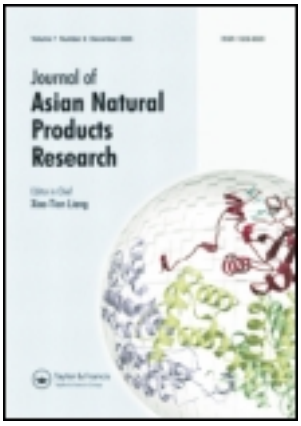

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Two new triterpenoid glycosides from *Curculigo orchioides*

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Two new cycloartane triterpenoid glycosides, named curculigosaponin N and curculigosaponin O, were isolated from rhizomes of *Curculigo orchioides* Gaertn. Their structures were elucidated on the basis of comprehensive spectroscopic analysis including IR, MS, 1D, and 2D NMR (HSQC and HMBC).

Keywords: *Curculigo orchioides*; cycloartane triterpenoid glycoside; curculigosaponins N—O

1. Introduction

Curculigo orchioides Gaertn., belonged to the Hypoxidaceae family and named 'Xian-Mao' in Pharmacopoeia of China, mainly distributed in Japan, China, Malaysia, India, and Australia [1]. Its rhizomes have been employed as an analeptic agent for the treatment of decline in strength, against jaundice and asthma [1]. Previous investigation on this plant revealed that it contained cycloartane triterpenoid saponins [2–5], phenolic glycosides [6–8], and chlorophenyl glycosides [9]. Some compounds from *C. orchioides* exhibited biological activities including stimulation of the immune response [10,11], antioxidative activities [8], antidepression activities [12], and cytotoxic activities [4,5]. Recently, several novel phenolic glycosides [13–16] in this genus have been elucidated by our group, meanwhile, some isolated phenolic derivatives displayed significant antidepressant effect [12] and anti-hepatitis B virus (HBV) activities [13]. To further find structurally new chemical constituents from this medicinal plant, we explored its phytochemical composition. Our reinvestigation resulted in the isolation of two new cycloartane triterpenoid glycosides, named curculigosaponin N and curculigosaponin O. This paper deals with the isolation and structural elucidation of the two new compounds (**1** and **2**; Figure 1).

2. Results and discussion

Compound **1** was isolated as an amorphous powder with $[\alpha]_D^{15.3}$ − 3.41 (*c* 0.18, MeOH); its molecular formula was determined to be $C_{42}H_{72}O_{14}$ based on HR-ESI-MS at *m/z* 799.4841 $[M - H]^-$. Negative mode FAB-MS exhibited quasi-molecular ion and fragment ions at *m/z* 799 $[M - H]^-$, 653 $[M - H - 146]^-$, and 491 $[M - H - 146 - 162]^-$, suggesting the presence of hexose moiety in the molecule of compound **1**. Acid hydrolysis of compound **1** with 2 M H_2SO_4 liberated rhamnose and glucose detected by comparison

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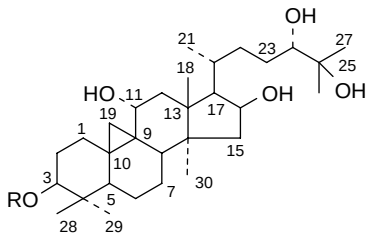

Curculigosaponin N (1) R = Rha(1-2)Glc

Curculigosaponin O (2) R = Glc(1-3)Glc(1-2)Glc

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

with authentic samples on paper chromatography (PC). IR showed the absorption bands for hydroxy (3438 cm^{-1}), saturated methenes (2926 cm^{-1}), and glycosidic bonds (1036 cm^{-1}). ^1H NMR spectrum of compound **1** revealed the presence of characteristic cyclopropane methylene proton resonances at δ_H 0.26 (1H, d, $J = 3.2$ Hz, H-19a) and 0.43 (1H, d, $J = 3.2$ Hz, H-19b), six singlet methyl resonances at δ_H 1.22, 1.26, 1.28, 1.30, 1.10, and 1.43 (each 3H, s, H-18, H-28, H-29, H-30, H-25, H-26) assignable to cycloartane-type aglycone, together with two anomeric proton signals at δ_H 4.93 (1H, d, $J = 6.9$ Hz, H-1′) and 6.58 (1H, br. s, H-1″), implying the presence of one β-linked glucosyl moiety and one α-linked rhamnosyl moiety. Rhamnosyl unit further supported by the methyl signal at δ 1.46 (3H, d, $J = 6.1$ Hz, H-6″). The ^{13}C NMR spectrum of compound **1** exhibited 42 carbon signals, of which aglycone included seven methyl carbon signals at δ_C 15.7, 17.4, 18.8, 22.0, 25.7, 25.8, and 26.5 and five oxygen-bearing carbons at δ_C 72.1, 72.5, 72.6, 77.1, and 88.6. The ^{13}C NMR data also confirmed the presence of two sugar moieties with anomeric carbons at δ_C 105.4 (d) and 101.8 (d), respectively. Above NMR data were similar to those of curculigosaponin L [2] except that C-25 in compound **1** was shifted downfield to δ_C 72.6, indicating that C-25 was substituted by a hydroxyl group. This inference was further supported by the correlations observed from H-26 and H-27 to C-25 in HMBC experiment. In addition, the HMBC correlations observed from H-1′ at δ_H 4.93 (1H, d, $J = 6.9$ Hz) to C-3 and from H-1″ at δ_H 6.58 (1H, br.s) to C-2′ demonstrated that the inner glucopyranosyl unit was attached to C-3 of aglycone, and the rhamnopyranosyl moiety was linked to C-2′ of the inner glucose (Figure 2). Based on the above evidence, compound **1** was elucidated as 25-hydroxyl-curculigosaponin L, and named curculigosaponin N (**1**; Figure 1).

Compound **2**, obtained as an amorphous powder with $[\alpha]_D^{17.8} + 4.62$ (*c* 0.15, MeOH), had the molecular formula $C_{48}H_{82}O_{20}$ as established from the positive-mode HR-ESI-MS at *m/z* 1001.5290 $[M + Na]^+$. Negative-mode FAB-MS spectrum showed quasi-molecular ion and fragment ions at *m/z* 977 $[M - H]^-$, 815 $[M - 162]^-$, 653 $[M - 162 - 162]^-$, and 491 $[M - 162 - 162 - 162]^-$, suggesting the presence of three hexoses in the molecule of compound **2**. Hydrolysis of **2** with 2 M H_2SO_4 in MeOH revealed glucose as the only sugar moiety identified by comparison with authentic sample on PC. IR spectrum indicated the presence of hydroxyl (3423 cm^{-1}) and glycosidic (1077 cm^{-1}) groups. The ^1H NMR spectrum displayed characteristic of the C-19 methylene protons of cyclopropane ring for a cycloartane triterpene at δ_H 0.47 (1H, d, $J = 3.6$ Hz, H-19a) and 0.56 (1H, d, $J = 3.6$ Hz, H-19b), seven methyl signals at δ_H 0.91, 1.00, 1.13, 1.17, 1.19 (each 3H, s, H-29, H-18, H-28, H-26, H-27), and 1.08–1.09 (6H, overlap, H-21, H-30) attributable to cycloartane-type aglycone, as well as three anomeric proton signals at δ_H 4.47 (1H, d, $J = 7.0$ Hz, H-1′), 4.58 (1H, d, $J = 7.8$ Hz, H-1″), and 4.76 (1H, d, $J = 7.7$ Hz, H-1′) assigned to three β-linked sugar moieties. ^{13}C NMR spectrum displayed 48 signals, of which the anomeric carbons of three sugar moieties at δ_C 105.2 (d), 105.2 (d), and 104.2 (d) were observed.

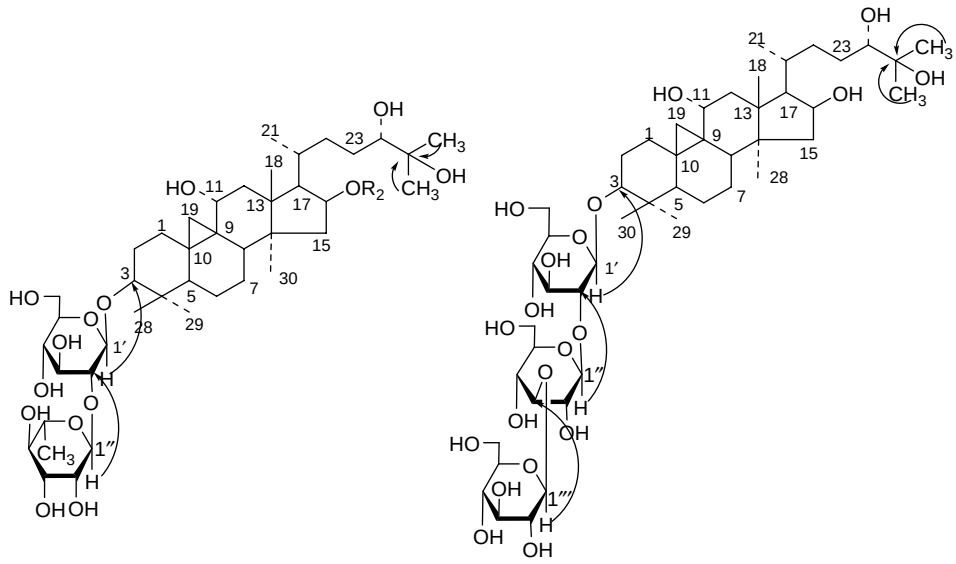

Figure 2. The key HMBC correlations of compounds **1** and **2**.

Comparing the NMR data of compound **2** with those of curculigosaponin K [2] indicated that they were analogous in structure except that C-25 was shifted downfield from δ_C 31.1 (d) to δ_C 73.8 (s), suggesting a hydroxyl linked to C-25; to further substantiate the location of the hydroxy group, HMBC experiment was conducted, in which the correlations from H-26 at δ_H 1.17 (3H, s) and H-27 at δ_H 1.19 (3H, s) to C-25 at δ_C 73.8 (s) were observed. Therefore, compound **2** was determined as 25-hydroxyl-curculigosaponin K and named curculigosaponin O (Figure 2).

3. Experimental

3.1 General experimental procedures

Optical rotations were performed on a Horiba SEPA-300 polarimeter (Tokyo, Japan). IR spectra were recorded on a Bio-Rad FTS-135 spectrometer (Richmond, VA, USA) with KBr pellets, ν in cm^{-1}. UV spectra were measured on a UV-210A spectrometer (Shimadzu, Japan); NMR spectra were conducted on Bruker AV-400 or DRX-500 spectrometer (Karlsruhe, Germany) with tetramethylsilane as internal standard; chemical shifts (δ) were expressed in ppm and coupling constants (J) in Hz. FAB-MS was recorded on a VG Autospec 3000 mass spectrometer (Manchester, England); ESI and HR-ESI-MS were taken on an API Qstar-Pulsar-1 mass spectrometer (Applied Biosystems/MDS Sciex, Vaughan, ON, Canada). Column chromatography (CC) separations were performed on silica gel (200–300 mesh, Qingdao Meigao Chemical Co., Ltd., Qingdao, China), Al_2O_3 (Shanghai Wusi Chemical Reagents Company, Shanghai, China), D_{101} macroporous resins (Tianjin Pesticide Chemical Company, Tianjin, China), Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden), and LiChroprep RP-18 (40–63 μm; Merck, Darmstadt, Germany). Fractions were monitored by thin-layer chromatography and visualized by spraying with 10% H_2SO_4 in EtOH followed by heating.

3.2 Plant material

The rhizomes of *C. orchioides* Gaertn. were collected in Wenshan county, Yunnan Province, 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) has been deposited in the Group of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried, powdered rhizomes of *C. orchioides* (200 kg) were extracted with 70% EtOH (each 1000 liters, 2 h) three times under reflux to yield an extract which was combined and concentrated to a small volume (600 liters) 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 (1–5). The fraction 4 (70% EtOH–H_2O eluted, 200 g) was subjected to Al_2O_3 CC subsequently eluted with EtOAc–EtOH–H_2O (8:2:0.2) and EtOAc–EtOH–H_2O (7:3:0.5) to afford fractions A and B.

Fraction A (60.0 g) was successively subjected to RP-18 CC eluted with MeOH–H_2O (4:6–7:3) to afford fractions A1–3. Fraction A2 (22.0 g) was performed on silica gel CC eluted with $CHCl_3$–MeOH–H_2O (8:2:0.3) to give a residue (2.0 g) that was submitted to Sephadex LH-20 CC developed with $CHCl_3$–MeOH (1:1) and further purified by silica gel CC with EtOAc–EtOH–H_2O (8:2:0.3) as solvent to yield compound **2** (290 mg). Fraction A3 (9.0 g) was applied to a silica gel CC eluted with $CHCl_3$–MeOH–H_2O (8.5:1.5:0.2) to give four portions. The fourth portion (1.3 g) was purified on RP-18 CC eluted with MeOH–H_2O (5:5) to afford compound **1** (221 mg).

3.3.1 Curculigosaponin N (*1*)

Amorphous powder; $C_{42}H_{72}O_{14}$; $[\alpha]_D^{15.3}$ − 3.41 (*c* 1.78, MeOH); UV (MeOH) λ_{max} (log ϵ): 250 (3.16) nm; IR (KBr) ν_{max}: 3438, 2926, 1629, 1429, 1036, 876, 537, 481 cm^{-1}; ^1H NMR (400 MHz, C_5D_5N) δ: 0.26 (1H, d, $J = 3.2$ Hz, H-19a), 0.43 (1H, d, $J = 3.2$ Hz, H-19b), 1.22, 1.26, 1.28, 1.30, 1.10, 1.43 (each 3H, s, H-18, H-28, H-29, H-30, H-25, H-26), 1.28–1.30 (3H, overlap, H-21), 4.93 (1H, d, $J = 6.9$ Hz, H-1′), 6.58 (1H, br. s, H-1″), 1.46 (3H, d, $J = 6.1$ Hz, H-6″), 3.41 (1H, dd, $J = 11.2$, 3.6 Hz, H-3), 3.95–3.97(1H, m, H-11), 3.90–3.91 (1H, m, H-16), 4.26–4.29 (1H, m, H-24); for ^{13}C NMR (100 MHz, C_5D_5N) spectral data, see Table 1. (−) FAB-MS: *m/z* 799 $[M - H]^-$, 653 $[M - H - 146]^-$, 491 $[M - H - 146 - 162]^-$; (−) HR-ESI-MS: *m/z* 799.4841 $[M - H]^-$ (cald for $C_{42}H_{71}O_{14}$, 799.4843).

3.3.2 Curculigosaponin O (*2*)

Amorphous powder; $C_{48}H_{82}O_{20}$; $[\alpha]_D^{17.8}$ + 4.62 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ): 202 (3.73) nm; IR (KBr) ν_{max}: 3423, 2936, 1641, 1457, 1384, 1077, 635 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ: 0.47 (1H, d, $J = 3.6$ Hz, H-19a), 0.56 (1H, d, $J = 3.6$ Hz, H-19b), 0.91, 1.00, 1.13, 1.17, 1.19 (each 3H, s, H-29, H-18, H-28, H-26, H-27), 1.08–1.09 (6H, overlap, H-21, H-30), 4.47 (1H, d, $J = 7.0$ Hz, H-1′), 4.58 (1H, d, $J = 7.8$ Hz, H-1″), 4.76 (1H, d, $J = 7.7$ Hz, H-1′), 3.27–3.35 (1H, m, H-3), 4.47–4.49 (1H, m, H-11), 3.82–3.89 (1H, m, H-16), 3.27–3.32 (1H, m, H-24); for ^{13}C NMR (100 MHz, CD_3OD) spectral data, see Table 1; (−) FAB-MS: *m/z* 977 $[M - H]^-$, 815 $[M - 162]^-$, 653 $[M - 162 - 162]^-$, 491 $[M - 162 - 162 - 162]^-$; (+) HR-ESI-MS: *m/z* 1001.5290 $[M + Na]^+$ (cald for $C_{48}H_{82}O_{20}Na$, 1001.5297).

3.4 Acid hydrolysis

Compounds **1** and **2** (2.0 mg) were dissolved in MeOH (1.0 ml) and 2 M H_2SO_4 (1.0 ml) solution and hydrolyzed under reflux for 2 h, respectively. The

Table 1. ^{13}C NMR (100 MHz) spectral data of compounds **1** and **2** (**1** in C_5D_5N, **2** in CD_3OD, δ in ppm, and J in Hz).

No.	1	2	No.	1	2	Glc		1			2		
1	33.3 (t)	33.0 (t)	16	72.1 (d)	73.8 (d)	1′	105.4 (d)	1′	105.2 (d)	1″	105.2 (d)	1‴	104.2 (d)
2	30.4 (t)	30.8 (t)	17	49.3 (d)	50.5 (d)	2′	78.2 (d)	2′	81.4 (d)	2″	75.5 (d)	2‴	75.6 (d)
3	88.6 (d)	90.9 (d)	18	22.0 (q)	21.9 (q)	3′	77.7 (d)	3′	78.0 (d)	3″	87.7 (d)	3‴	78.3 (d)
4	40.3 (s)	42.2 (s)	19	30.1 (t)	31.0 (t)	4′	72.1 (d)	4′	70.3 (d)	4″	71.5 (d)	4‴	71.5 (d)
5	47.1 (d)	47.6 (d)	20	28.4 (d)	29.3 (d)	5′	80.0 (d)	5′	78.3 (d)	5″	78.1 (d)	5‴	77.7 (d)
6	21.5 (t)	22.3 (t)	21	17.4 (q)	17.4 (q)	6′	62.9 (t)	6′	63.0 (t)	6″	62.8 (t)	6‴	62.6 (t)
7	26.8 (t)	27.5 (t)	22	30.9 (t)	33.2 (t)	Rha							
8	49.5 (d)	49.9 (d)	23	31.2 (t)	31.1 (t)	1″	101.8 (d)						
9	19.9 (s)	20.9 (s)	24	77.1 (d)	77.7 (d)	2″	72.2 (d)						
10	26.1 (s)	27.1 (s)	25	72.6 (s)	73.8 (s)	3″	72.5 (d)						
11	72.5 (d)	73.2 (d)	26	25.7 (q)	25.4 (q)	4″	74.2 (d)						
12	40.9 (t)	40.2 (t)	27	25.8 (q)	25.4 (q)	5″	69.7 (d)						
13	47.1 (s)	47.6 (s)	28	18.8 (q)	18.6 (q)	6″	18.6 (q)						
14	49.8 (s)	50.0 (s)	29	26.5 (q)	26.0 (q)								
15	50.1 (t)	50.3 (t)	30	15.7 (q)	15.5 (q)								

hydrolysate was allowed to cool, diluted with 2.0 ml H_2O, and extracted with 2.0 ml EtOAc. The aqueous layer was neutralized with aqueous $Ba(OH)_2$ and concentrated *in vacuo* to give a residue, in which glucose (from **1** and **2**) and rhamnose (from **1**) were identified by comparing with authentic samples on PC [BuOH–EtOAc–H_2O 4:1:5, upper layer, $R_f = 0.65$ (rhamnose); BuOH–EtOAc–H_2O 4:1:5, upper layer, $R_f = 0.45$ (glucose) on PC, respectively].

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