

Two New Phenolic Glycosides from *Hypoxis aurea* Lour

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Plants of the genus Hypoxidaceae are widely distributed in Torrid Zone in the world, and previous studies of the genus by our group revealed that it mainly contain phenolic glycosides.²⁻⁴ *Hypoxis aurea* Lour. belongs to the genus Hypoxidaceae, called as 'xiao jin mei' to treat hernia and warm kidney in China¹ and mainly distributed over the southern of China, south east of Asia and Japan. Chemical investigation of the rhizomes of *H. aurea* toward potentially bioactive secondary metabolites from this genus leading to the isolation of two new phenolic glycosides, aureaside A (1) and aureaside B (2), together with seven known compounds, curculigoside I (3),⁵ orcinol glucoside (4),⁶ curcapital (5),⁷ cimifugin prim-*O*- β -D-glucopyranoside (6),⁸ 2-*O*- β -D-apiofuranosyl (1 \rightarrow 6)-*O*- β -D-glucopyranoside (7),⁹ (2*R*,5*S*)-bornane-2,5-diol-2-*O*- β -D-glucopyranoside (8),⁹ and bornyl 7-*O*- β -D-apio-D-furanosyl (1 \rightarrow 6)-*O*- β -D-glucopyranoside (9)¹⁰ as shown in Figure 1. Their structures were elucidated on the basis of spectroscopic analysis and comparing spectral data with those known compounds reported in literatures. All these compounds were found in this plant for the first time. In this paper we reported the isolation and structural elucidation of two new compounds from *H. aurea* Lour.

Aureaside A (1) was isolated as colorless needles (CH₃OH). Its molecular formula was determined to be C₁₉H₂₆O₁₁Cl₂ with six unsaturation degrees by HRESIMS (*m/z* 535.0532

[M+Cl]⁻) and ¹³C NMR spectra. The IR spectrum showed the presence of hydroxyl group (3425 cm⁻¹), aromatic ring (1631, 1588, 1463 cm⁻¹), and the C-Cl bond (1100 ~ 1043 cm⁻¹). The ¹H NMR spectrum of compound 1 clearly showed one methyl at δ 2.38 (3H, s), one OCH₃ at δ 3.88 (3H, s), two anomeric protons of sugars at δ 5.20 (1H, d, *J* = 7.6 Hz) and 4.12 (1H, d, *J* = 7.0 Hz) and one aromatic proton at δ 6.85 (1H, s). The ¹³C NMR (DEPT) spectroscopic data (Table 1) revealed the presence of 2 methyls, 2 methylenes, 10 methines and 5 quaternary carbons, among which carbon signals at δ 150.0 (s), 121.9 (s), 137.2 (s), 155.5 (s), 116.8 (s) and 111.4 (d) indicated the presence of penta-substituted aromatic ring. Carbon signals at δ 104.6 (d), 74.6 (d), 77.6 (d), 71.5 (d), 66.5 (t) and 103.7 (d), 75.6 (d), 77.2 (d), 71.0 (d), 78.5 (d), 68.9 (t) suggested the presence of one xylose and 6-substituted glucose, which were further confirmed by its MS fragmentation peaks at *m/z* 369 [M-1-xy]⁻ and 207 [M-1-xy-glc]⁻. Acid hydrolysis of compound 1 also showed that it contains glucose and xylose. Comparison of these NMR data with those reported in the literature showed that compound 1 was similar to capitulatin A³ except for the difference of substituted position in the aromatic ring and one aromatic methine at C-4 (δ 111.4) in 1 replaced the corresponding quaternary carbon linked to a hydroxyl group in capitulatin A.

The linkages of OCH₃ to C-6 and two sugar moieties to C-1

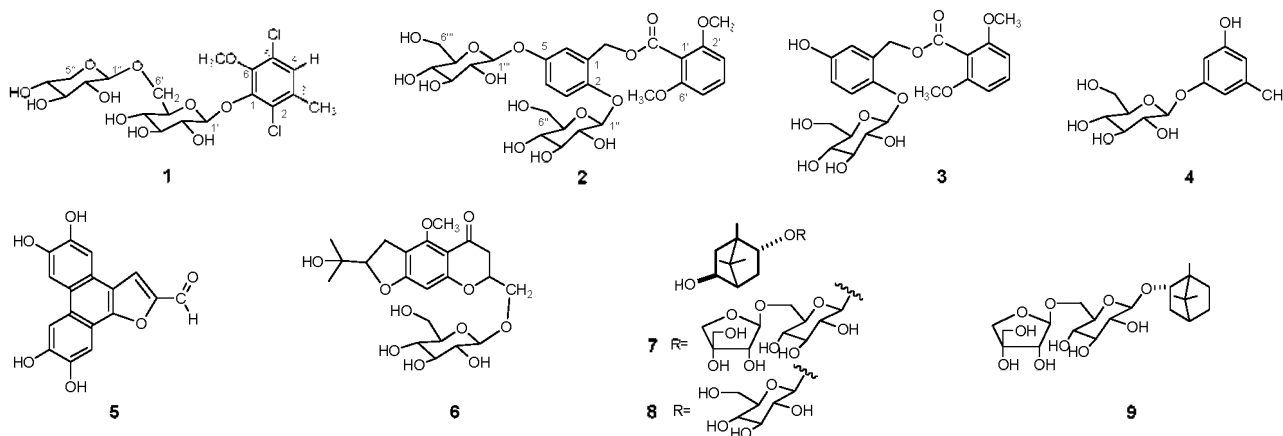


Figure 1. Structures of compounds 1-9.

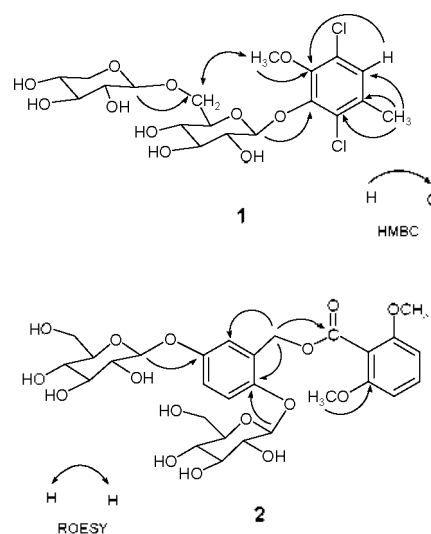
Table 1. ^1H ^{13}C NMR data of aureaside A (**1**) and aureaside B (**2**) in CD_3OD .

1			2		
Position	δ_{H}	δ_{C}	Position	δ_{H}	δ_{C}
1	-	150.0 (s)	1	-	128.5 (s)
2	-	121.9 (s)	2	-	151.7 (s)
3	-	137.2 (s)	3	7.17, d, 9.0 Hz	118.7 (d)
4	6.85, s	111.4 (d)	4	7.06, dd, 2.9 Hz, 9.0 Hz	118.7 (d)
5	-	116.8 (s)	5	-	154.4 (s)
6	-	155.5 (s)	6	7.23, d, 2.9 Hz	117.9 (d)
1'	5.20, d 7.6 Hz	103.7 (d)	1'	-	114.1 (s)
2'	3.57, m	75.6 (d)	2'	-	158.7 (s)
3'	3.04, m	77.2 (d)	3'	6.68, d, 8.5 Hz	105.2 (d)
4'	3.41, m	71.0 (d)	4'	7.35, t, 8.5 Hz	132.6 (d)
5'	3.39, m	78.5 (d)	5'	6.68, d, 8.5 Hz	105.2 (d)
6'	3.70, m, 3.91, m	68.9 (t)	6'	-	158.7 (s)
1''	4.12, d, 7.0 Hz	104.6 (d)	1''	4.83, d, 7.3 Hz	102.9 (d)
2''	3.00, m	74.6 (d)	2''	3.43, m	74.9 (d)
3''	3.41, m	77.6 (d)	3''	3.34, m	78.1 (d)
4''	3.31, m	71.5 (d)	4''	3.39, m	71.3 (d)
5''	3.77, m; 3.02, m	66.5 (t)	5''	3.43, m	78.0 (d)
6-OCH ₃	3.88, s	57.0 (q)	6''	3.86, m	62.5 (t)
3-CH ₃	2.38, s	20.9 (q)	1'''	4.84, d, 7.2 Hz	103.1 (d)
			2'''	3.40, m	74.9 (d)
			3'''	3.45, m	78.2 (d)
			4'''	3.39, m	71.2 (d)
			5'''	3.42, m	78.0 (d)
			6'''	3.86, m, 3.80, overlapped	62.3 (t)
			2', 6'-OCH ₃	3.82, s	56.6 (q)
			1-CH ₂ -	5.45, s	63.1 (t)
			1'-C=O	-	168.5 (s)

were determined by the HMBC correlations from OCH₃ (δ 3.88) to C-6 (δ 155.5) and H-1' (δ 5.20) to C-1 (δ 150.0) (Figure 1). The *o*-substitutions of 3-CH₃ with 2-Cl and 4-H in aromatic ring were established by the HMBC correlations from the CH₃ protons (δ 2.38) to C-4 (δ 111.4), C-3 (δ 137.2) and C-2 (δ 121.9) attached to a chlorine. The key cross-peak in ROESY between OCH₃ (δ 3.88) and H-6' (δ 3.70, 3.91) established the *o*-substitutions of 6-OCH₃ and 1-O-Glc in the aromatic ring. The linkage of the xylose to C-6' (δ 68.9) in glucose was determined by the key correlation of H-1'' (δ 4.12) with C-6' in the HMBC spectrum. Besides, the coupling constants of the anomeric protons of two sugar moieties indicated the β -type sugars. Based on the above evidences, compound **1** was identified as 2,5-dichloro-3-methyl-6-methoxyphenol-*O*- β -D-xylopyranosyl (1 \rightarrow 6)-*O*- β -D-glucopyranoside, named aureaside A.

Aureaside B (**2**) was deduced to have a molecular formula of C₂₈H₃₆O₁₆ from HRESIMS [M+Cl]⁻ ion peak at *m/z* 663.1684 and ^{13}C NMR spectra (Table 1). The IR spectrum showed the presence of hydroxyl groups (3395, 3448 cm⁻¹), carbonyl group (1718 cm⁻¹) and aromatic ring (1598, 1495, 1450 cm⁻¹). ^1H NMR spectrum showed one typical 1,2,4-trisubstituted aromatic ring according to the signals at δ 7.17 (1H, d, J = 9.0 Hz), 7.06 (1H, dd, J = 2.9, 9.0 Hz) and 7.23 (1H, d, J = 2.9 Hz),

one 1,2,3-trisubstituted aromatic ring at δ 6.68 (1H, d, J = 8.5 Hz), 7.35 (1H, t, J = 8.5 Hz) and 6.68 (1H, d, J = 8.5 Hz), and two methoxyls at δ 3.82 (6H, s). The ^{13}C NMR (DEPT) spectroscopic data (Table 1) revealed the presence of 2 methyls, 3

**Figure 2.** Key HMBC and ROESY correlations of compounds **1** and **2**.

methylenes, 16 methines and 7 quaternary carbons. Comparison of these NMR data with those reported in the literature showed that compound **2** was similar to curculigoside **1**⁵ except for one more additional glucose moiety attached to C-5 (δ 154.4) in **2**, which was confirmed by the key HMBC correlation of H-1'' at δ 4.84 (1H, d, $J = 7.2$ Hz) to C-5. The other glucose group linked to C-2 (δ 151.7) was determined by the HMBC correlation of H-1'' at δ 4.83 (1H, d, $J = 7.3$ Hz) to C-2. The coupling constants of two anomeric protons (7.3 and 7.2 Hz) suggested the two glucose moieties were β -type sugars. Acid hydrolysis of compound **2** further supported the presence of glucose moiety. The ¹³C NMR spectrum had signals for two aromatic rings, one benzylic carbon, one hexose unit and two aromatic methyl ethers. The assignment of **2** was further established by HMQC, ¹H-¹H COSY and other HMBC correlations (Figure 2). Based on the above evidences, compound **2** was identified as 5-*O*- β -D-glucopyranoside curculigoside **1**, named aureaside B.

Experimental Section

General experimental procedures. Melting points were measured on a XRC-1 micro-melting point apparatus and were uncorrected. MS spectra were obtained on a VG Auto Spec-3000 mass spectrometer. 1D and 2D NMR spectra were recorded on Bruker AM-400 MHz and DRX-500 MHz spectrometers, with chemical shifts (δ) in ppm relative to TMS as internal standard and coupling constants in hertz (Hz). IR spectra were measured with a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were measured on a Hitachi UV-3210 spectrophotometer. Silica gel (200 - 300 mesh) for column chromatography was product of the Qingdao Marine Chemical Ltd., Qingdao, P. R. China. Sephadex LH-20 for chromatography was purchased from Amersham Biosciences. Reversed-phase chromatography was with RP-18 (LiChroprep, 40 - 63 μ m, Merck, Darmstadt, Germany).

Plant materials. The whole plant of *H. aurea* was collected in Kunming, Yunnan Province, People's Republic of China, in September 2008, and authenticated by professor Hua Peng. A voucher specimen (KUN 0864822) has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered rhizomes of *H. aurea* (3.0 kg) were extracted three times each with 15 L of 95% EtOH under reflux for 3 h. The extracts were evaporated and the residue was resuspended in 15 L of H₂O and partitioned successively with petroleum ether (3 L \times 3), AcOEt (3 L \times 3) and *n*-BuOH (3 L \times 3) to yield a petroleum ether extract (49 g), AcOEt extract (70 g), *n*-BuOH extract (210 g), respectively. The *n*-BuOH extract was applied to a silica gel column chromatography (200 - 300 mesh) eluted with CHCl₃-CH₃OH-H₂O (15 : 3 : 0.5) to give five fractions. Fraction 1 was purified by column chromatography silica gel with CHCl₃-CH₃OH (4 : 1), then on RP-18 with 60% \rightarrow 100% aqueous CH₃OH, and on Sephadex LH-20 with CH₃OH : CH₂Cl₂ (1 : 1) to afford compounds **7** (12 mg), **8** (21 mg) and **9** (10 mg). Fraction 2 was subjected to column chromatography on silica gel with CHCl₃-CH₃OH-H₂O (10 : 3 : 0.5) and on Sephadex LH-20 with CH₃OH to yield compounds **5** (7 mg)

and **6** (36 mg). Fraction 5 was purified by column chromatography on RP-18 with 10% \rightarrow 100% aqueous CH₃OH and on Sephadex LH-20 with CH₃OH to obtain compounds **1** (25 mg), **2** (15 mg), **3** (29 mg) and **4** (27 mg).

Aureaside A: Colorless needles (CH₃OH), mp: 198 ~ 199 °C; $[\alpha]_D^{25}$ -38.9105° (*c* 0.170, CH₃OH); UV (CH₃OH) λ_{max} (*log* ϵ): 205 (4.604), 288 (3.138); IR (KBr) ν 3425, 2917, 1631, 1588, 1100, 1063, 1043, 1043 cm⁻¹; ¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (125 MHz, CD₃OD) see Table 1; HRESIMS (negative ion) *m/z* 535.0532 [M+Cl]⁻ (Calcd. for C₁₅H₂₆O₁₁Cl₂, 535.0540); FABMS (negative ion) *m/z* (%) 499[M-1]⁻ (58), 447 (38), 369 (5), 283 (22), 207 (100), 183 (35), 80 (14).

Aureaside B: White amorphous solid, mp: 137 ~ 138 °C. $[\alpha]_D^{25}$ -52.42° (*c* 1.240, CH₃OH); UV (CH₃OH) λ_{max} (*log* ϵ): 281 (3.656), 255 (3.418), 204 (4.544); IR (KBr) ν 3448, 3395, 1719, 1598, 1495, 1478, 1116, 1073 cm⁻¹; ¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (125 MHz, CD₃OD) see Tables 1; HRESIMS (negative ion) *m/z* 663.1684 [M+Cl]⁻ (Calcd for C₂₈H₃₆O₁₆Cl, 663.1691); FABMS (negative ion) *m/z* (%): 627[M-1]⁻ (5), 550 (17), 465 (5), 305 (14), 243 (60), 180 (100), 86 (10).

Acid hydrolysis of compound 1 and 2. Compound **1** (2 mg) and **2** (2 mg) in 2 N HCl (2 mL) were each heated at 95 °C for 2 h, then cooled for few minutes. The mixture was washed with EtOAc (2 mL \times 2). The H₂O layer was concentrated in vacuo to give a residue. The residue and authentic sugars were dotted to the plate developed with CHCl₃-CH₃OH-H₂O (5 : 3 : 0.5), and phenylene diamine-aniline-phosphoric acid used as spray reagent, followed by heating at 80 °C. From compound **1** glucose and xylose were detected; R_f: glucose 0.45, xylose 0.57. From compound **2** glucose was detected; R_f: glucose 0.45.

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