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A New Phloroglucinol Glucoside from the Whole Plants of *Glochidion eriocarpum*

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The genus *Glochidion* (Euphorbiaceac), composed of more than 300 species globally, is mainly growing in Asia and few in America and Africa, with about 28 species and two varieties distributed in the southwest of China. Some *Glochidion* plants have been used for treating cold, fever, cough, malaria, gastroenteritis, diarrhea, arthritis, bruises, gynecological diseases, hepatitis, stomatitis, ulcers and many other diseases among folks.[1] Previous investigations resulted in the isolation of flavonoids, sesquiterpenoids, triterpenoids, and coumarins from *Glochidion* spp..[2]

G. eriocarpum Champ. ex Benth. (Euphorbiaceae), a shrub up to 5 m height, is mainly found in the southern parts of China and also distributed in Vietnam. The whole plant, roots or leaves have been utilized medicinally for treating allergy caused by *Toxicodendron vernicifluum*, urticaria, enteritis, infectious diarrhea, *etc*.[1] Chemical studies on the leaves and stem barks of *G. eriocarpum* previously have revealed the occurrence of cytotoxic triterpenoids.[3-5] In our search for new bioactive secondary metabolites from medicinal plants, a new isovalerylphloroglucinol glucoside, 2-β-D-glucopyranosyloxy-4-methoxy-6-hydroxy-isovalero-phenone (**1**), was isolated from the whole plants of *G. eriocarpum*, together with eight ionon (**2**-**9**), six phenolic (**10**-**15**), and two lignan (**16**, **17**) glucosides (Figure 1). Their structures were elucidated by extensive spectroscopic analyses. Herein, we describe the structure determination of compound **1** by detailed spectroscopic analyses. The relative configurations of **2** and **3** were reported for the first time. In addition, the isolated compounds **4**, **7**-**9**, **13** and **15** were evaluated for their cytotoxic and antiviral activities, unfortunately none of them showed any activities.

Compound **1**, obtained as a white powder, has the molecular formula $C_{18}H_{26}O_9$ established by HREIMS *m/z* 386.1572 $[M]^+$ (calcd for $C_{18}H_{26}O_9$, 386.1577) and ^{13}C NMR (DEPT) data, requiring for six degrees of unsaturation. The ^1H NMR spectrum showed the existence of two doublet methyl signals (δ_H 0.97, d, J = 6.6 Hz, H-5'; δ_H 0.93, d, J = 6.6 Hz, H-4'), a multiplet methine (δ_H 2.24, m, H-3'), and two geminally coupled methylene protons (δ_H 2.87, dd, J = 15.9, 7.5 Hz H-2'a; δ_H 3.16, dd, J = 15.9, 6.4 Hz H-2'b), arising from an isobutyl group based on ^1H-^1H COSY correlations (Figure 2). Moreover, two *meta*-coupled aromatic (δ_H 6.30 and 6.11, each 1H, d, J = 2.4, H-3 and H-5) and one methoxy (δ_H 3.81, s) protons were observed in ^1H NMR spectrum, together with a set of proton signals at δ_H 5.06 (d, J = 7.8 Hz, H-1"), 3.54 (dd, J = 9.1, 7.8 Hz, H-2"), 3.47 (t, J = 9.1 Hz, H-3"), 3.37 (t, J = 9.1, H-4"), 3.48 (m, H-5"), 3.68 (dd, J = 12.1, 6.1 Hz, H-6"), and 3.90 (dd, J = 12.1, 2.1 Hz, H-6"), indicating the presence of a β-glucopyranosyl moiety. This was supported by the ^1H-^1H COSY data. The ^{13}C NMR (DEPT) spectra of **1** showed the presence of 18 carbons, identified as six aromatic carbons resonated between δ_C 94 to 168 due to a tetra-substituted benzene ring, a glucopyranosyl unit (δ_C 102.1, 74.9, 78.7, 71.4, 78.6, 62.5), one ketone (δ_C 207.6), one methoxy (δ_C 56.1), and four aliphatic carbons [δ_C 54.2 (CH_2), 26.1 (CH), 23.2 (CH_3) and 22.9 (CH_3)]. The aforementioned data (Table 1) of **1** exhibited close resemblance to those of an isovalerylphloroglucinol glucoside, 2-β-D-glucopyranosyloxy-4,6-di-hydroxyisovalerophenone isolated from strawberry *Fragaria ananassa*,[6] except the appearance of an additional methoxy signal (δ_H 3.81, δ_C 56.1) in **1**.

The HMBC correlations of the aliphatic H-4' (δ_H 0.93) and H-5' (δ_H 0.97) with C-2' (δ_C 54.2), and H-2' (δ_H 2.87 and

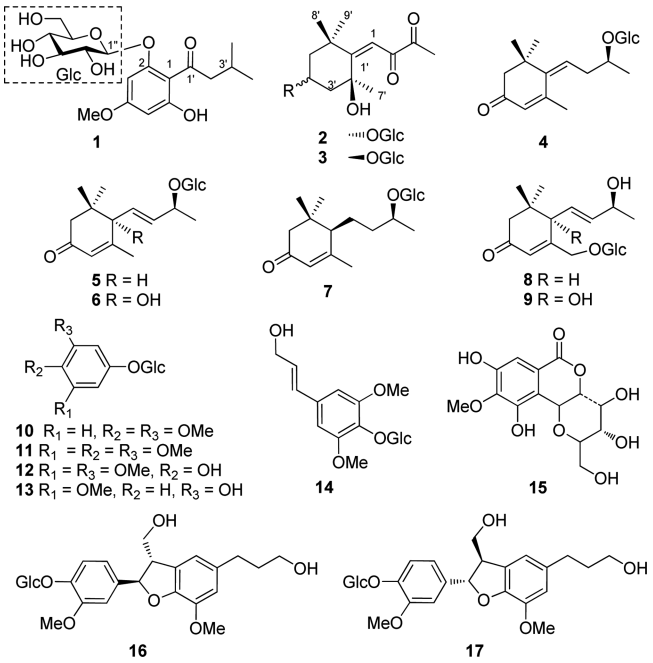

Figure 1. Chemical structures of compounds **1**-**17**.

Table 1. ^{13}C NMR and ^{1}H NMR data of **1** (CD_3OD, *J* in Hz)

No.	δ_C	δ_H
1	107.9, s[a]	
2	161.8, s	
3	94.8, d	6.30, d (2.4)
4	167.2, s	
5	96.6, d	6.11, d (2.4)
6	167.4, s	
1'	207.6, s[a]	
2'	54.2, t	3.16, dd (15.9, 6.4) 2.87, dd (15.9, 7.5)
3'	26.1, d	2.24, m
4'	22.9, q	0.93, d (6.6)
5'	23.2, q	0.97, d (6.6)
1"	102.1, d	5.06, d (7.8)
2"	74.9, d	3.54, dd (9.1, 7.8)
3"	78.7, d	3.47, t (9.1)
4"	71.4, d	3.37, t (9.1)
5"	78.6, d	3.48, m
6"	62.5, t	3.90, dd (12.1, 2.1) 3.68, dd (12.1, 6.1)
OCH_3	56.1, q	3.81, s

[a]Determined by HMBC experiment

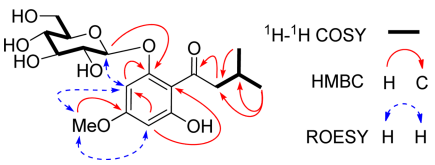

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and ROESY correlations of **1**.

3.16) with C-1' (δ_C 207.6, a keto carbon) supported the presence of an isovaleryl (3-methylbutanone) group in **1** (Figure 2). Extensive analysis of HSQC, ^{1}H–^{1}H COSY, and HMBC spectra revealed that **1** contained a phloroglucinol moiety linked with the isovaleryl unit at C-1, which caused the upfield shifting of C-3 and C-5.[7] In the HMBC spectrum of **1**, the glucopyranosyl anomeric proton at δ_H 5.06 and the methoxy protons at δ_H 3.81 showed significant correlations with the phloroglucinol aromatic carbons at δ_C 161.8 (C-2) and 167.4 (C-4), respectively. The locations of the methoxy and glucopyranosyl moieties were further confirmed on the phloroglucinol C-2 and C-4, respectively, by the ROESY experiment. In which, the methoxy protons displayed correlations with both aromatic protons at δ_H 6.30 (H-3) and 6.11 (H-5), while the anomeric proton at δ_H 5.06 only correlated with one aromatic proton at δ_H 6.30 (H-3). Thus, the structure of **1** was established as 2-β-D-gluco-pyranosyloxy-4-methoxy-6-hydroxyisovalerophenone.

Compounds **2** and **3** had the same molecular formula $C_{19}H_{30}O_9$, as established from the EIMS and NMR data, allowing five degrees of unsaturation. The ^{13}C-NMR spectra of both **2** and **3** revealed the presence of two ketones, one trisubstituted double bond, four methyls, two aliphatic methylenes, one oxymethine and two quaternary carbons, in addition to a hexosyl unit. The aforementioned NMR features

Table 2. ^{13}C NMR and ^{1}H NMR data of **2** and **3** (CD_3OD, *J* in Hz)

No.	**2**		**3**	
	δ_C	δ_H	δ_C	δ_H
1	101.3, s	5.83, s	102.8, s	5.88, s
2	211.7, s		211.5, s	
3	201.1, s		201.9, s	
4	26.7, q	2.16, s	26.7, q	2.18, s
1'	120.2, s		123.7, s	
2'	72.5, s		72.5, s	
3'	46,7, t	2.27, dd (11.5, 4.2) 1.35, dd (11.5, 4.6)	45.1, t	1.89, dd (13.0, 2.7) 1.47, dd (13.0, 10.5)
4'	72.6, d	4.25, m	73.5, d	4.06, m
5'	48.2, t	2.05, dd (11.6, 4.5) 1.38, dd (11.6, 4.6)	48.5, t	2.21[a] 1.70, dd (12.9, 10.0)
6'	37.2, s		35.7, s	
7'	31.0, q	1.30, s	30.5, q	1.49, s
8'	32.4, q	1.07, s	30.0, q	1.18, s
9'	29.6, q	1.29, s	32.6, q	1.10, s
1"	101.3, d	4.34, d (7.8)	102.7, d	4.32, d (7.8)
2"	75.2, d	3.05, m	75.2, d	3.06, m
3"	78.2, d	3.25[a]	78.1, d	3.25[a]
4"	72.5, d	3.21[a]	71.8, d	3.20[a]
5"	78.1, d	3.21[a]	78.0, d	3.20[a]
6"	62.8, t	3.77, m 3.59, m	62.9, t	3.79, dd (11.9, 1.9) 3.55, dd (11.9, 5.5)

[a]Overlapped

(Table 2) of **2** and **3** were identical with those of cannabiside D, an ioion glucoside previously reported from *Senecio cannabifolius* by Wu *et al.*, but the configuration of cannabiside D was not determined.[8] Herein we describe the relative configurations of **2** and **3** for the first time.

For compound **2**, the small coupling values of $J_{3,4}$ (4.6 Hz) and $J_{4,5}$ (4.5 Hz) indicated that H-4' was in equatorial orientation (Table 2). Combining the ROESY correlations (Figure 3) of H-1" with H-5'$_{eq}$, H-8' with both H-3'$_{ax}$ and H-4', and H-7' with H-9' confirmed that the 4'-*O*-Glc and 2'-OH were orientated to be α and β directions, respectively. On the other hand, the large coupling values of $J_{3,4}$ (10.5 Hz) and $J_{4,5}$ (10.0 Hz) observed in ^{1}H NMR spectrum of compound **3** (Table 1) indicated that H-4' of 3 was axially oriented. In the same case of **2**, the ROESY correlations (Figure 3) of H-1" with H-5'$_{ax}$, H-9' with both H-4' and H-7' and H-4' with H-7' further confirmed that both 4'-*O*-Glc and 2'-OH were located on β orientations. Therefore, the relative configurations of **2** and **3** were established as shown in Figure 1.

The ^{13}C and ^{1}H NMR spectra of compounds **2** and **3** measured in DMSO-d_6 (see Supporting Information) were compared with those of cannabiside D, indicating that **2** had the same structure as cannabiside D, whose relative configurations were reported for the first time. Moreover, compound **3** were determined to be 1-4'-β-(2'-hydroxy-2',6',6'-trimethyl-4'-β-D-glucopyranosyl-cyclohexylidene)-butane-2,3-dione, and named as glochidionionoside E.

The structures of the known compounds were elucidated as (*Z*)-4-[3'-(β-D-glucopyranosyloxy)butylidene]-3,5,5-tri-

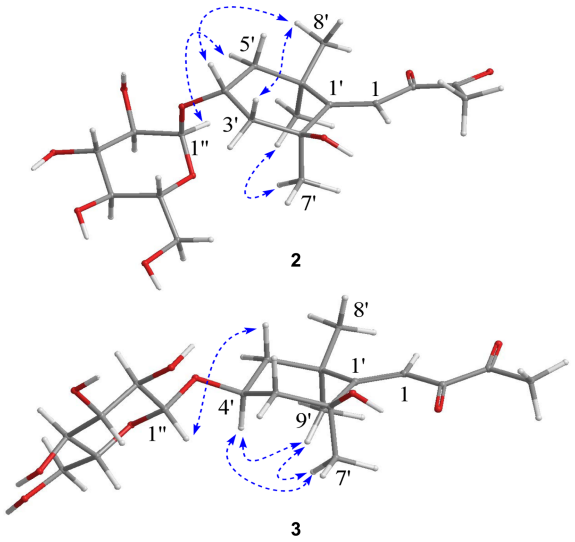

Figure 3. Key ROESY correlations of **2** and **3**.

methyl-2-cyclohexen-l-one (**4**),[9] (6*R*,9*S*)-3-oxo-α-ionol β-D-glucopyranoside (**5**),[10] (6*S*,9*S*) roseoside (**6**),[11] blumenol C glucoside (**7**),[12] glochidionionoside C (**8**),[13] glcochidionionoside A (**9**),[13] 1,2-dimethoxyphenyl-4-*O*-β-D-glucoside (**10**),[14] koaburaside monomethyl ether (**11**),[15] koaburaside (**12**),[15] 1-β-D-glucopyranosyloxy-3-methoxy-5-hydroxybenzene (**13**),[16] syringin (**14**),[17] bergenin (**15**),[18] 7*R*,8*S*- (**16**)[19] and 7*S*,8*R*- (**17**)[19] dihydrodehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside by comparing their spectroscopic data with those reported in literatures. Compounds **16** and **17** were isolated as a mixture of chiral isomers in a ratio of 1:1 as estimated by CD spectra and optical rotation.

The cytotoxicity of compounds **4**, **7**-**9**, **13** and **15** was tested on the ECA109 human esophagus cancer cell and Vero cell lines determined by an MTT assay. And these compounds were also evaluated for their anti HSV-1 effects using Acyclovir (ACV) as positive control. The EC_{50} value was calculated by MTT method. Unfortunately the results showed that none of the tested compounds displayed any activities at the concentration of 200 μM.

Experimental

General Procedures. Optical rotations were performed on a P-1020 polarimeter (JASCO, Tokyo, Japan). IR spectra were measured on a Bruker Tensor 27 spectrometer with KBr pellets. UV spectra were obtained on a 210A double-beam spectrophotometer (Shimadzu, Kyoto, Japan). 1D- and 2D-NMR spectra were run on Bruker DRX-500 and AV-600 instruments operating at 500 and 600 MHz for ^{1}H, and 125 and 150 MHz for ^{13}C, respectively. The MS data were recorded on a VG Auto Spec-3000 spectrometer (VG, Manchester, U.K.). HREIMS were recorded on a Waters Autospec Premier P776. Column chromatography (CC) was performed with Diaion HP20SS (Mitsubishi Chemical Industry, Ltd.), silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China), and Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co. Ltd. Japan). Thin-layer chromatography (TLC) was carried out on silica gel H-precoated plates (Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China) with $CHCl_3$/MeOH/H_2O (8.5:1.5:0.1, 8:2:0.2 or 7:3:0.5, v/v) as developing solvents. Spots were detected by spraying with 10% H_2SO_4 in EtOH followed by heating. Semi-preparative HPLC separation was performed on an Agilent 1260 liquid chromatography with a 5 μm Waters Sunfire-C18 column (10 × 250 mm, Waters, Sunfire TM, USA).

Plant Material. The whole plants of *G. eriocarpum* were collected from Yunnan Province, People's Republic of China, in May 2012. Voucher specimens (KUN_0186703) were deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The whole plants of *G. eriocarpum* (1.5 kg) were extracted with MeOH (3 times, each time 3h) under reflux at 60 °C. The resulting MeOH extract (64.5 g) was applied to Diaion HP20SS column chromatography (CC), eluting with MeOH/H_2O (0:1–1:0), to give five fractions (Fr. 1–5). Fr. 2 (7.6 g) was subjected to CC over Sephadex LH-20 (MeOH/H_2O, 0:100, 30:70, 60:90, 100:0) and silica gel ($CHCl_3$/MeOH/H_2O, 8:2:0.2–6:4:1) to give compounds **9** (6 mg), **13** (5 mg), and **15** (10 mg). Fr. 4 (15 g) was applied to CC over Sephadex LH-20 (MeOH/H_2O, 0:100, 30:70, 60:90, 100:0) and silica gel ($CHCl_3$/MeOH/H_2O, 8:2:0.2–6:4:1), and then the semi-preparative HPLC with a isocratic flow of H_2O/MeCN (flowing rate: 3 ml/min, 82:18) to afford compounds **4** (5 mg), **5** (13 mg), and **7** (13 mg). The residue was chromato-graphed over MCI-gel CHP-20P (MeOH/H_2O, 5% to 100% with a 10% increment), then purified by semi-preparative HPLC (flowing rate: 5 mL/min, 82:18) to afford **1** (2 mg), **6** (22 mg), **8** (5 mg), **3** (3 mg). Fr. 5 (8.2 g) was subjected to CC over Sephadex LH-20 (MeOH/H_2O, 0:100, 30:70, 60:90, 100:0) and silica gel ($CHCl_3$/MeOH/H_2O, 8:2:0.2–6:4:1), and then purified through the semi-preparative HPLC to afford compounds **2** (2 mg), **10** (3 mg), **11** (3 mg), **12** (5 mg), **14** (6 mg), **16** and **17** (3 mg).

2-β-D-Glucopyranosyloxy-4-methoxy-6-hydroxyisovalerophenone (1). Amorphous white powder, $[\alpha]_D^{24}$ −14.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.15), 224 (4.17), 284 (4.20); IR (KBr) ν_{max} 3450, 2925, 1627, 1597, 1427, 1384, 1276, 1220, 1167, 1083 cm^{-1}; HREIMS *m/z* 386.1572 $[M]^+$ (calcd for $C_{18}H_{26}O_9$, 386.1577); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) are given in Table 1.

Cannabiside D (2). Amorphous white powder, $[\alpha]_D^{26}$ −42.0 (*c* 0.10, MeOH); positive ESIMS *m/z* 425 $[M+Na]^+$, $C_{19}H_{30}O_9$; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) are given in Table 1.

Glochidionionoside E (3). Amorphous white powder, $[\alpha]_D^{25}$ −44.1 (*c* 0.10, MeOH); negative ESIMS *m/z* 437 $[M+Cl]^-$, $C_{19}H_{30}O_9$; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) are given in Table 1.

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Supporting Information. The ^{1}H NMR, ^{13}C NMR, DEPT and ROESY spectra measured in methanol-d_4 of **1**-**3**, the HSQC, HMBC, and ^{1}H-^{1}H COSY spectra of **1**, the ^{1}H and ^{13}C NMR data and spectra measured in DMSO-d_6 of **2** and **3** are provided.

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