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 China[1] 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→6)-*O*-*β*-D-glucopyranoside (**7**),[9] (*2R*,*5S*)-bornane-2,5-diol-2-*O*-*β*-D-glucopyranoside (**8**),[9] and bornyl 7-*O*-*β*-D-apio-D-furanosyl (1→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 (CH_3OH). Its molecular formula was determined to be $C_{19}H_{26}O_{11}Cl_2$ with six unsaturation degrees by HRESIMS (*m*/*z* 535.0532 $[M+Cl]^-$) and ^{13}C 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 OCH_3 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 ^{13}C 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 $[M-1\text{-xyl}]^-$ and 207 $[M-1\text{-xyl-glc}]^-$. 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 A[3] 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 OCH_3 to C-6 and two sugar moieties to C-1

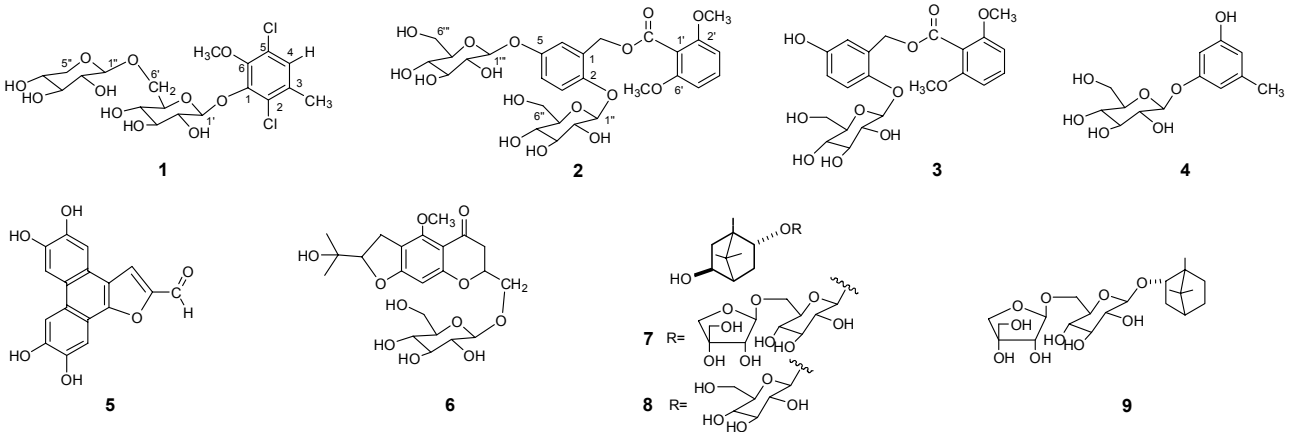

Figure 1. Structures of compounds **1**-**9**.

Table 1. ^{1}H ^{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 OCH_3 (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-CH_3 with 2-Cl and 4-H in aromatic ring were established by the HMBC correlations from the CH_3 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 OCH_3 (δ 3.88) and H-6′ (δ 3.70, 3.91) established the *o*-substitutions of 6-OCH_3 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-methoxylphenol-*O*-β-D-xylopyranosyl (1→6)-*O*-β-D-glucopyranoside, named aureaside A.

Aureaside B (**2**) was deduced to have a molecular formula of $C_{28}H_{36}O_{16}$ 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}). ^{1}H 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

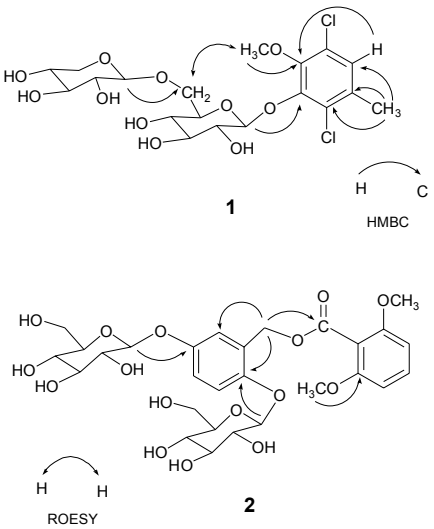

Figure 2. Key HMBC and ROSEY correlations of compounds **1** and **2**.

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 I[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 ^{13}C 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, ^{1}H-^{1}H COSY and other HMBC correlations (Figure 2). Based on the above evidences, compound **2** was identified as 5-*O*-β-D-glucopyrancoside curculigoside I, 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 μm, 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 H_2O and partitioned successively with petroleum ether (3 L × 3), AcOEt (3 L × 3) and n-BuOH (3 L × 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 $CHCl_3$-CH_3OH-H_2O (15 : 3 : 0.5) to give five fractions. Fraction 1 was purified by column chromatography silica gel with $CHCl_3$-CH_3OH (4 : 1), then on RP-18 with 60% → 100% aqueous CH_3OH, and on Sephadex LH-20 with CH_3OH : CH_3Cl (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 $CHCl_3$-CH_3OH-H_2O (10 : 3 : 0.5) and on Sephadex LH-20 with CH_3OH to yield compounds **5** (7 mg) and **6** (36 mg). Fraction 5 was purified by column chromatography on RP-18 with 10% → 100% aqueous CH_3OH and on Sephadex LH-20 with CH_3OH to obtain compounds **1** (25 mg), **2** (15 mg), **3** (29 mg) and **4** (27 mg).

Aureaside A: Colorless needles (CH_3OH), mp: 198 ~ 199 ^{o}C; [α] −38.9105^{o} (*c* 0.170, CH_3OH); UV (CH_3OH) λ_{max} (*log*ε): 205 (4.604), 288 (3.138); IR (KBr) ν 3425, 2917, 1631, 1588, 1100, 1063, 1043, 1043 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 1; HRESIMS (negative ion) *m*/*z* 535.0532 $[M+Cl]^-$ (Calcd. for $C_{19}H_{26}O_{11}Cl_3$, 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 ^{o}C, [α] −52.42^{o} (*c* 1.240, CH_3OH); UV (CH_3OH) 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}; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see Tables 1; HRESIMS (negative ion) *m*/*z* 663.1684 $[M+Cl]^-$ (Calcd for $C_{28}H_{36}O_{16}Cl$, 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 ^{o}C for 2 h, then cooled for few minutes. The mixture was washed with EtoAc (2 mL × 2). The H_2O layer was concentrated in vacuo to give a residue. The residue and authentic sugars were dotted to the plate developed with $CHCl_3$-CH_3OH-H_2O (5 : 3 : 0.5), and phenylene diamine-aniline- phosphoric acid used as spray reagent, followed by heating at 80 ^{o}C. From compound **1** gulcose and xylose were detected; R_f: glucose 0.45, xylose 0.57. From compound **2** gulcose was detected; R_f: glucose 0.45.

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