Phytochemistry Letters 28 (2018) 135–138

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Indole and flavonoid from the herbs of *Kalimeris shimadai*

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ARTICLE INFO

Keywords:
Kalimeris shimadai
Indole
Flavonoid
Cytotoxicity

ABSTRACT

A new indole, kalshiliod A (**1**), a new flavonoid glycoside, isorhamnetin-3-O-*β*-D- glucuronide-6″-ethyl ester (**2**), and ten known compounds (**3–12**) were isolated from the herbs of *Kalimeris shimadai*. The compounds' structures were elucidated through comprehensive spectroscopic techniques. All of the compounds were isolated from *K. shimadai* for the first time. Furthermore, the new compounds' cytotoxicities against five human cancer cell lines were evaluated through the SRB assay.

1. Introduction

Kalimer shimadai belonging to the genus *kalimeris* is widely distributed in Asia. This plant also has been used in traditional Chinese medicine and as food (Chinese Herbal Editorial Board, 2006). Previous investigations of *K. indica* have demonstrated the presence of terpenoids (Wang et al., 2017a,b), phenolic derivatives (Ji et al., 2014) and anthraquinones (Wang et al., 2010, 2015), and a series of sesquiterpenes (Wang et al., 2018) and flavonoids were isolated (Wang et al., 2017a) in studies of *K. integrifolia*. However, a survey of the literature indicates that limited chemical studies have been conducted on this plant. Therefore, this study investigated the structurally interesting and bioactive *K. shimadai*, leading to the isolation of an indole derivative and flavonoid glycoside, kalshiliod A (**1**) and isorhamnetin-3-O-*β*-D-glucuronide-6″-ethyl ester (**2**), respectively, from the whole herbs of *K. shimadai* and the identification of eleven known compounds, namely, 3-indolylglyoxylic acid (**3**) (Bao et al., 2014), 1*H*-indole-3-carboxylic acid, ethyl ester (**4**) (Lee et al., 2007), linchuniinone (**5**) (Zhang et al., 2006), olibanumol C (**6**) (Yoshikawa et al., 2009), phytol (**7**) (Fang et al., 2006), friedelin (**8**) (Xu et al., 2014), D:B-friedoolean-5-en-3*β*-ol (**9**) (Carvalho and Seita, 1993), spinasterol (**10**) (Zhang et al., 2005), methyl hematinate (**11**) (Chen et al. 2016) and (1*R*, 2*R*)-2-(5-methoxy-5-oxo-2-penten-1-yl)-3-oxo- cyclopentaneacetic acid methyl ester (**12**) (Haider et al., 2000), by comparing our data with the data in the literature. Thus, our investigation achieved the extraction, purification and identification of the isolated components and evaluated their anticancer activities.

2. Results and discussion

Kalshiliod A (**1**) was obtained as a yellow powder. The optical rotation of **1** was [α]23.8 D-38.7 (c = 0.05; MeOH).The molecular formula of **1** was determined as $C_{14}H_{14}N_2O_2$ by HR-ESI-MS (*m/z* 241.0988 (calc. 241.0983 for $C_{14}H_{14}N_2O_2$ $[M-H]^-$). According to the ^{13}C NMR and HR-ESI-MS results, the compound presents nine indices of hydrogen deficiency (IHD). The IR absorption bands at 3424, 1654 and 1633 cm^{-1} suggested the presence of amino and carboxyl groups. The ^{13}C NMR and DEPT spectra revealed the presence of fourteen carbons, including four methylenes, five methines and five quaternary carbons (Table 1). The ^1H-NMR spectrum indicated four characteristic proton signals at the low field, including δ_H 8.26 (1H, s), 8.21 (1H, d, J = 7.1 Hz), 7.47 (1H, d, J = 7.2 Hz), and 7.24 (2H, m), which are the feature of indole-type skeletons. Based on its IHD, a degree of unsaturation remains after calculating a potential predicted indole skeleton. As mentioned above, this evidence suggested that **1** is an indole-type skeleton with a tricyclic system. Moreover, the ^1H-^1H COSY correlations between δ_H 3.57 (H-3), 2.50 (H-4), and 2.14 (H-5) supported the presence of the methlyene group. HMBC correlations were observed between δ_H 3.57 (H-3) and δ_C 178.5 (C-2), 18.9 (C-4) and 49.8 (C-5). Together with one remaining IHD, the key HMBC and COSY correlations revealed the third cycle of **1**. Based on the presented IR absorption and MS data, the third cycle of **1** was identified as a pyrrolidonyl group.

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https://doi.org/10.1016/j.phytol.2018.10.002
Received 30 June 2018; Received in revised form 11 September 2018; Accepted 1 October 2018

Table 1
^{1}H and ^{13}C NMR spectroscopic data of **1** and **2** (δ in ppm, J in Hz).

No.	1		No.	2	
	δ_C[a]	δ_H[b]		δ_C[c]	δ_H[d]
2	178.5, s		2	158.8, s	
3	31.6, t	2.50 (t, 7.9)	3	135.2, s	
4	18.9, t	2.14 (m)	4	179.1, s	
5	49.8, t	3.57 (t, 7.1)	5	163.0, s	
1'	50.1, t	4.69 (s)	6	100.0, d	6.19 (s)
2'	191.0, s		7	166.3, s	
2"	134.7, d	8.26 (s)	8	94.9, d	6.39 (s)
3"	115.9, s		9	158.4, s	
3a"	126.9, s		10	105.6, s	
4"	122.7, d	8.21 (d, 7.1)	1'	122.7, s	
5"	123.4, d	7.24 (m)	1"	104.5, d	5.38 (d, 7.1)
6"	124.5, d	7.24 (m)	2'	114.5, d	7.96 (s)
7"	113.0, d	7.47 (d, 7.2)	2"	75.6, d	3.52 (m)
7a"	138.3, s		3'	148.3, s	
			3"	77.3, d	3.51 (m)
			4'	150.9, s	
			4"	72.9, d	3.57 (m)
			5'	115.9, d	6.88 (d, 8.4)
			5"	77.3, d	3.79 (d, 9.5)
			6'	123.6, d	7.54 (d, 8.4)
			6"	169.9, s	
			7"	62.5, t	4.10 (q, 7.1)
			8"	14.2, q	1.15 (t, 7.1)
			3'-OCH_3	56.7, q	3.96 (s)

[a] Recorded in 150 MHz.
[b] Recorded in 600 MHz.
[c] Recorded in 125 MHz.
[d] Recorded in 500 MHz; NMR solvent:CD_3OD.

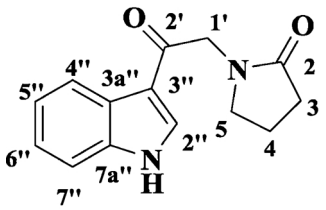
The linkage of the indolyl and pyrrolidonyl groups was confirmed by the HMBC correlation from δ_H 4.69 (H-1′) to δ_C 178.5 (C-2), 49.8 (C-5) and 191.0 (C-2′) assigned as the O = C − CH_2 bond, and δ_H 8.26 (H-2″) to δ_C 191.0 (C-2′) (Fig. 2). Eventually, the structure of **1** was determined to be indole-type alkaloid (Bao et al., 2014), 1-(2-(1H-indol-3-yl)-2-oxoethyl)pyrrolidin-2-one (Fig. 1) and was named kalshiliod A.

Isorhamnetin-3-O-β-D-glucuronide-6″-ethyl ester (**2**) was obtained as a yellow powder, and its optical rotation was [α]23.4 D-32.8 (c = 0.12; MeOH). According to the HR-ESI-MS spectrum, the molecular formula was determined as $C_{24}H_{24}O_{13}$ (*m/z* 519.1144, $[M-H]^-$; calcd. for 519.1141), indicating that this compound's IHD is thirteen. The IR absorption bands of two carbonyl and hydroxyl groups were observed at 1605, 1650 and 3427 cm^{-1}. The ^{13}C NMR and DEPT spectra of **2** exhibited eleven quaternary carbons, one methine, ten methylene and two methyl carbons. A series of characteristic aromatic proton signals of the ABX system were observed at δ_H 7.96 (1H, s, H-2′), 7.54 (1H, dd, J = 8.3, 2.1 Hz, H-6′), and 6.88 (1H, d, J = 8.4 Hz, H-5′)] (Table 1). In addition, two proton signals at the low field, δ_H 6.39 (1H, s, H-8), and 6.19 (1H, s, H-6), and the ABX system describe the basic flavonoid structural character. Certain proton signals of **2** were observed the sugar moiety, containing one doublet proton signal at 5.38 (1H, d, J = 7.1 Hz, H-1″) for the anomeric proton of the sugar moiety, at 3.79 (1H, d, J = 9.5 Hz), 3.57 (1H, m), 3.52 (1H, m), 3.51 (1H, m). The remaining proton signals were observed at 4.10 (2H, q, J = 7.1 Hz), 1.15 (3H, t, J = 7.1 Hz), corresponding to one methylene and one methyl group, respectively, indicating that this is an ethoxy group. Comparing these data with isorhamnetin-3-*O*-β-D-glucuronide-6″- methyl ester (He et al., 2017), it is clear that while **2** is similar to this compound, they differ by the ethoxy group. Based on the COSY and HMBC relationships (Fig. 2), the HMBC correlations of H-4″ to C-6″, and H-7″ to C-6″ identified the glucuronide ethyl ester moiety of **2**. The critical COSY and HMBC correlations of 2 are shown in Figure S1. The relative configuration was confirmed by the ROESY spectrum. In the ROESY spectrum of **2** (Fig. 3), H-1″ showed correlations with the proton signals H-3″ and H-5″. Taken together, the structure of **2** was elucidated as (2*S*, 3*S*, 4*S*, 5*R*, 6*S*)-ethyl 6-(5, 7- dihydroxy-2-(4- hydroxy-3-methoxyphenyl)-4- oxo-4H-chromen-3-yloxy)-3, 4, 5-trihydroxytetrahydro-2H-pyran -2-carboxylate (Fig. 1) and was named isorhamnetin-3-O-β-D- glucuronide-6″-ethyl ester.

3. Experimental

3.1. General experimental procedures

UV spectra were recorded using a UV-2401 A spectrophotometer (Shimadzu, Japan). Optical rotations were measured using a SEPA 300 polarimeter (Horiba, Japan). IR spectra were obtained using a Bruker FT-IR Tensor27 spectrometer (Bruker Corporation, Germany) using KBr pellets. HR-ESI–MS were recorded using an Agilent 6200 Q-TOF MS system (Agilent Technologies, USA). NMR spectra were recorded using a Bruker DRX-500 and AM-400 spectrometer (Karlsruhe, Germany). HPLC analyses were performed using an Agilent 1100 series and a 150 × 4.9 mm i.d., 5 μm, Agilent Zorbax SB C18 column as the ODS column (USA). The chiral column used was a 4.6 × 250 mm i.d., 5 μm, CHIRALPAK ASH column (Daicel Chiral Technologies Co., Ltd, Shanghai, China). Preparative HPLC was performed using an Agilent 1260 with an Agilent Zorbax SB C18 column (5 μm, 9.4 × 150 mm, USA) as the ODS column. Medium-pressure liquid chromatography (MPLC) was performed using a BÜCHI Sepacore System and columns packed with Chromatorex C-18 (40–75 mm, Fuji Silysia Chemical Ltd., Japan). Column chromatography (CC) was performed using a silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China), RP-18 (5 μm, Fuji Silysia Chemical Ltd., Japan), Sephadex LH-20 (Amersham Biosciences, Sweden), and the fractions were monitored by TLC (GF254, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China). Spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.



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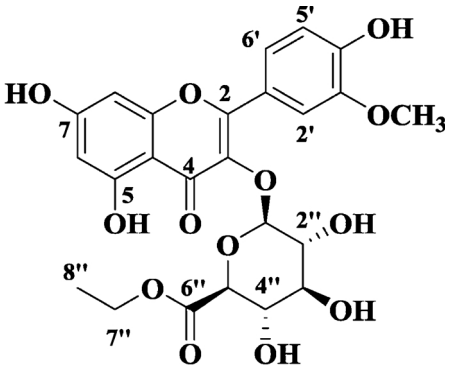

2

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