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Ursane triterpenoids from the bark of *Terminalia arjuna*

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ABSTRACT

Five ursane type triterpene glucosyl esters including a new one, 2α,3β-dihydroxyurs-12,18-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**) were isolated from the bark of *Terminalia arjuna*, along with two known phenolic compounds. It is the first report of ursane type triterpenoids from this species.

1. Introduction

Terminalia arjuna Wight & Arn (Combretaceae) is a 20–30 m tall tree distributed in India, Burma, and Sri Lanka. The bark of *T. arjuna* is a famous Indian folk medicinal plant used as a cardio-tonic in heart failure, ischaemic cardiomyopathy, atherosclerosis and myocardium necrosis [1–5]. Also, it is an essential ingredient of many Ayurvedic preparations which are sold as cardio-tonics [6]. It was reported that several species of *Terminalia* have been used in traditional treatment of cancer [7]. Previous phytochemical investigations showed triterpenoids, tannins and flavonoids were isolated from the bark of *T. arjuna* [8]. Many oleanane type triterpenoids have been reported from the title plant, some of which possess antitumoral, antioxidant, antiallergic, antiasthmatic, antifeedant and cardioprotective activities [9–13].

In continuing to search for new chemical and bio-markers from the medicinal plant *T. arjuna* for the quality control study of the related dietary supplements, one new ursane triterpene glucosyl ester, 2α,3β-dihydroxyurs-12,18-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**) and four known ursane triterpene glycosyl esters (Fig. 1), namely, 2α,3β,23-trihydroxyurs-12,18-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**2**) [14], quadranoside VIII (**3**) [15], kajiichigoside F1 (**4**) and 2α,3β,23-trihydroxyurs-12,19-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**5**) [14,16], as well as two known phenolic compounds, 3-*O*-methylellagic acid 4′-*O*-α-L-rhamnopyranoside and (−)-epicatechin were isolated [17,18]. Herein we report the isolation and structure elucidation of the new metabolite. Its structure was identified on the basis of extensive NMR experiments .

2. Experimental

2.1. General

Optical rotation: Rudolph Research AutoPol IV. UV: Hewlett–Packard 8453. IR: Bruker Tensor 27 FT-IR and MIRacle ATRFT-IR. HRESIMS: Agilent Series 1100 SL. NMR spectra: American Varian Mercury plus 400 (^{1}H 400 MHz, ^{13}C 100 MHz). HPLC: Waters LC Module I. HPLC column: Phenomenex Gemini C18 5 μ ODS column (10×250 mm).

2.2. Plant material

The bark of *T. arjuna* Wight & Arn. was purchased from Garry & Sun (Reno, Nevada, USA), and was authenticated by

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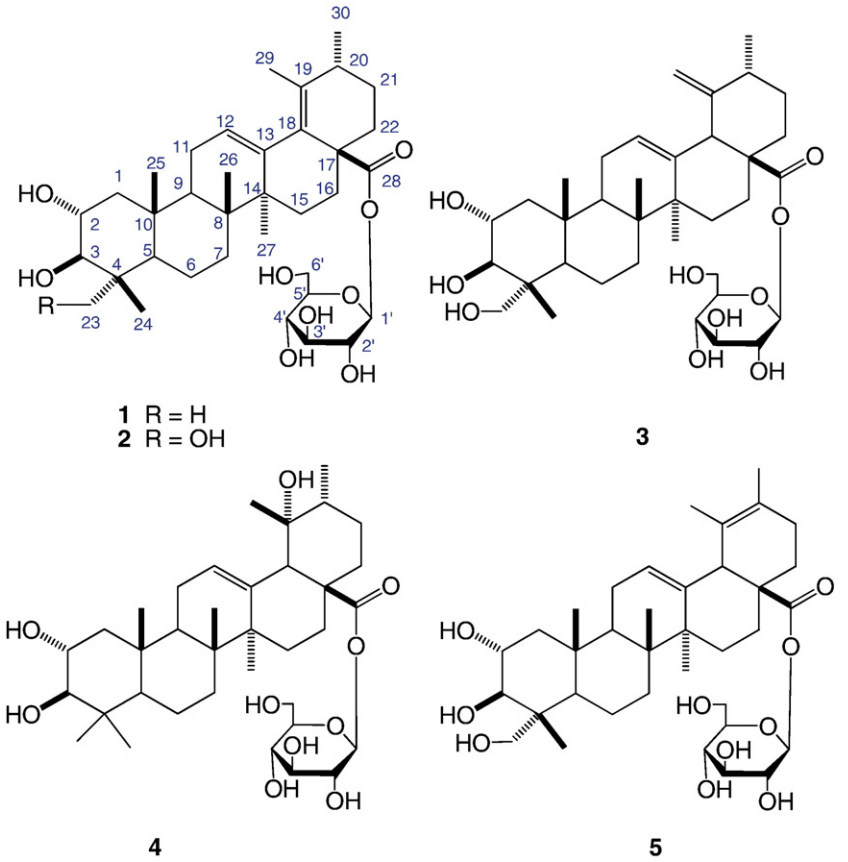

Fig. 1. Structures of **1**–**5**.

Dr. Vaishali C. Joshi (University of Mississippi). A voucher specimen (#3799) was deposited at the National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi, USA.

2.3. Extraction and isolation

The air-dried and powdered bark of *T. arjuna* (2 kg) was extracted by sonicating with MeOH (4×2 L) at room temperature for 2 h. The pooled MeOH solution was evaporated *in vacuo* to give a residue (645 g). The MeOH extract was suspended in H_2O (1.5 L) and then partitioned with hexanes (3×4 L), Et_2O (3×4 L) and EtOAc (3×4 L). The EtOAc layer afforded a residue (11 g), which was separated into 11 fractions by column chromatography (CC) on silica gel with a gradient elution of $CHCl_3$–MeOH (20:1–1:1). Fr. 7 was subjected to CC over reversed-phase C18 silica and eluted with MeOH/H_2O (2:3, v/v) to give (−)-epicatechin (42.5 mg). Fr. 9 was separated by reversed-phase C18 silica gel CC eluted with MeOH/H_2O (3:7, v/v) and further purified by semipreparative HPLC with MeCN/H_2O (33:67) as mobile phase (flow rate 6.0 mL/min) to afford **1** (5.2 mg), **4** (26.2 mg) and 3-*O*-methylellagic acid 4′-*O*-α-L-rhamnopyranoside (21.4 mg). Fr. 10 was treated in a similar manner to that of Fr. 9 to yield **2** (14.4 mg) and **5** (7.7 mg). Fr. 11 was subjected to a reversed-phase C18 silica gel column eluted with MeOH/H_2O (2:3, v/v) and purified by semipreparative HPLC with MeOH/H_2O (64:36) as mobile phase (flow rate 4.0 mL/min) to yield **3** (5.8 mg).

2α,3β-dihydroxyurs-12,18-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**): colorless gum; $[\alpha]D^{20}$: +59.2 (*c* 0.3, MeOH); UV, λ_{max} (MeOH) nm (logε): 250 (3.60) nm; IR (KBr), ν_{max} cm^{-1}: 3386, 2932, 2872, 1714, 1453, 1368, 1225, 1175, 1071, and 892; HR-ESI-MS *m/z* 655.3827 $[M+Na]^+$ (calcd for $C_{36}H_{56}O_9Na$, 655.3822); For ^1H and ^{13}C NMR data: see Table 1.

Acid hydrolysis of **1** and determination of D-glucose were carried out according to the method reported previously [19].

3. Results and discussion

Compound **1** was isolated as a colorless gum. The molecular formula of $C_{36}H_{56}O_9$ (positive mode *m/z* = 655.3827 $[M+Na]^+$ calcd. for $C_{36}H_{56}O_9Na$: 655.3822) was established by HR-ESI-MS and ^{13}C NMR spectra. The IR spectrum showed the bands at 3386 and 1714 cm^{-1} corresponding to the absorptions of the hydroxy and the carbonyl groups, respectively. The ^1H NMR spectrum (Table 1) showed six tertiary methyl [δ_H 1.04, 1.09 (×2), 1.18, 1.28 and 1.79], one secondary methyl (δ_H 1.03, d, *J* = 4.0 Hz) and the anomeric proton of a β-glucopyranosyl unit ($\delta_{H-1'}$ 6.35, d, *J* = 8.0 Hz). The ^{13}C NMR and DEPT (Table 1) spectra indicated the presence of 36 carbons, including one carboxyl (δ_C 175.1), four olefinic [δ_C 139.1 (s), 136.4 (s), 134.1 (s) and 126.9 (d)] and two oxymethine (δ_C 84.1 and 69.0) carbons in the low-field region and seven methyl, eight methylene, three