Phytochemistry Letters 25 (2018) 43–46

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

The first phytochemical investigation of *Rhododendron websterianum*: triterpenoids and their cytotoxic activity

Rui Zhang[a,b,c], Chunping Tang[a,c], Yan Li[a], Chang-Qiang Ke[a,c], Ge Lin[c,d], Hua Xie[a], Sheng Yao[a,c,*], Yang Ye[a,c,e,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] SIMM-CUHK Joint Research Laboratory for Promoting Globalization of Traditional Chinese Medicines between Shanghai Institute of Materia Medica, Chinese Academy of Sciences and The Chinese University of Hong Kong, Hong Kong SAR, China
[d] School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China
[e] School of Life Science and Technology, Shanghai Tech University, Shanghai 201210, China

ARTICLE INFO

Keywords:
Ericaceae
Rhododendron websterianum
Triterpenoids
Cytotoxicity

ABSTRACT

The first phytochemical investigation of the fruits of *Rhododendron websterianum* resulted in the isolation of two new triterpenoids, 11-dehydroxytrisnormogrol acid (**1**), 11-dehydroxytrisnormogrol (**2**), and 21 known analogues. Their structures were determined by extensive analysis of spectroscopic and mass data, as well as by comparison with those in literature. All isolates were assayed their cytotoxic activities on human hepatoma SMMC-7721 cells and human lung adenocarcinoma A549 cells. Three known compounds, dammarenediol II, lup-20(29)-ene-2α,3β-diol, and 19,24-dihydroxyurs-12-en-3-one-28-oic acid exhibited cytotoxic activities against these two human cancer cell lines with the IC_{50} values in a range of 27.4–42.8 μM.

1. Introduction

The genus *Rhododendron*, with nearly 960 species, is one of the biggest genera of the Ericaceae family, and mainly distributes in the northern hemisphere (Editorial Committee of Flora of China, 1999). Although toxicity was reported for some *Rhododendron* species, they have long been used as traditional medicines to treat asthma, cold, gastro-intestinal disorders, inflammation, and skin diseases (Popescu and Kopp, 2013). Phytochemical investigations of the genus *Rhododendron* resulted in the isolation of hundreds of secondary metabolites including triterpenoids, diterpenoids, magestigmane sesquiterpenoids, iridoid glycosides, flavonoids, and phenolic compounds (Li et al., 2013; Popescu and Kopp, 2013; Qiang et al., 2011). Diterpenoids are one of the characteristic secondary metabolites of the *Rhododendron* genus. To date, more than 100 diterpenoids and five dimeric grayanane diterpenoids had been reported (Li et al., 2013; Li et al., 2015; Liu et al., 2014; Niu et al., 2016; Qiang et al., 2011; Zhang et al., 2015; Zhang et al., 2017; Zhou et al., 2014; Zhou et al., 2017), and some of them exhibited significant bioactivities such as antinociceptive (Li et al., 2015), and insecticidal activities (Hu et al., 1993). In our previous studies, we obtained a series of diterpenoids from *R. molle* (Zhou et al., 2014; Zhou et al., 2017) and *R. pumilum* (Zhang et al., 2018). In ongoing endeavors to discover new bioactive molecules from the ericaceous plants, the fruits of *R. websterianum* was investigated.

R. websterianum is an evergreen shrub widespread in regions of Sichuan and Qinghai provinces of China. No phytochemical investigation of this plant had been carried out before. Our study is the first systematic investigation on this species, resulting in the isolation of two new triterpenoids (**1**–**2**) and 21 known analogues from the fruits of *R. websterianum*. All isolates were assayed for their cytotoxic activities on human hepatoma SMMC-7721 cells and human lung adenocarcinoma A549 cells. Herein, we report the isolation, structure elucidation, and cytotoxic activity of these compounds.

2. Results and discussion

The fruits of *R. websterianum* were extracted with 95% ethanol at room temperature. The ethanol extract was partitioned with petroleum ether (PE) and EtOAc (EA), successively. The EA extract was further separated using repeated column chromatography over silica gel, Sephadex LH-20, MCI gel, and preparative HPLC, affording two new and 21 known triterpenoids (Fig. 1).

Compound **1** was obtained as a white amorphous powder. The HRESIMS of **1**, recording a quasi-molecular ion at *m/z* 415.3224 $[M-H]^-$

* Corresponding authors at: State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China.
E-mail addresses: yaosheng@simm.ac.cn (S. Yao), yye@mail.shcnc.ac.cn (Y. Ye).

https://doi.org/10.1016/j.phytol.2018.03.015
Received 8 December 2017; Received in revised form 6 March 2018; Accepted 9 March 2018

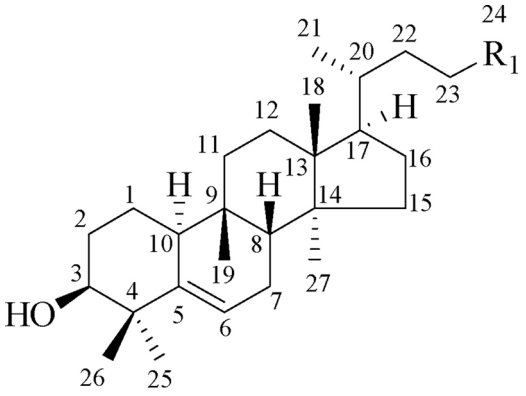

Fig. 1. Chemical structures of compounds **1**-**2**.

Table 1
^{1}H and ^{13}C NMR data for compounds **1**- **2** in $CDCl_3$.

Positions	1[a]		2[b]	
		δ_H(*J* in Hz)	δ_C	δ_H(*J*in Hz)
1	1.57, m	21.3	1.56, m	21.3
	1.44, m		1.45, m	
2	1.88, m	29.0	1.87, m	29.0
	1.72, m		1.72, m	
3	3.48, t (2.8)	76.8	3.47, br s	76.8
4	–	41.6	–	41.6
5	–	141.3	–	141.4
6	5.58, m	121.6	5.58, m	121.6
7	2.38, m	24.5	2.38, m	24.5
	1.78, m		1.80, m	
8	1.75, br d (7.9)	43.8	1.75, br d (7.8)	43.8
9	–	34.6	–	34.6
10	2.26, m	38.0	2.26, br d (12.7)	38.0
11	1.66, m	32.4	1.64, m	32.5
	1.42, m		1.42, m	
12	1.69, m	30.6	1.66, m	30.6
	1.47, m		1.50, m	
13	–	46.4	–	46.4
14	–	49.3	–	49.3
15	1.21, m	34.9	1.20, m	34.9
	1.14, m		1.13, m	
16	1.91, m	27.9	1.90, m	28.1
	1.51, m		1.29, m	
17	1.49, m	50.4	1.48, m	50.6
18	0.84, s	15.5	0.85, s	15.5
19	0.91, s	28.2	0.91, s	28.2
20	1.47, m	35.7	1.45, m	35.9
21	0.90, d (5.8)	18.5	0.90, d (6.0)	18.9
22	1.81, m	31.3	1.66, m	32.3
	1.31, m		1.05, m	
23	2.38, m	31.2	1.65, m	29.7
	2.26, m		1.46, m	
24	–	179.4	3.61, m	63.8
25	1.02, s	27.4	1.02, s	27.4
26	1.13, s	25.6	1.13, s	25.6
27	0.80, s	17.9	0.80, s	18.0

[a] Data were recorded at 600 MHz (^{1}H) and 125 MHz (^{13}C).
[b] Date were recorded at 400 MHz (^{1}H) and 125 MHz (^{13}C).

(cal. 415.3218), suggested a molecular formula of $C_{27}H_{44}O_3$ with six degrees of unsaturation. The IR absorption band at 3426 cm^{-1} indicated the presence of hydroxy group. The ^{1}H NMR data of **1** (Table 1) showed signals for five singlet methyls (δ_H 0.80, 0.84, 0.91, 1.02, 1.13), one doublet methyl (δ_H 0.90, d, J = 5.8), one triplet oxygenated methine (δ_H 3.48, t, J = 2.8), and one olefinic methine (δ_H 5.58, m). The ^{13}C NMR

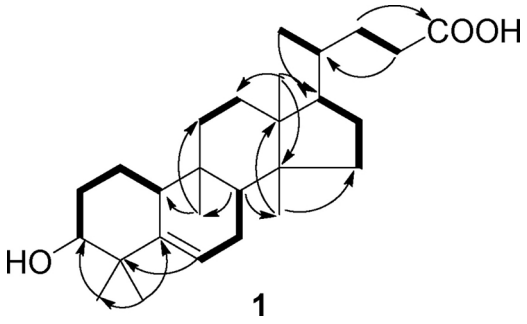

Fig. 2. Selected ^{1}H–^{1}H COSY (▬) and key HMBC correlations (H → C) of **1**.

and DEPT data (Table 1) revealed the existence of 27 carbon resonances including six methyls, nine methylenes, six methines (one oxygenated at δ_C 76.8), and six quaternary carbons (one carboxyl at δ_C 179.4). The NMR data were highly similar to those of the known compound (23*E*)-cucurbita-3*β*-hydroxy-5,23,25-triene (Zeng et al., 2016), except for the side chain. The chemical shifts of the side chain of **1**, however, were very close to those of the side chain of another known compound, 25,26,27-trisnor-3*α*-hydroxy-lanost-9(11)-en-24-oic acid (Wada and Tanaka, 2000). The evidence supported a structure with a nucleus of (23*E*)-cucurbita-3*β*-hydroxy-5,23,25-triene and a side chain of 25,26,27-trisnor-3*α*-hydroxy-lanost-9(11)-en-24-oic acid. Such elucidation was further supported by 2D NMR data. The ^{1}H–^{1}H COSY and HSQC spectra indicated the existence of the moieties: −CH(10)-CH_2(1)-CH_2(2)-CH(3)-, −CH(6)-CH_2(7)-CH(8)-, −CH_2(11)-CH_2(12)-, −CH_2(15)-CH_2(16)-CH(17)-, CH_3(21)-CH(20)-, −CH_2(22)-CH_2(23)- (Fig. 2). HMBC correlations from H_3-25 to C-3, H_3-26 to C-5 and C-25, H-6 to C-4, H-8 to C-19 and C-27, H_3-19 to C-10 and C-11, H_3-18 to C-12, C-14, and C-17, H_3-21 to C-17, H_2-22 to C-24, H_2-23 to C-20, and H_3-27 to C-13 and C-15 (Fig. 2) were clearly observed. The key HMBC correlations from H_3-21 to C-17 further linked the side chain to the tetracyclic nucleus (Fig. 2).

The relative configuration of the tetracyclic nucleus was deduced by the NOESY experiment (Fig. 3). A triplet of the oxygenated methine of H-3 (δ_H 3.48, t, J = 2.8) indicated an equatorial configuration of H-3 (Zeng et al., 2016). The correlations of H-3/H_3-25, H-10/H_3-25, H-10/H_3-27, and H-17/H_3-27 indicated the co-facial orientations of H-3, H_3-25, H-10, H-17, and H_3-27. The cross-peaks of H-8/H_3-18, H-8/H_3-19 suggested that these protons were on the other face of the molecule (Zeng et al., 2016). The stereochemistry of the side chain was inferred from a detailed comparison of the chemical shift and coupling constant pattern of H_3-21 with that of 25,26,27-trisnor-3*α*-hydroxy-lanost-9(11)-en-24-oic acid (Wada and Tanaka, 2000). The H_3-21 of both compounds appeared as a doublet at δ_H 0.90 with a J value around 5.9 Hz, suggesting that they shared the same side chain. Therefore, the whole structure of **1** was established.

Compound **2** was obtained also as a white amorphous powder. The HREIMS, together with the ^{13}C NMR data, revealed a molecular formula of $C_{27}H_{46}O_2$ with five indices of hydrogen deficiency. The IR

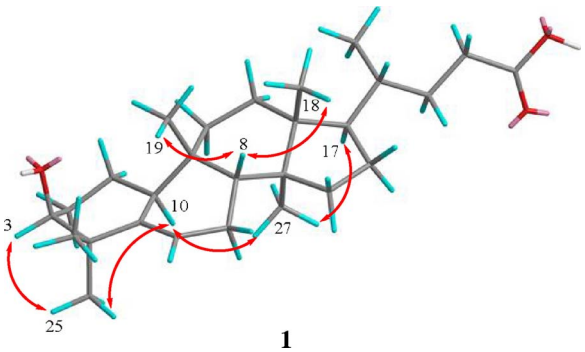

Fig. 3. Key NOESY correlations (H↔H) of **1**.