Chemical Constituents from Dried Aerial Parts of *Eclipta prostrata*

HAN Li-feng, ZHAO Jing, ZHANG Yi, KOJO Agyemang, LIU Er-wei, WANG Tao*

Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin 300193, China

Abstract: **Objective** To study the constituents in the dried aerial parts of *Eclipta prostrata*. **Methods** The constituents were isolated and purified by column chromatography and their structures were elucidated by spectroscopic methods (1D, 2D NMR, UV, IR, and HRESI-TOF-MS) and chemical analyses. **Results** Eight compounds were isolated and identified as 7-*O*-methylorobol-4′-*O*-β-*D*-glucopyranoside (**1**), 3′-hydroxybiochanin A (**2**), echinocystic acid 28-*O*-β-*D*-glucopyranoside (**3**), ecliptasaponin A (**4**), eclalbasaponin I (**5**), eclalbasaponin IV (**6**), echinocystic acid (**7**), and 3-oxo-16α-hydroxy-olean-12-en-28-oic acid (**8**). **Conclusion** **C**ompound **1** is a new compound and compound **3** is obtained from this genus for the first time.

Key words: Compositae; *Eclipta prostrata*; glycoside; isoflavonoid saponins; 7-*O*-methylorobol-4′-*O*-β-*D*-glucopyranoside
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Introduction

Eclipta prostrata L. (Compositae), known as *Mohanlian* in China, is wildly distributed in the tropical and subtropical areas of the world. It is one of the Chinese tonic medicines and has been used for the treatment of loose teeth, dizziness, tinnitus, spitting blood, hematuria, and uterine bleeding (Sun, Zhang, and Zhang, 2010). Several publications have reported its chemical constituents, such as coumarins (Zhang and Guo, 2001), triterpenoid saponins (Yahara, Ding, and Nohara, 1994), flavonoids (Wu *et al*, 2008), thiophenes (Wu *et al*, 2008), and steroids (Han *et al*, 1998; Cheng and Hu, 2010). In this paper, we reported the isolation and structural elucidation of a new isoflavonoid glycoside, 7-*O*-methylorobol-4′-*O*-β-*D*-glucopyranoside (**1**), together with seven known compounds, 3′-hydroxybiochanin A (**2**), echinocystic acid 28-*O*-β-*D*-glucopyranoside (**3**), ecliptasaponin A (**4**), eclalbasaponin I (**5**), eclalbasaponin IV (**6**), echinocystic acid (**7**), and 3-oxo-16α-hydroxy-olean-12-en-28-oic acid (**8**). Compound **3** was obtained from this genus for the first time.

Materials and methods

Instruments

The optical rotations were measured on a Rudolph Autopol® IV Automatic Polarimeter (l = 50 mm). The IR spectrum was recorded on a Varian 640-IR FT-IR spectrophotometer, and the UV spectra on a Varian Cary 50 UV-Vis Spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were determined on a Bruker 500 MHz NMR (Avance III 500MR) at 500 and 125 MHz with tetramethylsilane (TMS) as an internal standard. Negative-ion HRESI-TOF-MS was recorded on an Agilent Technologies 6520 Accurate-Mass Q-Tof LC/MS spectrometer.

Silica gel CC was obtained from Qingdao Haiyang Chemical Co., Ltd., (48—75 μm, Qingdao, China). Sephadex LH-20 was purchased from GE Healthcare Bio-Sciences Co., Ltd., Swiss. The preparative HPLC equipment is Agilent 1200 system including two G1361A Preparative Pumps, a Manual Sampler (7725i), a G1365D Multiple Wavelength Detector (VWD), and Agilent LC workstation. HPLC was performed on ODS (250 mm × 20 mm, Cosmosil 5 C_{18}-MS-II, Japan), and

* Corresponding author: Wang T Tel/Fax: +86-22-5959 6163 E-mail: wangt@263.net
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the flow rate was 10.0 mL/min. HPLC-ELSD (Alltech Grace Evaporative Light Scattering Detector 3300) was used to detect the purity of the extract with the temperature of 40 ℃, and gas flow of 1.8 L/min.

Plant materials

The dried aerial parts of *Eclipta prostrata* L. were collected from Hebei province, China, and identified by Prof. ZHANG Li-juan at Tianjin University of Traditional Chinese Medicine. The voucher specimen (20120903) was deposited at the Institute of Chinese Materia Medica of Tianjin University of Traditional Chinese Medicine (Tianjin, China).

Extraction and isolation

The dried aerial parts of *E. prostrata* (20 kg) were extracted with 70% ethanol under reflux. After removing of the solvent under reduced pressure at 45 ℃, the residue (2150 g) was suspended in water and partitioned successively with petroleum ether (60—90 ℃) and EtOAc. The EtOAc fraction (150 g) was subjected to silica gel CC (CH_2Cl_2-MeOH 1:0→0:1) to give 15 fractions, Frs. 1—15.

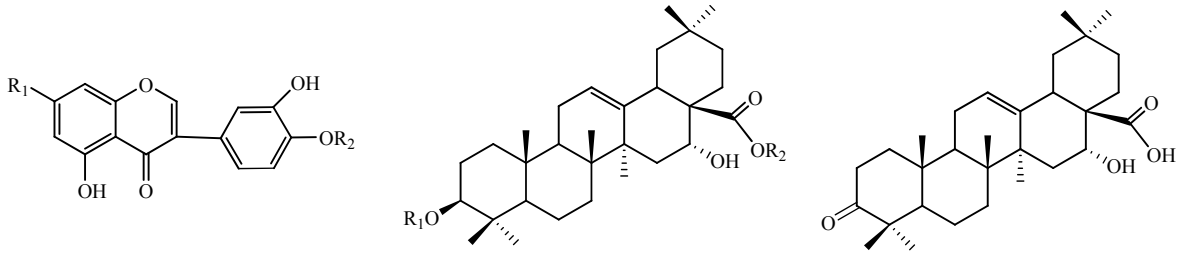
Fr. 5 (6.4 g) was subjected to flash ODS CC (0—180 min, 10%→100% MeOH) to yield 10 fractions (Frs. 5-1—5-10). Fr. 5-5 (430 mg) was separated by Sephadex LH-20, eluted with MeOH to yield eight fractions (Frs. 5-5-1—5-5-8). Fr. 5-5-8 (82 mg) was purified by preparative HPLC (65% MeOH) to yield compound **2** (49 mg). Fr. 5-7 (870 mg) was separated by Sephadex LH-20, eluted with MeOH to yield four fractions (Frs. 5-7-1—5-7-4). Fr. 5-7-3 (150 mg) was purified by preparative HPLC (80% MeOH) to yield compound **8** (109 mg), Fr. 5-7-4 (80 mg) was purified by preparative HPLC (85% MeOH) to yield compound **7** (43 mg).

Fr. 11 (8.6 g) was purified by flash ODS CC (0—180 min, 10%→100% MeOH) to yield 10 fractions (Frs. 11-1—11-10) and compound **4** (Fr. 11-9, 1290 mg) was obtained directly. Fr. 11-3 (520 mg) was separated by Sephadex LH-20, and eluted with MeOH to yield compound **1** (10 mg). Fr. 11-7 (580 mg) was purified by preparative HPLC (60% MeOH) to yield compound **3** (130 mg).

Fr. 13 (10.3 g) was purified by flash ODS CC (0—180 min, 10%→100% MeOH) to yield 10 fractions (Frs. 13-1—13-10), Fr. 13-4 (560 mg) was purified by preparative HPLC (45% MeOH) to yield compounds **5** (210 mg) and **6** (95 mg). The structures of compounds **1**—**8** were showed in Fig. 1.

Acid hydrolysis of compound 1

A solution of compound **1**, 7-*O*-methylorobol-4′-*O*-β-*D*-glucopyranoside (2.1 mg) in 1 mol/L HCl (1 mL) was refluxed for 3 h. After cooling, the reaction mixture was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following condition, respectively: HPLC column, Kaseisorb LC NH_2-60-5, 250 mm × 4.6 mm (Tokyo Kasei Co. Ltd., Japan); detection, optical rotation [Chiralyser (IBZ Messtechnik GMBH, Mozartstrasse 14-16 D-30173 Hannover, Germany)]; mobile phase, CH_3CN-H_2O (70:30); flow rate, 1.0 mL/min. The identification of *D*-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with that of authentic *D*-glusose (t_R: 17.9 min, positive optical rotation).



1 R_1=OCH_3 R_2=glc
2 R_1=OH R_2=CH_3

3 R_1=H R_2=glc
4 R_1=glc R_2=H
5 R_1=R_2=glc
6 R_1=glc-(1→2)-glc R_2=H
7 R_1=R_2=H

8

Fig. 1 Chemical structures of compounds 1—8

Results and discussion

Compound **1**: dark yellow powder with negative rotation ($[\alpha]_D^{25}$ −15.7º, *c* 0.01, MeOH). UV λ_{max}^{MeOH} (nm): 258 (3.07) and 282 (2.72). The IR spectrum indicated the presence of hydroxy (3335 cm^{-1}), α, β-unsaturated ketone (1653 cm^{-1}), aromatic ring (1616, 1574, and 1437 cm^{-1}), and an *O*-glycosidic linkage (1155 cm^{-1}). The molecular formula was determined to be $C_{22}H_{22}O_{11}$ by HRESI-TOF-MS (*m/z* 461.1079 $[M - H]^-$). Compound **1** was treated with 1 mol/L HCl liberated *D*-glucose, which was identified by HPLC (Yoshikawa *et al*, 2007). The ^{1}H-NMR (DMSO-d_6, 500 MHz) indicated that there were one isoflavonoid moiety [δ 6.42 (1H, d, *J* = 2.0 Hz, H-6), 6.67 (1H, d, *J* = 2.0 Hz, H-8), 6.96 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 7.09 (1H, d, *J* = 2.0 Hz, H-2′), 7.17 (1H, d, *J* = 8.0 Hz, H-5′), 8.45 (1H, s, H-2), and 12.94 (5-OH)], one anomeric proton of β-*D*-glucopyranose moiety [δ 4.73 (1H, d, *J* = 7.5 Hz, H-1″)], and one methoxyl moiety at δ 3.87 (3H, s). The ^{1}H-^{1}H COSY (Fig. 2) indicated the presence of the partial structure written in bold lines. The correlation between δ 8.45 (1H, s, H-2) and δ 154.8 (C-2) was observed clearly in the HSQC spectrum. In the HMBC spectrum, the long-range correlations were observed in the following proton and carbon pairs: i) δ 4.73 (H-1″) and δ 145.3 (C-4′), ii) δ 3.87 (7-OCH_3) and δ 165.2 (C-7), iii) δ 6.96 (H-6′) and δ 122.1 (C-3), and iv) δ 8.45 (H-2) and δ 125.1 (C-1′), δ 157.4 (C-9), δ 180.2 (C-4). The structure of compound **1** was elucidated to be 7-*O*-methylorobol- 4′-*O*-β-*D*-glucopyranoside.

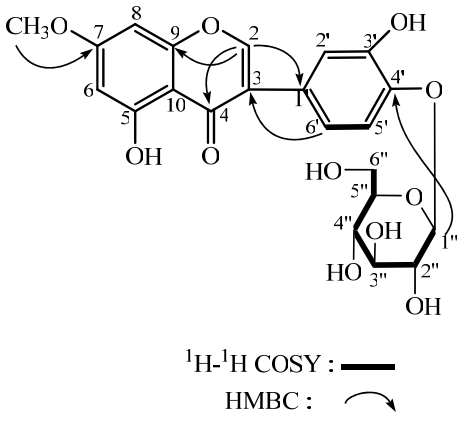


Fig. 2 Key ^{1}H-^{1}H COSY and HMBC correlation of compound 1

The physical, ^{1}H-NMR, and ^{13}C-NMR data of compound **1** were described as follows: dark yellow powder. $[\alpha]_D^{25}$ −15.7º (MeOH). UV λ_{max}^{MeOH} (nm): 258 (3.07), 282 (2.72). IR ν_{max}^{KBr} (cm^{-1}): 3335, 2922, 2852, 1653, 1616, 1574, 1437, 1362, 1279, 1198, 1155, 982, 825, 775. HRESI-TOF-MS *m/z* 461.1079 $[M - H]^-$ (Calcd. 461.1089 for $C_{22}H_{21}O_{11}$). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 8.45 (1H, s, H-2), 6.42 (1H, d, *J* = 2.0 Hz, H-6), 6.67 (1H, d, *J* = 2.0 Hz, H-8), 7.09 (1H, d, *J* = 2.0 Hz, H-2′), 7.17 (1H, d, *J* = 8.0 Hz, H-5′), 6.96 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 3.87 (3H, s, 7-OCH_3), 4.73 (1H, d, *J* = 7.5 Hz, H-1″), 3.32 (1H, m, overlapped, H-2″), 3.29 (1H, m, overlapped, H-3″), 3.18 (1H, m, H-4″), 3.34 (1H, m, overlapped, H-5″), 3.49 (1H, dd, *J* = 6.0, 12.0 Hz, H-6a″), and 3.73 (1H, d, *J* = 12.0 Hz, H-6b″); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 154.8 (C-2), 122.1 (C-3), 180.2 (C-4), 161.7 (C-5), 98.0 (C-6), 165.2 (C-7), 92.4 (C-8), 157.4 (C-9), 105.3 (C-10), 125.1 (C-1′), 116.6 (C-2′), 146.4 (C-3′), 145.3 (C-4′), 116.4 (C-5′), 119.9 (C-6′), 102.1 (C-1″), 73.2 (C-2″), 75.8 (C-3″), 69.7 (C-4″), 77.2 (C-5″), 60.6 (C-6″), 56.0 (7-OCH_3).

Compound **2**: dark yellow powder. HRESI-TOF-MS *m/z* 299.0530 $[M - H]^-$ (Calcd. 299.0561 for $C_{16}H_{11}O_6$). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 8.36 (1H, s, H-2), 6.23 (1H, d, *J* = 1.5 Hz, H-6), 6.39 (1H, d, *J* = 1.5 Hz, H-8), 7.14 (1H, d, *J* = 1.5 Hz, H-2′), 6.82 (1H, d, *J* = 8.5 Hz, H-5′), 6.99 (1H, dd, *J* =1.5, 8.5 Hz, H-6′), 3.80 (3H, s, 4′-OCH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 154.1 (C-2), 121.5 (C-3), 180.1 (C-4), 161.9 (C-5), 98.9 (C-6), 164.2 (C-7), 93.6 (C-8), 157.4 (C-9), 104.3 (C-10), 122.2 (C-1′), 113.2 (C-2′), 147.2 (C-3′), 146.6 (C-4′), 115.2 (C-5′), 121.6 (C-6′), 56.0 (4′-OCH_3). Compound **2** was identified as 3′-hydroxybiochanin A by comparison of the physical, ^{1}H-NMR and ^{13}C-NMR data with the reported data (Roberts *et al*, 2004).

Compound **3**: white powder. HRESI-TOF-MS *m/z* 679.4045 $[M + HCOO]^-$ (Calcd. 679.4063 for $C_{37}H_{59}O_{11}$). ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 3.44 (1H, dd, *J* = 5.0, 11.0 Hz, H-3), 5.63 (1H, brs, H-12), 5.29 (1H, brs, H-16), 1.21 (3H, s, H-23), 1.03 (3H, s, H-24), 0.97 (3H, s, H-25), 1.16 (3H, s, H-26), 1.82 (3H, s, H-27), 1.00 (3H, s, H-29), 1.05 (3H, s, H-30), and 6.31 (1H, d, *J* = 8.5 Hz, H-1 of glucose). Compound **3** was identified as echinocystic acid 28-*O*-β-*D*-glucopyranoside by comparison of the physical, ^{1}H-NMR and ^{13}C-NMR data with the reported data (Akai *et al*, 1985).

Compound **4**: white powder. HRESI-TOF-MS *m/z*

679.4053 [M + HCOO]$^-$ (Calcd. 679.4063 for $C_{37}H_{59}O_{11}$). ^{1}H-NMR (CD_3OD, 500 MHz) δ: 3.18 (1H, m, H-3), 5.29 (1H, brs, H-12), 4.46 (1H, brs, H-16), 1.05 (3H, s, H-23), 0.85 (3H, s, H-24), 0.96 (3H, s, H-25), 0.79 (3H, s, H-26), 1.37 (3H, s, H-27), 0.88 (3H, s, H-29), 0.97 (3H, s, H-30), and 4.32 (1H, d, J = 7.5 Hz, H-1 of glucose). Compound **4** was identified as ecliptasaponin A by comparison of the physical, ^{1}H-NMR and ^{13}C-NMR data with the reported data (Zhang and Chen, 1996).

Compound **5**: white powder. HRESI-TOF-MS *m/z* 841.4562 [M + HCOO]$^-$ (Calcd. 841.4591 for $C_{43}H_{69}O_{16}$). ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 3.43 (1H, m, H-3), 5.63 (1H, brs, H-12), 5.32 (1H, brs, H-16), 1.30 (3H, s, H-23), 1.01 (3H, s, H-24), 0.90 (3H, s, H-25), 1.15 (3H, s, H-26), 1.86 (3H, s, H-27), 1.01 (3H, s, H-29), 1.05 (3H, s, H-30), 4.96 (1H, d, J = 7.5 Hz, H-1′), and 6.34 (1H, d, J = 8.0 Hz, H-1″). Compound **5** was identified as eclalbasaponin I by comparison of the physical, ^{1}H-NMR and ^{13}C-NMR data with the reported data (Yahara, Ding, and Nohara, 1994).

Compound **6**: white powder. HRESI-TOF-MS *m/z* 841.4554 [M + HCOO]$^-$ (Calcd. 841.4591 for $C_{43}H_{69}O_{16}$). ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 3.31 (1H, m, H-3), 5.65 (1H, brs, H-12), 5.25 (1H, brs, H-16), 1.28 (3H, s, H-23), 1.10 (3H, s, H-24), 0.87 (3H, s, H-25), 1.03 (3H, s, H-26), 1.85 (3H, s, H-27), 1.07 (3H, s, H-29), 1.19 (3H, s, H-30), 4.92 (1H, d, J = 7.0 Hz, H-1′) and 5.37 (1H, d, J = 7.5 Hz, H-1″). Compound **6** was identified as eclalbasaponin IV by comparison of the physical, ^{1}H-NMR and ^{13}C-NMR data with the reported data (Yahara, Ding, and Nohara, 1994).

Compound **7**: white powder. HRESI-TOF-MS *m/z* 471.3470 [M – H]$^-$ (Calcd. 471.3480 for $C_{30}H_{47}O_4$). ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 3.64 (1H, dd, J = 4.0, 9.5 Hz, H-3), 5.68 (1H, brs, H-12), 5.27 (1H, brs, H-16), 1.14 (3H, s, H-23), 1.02 (3H, s, H-24), 0.95 (3H, s, H-25), 1.05 (3H, s, H-26), 1.81 (3H, s, H-27), 1.07 (3H, s, H-29), and 1.19 (3H, s, H-30). Compound **7** was identified as echinocystic acid by comparison of the physical, ^{1}H-NMR and ^{13}C-NMR data with the reported data (Yahara *et al*, 1994).

Compound **8**: white powder. HRESI-TOF-MS *m/z* 469.3315 [M – H]$^-$ (Calcd. 469.3323 for $C_{30}H_{45}O_4$). ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 5.65 (1H, brs, H-12), 5.24 (1H, brs, H-16), 1.24 (3H, s, H-23), 1.04 (3H, s, H-24), 0.94 (3H, s, H-25), 1.07 (3H, s, H-26), 1.86 (3H, s, H-27), 1.08 (3H, s, H-29), and 1.20 (3H, s, H-30). Compound **8** was identified as 3-oxo-16α-hydroxy-olean-12-en-28-oic acid by comparison of the physical, ^{1}H-NMR and ^{13}C-NMR data with the reported data (Bai *et al*, 2004).

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