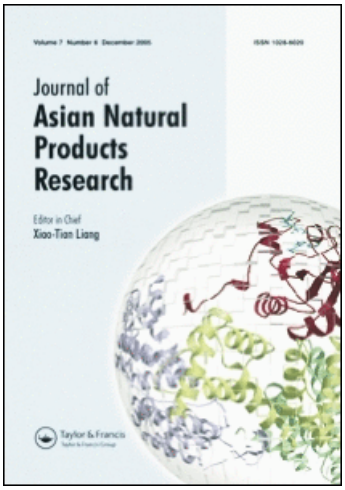

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A new diterpenoid glucopyranoside from *Erythrophleum fordii*

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A phytochemical investigation of the bark of *Erythrophleum fordii* furnished six compounds, of which one is a new diterpenoid glucopyranoside, named 15*Z*-[dodecahydro-18-hydroxymethyl-17,19,20-trimethyl-15(1*H*)-phenanthrenylidene]-acetic acid-16-*O*-β-D-glucopyranoside (**1**). Five known compounds were identified as (+)-taxifolin (**2**), (−)-robinetinidol (**3**), palmaitic acid (**4**), β-sitosterol (**5**) and baucosteyl (**6**), and were all isolated from the species for the first time. The structure of the new compound was elucidated on the basis of spectroscopic analyses and chemical methods.

Keywords: *Erythrophleum fordii*; Diterpenoid glucopyranoside; X-ray crystallography; Cytotoxic activities; KB cells; A2780 cells

1. Introduction

Erythrophleum fordii Oliv. is a Chinese traditional medicine whose main action is "invigoration and promoting blood circulation". To our knowledge, no previous chemical and pharmacological investigation has been reported on it.

During our screening program to search for cytotoxic herbs, an ethanol extract of the bark of *Erythrophleum fordii* was found to have significant cytotoxicities against cells of KB and A2780. Phytochemical investigation of the EtOAc fraction from this active extract led to the isolation and identification of six compounds. We report herein the isolation and structure elucidation of the new compound (**1**) by means of spectroscopic techniques and chemical methods.

2. Results and discussion

The 95% ethanol extract of the dried bark of *Erythrophleum fordii* was partitioned between EtOAc and H_2O to afford an EtOAc-soluble portion, which showed cytotoxic activities against KB cells with an IC_{50} of 0.08 μg mL^{-1}, A2780 cells with an IC_{50} of 3.1 μg mL^{-1}.

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The EtOAc-soluble fraction was subjected to column chromatography on silica gel to give twelve fractions (Fr. 1–12). The Fr. 3 and 7, which also showed exciting cytotoxic activities against KB cells and A2780 cells, were repeatedly chromatographed over silica gel and Sephadex LH-20 to give six compounds (**1**–**6**), including the new one (**1**). The known compounds (**2**–**6**) were identified by comparing the melting points and spectroscopic data (^{1}H NMR, ^{13}C NMR and MS) with those reported in the literature [1–4]. The structural elucidation of the new compound **1** is presented herein.

Compound **1** was obtained as a white amorphous powder, mp 143–145°C, $[\alpha]_D^{18}$ − 27 (CH_3OH, *c* 1.04). HR-FABMS gave a quasi-molecular ion at *m/z* 505.2734 $[M + Na]^+$, corresponding to a molecular formula of $C_{26}H_{42}O_8$. The ^{13}C NMR spectrum of **1** also confirmed that the molecule contained 26 carbons. Its IR spectrum indicated hydroxyl groups (3413, 1074 cm^{-1}) and a carbonyl group (1716 cm^{-1}). The ^{1}H NMR spectrum of **1** showed a signal for one anomeric proton at δ_H 5.49 (d, $J = 8.0$ Hz), indicating a β-stereochemistry of the glycoside bond. Acid hydrolysis of **1** liberated sapogenin (**1a**), and offered glucose that was determined by TLC comparison with authentic samples. The carbon signals of **1** at δ 95.2, 78.8, 78.1, 74.0, 71.1 and 62.4, which are not in the ^{13}C NMR spectrum of **1a**, also suggested the presence of glucose in **1**.

Compound **1a** was obtained as colorless prisms, mp 94–95°C, $[\alpha]_D^{18}$ − 16 (EtOAc, *c* 0.85). HR-FABMS gave a quasi-molecular ion at *m/z* 321.2445 ($[M + H]^+$) corresponding to a molecular formula of $C_{20}H_{32}O_3$. The ^{13}C NMR spectrum of **1a** and DEPT experiments confirmed that the molecule contained 20 carbons, including three methyl groups, eight methylenes (one oxygenated methylene), five methines (1 × CH═), and four quaternary carbons. A carbonyl and two olefinic signals in the ^{13}C NMR spectrum of **1a** accounted for two of the five degrees of unsaturation implied by the molecular formula and required this compound to be tricyclic.

Table 1. ^{13}C NMR (125 MHz) data for compounds **1** and **1a***.

Position	***1***	***1a***	*Position*	***1***
1	40.3 (2)	40.3 (2)	Glc	
2	19.2 (2)	19.3 (2)	G1	95.2 (1)
3	36.5 (2)	36.5 (2)	G2	74.0 (1)
4	38.6 (0)	38.6 (0)	G3	78.1 (1)
5	49.3 (1)	49.3 (1)	G4	71.1 (1)
6	22.5 (2)	22.5 (2)	G5	78.8 (1)
7	32.3 (2)	32.4 (2)	G6	62.4 (2)
8	41.4 (1)	41.3 (1)		
9	49.3 (1)	49.3 (1)		
10	37.9 (0)	37.8 (0)		
11	28.5 (2)	28.4 (2)		
12	33.9 (2)	33.8 (2)		
13	166.3 (0)	169.7 (0)		
14	37.1 (1)	36.8 (1)		
15	111.8 (1)	113.0 (1)		
16	173.2 (0)	170.4 (0)		
17	13.8 (3)	14.0 (3)		
18	72.0 (2)	72.0 (2)		
19	18.4 (3)	18.4 (3)		
20	15.2 (3)	15.2 (3)		

* Spectra determined in CD_3OD; carbon types determined by DEPT experiments and reported as 0 (quaternary), 1 (CH), 2 (CH_2), or 3 (CH_3).

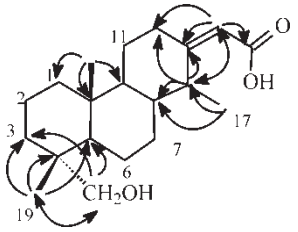
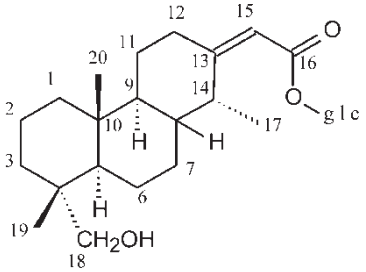


Figure 1. HMBC correlations of compound **1a**.

The skeleton of **1a** was established by 2D NMR experiments HMQC (^{13}C NMR assignments given in table 1), HMBC (figure 1) and ^{1}H–^{1}H COSY (figure 2).

The structure of **1a** was confirmed by a single crystal X-ray crystallographic analysis, which showed the double bond at C_{13}–C_{15} is in the *Z* form (figure 3). Compound **1a** is new and has been named 15*Z*-[dodecahydro-18-hydroxymethyl-17,19,20-trimethyl-15(1*H*)-phenanthrenylidene]-acetic acid.

In the HMBC spectrum of **1**, a correlation between H-1′ of glucose (δ_H 5.49) and C-16 (δ_C 173.2) indicated that glucose was connected to C-16. The glycosidation shift at C-16 also confirmed the position of this glucose. On the basis of above data, the structure of compound **1** was established as 15*Z*-[dodecahydro-18-hydroxymethyl-17,19,20-trimethyl-15(1*H*)-phenanthrenylidene]-acetic acid-16-*O*-β-D-glucopyranoside.



The structures of compounds **2**–**6** were determined by comparison of the physical and spectral data with literature values [1–4].

3. Experimental

3.1 General experimental procedures

Mps were determined on an X_4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a PE-241 MC polarimeter. IR spectra were recorded as KBr

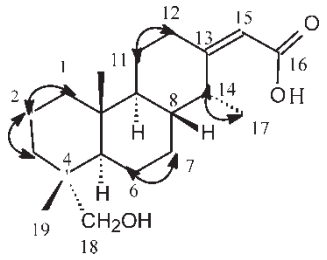


Figure 2. ^{1}H–^{1}H COSY correlations of compound **1a**.

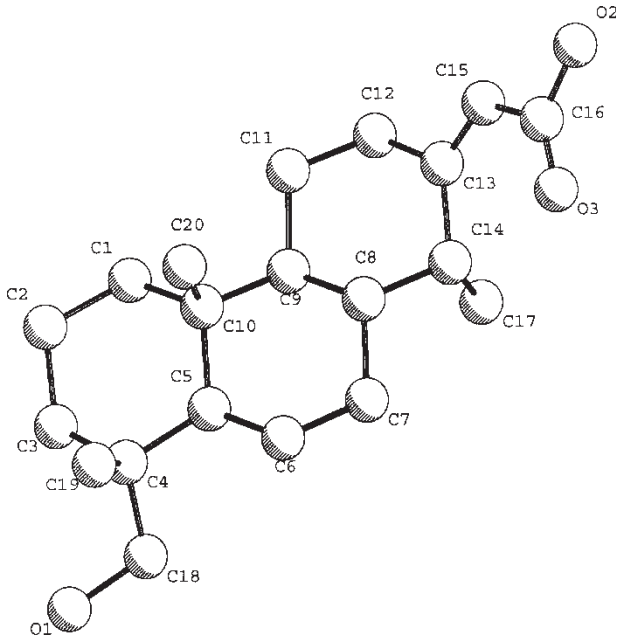


Figure 3. X-ray crystal structure of compound **1a**.

pellets on a Perkin–Elmer 683 IR spectrometer. ^{1}H and ^{13}C NMR spectra as well as 2D NMR experiments were recorded with TMS as internal standard on an Inova 500 FT-NMR spectrometer. High-resolution mass spectra were measured on an Autospec-ultima TOF (Micromass) spectrometer and low-resolution mass spectra were measured on a VG ZAB-2f spectrometer. X-ray structural analysis: MAC DIP 2030K diffractometer. Silica GF254 for TLC and silica gel (200–300 mesh) for CC were produced by the Qingdao Marine Chemical Company, Qingdao, China. Amersham Pharmacia Biotech AB, Sweden, produced Sephadex LH-20. Solvents and chemicals were of analytical grade and purchased from the Beijing Chemical Company, Beijing, China.

3.2 Plant material

The barks of *Erythrophleum fordii* were collected from Guangxi Province, China, and identified by Professor Shou-yang Liu (Guangxi College of Chinese Traditional Medicine, Guangxi Province, China) in August 2000. The barks were harvested and air-dried at room temperature. A voucher specimen has been deposited in the Institute of Materia Medica, Chinese Academy of Medical Sciences.

3.3 Extraction and isolation

The dried and chopped barks of *Erythrophleum fordii* (15 kg) were extracted three times with 95% hot EtOH. The combined EtOH extract was evaporated under reduced pressure to yield a dark brown syrup (2910 g), which was partitioned between EtOAc and H_2O. The EtOAc-soluble layer (400 g) was directly chromatographed over silica gel by eluting with a gradient of CH_2Cl_2–MeOH (20:1 to 1:1) to afford twelve fractions. Fraction 3 (47.7 g) was

chromatographed over silica gel with a gradient of light petroleum–EtOAc (9:1 to 1:1) to yield **4** (75 mg) and **5** (420 mg). Fraction 7 (32.7 g) was subjected to repeated column chromatography on silica gel with a gradient of CH_2Cl_2–MeOH (8:1 to 1:1) and Sephadex LH-20 (MeOH) to give **1** (270 mg), **2** (14 mg), **3** (119 mg) and **6** (36 mg).

3.3.1 15Z-[Dodecahydro-18-hydroxymethyl-17,19,20-trimethyl-15(1H)-phenanthrenylidene]-acetic acid-16-*O*-β-D-glucopyranoside (1). A white amorphous powder, mp 143–145°C; $[\alpha]_D^{18}$ − 27 (*c* 1.04, CH_3OH); IR (KBr) ν_{max} (cm^{-1}): 3413, 1716, 1074; ^1H NMR (500 MHz, CD_3OD) δ (ppm): 1.74 (1H, m, H-1), 1.91 (1H, m, H-11), 2.43 (1H, m, H-12), 2.13 (1H, m, H-12), 3.89 (1H, m, H-14), 5.63 (1H, s, H-15), 1.03 (3H, d, $J = 7$ Hz, CH_3-17), 3.01 (1H, $J = 11$ Hz, H-18), 0.78 (3H, s, CH_3-19), 0.89 (3H, s, CH_3-20), 5.49 (1H, d, $J = 8$ Hz, H-1 of Glc); ^{13}C NMR (125 MHz, CD_3OD), see Table 1; ESIMS *m/z*: 505 $[M+Na]^+$; HR-FABMS $[M+Na]^+$ *m/z*: 505.2734 (calcd. for $C_{26}H_{42}O_8$ Na, 505.2777).

3.3.2 15*Z*-[Dodecahydro-18-hydroxymethyl-17,19,20-trimethyl-15(1H)-phenanthrenylidene]-acetic acid (1a). Colorless prisms, mp 94–95°C; $[\alpha]_D^{18}$ − 16 (*c* 0.85, EtOAc); ^1H NMR (500 MHz, CD_3OD) δ (ppm): 0.89 (1H, m, H-1), 1.67 (1H, m, H-1), 1.05 (1H, m, H-11), 1.81 (1H, m, H-11), 2.01 (1H, m, H-12), 2.32 (1H, m, H-12), 3.77 (1H, m, H-14), 5.45 (1H, s, H-15), 0.94 (3H, d, $J = 7$ Hz, H-17), 2.93 (1H, $J = 11$ Hz, H-18), 3.29 (1H, $J = 11$ Hz, H-18), 0.71 (3H, s, H-19), 0.82 (3H, s, H-20); ^{13}C NMR (125 MHz, CD_3OD), see Table1; HR-FABMS *m/z* $[M + H]^+$ 321.2445 (calcd. for $C_{20}H_{33}O_3$, 321.2429).

3.3.3 (+)-Taxifolin (3,5,7,3′,4′-pentahydroxyflavanone) (2). Colorless prisms (130 mg), mp 239–241°C $[\alpha]_D^{18}$ + 39 (MeOH, *c* 0.90), IR ν_{max} (KBr) (cm^{-1}): 3359, 1641, 1082, 1018. EI-MS $[M]^+$ *m/z*: 304. ^1H NMR (500 MHz, CD_3OD): δ (ppm): 6.79 (1H, d, $J = 2.0$, 8.0 Hz, H-6′), 6.74 (1H, d, $J = 8.0$ Hz, H-5′), 6.90 (1H, d, $J = 2.0$ Hz, H-2′), 5.86 (1H, d, $J = 2.5$ Hz, H-8), 5.82 (1H, d, $J = 2.5$ Hz, H-6), 4.49 (1H, d, $J = 11.5$ Hz, H-3), 4.85 (1H, d, $J = 11.5$ Hz, H-2). ^{13}C NMR (500 MHz, C_5D_5N) δ (ppm): 85.1 (C-2), 73.7 (C-3), 198.4 (C-4), 165.3(C-5), 97.3(C-6), 168.7 (C-7), 96.3 (C-8), 164.5 (C-9), 101.8 (C-10), 129.9 (C-1′), 116.1 (C-2′), 146.3 (C-3′), 147.1 (C-4′), 115.9(C-5′), 120.9 (C-6′).

3.3.4 (−)-Robinetinidol (2R,trans-3′,4′,5′,7-tetrahydroxyflavan-3-ol) (3). Amorphous powder (210 mg), mp 163–164°C, $[\alpha]_D^{18}$ − 36 (CH_3COCH_3, *c* 1.02), IR ν_{max} (KBr) (cm^{-1}): 3408,3384, 1014. EI-MS $[M]^+$ *m/z*: 290. ^1H NMR (500 MHz, CD_3OD): δ (ppm): 6.79 (1H, d, $J = 8$ Hz, H-5), 6.33 (2H, s, H-2′, H-5′), 6.28 (1H, dd, $J = 8$, 2.5 Hz, H-6), 6.23 (1H, d, $J = 2.5$ Hz, H-8), 4.56 (1H, d, $J = 6.5$ Hz, H-2), 3.95 (1H, m, H-3), 2.79 (1H, dd $J = 15.5$, 4.5 Hz, H-4), 2.60 (1H, dd, $J = 15.5$, 7.5 Hz, H-4).

3.4 Acid hydrolysis of 1

Compound **1** (40 mg) was hydrolyzed with 7% HCl in aqueous EtOH (20 ml) in a H_2O bath (39°C) for 2 h. The contents were concentrated and allowed to cool, and then the residue was extracted with EtOAc. The aqueous layer was studied separately to identify any sugars, one

spot was detected, and this was identified as D-glucose, $[\alpha]_D^{25} + 52.5$ (H_2O, c 0.4), by comparison with authentic samples. The EtOAc layer was concentrated under reduced pressure and the residue was subjected to Sephadex LH-20 using MeOH to give compound **1a**.

3.5 X-ray crystallographic analysis of 1a

Crystals of **1a** suitable for X-ray diffraction analysis were obtained by recrystallization from EtOAc. A colorless crystal ($0.05 \times 0.05 \times 0.10$ mm) was selected for data collection. Crystal data: $C_{20}H_{32}O_3$; monoclinic, space group $P2_1$(#4), $Z = 2$, $a = 11.6310(9)$ Å, $b = 6.6970(3)$ Å, $c = 11.8480(9)$ Å, $\beta = 78.259(4)°$, V = 903.57(11) Å^3, $D_{cal} = 1.178$ Mg m^{-3}, $R = 0.064$, $R_W = 0.059$. The data were collected on a MAC DIP-2030K diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.7107$) by using the ω scan method at 298 K. The structure was solved by direct methods and refined by block matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at the calculated positions and refined isotropically.

3.6 Bioassays

Cytotoxicity against human cancer cell lines for the EtOAc-soluble portion was measured in a five-day MTT test at the Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, using A2780 human epithelial carcinoma cells and KB human epidermoid cancer cells [5]. Cells were seeded in 96-well plates (1000 cells/well per 0.2 ml of growth medium 1640 containing 10% NCS (newborn calf serum), and fresh medium with or without compounds was added 24 h later. After culturing for 5 days, 0.2 mg ml^{-1} of MTT (0.2 mg ml^{-1} in medium) was then aspirated. The cells were dissolved in DMSO (0.2 ml) and the absorbance at 540 nm was then measured in a microplate reader.

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