

Two New Phenolic Glycosides from Rhizomes of *Curculigo crassifolia*

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Curculigo crassifolia (Bak.) Hook. f. (Hypoxidaceae) is a tall perennial herb used as a folk medicine to treat child pneumonia by local people in Yunnan Province. Two new phenolic glycosides named crassifoside C (**1**) and crassifoside D (**2**) were isolated from the ethanolic extract of rhizomes of *Curculigo crassifolia*, and their structures were identified by 2D-NMR spectroscopic techniques and chemical transformations. In addition, seven known compounds (**3–9**) were isolated and identified by spectroscopic analysis and comparison of their spectral data with those reported previously.

Key words: *Curculigo crassifolia*, Hypoxidaceae, Phenolic Glycosides

Introduction

The genus *Curculigo* (Hypoxidaceae), comprising of about 20 species, are perennial herbs growing in the tropic and subtropic zones of Asia, Africa, South America and Australia, seven of them are found in the south of China [1]. The rhizomes of genus *Curculigo* are usually considered to be tonic medicines in China, such as *C. orchioides*, called as “Xianmao” in Chinese, a traditional anti-ageing medicine has been used since Tang dynasty [2]. *Curculigo crassifolia* (Bak.) Hook. f. (Hypoxidaceae) is a tall perennial herb with a coat of white hair on the back of leave, distributed in the western and southern of China, north of India and Nepal, its rhizomes were used as a folk medicines to treat child pneumonia by local people, such as Yi, Miao, Hui and Hani in Yunnan Province [1]. Previously, we reported several types of compounds from the rhizomes of plants of genus *Curculigo* [3–5]. Further investigation on *C. crassifolia* led to the isolation of two new phenolic glycosides (**1**, **2**) and seven known phenolic compounds (**3–9**). This paper described the isolation and structural elucidation of these compounds.

Results and Discussion

The 95% EtOH extract of air-dried and powdered rhizomes of *C. crassifolia* was suspended in H₂O and then passed through D101 resin column eluting with H₂O and EtOH to give EtOH eluted residue (500 g). Further repeated column chromatography of the EtOH residue on silica gel and Sephadex LH-20 led

to the isolation of two new compounds (**1**, **2**) together with seven known compounds identified as isonyasicoside (**3**) [6–8], pilosidine (**4**) [8], glucosyringic acid (**5**) [9], curculigoside I (**6**) [10], protocatechuic acid (**7**), 5-O-methylcurculigin (**8**) [11] and 5-O-methylisocurculigin (**9**) [11] by comparing data (MS, ¹H NMR, ¹³C NMR and [α]_D) with those previously reported or co-TLC with authentic samples (Fig. 1). Structural elucidation of the new compounds (**1**, **2**) was based on the spectroscopic and chemical evidence.

Compound **1** was obtained as white powder. The negative-ion HRFAB MS showed a quasi-molecular ion peak at *m/z* 491.1203 [M-H]⁻, in accordance with the molecular formula C₂₃H₂₄O₁₂-H (calcd. 491.1189). Its IR spectrum indicated the presence of hydroxyl groups (3439 cm⁻¹) and a carbonyl group (1652 cm⁻¹). The NMR data revealed the presence of a 3,4-di-substituted aromatic ring linked to the carbonyl. HMQC and COSY spectra revealed the presence of the fragment of =CHCH(O)CH(O)- (C-2 to C-4). In the HMBC spectrum of **1**, the correlations of H-2 to C-9 and C-11, H-3 to C-1 and C-10, and H-4 to C-5 and C-9 showed the presence of a benzo [9, 10] cyclohexatene linked to the carbonyl. In the HMQC spectrum, two singlet signals at δ = 7.11 (H-5) and 6.52 (H-8) had connectivities with carbon atoms at δ = 114.7 (C-5) and 114.4 (C-8). The ¹³C NMR spectrum indicated the presence of a hexose unit. The anomeric proton signal appeared as a doublet at δ = 4.47 (1H, d, *J* = 7.3 Hz) suggested a β-configured hexose unit. Glucose was detected by acidic hydrolysis

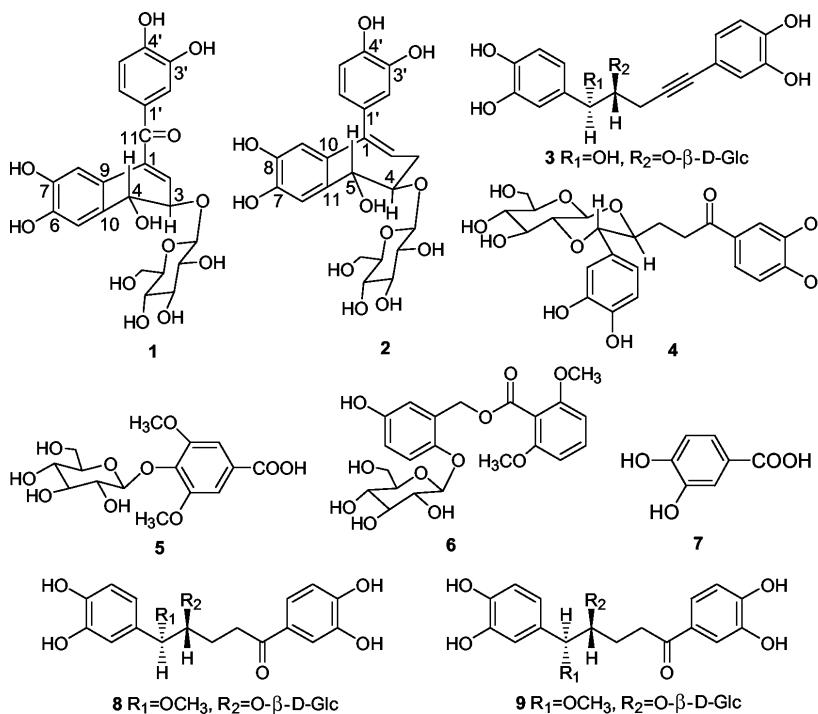


Fig. 1. Structures of compounds **1–9** isolated from *Curculigo crassifolia*.

of **1**. In the sugar moiety, the 1H - ^{13}C long-range correlations from the anomeric proton at $\delta = 4.47$ to C-3 ($\delta = 81.8$) ascertained that the sugar was attached to C-3 at the aglycone. The relative stereochemistry of **1** was determined by analysis of its coupling constants of H-3 at $\delta = 4.63$ (dd, $J = 10.1, 2.5$ Hz, 1H) and H-4 at 4.87 (d, $J = 10.1$ Hz, 1H), which indicated that 3- O - β -D-glucopyranosyl and 4-hydroxy group should be at 3 β , 4 α -orientation. On acetylation of **1** with acetic anhydride in pyridine, the positive-ion FAB MS analysis of the acetate of **1** showed m/z 493 + 9 × 42 ([M+1] $^+$ + 9Ac) (871), which indicated that **1** had five free hydroxyl groups located at C-4, C-6, C-7, C-3' and C-4', respectively, the other four acetyl groups were due to the glucose unit. Therefore, the chemical structure of compound **1** was deduced as 1-(3,4-dihydroxy-benzoyl)-3 β ,4 α ,6,7-tetrahydroxy-benzo[9,10]-1-cyclohexatene-3- O - β -D-pyranosylglucoside and named crassifoside C.

Compound **2** was obtained as brown powder. The negative-ion HRFAB MS showed a quasi-molecular ion peak at m/z 477.1401 [M-H] $^-$, in accordance with the molecular formula $C_{23}H_{26}O_{11}\text{-H}$ (calcd. 477.1396). Its IR spectrum indicated the presence of OH groups (3430 cm^{-1}). The NMR data revealed the presence of a 3,4-di-substituted aromatic ring.

The HMQC and COSY spectra revealed the presence of the fragment of =CHCH₂CH(O)CH(O)- (C-2 to C-5). In the HMBC spectrum of **2**, the correlations of H-2 to C-1 and C-10, H-3 to C-1, H-5 to C-6, C-10 and C-11, and H-9 to C-1 and C-11 showed the presence of a benzo [10,11] cycloheptene. In the HMQC spectrum, two singlet signals at $\delta = 8.21$ (H-6) and 7.29 (H-9) had connectivities with C-atoms at $\delta = 113.6$ (C-6) and 116.9 (C-9). Further 1H - ^{13}C long-range correlations of H-2' and H-6' to C-1 showed the 3,4-di-substituted aromatic ring was attached to C-1. The 1H and ^{13}C NMR spectra indicated the presence of a hexose unit. The anomeric proton signal appeared as a doublet at $\delta = 5.07$, attributed from the coupling constant of 7.7 Hz to a β -configurated anomeric proton. The glucose unit was detected by acidic hydrolysis of **2**. In the sugar moiety, the 1H - ^{13}C long-range correlations from the anomeric proton at $\delta = 5.07$ to C-4 ($\delta = 94.8$) ascertained that the sugar was attached to C-4 at the aglycone. The coupling constant of 8.8 Hz between H-4 and H-5 indicated that 4- O - β -D-glucopyranosyl and 5-hydroxy group should be at 4 β , 5 α -orientation. On acetylation of **2** with acetic anhydride in pyridine, the positive-ion FAB MS analysis of the acetate of **2** showed m/z 479 + 9 × 42 ([M+1] $^+$ + 9Ac) (857),

which indicated that **2** had five free hydroxyl groups located at C-5, C-7, C-8, C-3' and C-4', respectively and the other four acetyl groups were due to the glucose unit. Thus, **2** was identified as 1-(3,4-dihydroxy phenyl)-4 β ,5 α ,7,8-tetrahydroxy-benzo[10,11]-1-cycloheptene-4-*O*- β -D-pyranosylglucoside and named crassifoside D.

Experimental Section

General

The optical rotations were run on a JASCO-370 polarimeter. The UV spectra were carried on a UV-2401PC Spectrometer in MeOH. The IR spectra were recorded on a Bio-Rad FTS-35 spectrometer with KBr pellets. The MS data were performed on an Autospec-3000 spectrometer at a negative ion or positive ion model. 1D and 2D NMR spectra were measured on a Bruker AM-400 or a Bruker DRX-500 spectrometers with TMS as an internal standard. Column chromatography was performed on Sephadex LH-20 (25–100 μ m, Pharmacia Fine Chemical Co. Ltd.) and silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.). TLC was carried on silica gel G precoated plates (Qingdao Haiyang Chemical Co.) and spots were detected by 5% sulfuric acid reagents followed by heating.

Plant material

The plant material was collected in Eshan Prefecture, Yunnan Province, China, in October 2002 and identified as *Curculigo crassifolia* by Prof. Ping-hua YU, Kunming Institute of Botany, Chinese Academy of Science, where a voucher specimen was deposited.

Extraction and isolation

The air-dried and powdered rhizomes of *C. crassifolia* (10 kg) were extracted with 95% EtOH (3×50 l) at r.t., the combined extracts were evaporated *in vacuo* to afford a residue (562 g). The residue was suspended in H₂O and then passed through D101 resin column eluting with H₂O and EtOH. The EtOH eluent was concentrated *in vacuo* to give a residue (500 g), which was fractionated by CC (silica gel, 3000 g, 200–300 mesh; with CHCl₃-MeOH, 9 : 1) to afford 5 fractions (1–5). Fraction 2 (13 g) was refractionated on a silica gel column (220 g, CHCl₃-MeOH, 9.5 : 0.5, 1600 ml) to provide 8 fractions (2-1 to 2-8). Fraction 2-5 (448 mg) was purified by repeated Sephadex LH-20 (EtOH) to afford pure **7** (82 mg). Fraction 3 (40 g) was chromatographed on a silica gel column (600 g, CHCl₃-MeOH, 9 : 1, 2400 ml) to provide 9 fractions (3-1 to 3-9). Fraction 3-5 (364 mg) and fraction 3-8 (512 mg) were purified, respectively, by repeated Sephadex LH-20 (EtOH) to afford pure **5** (60 mg) and **6** (89 mg). Fraction 5 (210 g) was refractionated by Sephadex

LH-20 (EtOH-H₂O, 0 : 1 – 1 : 0; 2000 ml each eluent) to yield 12 crude fractions (5-1 to 5-12). Fraction 5-7 (4.34 g) was purified by Sephadex LH-20 (EtOH-H₂O, 0 : 1 – 1 : 0; 700 ml each eluent) to yield 6 fractions (5-7-1 to 5-7-6). Fraction 5-7-6 (572 mg) was repeated purified by Sephadex LH-20 (EtOH) to afford a mixture of **8** and **9** (112 mg). Fraction 5-9 (48 g) was purified by Sephadex LH-20 (EtOH-H₂O, 0 : 1 – 1 : 0; 1000 ml each eluent) to yield 10 fractions (5-9-1 to 5-9-10). Fr. 5-9-5 (2.96 g) was repeated purified by Sephadex LH-20 (EtOH) to afford pure **3** (700 mg), **4** (34 mg) and **2** (26 mg). Fraction 5-9-9 (140 mg) was purified by Sephadex LH-20 (EtOH) to afford pure **1** (60 mg).

Acetylation **1** and **2**

A soln. of each sample (1 mg) in pyridine (1 ml) was treated with Ac₂O (1 ml) and kept at 60–70 °C for 3–6 h. The reactants were condensed *in vacuo* to give a residue, which was purified on a preparative TLC plates with CHCl₃-MeOH (9 : 1) to obtain an acetate. The acetate was subjected to FAB MS analysis.

Acidic hydrolysis **1** and **2**

Each sample (3 mg) was dissolved, respectively, in a mixture of MeOH (2.0 ml) and 2 mol/l H₂SO₄ (2.0 ml) and refluxed on a boiling water bath for 2 h. The hydrolysate was allowed to cool, diluted twofold with distilled H₂O and partitioned between EtOAc and water. The aq. layer was neutralized with aq. Ba(OH)₂ and concentrated *in vacuo* to give a residue, from which glucose was identified by PC comparison with authentic sample (n-BuOH-HAc-H₂O, 5:1:5, upper layer).

Crassifoside C (1): White powder. $[\alpha]_D^{23} - 50.5^\circ$ (c 0.10, MeOH). – UV_{max} (MeOH): λ_{max} (lg ϵ) = 204 nm (3.95), 283 nm (4.45). – IR (KBr): ν = 3439 (OH), 1652 (C=O), 1456, 1296, 1105, 1074 cm⁻¹. – ¹H NMR (400 MHz, CD₃OD): δ = 6.07 (d, J = 2.5 Hz, 1H, H-2), 4.63 (dd, J = 2.5, 10.1 Hz, 1H, H-3), 4.87 (d, J = 10.1 Hz, 1H, H-4), 7.11 (s, 1H, H-5), 6.52 (s, 1H, H-8), 7.40 (d, J = 2.0 Hz, 1H, H-2'), 6.81 (d, J = 8.3 Hz, 1H, H-5'), 7.38 (dd, J = 2.0, 8.3 Hz, 1H, H-6'), Glc: 4.47 (d, J = 7.3 Hz, 1H, H-1''), 3.35 (m, 1H, H-2''), 3.34 (m, 1H, H-3''), 3.28 (m, 1H, H-4''), 3.40 (m, 1H, H-5''), 3.62 (dd, J = 5.3, 11.8 Hz, 1H, H-6''a), 3.87 (dd, J = 1.8, 11.8 Hz, 1H, H-6''b). – ¹³C {¹H} NMR (100 MHz, CD₃OD): δ = 140.0 (C-1), 129.3 (C-2), 81.8 (C-3), 72.9 (C-4), 114.7 (C-5), 145.4 (C-6), 146.9 (C-7), 114.4 (C-8), 123.8 (C-9), 129.9 (C-10), 197.8 (C-11), 130.3 (C-1'), 117.6 (C-2'), 146.5 (C-3'), 153.0 (C-4'), 115.9 (C-5'), 125.6 (C-6'), Glc: 103.5 (C-1''), 71.6 (C-2''), 78.1 (C-3''), 74.9 (C-4''), 77.9 (C-5''), 62.7 (C-6''). – FAB MS *m/z*: 491 [M-H]⁻, – HRFAB MS *m/z*: 491.1203 [M-H]⁻, calcd. for [C₂₃H₂₃O₁₂]⁻: 491.1189.

Crassifoside D (2): Brown powder. $[\alpha]_D^{29} - 145.8^\circ$ (c 0.27, MeOH). – UV_{max} (MeOH): $\lambda_{\text{max}} (\lg \epsilon) = 208 \text{ nm}$ (4.49), 363 nm (2.58), 375 nm (2.39). – IR (KBr): $\nu = 3430$ (OH), 1543, 1510, 1074, 1021 cm^{-1} . – ¹H NMR (400 MHz, C₅D₅N): $\delta = 6.40$ (t, $J = 6.9 \text{ Hz}$, 1H, H-2), 2.27 (m, 1H, H-3a), 2.72 (m, 1H, H-3b), 4.63 (m, 1H, H-4), 5.24 (d, $J = 8.8 \text{ Hz}$, 1H, H-5), 8.21 (s, 1H, H-6), 7.29 (s, 1H, H-9), 7.35 (d, $J = 1.9 \text{ Hz}$, 1H, H-2'), 7.20 (d, $J = 8.2 \text{ Hz}$, 1H, H-5'), 6.91 (dd, $J = 1.9, 8.2 \text{ Hz}$, 1H, H-6'), Glc: 5.07 (d, $J = 7.7 \text{ Hz}$, 1H, H-1''), 4.21 (m, 1H, H-2''), 4.04 (m, 1H, H-3''), 4.06 (m, 1H, H-4''), 4.26 (m, 1H, H-5''), 4.34 (dd, $J = 5.5, 11.0 \text{ Hz}$, 1H, H-6''a), 4.56 (dd, $J = 1.6, 11.0 \text{ Hz}$, 1H, H-6''b). – ¹³C {¹H} NMR (100 MHz, C₅D₅N): $\delta = 143.8$ (C-1), 123.0 (C-2), 31.6 (C-3), 94.8 (C-4), 74.6 (C-5), 113.6 (C-6), 145.6 (C-7), 146.7 (C-8), 116.9 (C-9), 129.5 (C-10), 134.4 (C-11), 134.4 (C-1'), 117.7 (C-2'), 146.9 (C-3'), 147.0 (C-4'), 116.4 (C-5'), 120.2 (C-6'), Glc: 103.6 (C-1''), 71.9 (C-2''), 78.7 (C-3''), 75.1 (C-4''), 78.5 (C-5''), 62.7 (C-6''). – FAB MS m/z : 477 [M-H]⁻, – HR FAB MS m/z : 477.1401 [M-H]⁻, calcd. for [C₂₃H₂₅O₁₁]⁻: 477.1396.

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