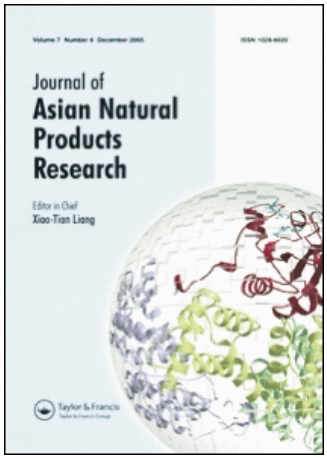

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Two new norbisabolane sesquiterpenoid glycosides from *Glochidion coccineum*

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Two new norbisabolane sesquiterpenoid glycosides, glochicoccinosides A (**1**) and B (**2**), together with two known compounds, have been isolated from the rhizomes of *Glochidion coccineum*. Their structures were elucidated by the combination of 1D NMR, 2D NMR, and MS spectral analysis, as well as chemical evidence. Cytotoxic activities and the antioxidant effect of these compounds were evaluated, but none of them showed activity.

Keywords: Euphorbiaceae; *Glochidion coccineum*; Norbisabolane sesquiterpenoid glycosides; Glochicoccinoside A; Glochicoccinoside B

1. Introduction

Glochidion coccineum (Buch-Ham.) Muell. Arg, belonging to the genus *Glochidion* of the family Euphorbiaceae, is a folk medicinal plant in China and has been used to treat influenza, dysentery, impaludism, rheumatoid arthritis, and dyspepsia for a long time [1]. In order to search for its bioactive components, we investigated the chemical constituents of this plant, which resulted in the isolation of two new norbisabolane sesquiterpenoid glycosides, glochicoccinosides A (**1**) and B (**2**), together with two known compounds, 4,4′-dimethoxy-3′-hydroxy-7,9′:7′,9-diepoxylignan-3-*O*-D-glucopyranoside (**3**) [2] and glochidioside (**4**) [3]. We herein report the isolation and structure elucidation of two new norbisabolane sesquiterpenoid glycosides, as well as their cytotoxic and antioxidant activities.

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2. Results and discussion

Glochicoccinoside A (**1**) was obtained as white amorphous powder. The positive HRESI-MS exhibited an accurate ion peak at *m/z* 783.2359 ($[M + Na]^+$) in accordance with the moleculer formula $C_{33}H_{44}O_{22}$. The IR spectrum showed absorption bands due to hydroxyl (3425 cm^{-1}), carbonyl (1777, 1740, 1690 cm^{-1}), and phenyl (1609, 1515, 1449 cm^{-1}) groups. The ^1H NMR signals due to aromatic protons at δ 8.02 (2H, d, $J = 8.7$ Hz), 6.91 (2H, d, $J = 8.7$ Hz), as well as one ester carbonyl carbon at δ 168.0 in the ^{13}C NMR spectrum, suggested the presence of one *p*-hydroxy benzoyl moiety. The ^1H NMR and ^{13}C NMR spectra of **1** (table 1) also revealed the presence of two glucopyranose moieties. The β-anomeric configurations for the glucopyranoses were determined from the anomeric proton signals at δ 4.21 (1H, d, $J = 7.8$ Hz) and 5.59 (1H, d, $J = 8.1$ Hz). On acid hydrolysis, **1** afforded glucose, which was identified by high-performance thin-layer chromatography (HPTLC) with authentic sample. The glucosyl-(1 → 2)-glucosyl linkage of the glycoside

Table 1. The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for **1** and **2** (CD_3OD, δ, ppm).

	1		***2***	
No.	δ_C	δ_H	δ_C	δ_H
1	71.5 (CH)	3.91 m[a]	71.7 (CH)	3.93 m
2	32.2 (CH_2)	2.02 m	32.1 (CH_2)	2.10 m
		1.75 m		1.78 m
3	32.1 (CH)	2.89 m	32.3 (CH)	3.01 m
4	29.3 (CH_2)	2.34 d (14.5)	29.3 (CH_2)	2.55 d (14.5)
		1.86 m		1.89 m
5	76.2 (CH)	4.27 br s	76.5 (CH)	4.30 br s
6	75.5 (C)		75.4 (C)	
7	213.8 (C)		214.0 (C)	
8	100.6 (C)		100.6 (C)	
9	32.8 (CH_2)	2.23 dd (14.7, 3.0)	32.9 (CH_2)	2.16 m[h]
		1.95 dd (14.7, 3.0)		2.02 m
10	70.4 (CH)	5.29 m	70.1 (CH)	5.36 m
11	34.3 (CH)	2.14 m	34.3 (CH)	2.14 m[h]
12	63.5 (CH_2)	4.00 t (11.2)	63.5 (CH_2)	3.99 m[d]
		3.55 m[b]		3.54 m[e]
13	176.0 (C)		176.9 (C)	
14	13.1 (CH_3)	0.87 d (7.0)	12.9 (CH_3)	0.86 d (7.0)
1′	168.0 (C)		167.9 (C)	
2′	123.1 (C)		122.9 (C)	
3′, 7′	133.1 (CH)	8.02 d (8.7)	133.3 (CH)	8.04 d (8.7)
4′, 6′	116.6 (CH)	6.91 d (8.7)	116.7 (CH)	6.99 d (8.7)
5′	163.2 (C)		163.5 (C)	
1″	93.8 (CH)	5.59 d (8.1)	73.1 (CH)	3.44 m[f]
2″	83.1 (CH)	3.49 m	72.0 (CH)	4.02 m[d]
3″	77.9 (CH)	3.24 m[c]	73.0 (CH)	4.81dd (10.0, 2.5)
4″	70.7 (CH)	3.52 m	82.7 (CH)	4.02 m[d]
5″	79.0 (CH)	3.41 m	76.5 (CH)	3.42 m[f]
6″	62.3 (CH_2)	3.91 m[a]	73.6 (CH)	3.73 t (9.5)
		3.78 dd (12.0, 5.5)		
1‴	106.1 (CH)	4.21 d (7.8)	106.3 (CH)	4.24 d (7.5)
2‴	77.9 (CH)	3.24 m	76.0 (CH)	3.09 m
3‴	76.0 (CH)	3.11 m	78.2 (CH)	3.18 m[g]
4‴	70.6 (CH)	3.24 m[c]	70.6 (CH)	3.18 m[g]
5‴	77.6 (CH)	2.75 m	77.4 (CH)	2.47 m
6‴	61.9 (CH_2)	3.56 m[b]	61.8 (CH_2)	3.52 m[e]
		3.54 m[b]		3.48 m

[a–h]Overlapping signals.

moiety was assigned from the cross-peaks observed between terminal glucose H-1‴ (δ 4.21) and inner glucose C-2″ (δ 83.1) in the HMBC spectrum, and the downfield shift of C-2″ (δ 83.1), comparing with the non-substituted C-2″ (δ 74.0) of glucose [4].

Except for the characteristic signals of glucose moiety, the ^{13}C NMR spectrum of **1** showed 21 signals for the aglycon portion. A detailed comparison of the NMR spectral data of the aglycon with those of phyllaemblic acid, which was isolated from *Phyllanthus emblica* of the family Euphorbiaceae [5], indicated that both of them were almost identical, except for the benzoyl unit being replaced by a *p*-hydroxy benzoyl unit in the aglycon of **1**. The linkage of glycosidation was revealed to be at C-13 by an HMBC experiment, which showed a long-range correlation between C-13 (δ 176.0) and the anomeric proton H-1″ (δ 5.59) of glucose. Based on the above results, the structure of glochicoccinoside A (**1**) was established as 5′-hydroxy-phyllaemblic acid *O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside ester.

Glochicoccinoside B (**2**) was assigned the same molecular formula, $C_{33}H_{44}O_{22}$, as **1**, from the positive HRESI-MS (*m*/*z* 783.2331 $[M + Na]^+$), which was confirmed by the ^{13}C and DEPT NMR spectral data. Detailed analysis of the ^{1}H NMR and ^{13}C NMR spectra (table 1) revealed that the structure of **2** has the same aglycone as **1**, as well as the presence of glucopyranose and inositol moieties. A triplet at δ 3.73 with the coupling constant value 9.5 Hz, revealed H-6″ and its vicinal protons (H-1″ and H-5″) all in axial orientations, and the coupling constant value of H-3″ (δ 4.81, dd, J = 10.0, 2.5 Hz), also revealed it in an axial orientation. The ROESY spectrum of **2** revealed that H-1″, H-2″, H-3″, H-5″ were on the same face of the skeleton, and the correlation of H-4″ with H-6″, suggested an axial orientation of H-4″. Meanwhile, the coupling constant value between H-3″ and H-2″ ($J_{2''/3''}$ = 2.5 Hz), implied H-2″ in an equatorial orientation. On the basis of these data, the possible chair conformation (in MeOH) of the inositol moiety was therefore proposed as showed in figure 2, which was the same as that of *O*-α-D-galactopyranosyl-(1 → 3)-4-*O*-methyl-D-*myo*-inositol [6] (figure 2). The β configuration for glucopyranose was determined from the coupling constant value of the aromatic proton (7.5 Hz), and the glucosyl-(1 → 4)-inositol linkage of the glycoside moiety was assigned from the cross-peaks observed between glucose H-1‴ (δ 4.24) and inositol C-4″ (δ 82.7) in the HMBC spectrum (figure 1); also in the ROESY spectrum (figure 2) of **2**, a correlation between glucose H-1‴ (δ 4.24) and inositol H-4″ (δ 4.02) was obvious. The linkage of glycosidation was also revealed to be at C-13 by HMBC experiment which showed a long-range correlation between H-3″ (δ 4.81) of inositol and C-13 (δ 176.9). Glochicoccinoside B (**2**) therefore was concluded to be 5′-hydroxy-phyllaemblic acid, *O*-β-D-glucopyranosyl-(1 → 4)-3-*O*-D-*myo*-inositol ester.

Both new compounds are highly oxygenated norbisabolane-type sesquiterpenoid glycosides, which were isolated from the genus *Glochidion* for the first time. Cytotoxic activities and the antioxidant effect were also evaluated, but the results showed that two compounds were inactive against human lung adenocarcinoma cell line A-549, mice leucocythaemia cell line P-388, human leucocythaemia cell line HL-60 and human liver cancer cell line BEL-7402, while there was no activity on DPPH radical scavenging.

3. Experimental

3.1 General experimental procedures

HPLC was performed on an Agilent 1100, with XTerra Prep RP-18 column (10 μm, 7.8 × 150 mm). Optical rotations were measured with a Horiba SEPA-300 polarimeter or

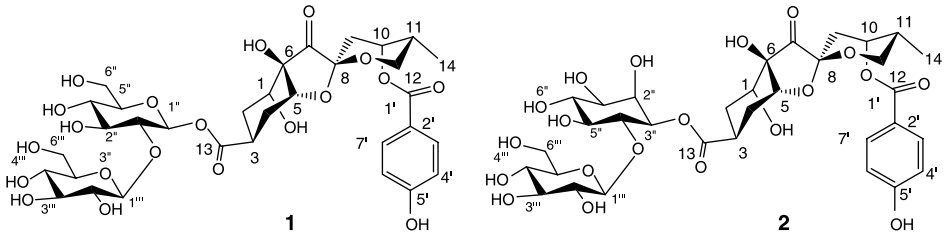

Figure 1. The structures of **1** and **2**.

JASCO DIP-370 digital polarimeter. UV spectra were obtained using a Shimadzu UV-2401PC spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr disks. ^{1}H NMR and ^{13}C NMR experiments were performed on a DRX-500 NMR spectrometer with TMS as internal standard. ESI-MS and HRESI-MS were measured on a VG Auto Spec 3000 spectrometer. Column chromatography was performed with silica gel (200–300 mesh; Qingdao Marine Chemical Inc. Qingdao, China), silica gel H (10–40 μm; Qingdao), and Lichroprep RP-18 gel (40–63 μm; Merck, Darmstadt, Germany). TLC plates (0.20–0.25 mm; Qingdao) were visualised under UV light or by spraying with 10% H_2SO_4 in 95% EtOH, followed by heating.

3.2 Plant material

The rhizomes of *Glochidion coccineum* were obtained from Guiyang, Guizhou Province, China, in May 2005. The plant was identified by Professor De-Yuan Chen, Guiyang College of Traditional Chinese Medicine, Guiyang, where a voucher specimen (GTCM No: 050517) is deposited.

3.3 Extraction and isolation

The dried rhizomes of *G. coccineum* (12 kg) were extracted (3 × 18 L) with 90% EtOH for 2 h under reflux. After the removal of EtOH *in vacuo*, the viscous extract was partitioned with EtOAc/H_2O. The water-soluble fraction (78 g) was subjected to macroporous absorption resin D-101, eluting with MeOH/H_2O (0:1, 3:7, 1:1, 1:0), to give four parts. The third part was further chromatographed on silica gel, eluting with $CHCl_3$/MeOH (10:1, 9:1, 8:2, 7:3, 1:1), to

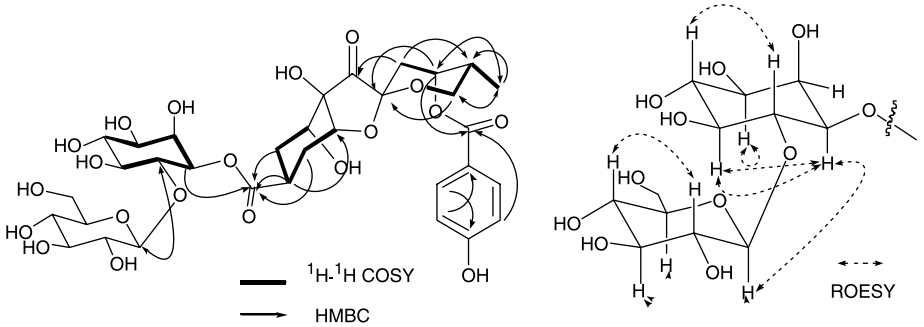

Figure 2. The key ^{1}H-^{1}H COSY, HMBC and ROESY correlations of **2**.

give five fragments (I–V). Then fraction II was subjected to RP-18 column, eluting with MeOH/H_2O (0:1, 1:3, 1:1, 3:1, 1:0), to yield five subfractions (II_a–II_e), Fractions II_c and II_d were finally purified by HPLC [ODS C-18; Mobile phase: CH_3CN/H_2O (30:70)], to afford compounds **3** (21 mg) and **4** (13 mg), respectively. Fraction III was subjected to RP-18 column as well as II, to get five subfractions (III_a–III_e). Fraction III_c was also purified by HPLC [ODS C-18; Mobile phase: CH_3CN/H_2O (22:78)], to afford compounds **1** (30 mg) and **2** (5 mg).

3.3.1 Glochicoccinoside A (1). Amorphous powder, $[\alpha]_D^{26}$ + 5.40 (*c* 1.05, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 258 (3.61) nm; IR (KBr) ν_{max} 3425, 2932, 1777, 1740, 1690, 1609, 1515, 1449, 1280, 1169, 1076, 1007, 851, 773 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectral data: see table 1; ESI-MS *m/z* 783 $[M + Na]^+$, HRESI-MS *m/z*: 783.2331 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{20}Na$, 783.2323).

3.3.2 Glochicoccinoside A (2). Amorphous powder, $[\alpha]_D^{28}$ −4.94 (*c* 0.81, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 258 (3.60), 320 (1.56), 374 (1.37) nm; IR (KBr) ν_{max} 3423, 2928, 1777, 1691, 1608, 1514, 1448, 1279, 1169, 1076, 1009, 948, 852, 701 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectral data: see table 1; ESI-MS *m/z*: 783 $[M + Na]^+$, HRESI-MS *m/z*: 783.2329 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{20}Na$, 783.2323).

3.4 Acid hydrolysis of 1

A solution of glochicoccinoside A (**1**) (2 mg) in 0.5 N HCl (2 ml) was heated at 95°C for 3 h in water bath. The mixture was then neutralised with $NaHCO_3$ and concentrated to dryness under reduced pressure. The residue was compared with authentic samples by HPTLC ($CHCl_3/CH_3OH/H_2O/CH_3COOH$, 16:9:2:1, detection with spray agent: 10% H_2SO_4 in 95% EtOH). The R_f value of glucose was 0.40.

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