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A new phenolic glycoside from *Saprosma merrillii*

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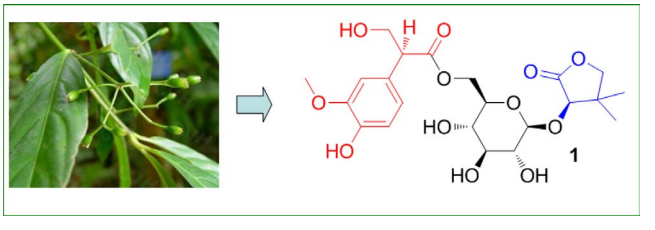
ABSTRACT

A new phenolic glycoside derivative, saproglucoside (**1**), along with five known phenolic glycoside derivatives (**2–6**) were isolated from the stems of *Saprosma merrillii*. The structure of the new compound **1** was determined by 1D and 2D NMR as well as by HRESIMS and hydrolysis. The inhibitory activities of all compounds against seven pathogenic bacteria and two cancer cell lines were evaluated.



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1. Introduction

The genus *Saprosma* (Rubiaceae) consists of about 30 species, which are mainly distributed in the tropical Asian region, and has been used as a traditional medicine for the treatment of fever (Ling et al. 2002; Lu et al. 2010; Quang et al. 2002; Singh et al. 2006; Wang et al. 2011, 2014). *Saprosma merrillii* is an endemic medicinal plant in Hainan, P.R. China. Our group recently reported the isolation of three new ursane-type triterpenoids and many anthraquinone derivatives, which showed antibacterial and cytotoxic activity (Li et al. 2015; Zhang et al. 2014, 2013). In our ongoing investigation of natural antibacterial products from the *S. merrillii*, a new phenolic glycoside derivative, saproglucoside (**1**), along with five known phenolic glycoside derivatives (Figure 1), isotachioside (**2**) (Inoshiri et al. 1987), tachioside (**3**) (Inoshiri et al. 1987), canthoside D (**4**) (Kanchanapoom et al. 2002), canthoside C (**5**) (Kanchanapoom et al. 2002) and koaburaside (**6**) (Ogawa et al. 1973; Kosuge et al. 1994) were isolated from the stems of *S. merrillii*. Known compounds **2–6** were isolated from *Saprosma* for the first

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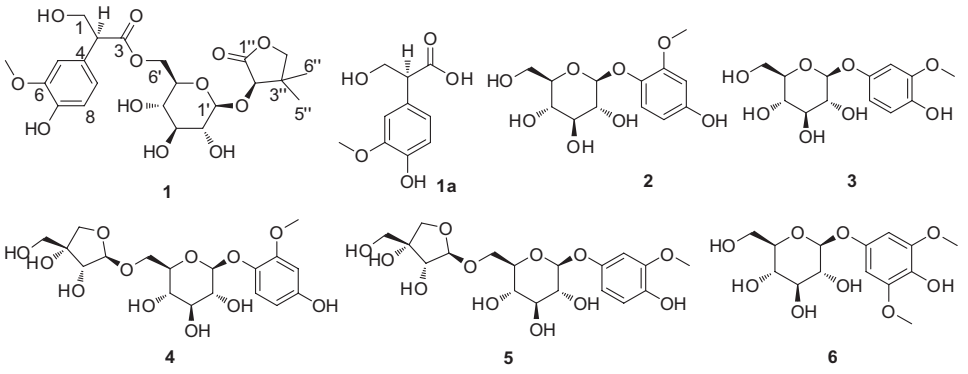

Figure 1. Structures of compounds **1**–**6** and 1**a**.

time. The isolation and structure elucidation of **1** and the inhibitory activities of all compounds against six pathogenic bacteria and two cancer cell lines are reported herein.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with a molecular formula of $C_{22}H_{30}O_{12}$ (8° of unsaturation), as deduced from HRESIMS (*m/z* 485.1668 for **1** $[M - H]^-$; calc'd 485.1659) and ^{13}C NMR data. In the ^1H NMR spectrum, three phenyl proton signals δ_H 6.74 (d, $J = 8.0$ Hz, H-8), δ_H 6.76 (d, $J = 8.0$ Hz, H-9) and δ_H 6.90 (br s, H-5), one methoxy group at δ_H 3.84 (s), two singlet methyl groups at δ_H 1.13 and 1.25 (s), together with many protons arising from sugar moiety were observed. The ^{13}C NMR data showed 22 resonances, including two ester carbonyl carbons (δ_C 178.0 and 176.8), six olefinic carbons (δ_C 149.0, 129.3, 121.9, 116.3, 112.8 and 104.4), nine *O*-bearing carbons, one methoxy carbon (δ_C 56.4), one methyne carbon (δ_C 55.5), one sp^3 quaternary carbon (δ_C 41.9) and two methyl carbons (δ_C 23.0 and 19.6). These spectroscopic features suggested that **1** comprised of one 6-methoxyl-7-hydroxytropic acid (Li & Kuo 1998; Watson & Youngson 1972) subunit, one glucose subunit and one pantolactone subunit. The location of the glucose at C-2′′ and C-3 was confirmed by the HMBC correlation from H-1′ to C-2′′ (as well as H-2′′ to C-1′) and H-6′ to C-3. The location of the methoxy group at C-6 was confirmed by the HMBC correlation between 6-OMe, H-5, H-8 and C-6 (Figure S1). The glycone part of **1**, a glucose moiety, was identified and characterised by the anomeric proton doublet at δ_H 4.45 (1H, d, $J = 7.6$ Hz, H-1′). These data suggested that the glycosyl moiety was *β*-glucose. The acidic hydrolysis of **1** afforded D-(-)-pantolactone ($[\alpha]^{25}_D$ -49.6, *c* 0.1, CH_3OH). Further basic hydrolysis of the remainder afforded glucose and 6-methoxyl-7-hydroxytropic acid (**1a**). The glucose as the sole sugar identified on the basis of thin-layer chromatography (TLC) by comparing with an authentic sugar sample. The glucose isolated from the hydrolysate gave a positive optical rotation, $[\alpha]^{24}_D$ + 19.2 (*c* 0.1, MeOH) and indicated that they are D-glucose. The **1a** ($[\alpha]^{25}_D$ −21.2, *c* 0.1, MeOH) has the (*S*)-form because of the negative specific rotation based on Y.C. Li confirmation on the *S*-configuration of the compound ficusol (Li & Kuo 1998). Thus, compound **1** was identified as an uncommon phenolic glycoside derivative which comprised of (2*S*)-6-methoxyl-7-hydroxytropic acid, *β*-D-glucose and D-(-)-pantolactone. And we named compound **1** as saproglucoside.

The structures of known compounds **2**–**6** were identified by comparison with their $^{1}H/^{13}C$ NMR spectra and $[\alpha]^{25}_{D}$ with those in the literature.

The isolated compounds were evaluated for cytotoxic activities against the mouse melanoma cell line (B16F10) and the human lung adenocarcinoma cell lines (A549). However, the IC_{50} values of all compounds were higher than 10 μM in the assay

The antibacterial activities of all compounds were determined against seven terrestrial pathogenic bacteria *Micrococcus tetragenus, Escherichia coli, Staphylococcus albus, Bacillus cereus, Saphylococcus. aureus, Micrococcus luteus and Bacillus subtilis*. However, the MIC values of all compounds were higher than 20 μg/mL in the assay.

3. Experimental

3.1. General

IR spectra were recorded on a Nicolet 6700 spectrophotometer. UV spectra were recorded on a Beckman DU 640 spectrophotometer. Optical rotations were measured on a JASCO P-1020 digital polarimeter. NMR spectra were recorded on a Bruker AV spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). TMS was used as an internal standard. HRESIMS spectra were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh) and octadecylsilyl silica gel (YMC; 12 nm–50 μM) were used for column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for TLC. Semi-preparative HPLC was performed on an Agilent 1260 LC series with a DAD detector using an Agilent Eclipse XDB-C18 column (9.4 × 250 mm, 5 μM).

3.2. Plant material

The stems of *S. merrillii* were collected from Sanya, Hainan Province, China, in June 2014 and identified by Prof. Qiong-Xin Zhong, College of Life Science, Hainan Normal University. A voucher specimen (No. GFM20140615) has been deposited at the Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University.

3.3. Extraction and isolation

The air-dried and powdered stems (4.5 kg) of *S. merrillii* were extracted three times with 85% EtOH (30 L) at room temperature (3 × 3d). After concentrated under reduced pressure, the water soluble residue was partitioned successively by petroleum ether and EtOAc and then fractionated to yield an ethyl acetate extract (95 g), petroleum ether extract (60 g) and aqueous layer (100 g). The aqueous layer was subjected to silica gel CC (EtOAc, MeOH v/v, gradient) to generate five fractions (Frs. 1–5). Frs. 4 (12 g) was applied to silica gel CC eluted with EtOAc–MeOH (from 20:1 to 1:1) to afford three subfractions (3a–3c). Subfraction 3c was further purified by using octadecylsilyl silica gel (10% MeOH/H_2O) to obtain **1** (31 mg) and **6** (22 mg). Subfractions 3b was further separated by semi-preparative HPLC (CH_3CN/H_2O, 7:93 *v/v*) to obtain **2** (8 mg), **3** (7 mg), and **4** (8 mg) and **5** (6 mg), respectively.

3.3.1. Saproglucoside (1)

White amorphous powder; $[\alpha]^{25}_{D}$ -21.8 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.2) nm; IR (KBr) ν_{max}^{-1} 3258, 1733, 1610, 1448 and 1288 cm^{-1}; HR-ESI-MS *m/z* 485.1668 $[M - H]^-$ ($C_{22}H_{29}O_{12}$; calc'd 485.1659); ^{1}H NMR (MeOD-d_4, 400 MHz) δ: 6.90 (1H, br s, H-5), 6.76 (1H, d, *J* = 8.0 Hz, H-9), 6.74 (1H, d, *J* = 8.0 Hz, H-8), 4.58 (1H, s, H-2′′), 4.45 (1H, d, *J* = 7.6 Hz, H-1′), 4.04 (1H, dd, *J* = 9.2, 8.0 Hz, H-1α), 4.02 (1H, d, *J* = 10.4, H-4′′a), 4.00 (1H, d, *J* = 10.4, H-4′′b), 3.87 (1H, dd, *J* = 5.6, 3.2, H-6′β), 3.84 (3H, s, 6-OMe), 3.70 (1H, dd, *J* = 5.6, 3.6 Hz, H-6′α), 3.67 (1H, d, *J* = 9.2 Hz, H-1β), 3.63 (1H, d, *J* = 8.0 Hz, H-2), 3.37 (1H, m, H-3′), 3.35 (1H, m, H-4′), 3.30 (1H, m, H-5′), 3.29 (1H, m, H-2′), 1.25 (3H, s, H-5′′), 1.13 (3H, s, H-6′′); ^{13}C NMR (400 MHz, MeOD-d_4) δ: 178.0 (C-1′′), 176.8 (C-3), 149.0 (C-6), 147.0 (C-7), 129.3 (C-4), 121.9 (C-9), 116.3 (C-8), 112.8 (C-5), 104.4 (C-1′), 81.1 (C-2′′), 78.3 (C-5′), 77.8 (C-3′), 77.4 (C-4′′), 74.7 (C-2′), 71.2 (C-4′), 65.4 (C-1), 62.6 (C-6′), 56.4 (6-OMe), 55.5 (C-2), 41.9 (C-3′′), 23.0 (C-5′′), 19.6 (C-6′′).

3.3.2. (2S)-6-methoxyl-7-hydroxytropic acid (1a)

White amorphous powder; $[\alpha]^{25}_{D}$ -21.2 (*c* 1.0×10^{-3}, MeOH); ^{1}H NMR (MeOD-d_4, 400 MHz) δ: 7.05 (1H, d, *J* = 1.2 Hz, H-5), 6.79 (1H, dd, *J* = 8.0, 1.2 Hz, H-9), 6.70 (1H, d, *J* = 8.0 Hz, H-8), 4.06 (1H, dd, *J* = 11.6, 9.2 Hz, H-1α), 3.85 (3H, s, 6-OMe), 3.72 (1H, dd, *J* = 11.6, 3.6 Hz, H-1β), 3.58 (1H, dd, *J* = 9.2, 3.6 Hz, H-2); ^{13}C NMR (400 MHz, MeOD-d_4) δ: 177.2 (C-3), 147.9 (C-6), 147.2 (C-7), 129.1 (C-4), 122.6 (C-9), 116.5 (C-8), 112.6 (C-5), 64.9 (C-1),56.5 (6-OMe) 54.8 (C-2).

3.4. Hydrolysis of Saproglucoside (1)

Acid hydrolysis of Saproglucoside (**1**): a solution of **1** (20 mg) in 5% H_2SO_4 (2 mL) was heated under reflux for 3 h and then evaporated to dryness. The mixture was separated by the semi-preparative HPLC to obtain a D-(-)-pantolactone (4 mg) and the remainder (13 mg). The remainder further basic hydrolysis in 20% KOH (1.5 mL) was heated under reflux for 2 h. The mixture was neutralised with 1 N HCl and then evaporated to dryness. The mixture was extracted with ethyl acetate. After evaporation, the residue was purificated by the semi-preparative HPLC to give 6-methoxyl-7-hydroxytropic acid (**1a**) (7 mg). The water soluble residue evaporated to dryness (contained glucose). The contained glucose was purified by the semi-preparative HPLC.

3.5. Biological assays

Cytotoxic activity was evaluated by the MTT method as described previously (Scudiero et al. 1988). Two cancer cell lines, ECA-109 and A549 cell lines, were used. Epirubicin was used as a positive control. Antibacterial activity was determined by the conventional broth dilution assay (Pierce et al. 2008). Seven terrestrial pathogenic bacteria *M. tetragenus, E. coli, S. albus, B. cereus, S. aureus*, *M. luteus* and *B. subtilis* were used, and ciprofloxacin was used as a positive control.

Disclosure statement

No potential conflict of interest was reported by the authors.

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