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Isolation of a New Flavonone Glycoside from *Eria marginata*

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Eria marginata Rolfe (Orchidaceae) is distributed in China, Burma and Thailand.[1] In China, plants of the *Eria* genus have been used in folk medicine as a substitute or an adulterant of "Shi-hu", an important Chinese herb prepared from the dried stems of species of *Dendrobium* used as a tonic to nourish the stomach, promote secretion of saliva, and reduce fever.[2] Previously chemical constituents of *E. carinata*, *E. confusa*, *E. convallarioides*, *E. flava*, *E. spicata* and *E. stricta* have been investigated and nine phenanthrenes, one bibenzyl, two phenethylamines, four sterols, 3-(4-hydroxy-3-methoxyphenyl)-propenal and one uncharacterized fatty alcohol were isolated.[3-7] Erianin, a bibenzyl isolated from *E. carinata* showed significant antitumor activities.[8] However, there was no report so far on the chemical constituents and biological activity of the secondary metabolites from *E. marginata*. As a part of our research of structurally unique and biologically active compounds from medicinal plants of Yunnan, China, we have isolated and identified a new flavonone glycoside (**1**), as well as thirteen known compounds (**2-14**) from *E. marginata*. Our procedures and findings are reported in this paper (Figure 1).

The chemical structures of known compounds including two flavanones, pinocembrin (**2**)[9] and naringenin (**3**),[10] three 9,10-dihydrophenanthrenes, erianthridin (**4**),[11] coelonin (**5**)[12] and 4-methoxy-9,10-dihydrophenanthrene-1,2,7-triol (**6**),[13] a phenanthrene, nudol (**7**),[11] two bibenzyls, 3,4'-dihydroxy-5-methoxy bibenzyl (**8**)[14] and batatasin III (**9**),[15] a oleanane triterpenod, β-amyrin (**10**),[16] two sterols, β-sitosterol (**11**) and β-sitosteryl-3-*O*-β-D-glucopyranoside-2'-*O*-palmitate (**12**),[17] vanillin (**13**)[18] and mannitol (**14**)[19] were determined by comparison of the obtained spectroscopic data with those reported in the literature. Compounds **2**, **3**, **6**, **8**, **9**, **10**, and **12-14** were isolated from the *Eria* genus for the first time and the others were originally obtained from *E. marginata*.

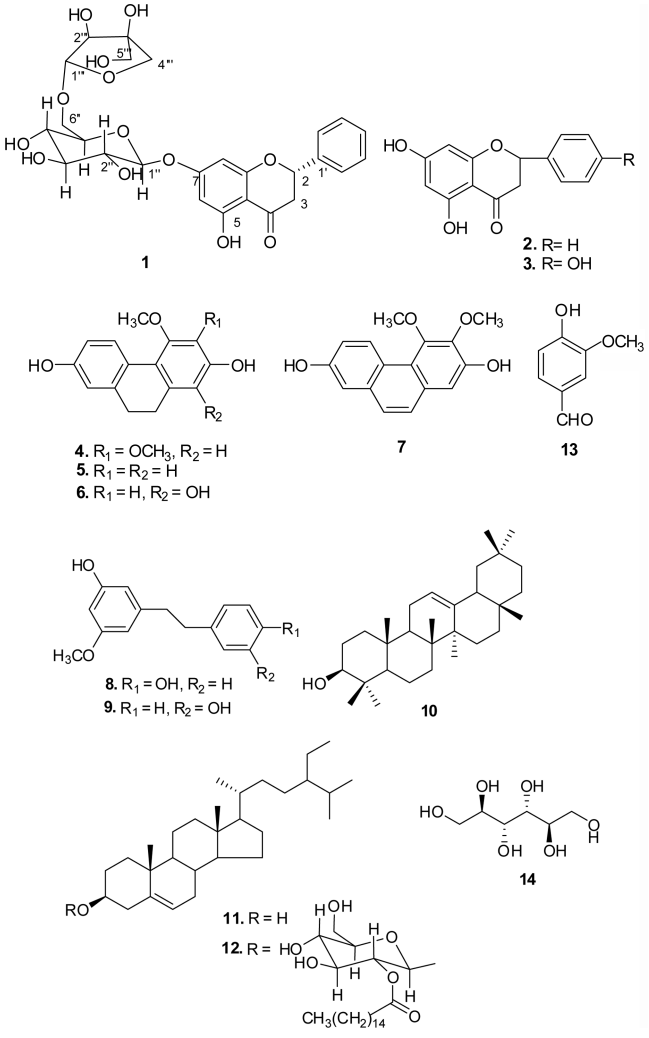

Figure 1. Structure of compounds isolated from *Eria marginata*.

Compound **1** was obtained as a colorless amorphous powder. The molecular formula of **1** was determined to be $C_{26}H_{30}O_{13}$ by HR-EI-MS ([M]$^+$ 550.1679, calcd for 550.1686). Its UV absorption bands at 330 (sh) and 284 nm indicated the presence of a flavanone skeleton.[20] Its IR absorptions at 3424 and 1648 cm^{-1} inferred the hydroxyl and carbonyl groups, respectively. The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of three mutually coupled protons at δ_H 5.49 (1H, dd, J = 13.0, 3.0 Hz), 3.15 (1H, dd, J = 17.5, 13.0 Hz) and 2.85 (1H, dd, J = 17.5, 3.0 Hz), corresponding to the moiety of flavanone H-2 and H-3. The presence of mono-substituted flavanone B-ring was inferred by five mutually coupled protons at δ_H 7.55 (2H, d, J = 7.0 Hz), and 7.43 (2H, dd, J = 7.0, 7.0 Hz), 7.36 (1H, dd, J = 7.0, 2.0 Hz). Besides, meta-coupled H-6 at δ_H 6.62 (1H, d, J = 2.5 Hz) and H-8 at δ_H 6.53 (1H, d, J = 2.5 Hz) of ring A in **1** were observed, together with a series of sugar signals at δ_H 4.10–4.65, with two anomeric protons at δ_H 5.62 (1H, d, J = 7.5 Hz) and 5.68 (1H, d, J = 2.5 Hz).

The ^{13}C NMR spectra (Table 1) also showed **1** to be a flavanone diglycoside possessing a furanosyl and a pyranosyl

Table 1. ^{1}H and ^{13}C NMR data of Compound **1** isolated from *Eria marginata* (measured in C_5D_5N)

No	δ_H	δ_C
2	5.49 (1H, dd, *J* = 13.0, 3.0 Hz)	79.3 d
3	3.15 (1H, dd, *J* = 17.5, 13.0 Hz)	43.1 t
	2.85 (1H, dd, *J* = 17.5, 3.0 Hz)	
4		196.5 s
5		164.2 s
6	6.62 (1H, d, *J* = 2.5 Hz)	97.7 d
7		166.3 s
8	6.53 (1H, d, *J* = 2.5 Hz)	96.3 d
9		163.1 s
10		104.5 s
1'		138.9 s
2',6'	7.55 (2H, d, *J* = 7.0 Hz)	126.7 d
3',5'	7.43 (2H, dd, *J* = 7.0, 7.0 Hz)	128.9 d
4'	7.36 (1H, dd, *J* = 7.0, 2.0 Hz)	128.9 d
Glc-1"	5.62 (1H, d, *J* = 7.5 Hz)	101.2 d
2"	4.25 (1H, m)	74.2 d
3"	4.32 (1H, m)	78.1 d
4"	4.12 (1H, m)	71.0 d
5"	4.21 (1H, m)	77.1 d
6"	4.65 (1H, d, *J* = 10 Hz)	68.5 t
	4.09 (1H, d, *J* = 10 Hz)	
Api-1'"	5.68 (1H, d, *J* = 2.5 Hz)	110.9 d
2'"	4.31 (1H, d, *J* = 5.5 Hz)	77.4 d
3'"		80.1 s
4'"	4.55 (1H, d, *J* = 9.5 Hz)	74.9 t
	4.31 (1H, d, *J* = 9.5 Hz)	
5'"	4.10 (2H, d, *J* = 6.0 Hz)	65.6 t

moiety. By acid hydrolysis, **1** afforded D-glucose and apiose, which was detected by direct co-TLC comparison with authentic samples. The glucose moiety was determined to have a β-configuration at H-1" due to a large coupling constant for the anomeric proton of the sugar unit at δ_H 5.62 (1H, d, *J* = 7.5 Hz),[20] and the apiose unit was also determined to have a β-configuration at C-1'" due to the chemical shift of its anomeric carbon signal in the ^{13}C NMR at δ_C 110.9.[21,22] Comparison of the ^{13}C NMR spectra of **1** with those of (2*S*)-pinocembrin 7-*O*-[β-D-apiosyl(1→2)]-β-D-glucoside revealed they were very similar, except for the glucose C-6" signal in **1** was downfield from δ_C 61.0 to 68.5, suggesting that the interglycosyl linkage is apiosyl-(1→6)-glucose,[20,23] which was confirmed by the presence of the HMBC correlation between H-1'" and C-6" (Figure 2). The glycosidation position was further determined by the presence of the three-bond HMBC correlation between the glucosyl anomeric proton H-1" and C-7 of ring A. The complete assignment of all protons and carbons were established by extensive use and interpretation of ^{1}H-^{1}H COSY, HMQC, and HMBC spectra. By the rotation comparison with (2*S*)-pinocembrin 7-*O*-[β-D-apiosyl(1→2)]-β-D-glucoside,[20] C-2 was assigned as the *S*-configuration, which was further confirmed by the CD spectrum of **1** exhibiting a positive Cotton effect at 330 nm and a negative Cotton effect at 284 nm, coincide with that of (2*S*)-pinocembrin 7-*O*-[β-D-apiosyl(1→2)]-β-D-glucoside. Consequently, the structure of compound **1** was assigned as (2*S*)-pinocembrin 7-*O*-[β-D-apiosyl(1→6)]-β-D-glucoside.

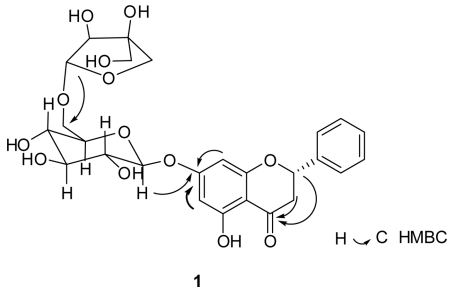

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Figure 2. Key HMBC (H→C) correlations for **1**.

Experimental

General Experimental Proceduces. Optical rotations were determined on a Horiba SEAP-300 spectropolarimeter. UV data were obtained on a Shimadzu UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). CD spectra were recorded on a Chirascan Circular Dichroism spectrometer (Applied Photophysics, Surrey, United Kingdom). IR was measured on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Bruker DRX-AV-500 spectrometer at 500 MHz for 1H and 125 MHz for 13C using standard pulse sequence programs. All chemical shifts were recorded with respect to TMS as an internal standard. MS was obtained on Finnigan MAT 95 instrument and VG Auto Spec-3000 spectrometer. Column chromatography was carried out on silica gel (200-300 mesh, Qingdao Haiyang Chemical Factory, Qingdao, China), MCI gel CHP20P (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (25-100 mm, Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden).TLC was performed on silica gel GF254 (Yantai Jiangyou Silica Gel Co. Ltd, Yantai, China). Semi-preparative HPLC was carried out using a system composed of a Waters 600 pump, with an Agilent 1100 detector and Sunfire C18 reversed phase column (10 × 150 mm, detected at UV of 254 nm). Solvents were of industrial purity and distilled prior to use.

Plant Material. The whole plants of *E. marginata* were collected from Pingbian County of Yunnan Province, China in December, 2008 and identified by Dr. Guangwan Hu, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China and the voucher specimen (No. 0812004) is deposited at the Herbarium of School of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming, China.

Extraction and Isolation. The air-dried powdered whole plants of *E. marginata* (0.2 Kg) were extracted with MeOH (2L × 4) at room temperature and the concentrated extract (31 g) was subjected to MCI gel chromatography and eluted with 80% aq. MeOH, 90% aq. MeOH, MeOH and acetone successively to provide 4 fractions, in order of elution. Frac-

tion 1 (16.8 g) was isolated on silica gel chromatography eluting with gradient $CHCl_3$:MeOH to afford 6 subfractions (1a-1f). Subfraction 1a (198 mg) was purified on Sephadex LH-20 eluting with 60% $CHCl_3$ in MeOH to give **4** (20 mg) and **7** (25 mg). Subfraction 1b (541 mg) was separated on Sephadex LH-20 eluting with 60% $CHCl_3$ in MeOH to produce 3 subfractions (1b1-1b3). Subfraction 1b1 (101 mg) was purified by reversed-phase HPLC with 57% aq. MeOH to yield **9** (6 mg) and **13** (3 mg). Subfraction 1b2 (57 mg) was isolated on Sephadex LH-20 (MeOH) to produce **5** (9 mg). Subfraction 1b3 (83 mg) was purified by reversed-phase HPLC with 66% aq. MeOH to afford **6** (8 mg) and **8** (3 mg). Subfraction 1c (157 mg) was purified by Sephadex LH-20 (MeOH) to obtain **3** (3 mg). **1** (2.1 g) and **14** (200 mg) were obtained from 1d (4.3 g) and 1e (476 mg) respectively by repeated recrystallization. Fraction 2 (2.5 g) was subjected to silica gel chromatography eluting with $CHCl_3$: acetone (30:1→0:1) to produce 2 subfractions (2a and 2b). Subfraction 2a (1.9 g) was purified by Sephadex LH-20 (60% $CHCl_3$ in MeOH) to obtain **2** (1.3 g). Fraction 3 (3.5 g) was separated on silica gel chromatography eluting with gradient petroleum ether:acetone to afford 4 subfractions (3a-3d). Subfraction 3b (296 mg) was isolated on silica gel chromatography eluting with petroleum ether:acetone (5:1) to afford **10** (6 mg). Subfraction 3c (147 mg) was purified on Sephadex LH-20 (60% $CHCl_3$ in MeOH) to give **11** (20 mg) and **12** (3 mg).

(2*S*)-Pinocembrin 7-*O*-[β-D-apiosyl(1→6)]-β-D-glucoside (1): A colorless amorphous powder, mp 112-114 °C, $[\alpha]_D^{25}$ −99.9 (c 0.0038, MeOH). ESI-MS *m/z* 573 $(M+Na)^+$, HR-EI-MS *m/z* 550.1679 $[M]^+$ (calcd for $C_{26}H_{30}O_{13}$: 550.1686); UV λ_{max} nm: 330 (sh) (3.31), 284 (4.16); IR (KBr): 3424, 1648, 1577, 1294, 1087, 1012; ^1H and ^{13}C NMR, see Table 1. CD (*c* 3.22 × 10^{-4}, MeOH): $[\theta]_{330}$ +5483, $[\theta]_{284}$ −36347.

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