Three New Dimeric Orcinol Glucosides from *Curculigo orchioides*

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Three new phenolic glucosides named orcinosides A, B, and C (**1**, **2**, and **3**, resp.) were isolated in low yields (4.0×10^{-6}, 11.5×10^{-6}, 4.5×10^{-6}%, resp.) from the rhizomes of *Curculigo orchioides*. Their structures were elucidated by comprehensive spectroscopic analyses including FAB-MS, HR-ESI-MS, IR, and 1D- and 2D-NMR (HSQC, HMBC) data. Compounds **1**–**3** contained two orcinol-glucoside moieties linked through a CH_2 group.

Introduction. – *Curculigo orchioides* GAERTN., which belongs to the Amaryllidaceae family and named 'Xian-Mao' in Pharmacopoeia of China [1], is a multipurpose drug with numerous pharmacological activities. It has been employed as an analeptic agent for the treatment of decline in strength, and against jaundice and asthma [2]. Previous studies on the rhizomes of this species revealed the presence of cycloartane saponins [3], phenolic glycosides [4], and chlorophenyl glucosides [5]. *Lakshmi et al.* reported that phenols and phenolic glycosides from this plant were responsible for the stimulation of the immune response by acting both on macrophages and lymphocytes [6]. In addition, *Wu et al.* reported the potent antioxidative activities of some phenolic glycosides [7]. We had found that orcinol derivatives from *C. orchioides* showed antidepression activity [8]. The interest in biologically active substances from this medicinal plant encouraged us to further explore its phytochemical composition. Our investigation resulted in the isolation of three new phenolic glucosides in trace amounts, orcinosides A, B, and C (**1**, **2**, and **3**, resp.). We describe the isolation and structural elucidation of **1**–**3** (*Fig. 1*).

Results and Discussion. – The 70% EtOH extract of the roots of *C. orchioides* was applied to D_{101} macroporous resin eluted with 10% EtOH/H_2O. Further fractionation by a combination of column chromatography on silica gel, *PR-18*, and *Sephadex LH-20* afforded compounds **1**–**3** in low yields.

Compound **1** was obtained as colorless needles with an optical rotation of $[\alpha]_D^{28.0} = -65.6$ ($c = 0.61$, MeOH). The FAB mass spectrum (negative-ion mode) exhibited a *quasi*-molecular-ion peak and fragment-ion peaks at m/z 583 ($[M-H]^-$), 421 ($[M-C_6H_{10}O_5]^-$), and 259 ($[M-C_{12}H_{20}O_{10}]^-$), suggesting the presence of two hexose moieties. The HR-ESI-MS (negative-ion mode) analysis provided the molecular

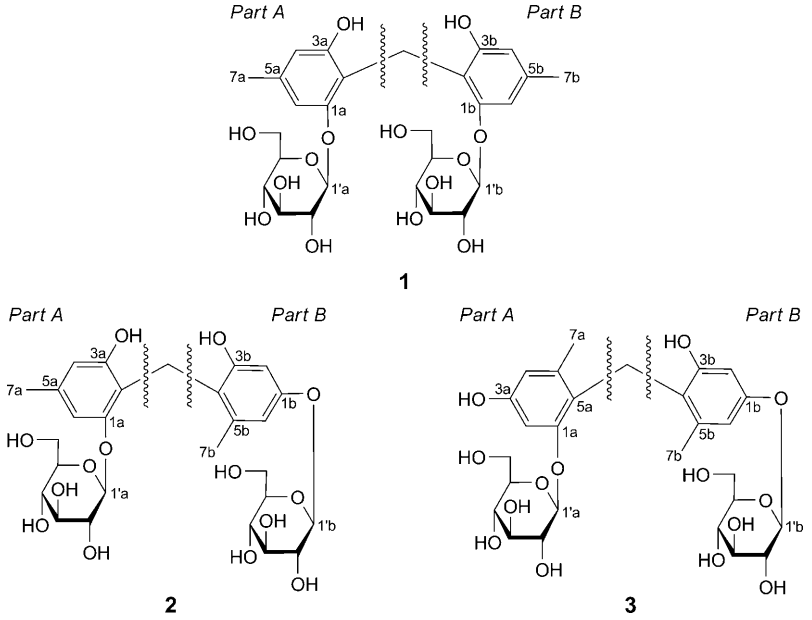

Fig. 1. *The structures of compounds* **1**–**3**

formula $C_{27}H_{36}O_{14}$ from the *quasi*-molecular-ion peak at *m*/*z* 583.2025 ($[M-H]^-$). The IR spectrum showed absorptions for OH groups (3417 cm^{-1}) and aromatic rings (1621, 1590 cm^{-1}), and a strong absorption at 1074 cm^{-1} due to a glucosidic linkage in the molecule. Hydrolysis of compound **1** with 10% H_2SO_4 in MeOH furnished glucose, which was identified by comparison with an authentic sample on PC. In the ^{1}H-NMR spectrum (*Table 1*), two aromatic H-atom signals (δ(H) 6.49 (br. *s*), 6.37 (br. *s*)), a CH_2 (δ(H) 3.93 (*s*)), and a Me signal (δ(H) 2.19 (*s*)) were observed, together with a signal of an anomeric H-atom at δ(H) 4.72 (*d*, *J* = 7.6) due to the β-linked glucose moiety in the molecule. The ^{13}C-NMR spectrum (*Table 1*) displayed 14 C-atom signals, including those for one Me and one CH_2 group, and six aromatic C-atoms assignable to a benzene ring, and a set of β-D-glucopyranose C-atom signals [9]. The above NMR data were similar to those of orcinol glucopyranoside [10] except that there were signals of a

Table 1. *^{1}H- and ^{13}C-NMR Data of Compound* **1**. At 500/125 MHz, in CD_3OD, δ in ppm, *J* in Hz. For positions, see *Fig. 1*.

Position	δ(C)	δ(H)	Position	δ(C)	δ(H)
1a (1b)	158.3 (*s*)	–	1′a (1′b)	103.1 (*d*)	4.72 (*d*, *J* = 7.6)
2a (2b)	115.3 (*s*)	–	2′a (2′b)	74.6 (*d*)	3.45–3.46 (*m*)
3a (3b)	156.1 (*s*)	–	3′a (3′b)	78.1 (*d*)	3.37–3.39 (*m*)
4a (4b)	108.8 (*d*)	6.49 (br. *s*)	4′a (4′b)	71.3 (*d*)	3.34–3.36 (*m*)
5a (5b)	138.2 (*s*)	–	5′a (5′b)	77.4 (*d*)	3.47–3.48 (*m*)
6a (6b)	111.5 (*d*)	6.37 (br. *s*)	6′a (6′b)	62.4 (*t*)	3.86 (*dd*, *J* = 12.4, 1.5), 3.68 (*dd*, *J* = 12.1, 4.0)
7a (7b)	21.5 (*q*)	2.19 (*s*)	CH_2	18.3 (*t*)	3.93 (*s*)

quaternary C-atom at δ(C) 115.3 and of a CH_2 group at δ(C) 18.3 in the ^{13}C-NMR spectrum of compound **1**. Analyses of the FAB-MS, HR-ESI-MS and the NMR data indicated that compound **1** was composed of two orcinol glucopyranoside units and a CH_2 group, with a symmetrical structure. The HMBCs between δ(H) 3.93 (br. *s*, CH_2), and δ(C) 158.3 (C(1a) and C (1b)), 115.3 (C(2a) and C (2b)), and 156.1 (C(3a) and C (3b)) established the connection between the two orcinol glucopyranoside units through the CH_2 group. Therefore, the structure of compound **1** was assigned as shown in *Fig. 1* and named orcinoside A (**1**).

Compound **2** was isolated as colorless needles and had a molecular formula of $C_{27}H_{36}O_{14}$ as determined by the HR-ESI-MS (negative-ion mode) peak at *m*/*z* 583.2040 ($[M-H]^-$). The FAB-MS showed *quasi*-molecular-ion peak and fragment-ion peaks at *m*/*z* 583 ($[M-H]^-$), 421 ($[M-C_6H_{11}O_4]^-$), and 259 ($[M-C_{12}H_{21}O_{10}]^-$), similarly to compound **1**. The IR spectrum displayed absorptions at 3417 (OH), 1613, 1593 (aromatic ring), and 1075 cm^{-1} (glucosidic bond). Acidic hydrolysis of compound **2** with 10% H_2SO_4 liberated glucose identified by comparison with an authentic sample on PC. The ^{1}H-NMR spectrum (*Table 2*) exhibited signals for four aromatic H-atoms at δ(H) 6.40 (br. *s*, 1 H), 6.31 (br. *s*, 1 H), 6.44 (*d*, $J=2.4$, 1 H), 6.25 (*d*, $J=2.4$, 1 H), two Me-group *singlet*s at δ(H) 2.20, 2.17, and signals for two β-linked anomeric H-atoms at δ(H) 4.78 (*d*, $J=7.7$) and 4.79 (*d*, $J=7.6$). The ^{13}C-NMR spectrum (*Table 2*) displayed 27 C-atom signals, corresponding to two Me and one CH_2 groups, and twelve aromatic C-atoms and two sets of β-D-glucopyranose C-atoms. Comparison of the NMR data of compound **2** with those of compound **1** revealed a high similarity except that signals of all C-atoms in the ^{13}C-NMR spectrum of compound **2** appeared in pairs, suggesting that compound **2** should also consist of two orcinol glucopyranoside units and one CH_2 group, but that these were arranged asymmetrically. Compound **2** differed from **1** mainly in the linkage mode between the two orcinol glucopyranoside units and the CH_2 group. As shown in *Fig 2*, the HMBC correlations of the CH_2 H-atoms (δ(H) 3.97 (*d*, $J=9.4$), 3.87 (*d*, $J=9.4$)) with C(1a), C(2a), C(3a), C(3b), C(4b), and C(5b) indicated that the C(2a) of part A was linked to C(4b) of part B through the CH_2 group. Consequently, the structure of compound **2** was determined as depicted in *Fig. 1* and named orcinoside B (**2**).

Compound **3** was obtained as colorless needles. Its molecular formula $C_{27}H_{36}O_{14}$, deduced from the HR-ESI-MS peak at *m*/*z* 583.2041 ($[M-H]^-$), was the same as those of compounds **1** and **2**. Analyses of the NMR data (*Table 2*) revealed that compound **3** also contained two orcinol glucopyranoside units and one CH_2 group. The main difference between compounds **3** and **2** were the linkage positions of the two orcinol glucopyranoside units through the CH_2 group. The correlations of the CH_2 group (δ(H) 4.02 (*d*, $J=9.1$), 3.92 (*d*, $J=9.1$)) with C(3a), C(4a), C(5a), C(1b), C(5b), and C(6b) in the HMBC experiment (*Fig. 2*) suggested that the linkage between the two orcinol glucopyranoside fragments and the CH_2 group was C(6a)–CH_2–C(4b). The other correlations in the HMBC spectrum (*Fig. 2*) confirmed the structure. Thus, the structure of compound **3** was characterized as displayed in *Fig. 1* and named orcinoside C (**3**).

Compounds **1**–**3** were obtained in trace amount from the rhizome of *C. orchioides*. The three compounds had the same molecular formula and almost the same NMR spectra. They differ in the linkage positions of the two orcinol glucopyranoside units

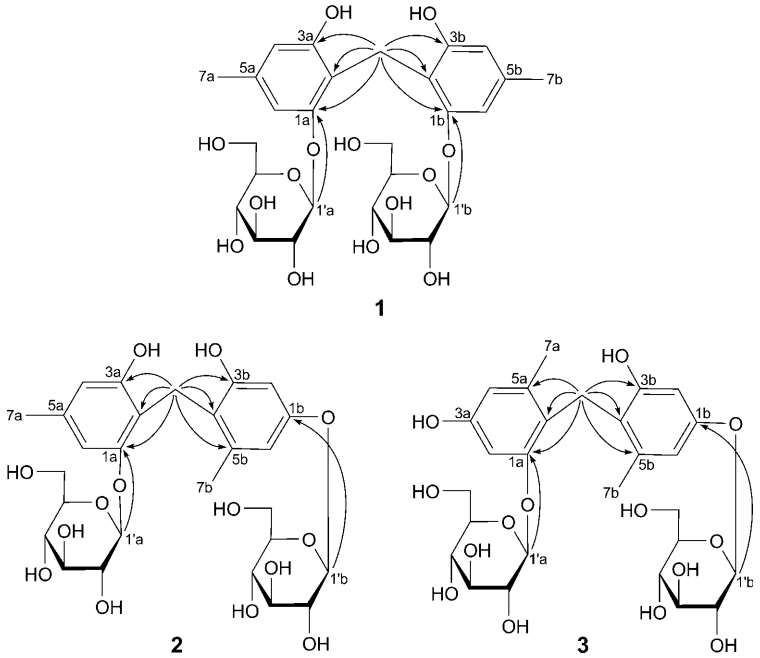

Fig. 2. *The key HMBCs of compounds* **1**–**3**

with the CH_2 group. As shown in *Fig. 1*, compounds **1** and **2** possessed the same part A moieties, and compounds **2** and **3** shared the same part B. Orcinol glucoside is the main phenolic glycoside in this plant [10]. Additionally, several orcinol derivatives had been isolated from *C. orchioides* [11]. However, dimeric orcinol glucosides were isolated for the first time from this plant and even from the Amaryllidaceae family. From the combinatorial view, the other three orcinol glucopyranoside derivatives with C(2a)–CH_2–C(6b), C(4a)–CH_2–C(4b), and C(6a)–CH_2–C(6b) connections may also exist in this plant, although we could not detect them during our investigation.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 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.*), and *Lichroprep RP-18* (40–63 μm; *Merck*, D-Darmstadt). M.p.: *XRC-1* micro-melting-point apparatus (Sichuang University, P. R. China); uncorrected. Fractions were monitored by TLC, visualizing by spraying with 10% H_2SO_4 in EtOH followed by heating. Paper chromatography (PC):

Table 2. *^{1}H- and ^{13}C-NMR Data of Compounds* **2** *and* **3**. At 400 and 100 MHz, respectively, in CD_3OD, δ in ppm, J in Hz. For positions, see *Fig. 1*.

Position	**2**		**3**		Position	**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)		δ(C)	δ(H)	δ(C)	δ(H)
1a	157.9 (*s*)	–	157.1 (*s*)	–	1b	157.7 (*s*)	–	158.0 (*s*)	–
2a	115.5 (*s*)	–	101.5 (*d*)	6.52 (*d*, *J* = 2.4)	2b	100.8 (*d*)	6.44 (*d*, *J* = 2.4)	102.5 (*d*)	6.50 (*d*, *J* = 2.4)
3a	156.5 (*s*)	–	156.5 (*s*)	–	3b	156.8 (*s*)	–	156.5 (*s*)	–
4a	107.8 (*d*)	6.40 (br. *s*)	121.1 (*s*)	–	4b	121.9 (*s*)	–	111.5 (*d*)	6.37 (*d*, *J* = 2.4)
5a	137.7 (*s*)	–	140.5 (*s*)	–	5b	140.7 (*s*)	–	140.3 (*s*)	–
6a	111.1 (*d*)	6.31 (br. *s*)	112.3 (*d*)	6.30 (*d*, *J* = 2.4)	6b	112.2 (*d*)	6.25 (*d*, *J* = 2.4)	122.5 (*s*)	–
7a	21.6 (*q*)	2.20 (*s*)	20.5 (*q*)	2.11 (*s*)	7b	20.5 (*q*)	2.17 (*s*)	20.4 (*q*)	2.00 (*s*)
1′a	101.8 (*d*)	4.78 (*d*, *J* = 7.7)	102.2 (*d*)	4.85 (*d*, *J* = 7.6)	1′b	102.8 (*d*)	4.79 (*d*, *J* = 7.6)	102.9 (*d*)	4.77 (*d*, *J* = 7.5)
2′a	74.4 (*d*)	3.38–3.39 (*m*)	74.7 (*d*)	3.43–3.44 (*m*)	2′b	74.6 (*d*)	3.39–3.40 (*m*)	74.8 (*d*)	3.43–3.44 (*m*)
3′a	77.8 (*d*)	3.36–3.38 (*m*)	77.9 (*d*)	3.42–3.43 (*m*)	3′b	78.0 (*d*)	3.40–3.41 (*m*)	77.9 (*d*)	3.44–3.45 (*m*)
4′a	71.2 (*d*)	3.34–3.35 (*m*)	71.3 (*d*)	3.40–3.41 (*m*)	4′b	71.4 (*d*)	3.28–3.29 (*m*)	71.2 (*d*)	3.40–3.41 (*m*)
5′a	77.8 (*d*)	3.30–3.31 (*m*)	77.6 (*d*)	3.44–3.46 (*m*)	5′b	77.8 (*d*)	3.37–3.39 (*m*)	77.9 (*d*)	3.44–3.46 (*m*)
6′a	62.5 (*t*)	3.65 (*dd*, *J* = 15.0, 6.7), 3.84–3.85 (*m*)	62.4 (*t*)	3.91 (overlapped), 3.73–3.74 (*m*)	6′b	62.3 (*t*)	3.75 (*dd*, *J* = 15.3, 5.0), 3.93–3.94 (*m*)	62.4 (*t*)	3.91 (overlapped), 3.74–3.75 (*m*)
$-CH_2-$	21.1 (*t*)	3.97 (*d*, *J* = 9.4), 3.87 (*d*, *J* = 9.4)	23.4 (*t*)	4.02 (*d*, *J* = 9.1), 3.92 (*d*, *J* = 9.1)					

visualization by spraying with phthalic acid/aniline reagent, followed by heating. Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *UV-210A* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Shimadzu IR-450* instrument; KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker AV-400* or *DRX-500* spectrometers; with TMS as internal standard; δ in ppm, J in Hz. FAB-MS (neg.): *VG-Auto-spec-3000* mass spectrometer, glycerol as matrix. ESI- and HR-ESI-MS: *API Qstar-Pulsar-1* mass spectrometer; in *m/z* (rel. %).

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. There were no microbial contamination or other impurities found in the collected samples. A voucher specimen (No. 20051106) had been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, 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 three times under reflux (each 1000 l, 2 h). The extract was concentrated to a small volume (200 l) and submitted to CC on D_{101} macroporous resin with gradient elution ($H_2O \rightarrow$ 10% EtOH/$H_2O \rightarrow$ 40% EtOH/$H_2O \rightarrow$ 70% EtOH/$H_2O \rightarrow$ 90% EtOH/H_2O) to afford five fractions: *Frs. I–V. Fr. II* (10% EtOH/H_2O fraction, 8000 g) was subjected to Al_2O_3 CC and eluted with AcOEt/EtOH/H_2O (90 : 10 : 1 → 80 : 20 : 2 → 70 : 30 : 3) to afford *Frs. II.A–II.C. Fr. II.A* (100 g) was subjected to SiO_2 CC with $CHCl_3$/MeOH/H_2O 85 : 15 : 1.5 to give three fractions *Fr. II.A.1–II.A.3*. *Fr. II.A.3* was separated by *RP-18* CC (MeOH/H_2O 10 : 90), and subsequently subjected to SiO_2 CC with AcOEt/MeOH/H_2O 80 : 20 : 1 and purified by *Sephadex LH-20* (MeOH) to yield compounds **1** (8 mg), **2** (23 mg), and **3** (9 mg).

Orcinoside A (=*2-[2-(β-*D*-Glucopyranosyloxy)-6-hydroxy-4-methylbenzyl]-3-hydroxy-5-methylphenyl β-*D*-Glucopyranoside*; **1**). Colorless needles. M.p. 179–180°. $[\alpha]_D^{28.0} = -65.6$ ($c = 0.61$, MeOH). UV (MeOH): 270 (3.68). IR (KBr): 3417, 2921, 2883, 1621, 1590, 1515, 1458, 1420, 1074, 1034, 894, 534. NMR: *Table 1*. FAB-MS (neg.): 583 ($[M-H]^-$), 421 ($[M-H-Glc]^-$), 259 ($[M-H-2\,Glc]^-$). HR-ESI-MS (neg.): 583.2025 ($[M-H]^-$, $C_{27}H_{35}O_{14}^-$; calc. 583.2026).

Orcinoside B (=*2-[4-(β-*D*-Glucopyranosyloxy)-2-hydroxy-6-methylbenzyl]-3-hydroxy-5-methylphenyl β-*D*-Glucopyranoside*; **2**). Colorless needles. M.p. 175–177°. $[\alpha]_D^{27.2} = -231.5$ ($c = 0.72$, MeOH). UV (MeOH): 278 (3.63). IR (KBr): 3417, 2921, 2887, 1613, 1593, 1492, 1075, 1033, 531. NMR: *Table 2*. FAB-MS (neg.): 583 ($[M-H]^-$), 421 ($[M-H-Glc]^-$), 259 ($[M-H-2\,Glc]^-$). HR-ESI-MS (neg.): 583.2040 ($[M-H]^-$, $C_{27}H_{35}O_{14}^-$; calc. 583.2026).

Orcinoside C (=*2-[4-(β-*D*-Glucopyranosyloxy)-2-hydroxy-6-methylbenzyl]-5-hydroxy-3-methylphenyl β-*D*-Glucopyranoside*; **3**). Colorless needles. M.p. 210–211°. $[\alpha]_D^{27.4} = -26.9$ ($c = 0.62$, MeOH). UV (MeOH): 280 (3.73). IR (KBr): 3418, 2922, 1612, 1592, 1489, 1460, 1074, 1036, 531. NMR: *Table 2*. FAB-MS (neg.): 583 ($[M-H]^-$). HR-ESI-MS (neg.): 583.2041 ($[M-H]^-$, $C_{27}H_{35}O_{14}^-$; calc. 583.2026).

Acidic Hydrolysis. A soln. of **1**–**3** (each 3 mg) in a mixture of MeOH (1.0 ml) and 10% H_2SO_4 (1.0 ml) was refluxed for 2 h. The hydrolysate was allowed to cool, diluted with 2 ml of H_2O, and extracted with 4 ml of AcOEt. The aq. layer was neutralized with aq. $Ba(OH)_2$ and concentrated in vacuum to give a residue, in which glucose (from **1**–**3**) was identified by comparison with an authentic sample (BuOH/AcOEt/H_2O 4 : 1 : 5, upper layer, R_f 0.45; PhOH/H_2O 4 : 1, R_f 0.50) on PC: visualized by spraying with phthalic acid/aniline reagent (1.66 g phthalic acid and 0.93 g aniline dissolved in 100 ml H_2O/sat. BuOH), followed by heating.

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