Asian Journal of Chemistry; Vol. 26, No. 13 (2014), 4000-4002

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16989

Chemical Constituents from *n*-Butanol Extract of *Rabdosia japonica* var. *glaucocalyx*

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Received: 6 January 2014; *Accepted*: 26 March 2014; *Published online*: 23 June 2014; AJC-15392

The *n*-butanol fraction of the dried whole plants of *Rabdosia japonica* var. *glaucocalyx* was isolated and purified by means of chromatography. Six compounds were obtained and their structures were identified by spectral analysis as glaucocalyxin G (**1**), arjunglucoside (**2**), kaempferol-3-*O*-rutinoside (**3**), quercetin-3-*O*-α-L-rhamnoside (**4**), rutin (**5**) and acacetin-7-*O*-β-D-glucoside (**6**). Compound **1** was a new *ent*-kaurane diterpenoid glycoside, compounds **2** and **3** have not been reported before from the plant source.

Keywords: *Rabdosia japonica* var. *glaucocalyx*, Chemical constituents, Glaucocalyxin G, *n*-Butanol fraction.

INTRODUCTION

The genus *Rabdosia japonica* (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara is a member of the family Labiatae, subfamily Ocimoideae, tribe Plectrantheae and is mainly distributed in northeast Asia, such as China, Russian, Korea and Japan[1]. It possesses some pharmacological functions, such as antitumor, antioxidation, antiinflammatory and antibacteria[2]. Results of toxicological tests indicated that the *Rabdosia japonica* var. *glaucocalyx* is safe to human within the scope of the experimental dose[3]. As for the chemical constituents of the plant, diterpenoids[4-8], flavonoids[9-11] and triterpenoids[12,13] are the major constituents in the whole plant. In the course of further studies, six compounds were obtained and their structures were identified as glaucocalyxin G (**1**), arjunglucoside (**2**)[14] , kaempferol-3-*O*-rutinoside (**3**)[15], quercetin-3-*O* -α-L-rhamnoside (**4**)[16], rutin (**5**)[16] and acacetin-7-*O*-β-D -glucoside (**6**)[11] on the basis of their NMR spectral data and by comparison of their physical properties with those reported in the literatures. Compound **1** was a new *ent*-kaurane diterpenoid glycoside from *R. japonica* var. *glaucocalyx*, though the diterpenoids have been regarded as the marking composition of tribe Plectrantheae, there was few report about diterpenoids glycoside of the tribe. compounds **2** and **3** have not been reported before from the plant source.

EXPERIMENTAL

ESI-MS and HRESI-MS were performed with a Mat-212 and a Micro mass Auto Spec Q-TOF spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer with tetramethylsilane as an internal standard and DMSO-d_6 as solvents. Chemical shifts were given in (ppm) values. Optical rotations was determined using a Perkin-Elmer 341 automatic polarimeter.

The plant materials were collected in June 2009 from Antu, Jilin province and identified as the dried whole plants of *Rabdosia japonica* var. *glaucocalyx* by Prof. Hanming Zhang, College of Pharmacy, Second Military Medical University. A voucher specimen (No. 20090603) has been deposited in the herbarium of College of Bio-information, Chongqing University of Posts and Telecommunications, Chongqing.

Extraction and isolation: The dried whole plants (8 kg) were chopped and extracted with 80 % EtOH three times under reflux and concentrated under vacuum to yield an EtOH extract (500 g). The extract was suspended in water and extracted successively with petroleum ether, ethyl acetate and *n*-butanol to obtain petroleum ether fraction (20 g), ethyl acetate fraction (220 g) and *n*-butanol fraction (60 g). The *n*-butanol fraction (55 g) was fractionated *via* silica gel column chromatography eluting with $CHCl_3$-MeOH-H_2O (3:1:0 to 6:4:1) to give six major fractions A_1-A_6. Fraction A_2 (3 g) was subjected to ODS column eluting with MeOH-H_2O (10:90 to 70:30) to give four major fractions B_1-B_4. Fraction B_2 (180 mg) was purified over silica gel and Sephadex LH-20 with MeOH repeatedly to afford compounds **1** (48 mg), **4** (30 mg) and **6** (25 mg). Fraction A_3 (1.7 g) was subjected to ODS column eluting with MeOH-H_2O (10:90 to 70:30) to give five major fractions C_1-C_5. Fraction C_2 (122 mg) was purified over silica gel and Sephadex LH-20 with MeOH repeatedly to afford compound **2** (55 mg). Fraction C_3 (95 mg) was purified over silica gel and Sephadex LH-20 with MeOH repeatedly to afford compounds **3** (37 mg) and **5** (20 mg).

RESULTS AND DISCUSSION

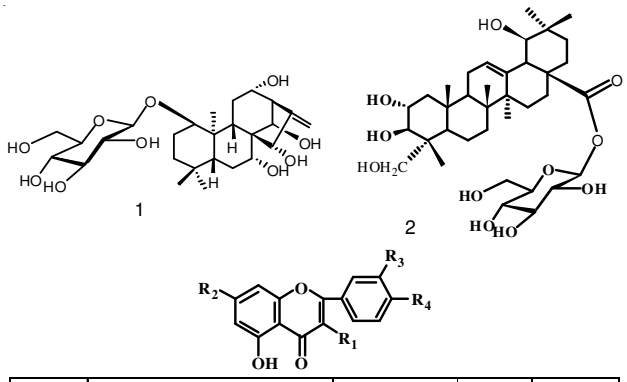
Compound **1** was isolated as a grey powder with an optical rotation $[\alpha]_{20}^{D}$ -78 (c 0.1, MeOH), exhibited a quasi-molecularion peak at *m/z* 537 $[M + Na]^+$ by ESI-MS and the molecular formula $C_{26}H_{42}O_{10}$ was determined by HRESI-MS (*m/z* 514.2777, calcd. 514.2778). And the result of its molish reaction was positive. Furthermore, six proton signals at δ_H 3.40-4.55 suggested the existence of a sugar moiety and the ^{13}C NMR and DEPT spectra displayed six aliphatic carbon signals (δ_C 103.1(d), 74.2(d), 76.7(d), 70.0(d), 76.8(d) and 61.1(t)) due to one sugar moiety. The signal at δ_H 4.55 (d, 1H, J = 7.5 Hz, H-1') was attributed to an anomeric proton, The coupling constant (J = 7.5 Hz) of the anomeric proton demonstrated that the glucose was in β-orientation.

The ^{13}C NMR and DEPT spectra of **1** (Table-1) gave twenty six carbon signals for three methyl, six methylene, thirteen methine and four quaternary carbons, including one exocyclic carbon-carbon double bond (δ_C 155.9 (s) and 106.3 (t)). the ^1H NMR spectrum of **1** revealed the presence of three angular methyl groups (δ_H 0.83 (s, 3H), 0.81 (s, 3H) and 1.27 (s, 3H)). Except for the sugar moiety, there were five oxygenated methines at δ_H 3.17 (dd, 1H, 9.5, 4.0), 3.52 (s, 1H), 3.56 (m, 1H), 4.64 (s, 1H) and 4.86 (m, 1H), respectively. Considering the structures of diterpenoids previously isolated from the plant[4-8] and structure characteristics of the *ent*-kaurane diterpenoid, compound **1** was assigned an *ent*-kaurane diterpenoid glycoside and the *ent*-kaurane diterpenoid has five hydroxy groups and one of them with a glucose generated the glycoside. The key work was to comfirm the positions of the five hydroxy groups.

TABLE-1
^1H NMR AND ^{13}C NMR DATA OF COMPOUND **1**
(500 AND 125 MHz, IN DMSO-d_6)

Position	δ_H (mult., J in Hz)	δ_C (mult.)
1	3.17 (dd, 1H, 9.5, 4.0)	89.8 (d)
2	1.58 (m, 1H); 1.72 (m, 1H)	29.5 (t)
3	1.16 (m, 1H); 1.32 (m, 1H)	38.8 (t)
4		32.4 (s)
5	0.74 (d,1 H, 11.5)	51.2 (d)
6	1.63 (m, 1H); 1.90 (m, 1H)	26.6 (t)
7	3.56 (m, 1H)	73.2 (d)
8		53.4 (s)
9	1.74 (s, 1H)	49.2 (d)
10		43.0 (s)
11	1.61 (m, 1H); 2.45 (d, 1H, 16.5)	26.2 (t)
12	4.86 (m, 1H)	71.7 (d)
13	2.39 (d, 1H, 3.5)	56.6 (d)
14	4.64 (s, 1H)	74.2 (d)
15	3.52 (s, 1H)	72.3 (d)
16		155.9 (s)
17	4.86 (s, 1H); 5.03 (s, 1H	106.3 (t)
18	0.83 (s, 3H)	32.3 (q)
19	0.81 (s, 3H)	21.6 (q)
20	1.27 (s, 3H)	13.9 (q)
1'	4.55 (d, 1H, 7.5)	103.1 (d)
2'	3.48 (m, 1H)	74.2 (d)
3'	3.58 (m, 1H)	76.7 (d)
4'	3.44 (m, 1H)	70.0 (d)
5'	3.40 (m, 1H)	76.8 (d)
6'	3.84 (dd, 1H, 11.5, 5.5); 3.70 (d, 1H, 11.5)	61.1 (t)

Three angular methyl groups were confirmed at C-18 (δ_C 32.3(q)), C-19 (δ_C 21.6(q)) and C-20 (δ_C 13.9(q)), respectively, according to HMQC and HMBC spectra. The characteristics signals of three methines (C-5, C-9 and C-13) in the *ent*-kaurane diterpenoid were very clear at C-5 (δ_C 51.2(d)), C-9 (δ_C 49.2(d)) and C-13 (δ_C 56.6(d)) and four quaternary carbons were easily confirmed at C-4 (δ_C 32.4(s)), C-8 (δ_C 53.4(s)), C-10 (δ_C 43.0(s)) and C-16 (δ_C 155.9 (q)), respectively in HMQC and HMBC spectra, So the positions of oxygenated methines may be C-1, 2, 3, 6, 7, 11, 12, 14 and 15. The proton of oxygenated methine, having HMBC correlations with C-5, C-10 and C-20, respectively, could be confirmed as H-1. Similarly, the correlation between H-15 (3.52 (s, 1H)) and C-17 (δ_C 106.3(t)) in the HMBC spectrum proved that C-15 was oxygenated methine. The correlations between H-14 (δ_H 4.64 (s, 1H)) and C-15 (δ_C 72.3(t)), C-16 (δ_C 155.9(t)) in the HMBC spectrum proved that C-14 was oxygenated methine. The correlations between C-7 (δ_C 73.2(d)) and H-5 (δ_H 0.74 (d, 1H, 11.5)), H-9 (δ_H 1.74 (s, 1H)), respectively in the HMBC spectrum proved that C-7 was oxygenated methine. The correlations between H-12 (δ_H 4.86 (m, 1H)) and C-9 (δ_C 49.2 (d)), C-16 (δ_C 155.9(t)) in the HMBC spectrum proved that C-12 was oxygenated methine. Furthermore, the correlation between H-1' (δ_H 4.55 (1H, d, J = 7.5 Hz)) and C-1 (δ_C 89.8 (d)) in the HMBC spectrum, proved that C-1 was connected with a glucose (Fig. 2). Thus, the structure of **1** was elucidated as shown in Fig. 1 and named glaucocalyxin G. The stereochemistry of **1** was deduced from its NOESY spectrum. Spatial correlations were observed between H-9 and H-12, H-20 and H-14, H-5 and H-1 as well as H-15 and H-7 (Fig. 2). Therefore, compound **1** was denominated to 7α,12α,14β,15α-tetrahydroxyl-*ent*-kaur-16-ene-1-*O*-β-D-glucopyranoside.



No.	R_1	R_2	R_3	R4
3	O-Rha-(1-6)-Glc	OH	H	OH
4	O-Rha	OH	OH	OH
5	O-Rha-(1-6)-Glc	OH	OH	OH
6	H	O-Glc	H	OCH_3

Fig. 1. Structures of compounds **1-6**

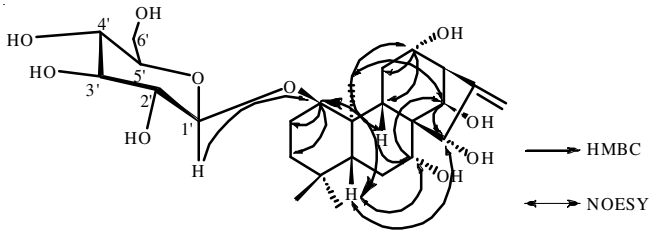


Fig. 2. Key HMBC and NOESY correlations of compound **1**

ACKNOWLEDGEMENTS

This work was supported financially by the National Natural Science Foundation of China (No. 81102790) and Natural Science Foundation for Post-Doctoral Scientists of China (No. 20090460111).

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