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Three new phenolic glycosides from *Curculigo orchioides* G.

Ai-Xue Zuo [a,c], Yong Shen [a,c], Zhi-Yong Jiang [a], Xue-Mei Zhang [a], Jun Zhou [a], Jun Lü [b], Ji-Jun Chen [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China
[b] Kunming Jingbiao Biosciences R&D Co. Ltd., Kunming, Yunnan, 650000, China
[c] Graduate University of Chinese Academy of Sciences, Beijing 100039, China

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ABSTRACT

Three new phenolic glycosides, curculigosides F–H (**1–3**), were isolated from rhizomes of *Curculigo orchioides* Gaertn. Their structures were elucidated based on comprehensive spectroscopic analyses including IR, MS, 1D- and 2D NMR (HSQC, COSY, and HMBC). Curculigosides F–H (**1–3**) were evaluated for their anti-HBV activity *in vitro* using the HBV transfected Hep G2.2.15 cell line. Compound **1** exhibited weak activity with an IC_{50} value of 2.08 mM on hepatitis B virus (HBV) e antigen (HBeAg) secretion of the HepG2.2.15 cell line.

1. Introduction

Curculigo orchioides Gaertn., belonging to the family Amaryllidaceae, widely distributed in southern part of China, Japan, Malaya, India, and Australia. Its rhizomers, known as "Xian-Mao" in traditional Chinese medicine, have been used for treatment of impotence, enuresis, cold sperm, cold pain of back and knee, numbness of the limbs, and decline in physical strength [1,2]. Previous phytochemical investigations on this species revealed the presence of phenolic glycosides [3], chlorophenolic glycosides [4], and cycloartane saponins [5]. Rhizomes of *C. orchioides* were a multipurpose drug with numerous pharmacological activities including hepatoprotective effect [6], immunostimulatory effect [7,8], and estrogenic activity [9]. As a part of our continuous exploring anti-HBV active compounds from natural sources [10,11], phytochemical investigation on the rhizomes of *C. orchioides* was conducted. As a result, three new phenolic glycosides named curculigosides F–H (**1–3**) were isolated from 70% EtOH extract of *C. orchioides*. This paper deals with the isolation and elucidation of three new phenolic glycosides using IR, MS, ^{1}H- and ^{13}C NMR, COSY, HSQC and HMBC techniques along with their anti-HBV activity.

2. Experimental

2.1. General experimental procedures

Optical rotations were performed on a Jaso-DIP 370 polarimeter (Spectroscopic Co., Ltd., Japan). IR spectra were recorded on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Richmond, Canada) with KBr pellets, ν in cm^{-1}. UV spectra were measured on SHIMADZU UV-2401PC spectrometer (Shimadzu Corporation, Tokyo, Japan); NMR spectra were conducted on Bruker AV-400 or DRX-500 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 (VG, 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 Makall Chemical Co., Ltd., Qingdao, P.R. China), Al_2O_3 (Shanghai Wusi Chemical Reagents Company, P.R. China), D_{101} macroporous resins

* Corresponding author. Tel.: +86 871 5223267; fax: +86 871 5223265.
E-mail address: zuoaixue@mail.kib.ac.cn (A.-X. Zuo).

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(Tianjin Pesticide Chemical Company, P.R. China), Sephadex LH-20 (Pharmacia, Fine Chemical Co. Ltd. Sweden), and Lichroprep RP-18 (40–63 μm; Merck, Darmstadt, Germany); Fractions were monitored by TLC and visualization by spraying with 10% H_2SO_4 in EtOH followed by heating.

2.2. 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.

2.3. Extraction and isolation

The air-dried and powdered rhizomes of *C.orchioides* (200 kg) were extracted with 70% EtOH three times (each 1000 L 2 h) 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, 40% EtOH-H_2O, 70% EtOH-H_2O, and 90% EtOH-H_2O to afford five fractions: (Frs. I–V). Fr. III (780 g, eluted with 40% EtOH-H_2O) was subjected to silica gel CC (8 kg, 14×110 cm) subsequently eluted with CH_3Cl-MeOH (9.5:0.5), CH_3Cl-MeOH-H_2O (9:1:0.1), and CH_3Cl-MeOH-H_2O (8.5:1.5:0.15) to afford fractions A–C. Fraction C (160 g) was subjected to silica gel CC (1 kg, 8×56 cm) eluted with CH_3Cl-MeOH-H_2O (9:1:0.1→8:2:0.2) to afford fractions A 1–2; The fraction A-1 (60 g) was successfully purified over silica gel CC EtOAc-CH_3COCH_3-H_2O (9:1:0.1), Sephadex LH-20 ($CHCl_3$-MeOH, 1:1), and RP-18 CC (MeOH-H_2O (1.5:8.5) to afford compounds **1** (429 mg), **2** (230 mg). The fraction A-2 (70 g) was applied to a silica gel CC (700 g, 8.4×27 cm) eluted with $CHCl_3$-MeOH-H_2O (8.5:1.5:0.15) then further subjected to silica gel CC (150 g, 4.8×45 cm) with EtOAc-MeOH-H_2O (9:1:0.2) as solvent to yield samples, which were purified by RP-18 CC (120 g, 2.5×33 cm) eluted with MeOH-H_2O (1:9) to gave compound **3** (712 mg).

Curculigoside F (**1**), colorless needles (MeOH). $[\alpha]_D^{27.1}$+4.10 (c=0.22, MeOH). UV λ_{max} (log ε) (MeOH) 278 (3.74) nm. IR (KBr) cm^{-1}: 3396, 2938, 1722, 1598, 1476, 1298, 1256, 1113, 1072, 784, 759, 632. NMR: see Table 1. Negative-ion fast atom bombardment-mass spectrometry FAB-MS *m/z*: 465 $[M-H]^-$; Negative-ion high resolution electrospray ionization mass spectrometry HR-ESI-MS *m/z*: 465.1402 ($[M-H]^-$, $C_{22}H_{25}O_{11}^-$; calcd. for 465.1396).

Curculigoside G (**2**), yellow powder. $[\alpha]_D^{26.8}$−50.12 (c=0.25, MeOH). UV λ_{max} (log ε) (MeOH) 278 (3.60). IR (KBr) cm^{-1}: 3396, 2938, 1731, 1597, 1476, 1297, 1258, 1113, 1074, 786, 762, 635. NMR: see Table 1. Negative-ion FAB-MS *m/z*: 449 $[M-H]^-$, 287 $[M-H-Glc]^-$; Negative-ion HR-ESI-MS *m/z*: 449.1449 ($[M-H]^-$, $C_{22}H_{25}O_{10}^-$; calcd. for 449.1447).

Curculigoside H (**3**), yellow powder. $[\alpha]_D^{23.7}$−42.33 (c=0.56, MeOH). UV λ_{max} (log ε) (MeOH) 282 (3.76). IR (KBr) cm^{-1}: 3424, 2934, 1714, 1598, 1499, 1477, 1299, 1257, 1211, 1112, 1072, 789, 760, 730, 633,580. NMR: see Table 1. Negative-ion FAB-MS *m/z*: 627 $[M-H]^-$, 465 $[M-H-Glc]^-$, 302 $[M-H-Glc-Glc]^-$; Negative-ion HR-ESI-MS *m/z*: 627.1909 ($[M-H]^-$, $C_{28}H_{35}O_{16}^-$; calcd. for 627.1925).

2.4. Acid hydrolysis

Each of compounds **1**–**3** (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=0.60; BuOH-EtOAc-H_2O 4:1:5, upper layer, R_f=0.45; PhOH-H_2O, 4:1, R_f=0.55 on PC, respectively).

2.5. Anti-HBV assay

Curculigosides F–H (**1**–**3**) were evaluated for their anti-HBV activity *in vitro* using the Hep G2.2.15 cell line stably transfected with the HBV genome as reported previously [12]. Curculigoside F exhibited weak activity with an IC_{50} value of 2.08 mM (SI=0.65) on hepatitis B virus e antigen (HBeAg) secretion of the HepG2.2.15 cell line; The other two compounds showed no activity (Table 2).

3. Results and discussion

The phytochemical investigation of 70% EtOH extract obtained from the rhizomers of *C orchioides*. afforded three new phenolic glycosides curculigosides F–H (**1**–**3**) (Fig. 1).

Compound **1**, obtained as a colorless needle (MeOH) with an optical rotation $[\alpha]_D^{27.1}$ =+4.10 (c=0.22, MeOH), had the same molecular formula $C_{22}H_{26}O_{11}$ as those of curculigoside [13,14] as inferred from negative HR-ESI-MS at *m/z* 465.1402 $[M-H]^-$ (calcd. 465.1396). IR spectrum showed the presence of hydroxyl group (3396 cm^{-1}), conjugated carbonyl group (1722 cm^{-1}), and aromatic ring functional groups (1598, 1476 cm^{-1}). Acidic hydrolysis of compound **1** liberated glucose which was determined by comparing with authentic sample on Paper Chromatography (PC). The ^1H NMR spectrum of compound **1** demonstrated the signals for two methoxyls δ_H 3.78 (6H, s, OMe-2′, OMe-6′), one methlene δ_H 5.58 (1H, d, J=13.4 Hz, H-7a), 5.54 (1H, d, J=13.4 Hz, H-7b), one anomeric proton δ_H 4.61 (1H, d, J=8.0 Hz, H-1″) due to the β-linkaged glucose moiety, together with two aromatic rings: ring A at δ_H 6.96 (1H, dd, J=7.8, 1.4 Hz, H-3), 7.02 (1H, t, J=7.8 Hz, H-4), 6.85 (1H, dd, J=7.8, 1.4 Hz, H-5) and ring B at δ_H 6.65 (2H, d, J=8.5 Hz, H-3′, H-5′), 7.32 (1H, t, J=8.5 Hz, H-4′). ^{13}C NMR spectrum of compound **1** showed 22 signals ascrible to 2 aromatic rings, 1 ester carbonyl (δ_C 168.6), 1 methylene (δ_C 63.5), 2 methoxyls (δ_C 56.5), as well as one set of β-D-glucopyranosyl moiety [δ_C 107.3 (CH), 75.4 (CH), 78.4 (CH), 70.9 (CH), 77.9 (CH), 62.3 (CH_2)] matching to those of methyl-β-D-glucopyranoside [15]. Comparison of the NMR data of compound **1** with those of curculigoside indicated that they shared identical basic skeleton except the substitute patterns of aromatic ring A. Signals for ring A at δ_H 6.96 (1H, dd, J=7.8, 1.4 Hz), 7.02 (1H, t, J=7.8 Hz), and 6.85 (1H, dd, J=7.8, 1.4 Hz) in the ^1H NMR spectrum of compound **1** showed that substituting mode for ring A was 1,2,6-trisubstitute instead of

Table 1
^{1}H- and ^{13}C-NMR data of compounds **1–3** at 400/100 MHz in CD_3OD (δ in ppm, J in Hz).

No.	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		132.1 (s)		139.1 (s)		128.5(s)
2		150.8 (s)		159.2 (s)		153.8 (s)
3	6.96 (1H, dd, 7.8, 1.4)	120.5 (d)	7.06 (overlapped)	122.8 (d)	7.11 (1H, d, 8.8)	118.7 (d)
4	7.02 (1H, t, 7.8)	126.6 (d)	7.27 (1H, t, 8.0)	130.5(d)	6.72 (1H, dd, 8.8, 3.0)	116.4 (d)
5	6.85 (1H, dd, \7.8, 1.4)	117.5 (d)	7.06 (overlapped)	117.1 (d)		149.5 (s)
6		144.3 (s)	7.17 (1H, m)	117.4 (d)	6.91 (1H, d, 3.0)	116.4 (d)
7	5.58 (1H, d, 13.4)	63.5 (t)	5.28 (2H, s)	67.5 (t)	5.44 (1H, d, 13.4)	63.2 (t)
	5.54 (1H, d, 13.4)				5.39 (1H, d, 13.4)	
1′		114.2 (s)		115.1 (s)		114.3 (s)
2′		158.7 (s)		158.8 (s)		158.7 (s)
3′	6.65 (1H, d, 8.5)	105.7 (d)	6.66 (1H, d, 8.4)	105.1 (d)	6.66 (1H, d, 8.5)	105.1 (d)
4′	7.32(1H, t, 8.5)	132.6 (d)	7.34 (1H, t, 8.4)	132.6 (d)	7.34 (1H, t, 8.5)	132.6 (d)
5′	6.65 (1H, d, 8.5)	105.7 (d)	6.66 (1H, d, 8.4)	105.1 (d)	6.66 (1H, d, 8.5)	105.1 (d)
6′		158.7 (s)		158.8 (s)		158.7 (s)
7′		168.6 (s)		168.4 (s)		168.6 (s)
2′-OMe	3.78 (3H, s)	56.5 (q)	3.78 (3H, s)	56.5 (q)	3.80 (3H, s)	56.5 (q)
6′-OMe	3.78 (3H, s)	56.5 (q)	3.78 (3H, s)	56.5 (q)	3.80 (3H, s)	56.5 (q)
Glc-1						
1″	4.61 (1H, d, 8.0)	107.3 (d)	4.92 (1H, d, 7.6)	102.3 (d)	4,77 (1H, d, 7.1)	103.9 (d)
2″	3.50-3.53 (1H, m)	75.4 (d)	3.41-3.47 (overlapped)	74.9 (d)	3.22-3.49 (overlapped)	75.1 (d)
3″	3.24-3.30 (1H, m)	78.4 (d)	3.41-3.47 (overlapped)	78.1 (d)	3.22-3.49 (overlapped)	77.9 (d)
4″	3.41-3.44 (overlapped)	70.9 (d)	3.29-3.30 (overlapped)	71.3 (d)	3.22-3.34 (overlapped)	71.6 (d)
5″	3.41-3.44 (overlapped)	77.9 (d)	3.41-3.47 (overlapped)	78.0 (d)	3.59-3.65 (overlapped)	77.2 (d)
6″	3.84-3.86 (1H, m)	62.3 (t)	3.80-3.84 (1H, m)	62.4 (t)	3.81-3.83 (overlapped)	69.8 (t)
	3.72-3.75 (1H, m)		3.71-3.73 (1H, m)		4.12-4.17 (overlapped)	
Glc-2						
1‴					4.37 (1H, d,7.7)	104.7 (d)
2‴					3.22-3.49 (overlapped)	74.9 (d)
3‴					3.22-3.49 (overlapped)	77.2 (d)
4‴					3.22-3.34 (overlapped)	71.3 (d)
5‴					3.59-3.65 (overlapped)	77.8 (d)
6‴					3.81-3.83 (overlapped)	62.7 (t)

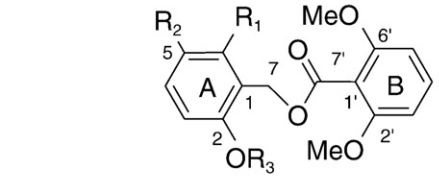
1,2,5-trisubstituted in curculigoside; Namely, the OH for ring A was assigned at the C-6 in compound **1**. This was supported by the HMBC correlations from H-7 (δ_H 5.58, 5.54, each 1H, d, J = 13.4 Hz) to C-1 (δ_C132.1, C), C-2 (δ_C150.8, C), and C-6 (δ_C 144.3, C) (Fig. 2). Therefore, compound **1** was elucidated as 6-hydroxyl-2-O-β-D-glucopyranosyl benzyl-2.6-dimethoxy benzoate as described in Fig. 1 and named as curculigoside F (**1**).

Compound **2** was isolated as yellow powder. Based on negative HR-ESI-MS at m/z 449.1449 $[M-H]^-$ (calc. 449.1447), its molecular formula was deduced to be $C_{22}H_{26}O_{10}$, which was less 16 mass unit than compound **1**. IR spectrum exhibited absorption bands due to hydroxyl (3396 cm^{-1}), conjugated carbonyl (1731 cm^{-1}) and aromatic ring (1597, 1476 cm^{-1}) groups. Acidic hydrolysis of compound **2** furnished glucose identified by comparison with the authentic sample on PC. Moreover, negative FAB-MS exhibited fragment-ion peak at 287 $[M-H-162]^-$ suggesting glucose in the molecule of compound **2**, besides quasimolecule ion peak at m/z 449 $[M-H]^-$; Comparison of NMR data of compound **2** with those of compound **1** indicated

Table 2
Anti-HBV activity of compounds **1–3**.

Compounds	CC_{50}	HBsAg [a]		HBeAg [b]	
	(mM)	IC_{50} (mM)	SI [c]	IC_{50} (mM)	SI
1	1.35	2.08	0.65	>2.79	>1.48
2	>2.98	>2.98	-	>2.13	>1.40
3	>2.55	>2.55	-	>2.55	-
3TC [d]	41.27	31.70	1.30	5.68	2.29

[a] HBsAg, HBV surface antigen.
[b] HBeAg, HBV e antigen.
[c] $SI = CC_{50}/IC_{50}$.
[d] 3TC: Lamivudine, an antiviral agent used as positive control.



	R_1	R_2	R_3
1 curculigoside F	OH	H	Glc
2 curculigoside G	H	H	Glc
3 curculigoside H	H	OH	Glc1→6Glc

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

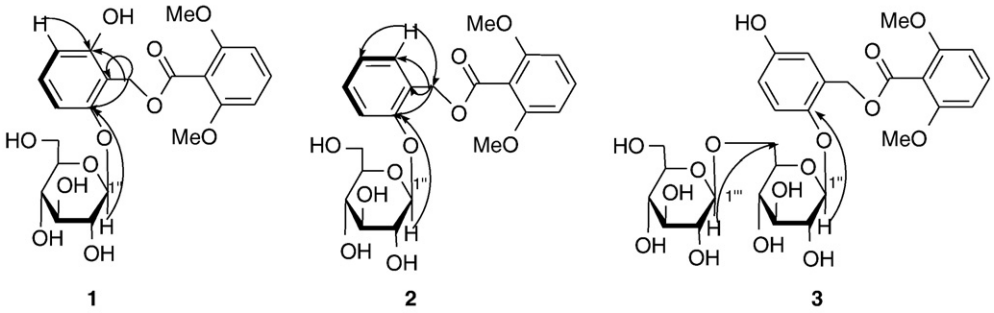


Fig. 2. The key correlations in HMBC(→) and COSY (—) of compounds (**1**–**3**).

that compound **2** was analogue of compound **1**. Compound **2** differed from **1** mainly in the substituting mode of ring A. Singnals of ring A at 7.06 (overlapped, H-3), 7.27 (1H, t, $J=8.0$ H-4), 7.06 (overlapped H-5) and 7.17 (1H, m H-6) in the ^{1}HMR spectrum of compound **2** suggested that substituting mode of ring A was 1,2-disubstituted and C-6 in ring A was connected by H atom instead of OH in **1**. This assignment was in accord with the observed changes of the less 16 mass unit in compound **2** than **1** and confirmed by the correlation from H-7 (δ_H 5.28, 2H, s) to C-6 (δ 117.4, CH) in HMBC plot (Fig. 2). Therefore, compound **2** was characterized as 2-O-β-D-glucopyranosyl benzyl-2,6-dimethoxybenzoate as depicted in Fig. 1 and named to be curculigoside G (**2**).

Compound **3** obtained as yellow powder with an optical rotation $[\alpha]_D^{23.7}=-42.33$ ($c=0.56$, MeOH). Its molecular formula $C_{28}H_{36}O_{16}$ was deduced from negative HR-ESI-MS spectrum at m/z 627.1909 $[M-H]^-$ (calcd. 627.1925); IR spectrum showed the presence of hydroxyl (3424 cm^{-1}), conjugated carbonyl (1714 cm^{-1}) and aromatic ring (1598, 1477 cm^{-1}) groups. Negative FAB-MS displayed quasimolecule ion peak at m/z 627 $[M-H]^-$, besides fragment-ion peak at m/z 465 $[M-H-162]^-$ and 302 $[M-H-162-162]^-$ suggesting the presence of two hexose in the molecule; Hydrolysis of compound **3** gave glucose which was identified by comparison with the authentic sample on PC. Analysis of ^{1}H- and ^{13}C NMR data of compound **3** revealed that structure of compound **3** was very similar to that of curculigoside except that compound **3** had one more glucopyranosyl moiety than that of curculigoside. Chemical shift for C-6″ from δ_C 62.6 shifted downfield to δ_C 69.8 in compound **3** suggested that the additional glucopyranosyl unit was linked to the C-6″ in compound **3**. This was further confirmed by the HMBC correlation from H-1‴(δ_H 4.37, 1H, d, $J=7.7$ Hz) to C-6″ (δ_C 69.8) (Fig. 2). Hence, compound **3** was concluded as 5-hydroxyl-2-[β-D-glucopyranosyl -(1→6)-β-D-glucopyranosyl]-benzyl-2,6-dimethoxy benzoate (Fig. 1) and named as curculigoside H (**3**).

Acknowledgements

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