

No.			Glc-1′		Glc-1″	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		160.6 (s)	5.62 (1H, d, 7.6)	102.2 (d)	5.41 (1H, d, 7.9)	106.4 (d)
2	7.07 (1H, br. s)	102.6 (d)	4.34-4.35 (overlapped)	74.3 (d)	4.12-4.16 (1H, m)	76.2 (d)
3		160.3 (s)	4.38-4.40 (overlapped)	88.7 (d)	3.88-4.02 (1H, m)	78.8 (d)
4	6.80 (1H, br. s)	109.2 (d)	4.26-4.28 (1H, m)	69.9 (d)	4.23-4.24 (1H, m)	72.1 (d)
5		140.0 (s)	4.28-4.30 (1H, m)	79.2 (d)	4.28-4.30 (1H, m)	78.8 (d)
6	6.78 (1H, br. s)	111.7 (d)	4.38-4.40 (overlapped) 4.28-4.30 (1H, m)	62.4 (t)	4.59 (1H, m) 4.34-4.35 (overlapped)	63.0 (t)
7	2.19 (3H, s)	22.2 (q)				

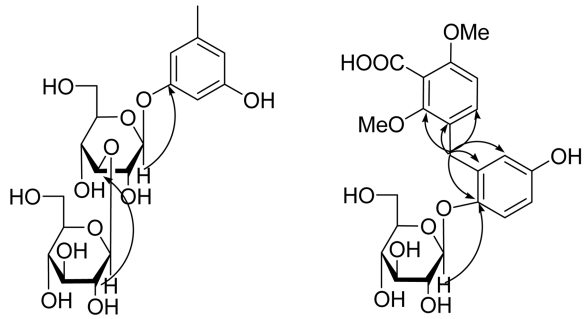


Figure 2. The key HMBC correlations of compounds **1-2**.

elucidated as 3-hydroxyl-5-methyphenol-1-*O*-[*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside.

Compound **2**, a white amorphous powder, gave a molecular formula of $C_{22}H_{26}O_{11}$ deduced by HR-EI-MS at *m/z* 465.1400 ([M-H]$^-$; calcd. for $C_{22}H_{25}O_{11}$, 465.1396); In the IR spectrum, the absorption bonds 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 ^{1}H-NMR demonstrated one typical 1,3,4-trisubstitued 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-substitued 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 methoxyls 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. ^{1}H - 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 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 1000L 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 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); $C_{19}H_{28}O_{12}$; $[\alpha]_D^{24.1}$ −42.42 (*c* 0.32, C_5H_5N); UV (MeOH) λ_{max} (log ε) 273 (3.30) nm; IR (KBr) ν_{max} 3364, 2862, 1601, 1507, 1458, 1086, 1058, 574 cm^{-1}; ^{1}H- and ^{13}C-NMR see Table 1; (–) ESI-MS *m/z* 447 $[M-H]^-$; (–) HR-ESI-MS *m/z* 447.1501 $[M-H]^-$ (calcd for $C_{19}H_{27}O_{12}$, 447.1502).

Compound (2): Amorphous powder; $C_{22}H_{26}O_{11}$; $[\alpha]_D^{21.4}$ −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}; ^{1}H- and ^{13}C-NMR see Table 2; (–) FAB-MS *m/z* 465 $[M-H]^-$, 451 $[M-Me]^-$, 413 $[M-Me-CO]^-$, 399 $[M-Me-CO-CH_2]^-$, 381 $[M-Me-CO-CH_2-H_2O]^-$; (–) HR-ESI-MS *m/z* 465.1400 ($[M-H]^-$; calcd. for $C_{22}H_{25}O_{11}$, 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. $Ba(OH)_2$ and concentrated in *vacuum* to give a residue, in which glucose was identified by comparing with authentic sample on PC [BuOH-EtOAc-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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