A new *β*-naphthalenecarboxylic acid biglycoside from *Chirita longgangensis* var. *hongyao*

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Abstract: To investigate the chemical constituents in the stems of *Chirita longgangensis* var. *hongyao*, methanol extract of the stems was subjected to column chromatography with various chromatographic techniques. One new *β*-naphthalenecarboxylic acid biglycoside, 1, 4-dihydroxy-2-naphthalenecarboxylic acid methyl ester-4-*O*-*α*-*L*-rhamnopyranosyl-(1→6)-*β*-*D*-glucopyranoside (**1**) was isolated, along with two known compounds: isotaxiresinol 4-*O*-methyl ether (**2**) and (*R*)-7-hydroxy-*α*-dunnione (**3**). Compound **2** was first obtained from *Chirita* genus and compound **3** was isolated from this plant for the first time. All structures were elucidated on the basis of spectral and chemical evidence, and the NMR spectroscopic data of compound **2** was published for the first time.

Key words: *Chirita longgangensis* var. *hongyao*; Gesneriaceae; *β*-naphthalenecarboxylic acid biglycoside
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红药中一个新的 *β*-萘甲酸双糖苷类化合物

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摘要: 为了研究红药 (*Chirita longgangensis* var. *hongyao*) 茎的化学成分, 运用多种色谱方法进行分离纯化, 从其甲醇提取物中分离得到 3 个化合物, 并根据理化性质和波谱数据鉴定其结构分别为 1, 4-dihydroxy-2-naphthalenecarboxylic acid methyl ester-4-*O*-*α*-*L*-rhamnopyranosyl-(1→6)-*β*-*D*-glucopyranoside (**1**), isotaxiresinol 4-*O*-methyl ether (**2**) 和 (*R*)-7-hydroxy-*α*-dunnione (**3**)。其中, 化合物 **1** 为新的 *β*-萘甲酸双糖苷化合物, 化合物 **2** 为首次从该属植物中分离得到, 且首次提供了化合物 **2** 的核磁波谱数据, 化合物 **3** 为首次从该植物中分离得到。

关键词: 红药; 苦苣苔科; *β*-萘甲酸双糖苷

Chirita longgangensis W. T. Wang var. *hongyao* S. Z. Huang (Gesneriaceae) is distributed in Guangxi Province, China. The stems of *C. longgangensis* var. *hongyao* have long been used as a folk medicine in China for the treatment of arthritis, anemia and fracture[1, 2]. Previous chemical investigation has resulted in five phenylethanoid glycosides[3], two anthraquinones and five other compounds from this plant[4]. The current study was carried out to search for bioactive metabolites from *C. longgangensis* var. *hongyao*, leading to the isolation of a new compound, *β*-naphthalenecarboxylic acid biglycoside, 1,4-dihydroxy-2-naphthalenecarboxylic acid methyl ester-4-*O*-*α*-*L*-rhamnopyranosyl-(1→6)-*β*-*D*-glucopyranoside (**1**) and two known compounds, isotaxiresinol 4-*O*-methyl ether (**2**) and (*R*)-7-hydroxy-*α*-dunnione (**3**). Compound **2** was first obtained from *Chirita* genus and compound **3** was isolated from this

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plant for the first time. The chemical structures of compounds **1**−**3** are shown in Figure 1. In this paper, we report the isolation and structure elucidation of these compounds.

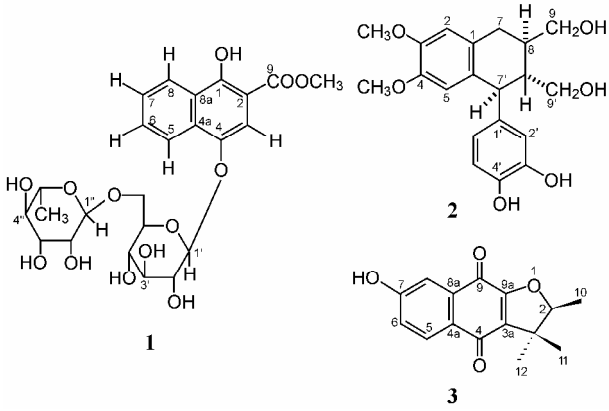

Figure 1 The chemical structures of compounds **1**, **2** and **3**

Results and discussion

Compound **1** was obtained as white amorphous powder that analyzed for the molecular formula $C_{24}H_{30}O_{13}$ by HR-ESI-MS at *m/z* 525.161 0 $[M-H]^-$. The IR (KBr) spectrum of **1** showed broad absorption for multiple hydroxyl groups (3 399 cm^{-1}), an ester carbonyl (1 695 cm^{-1}), and aromatic rings (1 635 and 1 603 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** (Table 1) showed an aromatic singlet at δ 7.38, a 1, 2-disubsititued aromatic ring at δ 8.36 (1H, d, J = 8.0 Hz, H-5), 7.72 (1H, m, H-6), 7.64 (1H, m, H-7), 8.29 (1H, d, J = 8.0 Hz, H-8), an *O*-methyl group at δ 3.98 (3H, s, OC$\underline{H}_3$-9), and a phenolic OH proton at δ 11.7 (1H, s, OH-1). Acid hydrolysis of **1** afforded glucose and rhamnose, which were identified by TLC comparison with authentic samples. One doublet and a broaden singlet due to anomeric protons at δ 4.82 (1H, d, J = 7.5 Hz, H-1') and 4.54 (1H, br s, H-1''), together with a methyl doublet at δ 1.07 (3H, d, J = 6.5 Hz, H-6''), as well as partially overlapped signals attributable to oxymethylenes and oxymethines between at δ 3.14 and 3.90, indicated that there were a β-glucopyranosyl and an α-rhamnopyranosyl groups. The configuration of the glucopyranosyl and rhamnopyranosyl was assigned as β-*D*- and α-*L*- on the basis of the coupling constant of the anomeric proton and of the abundance of the β-*D*-glucopyranosyl and α-*L*-rhamnopyranosyl units in natural products. Moreover, the ^{13}C NMR data of the sugar unit are consistent with those in literature[5]. The ^{13}C NMR spectrum of **1** (Table 1) showed carbon signals corresponding to the above structural units and one conjugated ester carbonyl at δ 170.5. 2D NMR experiments were carried out to construct the structure of **1**. Analyses of the ^1H-^1H COSY and HMQC spectra of **1** led to unambiguous assignment of proton and corresponding carbon signals in the NMR spectra (Table 1). HMBC correlations of H-8 with C-1, C-8a, C-7, and C-6, H-5 with C-4, C-4a, C-6 and C-7, and H-3 with C-4, C-4a, C-2, and C-1, in combination with chemical shifts of these protons and carbons, provided evidence for a 1, 2, 4-trisubstituted naphthalene moiety. The downfield chemical shift of phenolic OH proton at δ 11.7 suggested **1** possessed one intramolecular hydrogen bond structure skeleton. HMBC correlations of the carbonyl (C-9) with H-3 and *O*-methyl protons, and the phenolic OH proton with C-1, C-2 and C-8a, clearly located a hydroxyl and a methyl ester at C-1 and C-2, respectively. The signals assigned to the aglycone moiety were in good agreement with the published data in the literature[6]. In addition, HMBC correlation between the anomeric proton of rhamnopyranosyl (H-1'') and C-6 of the glucopyranosyl indicated a rhamnopyranosyl (1→6) glucopyranosyl linkage. Finally, the sugar chain was positioned at C-4 on the basis of HMBC correlations of the anomeric proton of glucopyranosyl (H-1') with C-4. Therefore, the structure of **1** was determined as 1, 4-dihydroxy-2-naphthalenecarboxylic acid methyl ester-4-*O*-α-*L*-rhamnopyranosyl-(1→6)-β-*D*-glucopyranoside. The key HMBC and ^1H-^1H COSY correlations of compound **1** are shown in Figure 2.

Table 1 ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data for compound **1** (DMSO-d_6, J in Hz). [a]Signal patterns were unclear due to overlapping

No.	δ_H	δ_C	No.	δ_H	δ_C
1		155.2	Glc-1'	4.82 (d, 7.5)	102.3
2		104.5	2'	3.40[a]	73.4
3	7.38 (s)	107.7	3'	3.31[a]	76.2
4		145.3	4'	3.14[a]	70.0
4a		129.9	5'	3.48[a]	75.7
5	8.36 (d, 8.0)	122.4	6'	3.42, 3.88 (d, 7.5)	66.6
6	7.72 (m)	129.4	Rha-1''	4.54 (br s)	100.6
7	7.64 (m)	126.7	2''	3.60 (m)	70.3
8	8.29 (d, 8.0)	123.1	3''	3.43[a]	70.7
8a		124.6	4''	3.16[a]	72.0
9		170.5	5''	3.40[a]	68.2
9-OCH_3	3.98 (s)	52.8	6''	1.07 (d, 6.5)	17.8
1-OH	11.7 (s)				

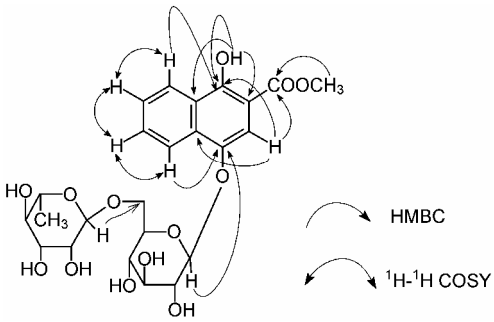

Figure 2 The key HMBC and ^{1}H-^{1}H COSY correlations of compound **1**

Compound **2** was firstly reported as a new lignan by Erdtman H. and Tsuno K. in 1969[7]. No NMR spectroscopic data for this compound had been reported, although some data of its structure-similar compounds have been described in the later literature[8−10]. Therefore, the NMR data of **2** assigned by interpretation of its 2D NMR spectra are included in this report.

Experimental

1 Generals

Optical rotations were measured on a JASCO P-1020 polarimeter. UV and IR spectra were recorded on Shimadzu UV-2500PC and Shimadzu IR Prestige-21 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM-500 spectrometer. Proton detected heteronuclear correlations were measured using HMQC and HMBC. HR-ESI-MS analysis was carried out on a Bruker microTOF-Q instrument. Column chromatography was performed using silica gel (60−120 and 300−400 mesh); TLC: precoated silica gel plates 60 GF_{254} or RP-C_{18} F_{254} plates with 0.5 or 1 mm film thickness (Merck). Spots were visualized under UV light or by spraying with H_2SO_4-EtOH or anisaldehyde-H_2SO_4 followed by heating.

2 Plant material

The stems of *C. longganesis* var. *hongyao* were collected in Tiandeng County, Guangxi Province, China, in 2005, and identified by Prof. Bin Dai, Guangxi Institute of National Medical Research, China, where a voucher specimen was deposited.

3 Extraction and isolation

The air-dried stems of *C. longganesis* var. *hongyao* (10 kg) were powdered and consecutively extracted with MeOH at room temperature. The combined extracts were concentrated in vacuum to yield a dark red residue that was suspended in water and then partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH extract (200 g) was applied to a Diaion D101 macroporous adsorbent resin column. Successive elution of the column with 20% EtOH, 50% EtOH and 95% EtOH yielded three corresponding fractions after removing solvents. The fraction eluted with 50% EtOH (42.7 g) was chromatographed over silica gel, eluting with a gradient of increasing MeOH (0−100%) in EtOAc, to give six fractions (1−6). Fraction 2 (6.3 g) was firstly separated after Sephadex LH-20 CC eluting with a step gradient from 10% to 50% MeOH in H_2O and then purified by ODS CC eluting with gradient mixtures of MeOH-H_2O [from MeOH-H_2O (1∶1, *v*/*v*) to MeOH-H_2O (85∶15, *v*/*v*)] to yield compound **1** (13.5 mg). The EtOAc extract (80.3 g) was subjected to CC on silica gel, and eluted with a gradient of increasing MeOH (0−50%) in $CHCl_3$, to afford eight fractions (I−VIII) based on TLC analysis. Fraction VII (11 g) was further purified by silica gel CC (300 g), eluting with a gradient of increasing MeOH (20%−40%) in $CHCl_3$, to give compound **2** (16.4 mg). Compound **3** (23.2 mg) was isolated from fraction VI (19.7 g) by repeated CC over silica gel using a gradient of increasing EtOAc (5%−50%) in petroleum ether as eluting solvent.

4 Structure identification

Compound 1 white amorphous powder (CH_3OH), mp 143−145 ℃ and $[\alpha]_D^{21}$ −130 (*c* 0.1, CH_3OH). UV λ_{max} (CH_3OH) nm: 215 (sh), 255, 351.5. IR bands (KBr) cm^{-1}: 3 399 (br), 2 945, 1 695, 1 635, 1 603, 1 098, 1 067, 1 053, 976. Negative HR-ESI-MS *m/z*: 525.161 0 $[M-H]^-$ (calcd. for $C_{24}H_{29}O_{13}$ 525.160 3). ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data were shown in Table 1.

Compound 2 white amorphous powder (CH_3OH), mp 172−173 ℃. UV λ_{max} (MeOH) nm: 282.5. IR bands (KBr) cm^{-1}: 3 406 (br), 2 361, 1 609, 1 514, 1 445, 1 275, 1 121, 1 028. EI-MS (70 eV): *m/z* (rel. int. %) 360 $[M]^+$ (83), 311 $[M-H_2O-OCH_3]^+$ (100); negative HR-ESI-MS *m/z*: 359.146 2 $[M-H]^-$ (calcd. for $C_{20}H_{23}O_6$ 359.148 9). ^{1}H NMR (500 MHz, CD_3OD) δ: 6.73 (1H, d, *J* = 8.0 Hz, H-5'), 6.67 (1H, d, *J* = 2.0 Hz, H-2'), 6.65 (1H, s, H-2), 6.61 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.18 (1H, s, H-5), 3.79 (3H, s, OC$\underline{H}_3$-4), 3.78 (1H, m, H-7'), 3.76 (3H, s, OC$\underline{H}_3$-3), 3.68 (1H, m, H-9), 3.66 (1H, m, H-9'), 3.64 (1H, m, H-9), 3.39 (1H, dd, *J* = 11.0, 4.0 Hz, H-9'), 2.76 (2H, d, *J* = 7.5 Hz, H-7), 1.99 (1H, m, H-8), 1.76 (1H, m, H-8'). ^{13}C NMR (125 MHz, CD_3OD) δ: 129.1 (C-1), 112.5 (C-2), 147.2 (C-3), 145.3 (C-4), 117.4 (C-5), 134.2 (C-6), 33.6 (C-7), 40.1 (C-8), 66.0 (C-9), 138.6 (C-1'), 113.9 (C-2'), 149.0 (C-3'), 146.0

(C-4'), 116.0 (C-5'), 123.2 (C-6'), 48.1 (C-7'), 48.7 (C-8'), 62.3 (C-9'), 56.4 (O$\underline{C}H_3$-3), 56.5 (O$\underline{C}H_3$-4). The ^{1}H and ^{13}C NMR data assigned by interpretation of its 2D NMR spectra are in good accordance with its structure-similar compounds in the literature[8−10], so compound **2** was identified as isotaxiresinol 4-*O*-methyl ether.

Compound 3 red needle crystals ($CHCl_3$), mp 133−134 ℃. EI-MS (70 eV): *m/z* (rel. int. %) 258 $[M]^+$ (50), 243 $[M-CH_3]^+$ (100); HR-EI-MS *m/z*: 258.088 8 $[M]^+$ (calcd. for $C_{15}H_{14}O_4$ 258.089 2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.94 (1H, d, J = 8.5 Hz, H-5), 7.48 (1H, d, J = 2.5 Hz, H-8), 7.12 (1H, dd, J = 8.5, 2.5 Hz, H-6), 4.55 (1H, q, J = 6.5 Hz, H-2), 1.46 (3H, s, H-11), 1.41 (3H, d, J = 6.5 Hz, H-10), 1.26 (3H, s, H-12). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 91.6 (C-2), 45.2 (C-3), 131.0 (C-3a), 182.1 (C-4), 126.8 (C-4a), 128.6 (C-5), 120.6 (C-6), 160.1 (C-7), 112.7 (C-8), 133.5 (C-8a), 178.6 (C-9), 158.5 (C-9a), 14.2 (C-10), 25.8 (C-11), 20.6 (C-12). The ^{1}H and ^{13}C NMR data are consistent with those in literature[11], and then compound **3** was deduced as (*R*)-7-hydroxy-α-dunnione.

5 Acid hydrolysis of compound 1: determination of the sugar

A solution of compound **1** (2 mg) was heated with 2 mol·L^{-1} HCl (2 mL) in a sealed tube at 100 ℃ for 4 h. The reaction mixture was extracted with ethyl acetate. After evaporating off the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and lyophilized. The lyophilized residue was dissolved in pyridine (0.2 mL), and co-eluted (TLC) with the authentic samples developed with EtOAc-*n*-BuOH-H_2O (20∶70∶10, *v*/*v*). The plates were sprayed with naphthoresorcinol reagent by heating at 100 ℃. Glucose and rhamnose were identified.

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