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Diterpenoids from *Rabdosia Japonica*

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Two new diterpenoids were isolated along with 5 known diterpenoids from the whole plant of *Rabdosia japonica* (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara and their structures were elucidated on the basis of spectroscopic methods and the two new diterpenoids named glaucocalyxin F (**1**) and glaucocalyxin X (**2**), respectively.

Key Words: *Rabdosia japonica* (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara, Diterpenoid, Glaucocalyxin X, Glaucocalyxin F.

INTRODUCTION

The dried whole plant of *Rabdosia japonica* (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara has been used as folk medicine for the treatment of hepatitis, gastricism, mastitis and coughing in China[1]. The chemical constituents of the plant, the occurrence of diterpenoids[2-6], triterpenoids[6-7] and flavonoids[5,8] in the whole plant were reported. Further investigation of *Rabdosia japonica* resulted in the isolation of two new diterpenoids, named glaucocalyxin F (**1**) and glaucocalyxin X (**2**), respectively, together with 5 known diterpenoids. Herein, the structure elucidation of the two new compounds is reported.

EXPERIMENTAL

Optical rotations were measured with a Perkin-Elmer 341 automatic polarimeter. ESI-MS and HRESI-MS were performed with a Mat-212 and a Micromass Auto Spec Q-TOF spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-600 spectrometer with tetramethylsilane (TMS) as an internal standard and $CDCl_3$ as solvents. Chemical shifts were given in δ (ppm) values.

The plant material was collected in October 2001 from Anshan, Liaoning province of China and identified as *Rabdosia japonica* (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara by Prof. Zhang Hanming, College of Pharmacy, Second Military Medical University. A voucher specimen (No. 20011018) has been deposited in the herbarium of College of Pharmacy, Second Military Medical University, Shanghai.

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Extraction and isolation: The dired whole plant (8 kg) were chopped and extracted with 80 % EtOH three times under reflux and concentrated under vacuum to yield an EtOH extract (330 g). The extract was suspended in water and extracted successively with petroleum ether, ethyl acetate and *n*-butanol to obtain petroleum ether reidue (30 g), ethyl acetate residue (140 g) and *n*-butanol residue (45 g). The ethyl acetate extract (140 g) was subjected to column chromatography over silica gel with the gradient solvent system $CHCl_3$-CH_3OH (50:1; 10:1; 1:1; 0:1) to afford four fractions (C-1, C-2, C-3, C-4). The fraction C-1, eluted by petroleum ether-EtOAc (10:1), was purified by recrystallization (petroleum ether-EtOAc 2:1) to obtain compound **4** (1.2 g) and the filter liquor, concentrated under vacuum, was further chromatographed over Sephadex LH-20 using $CHCl_3$-MeOH (1:1) to yield compound **5** (65 mg). the fraction C-2 was subjected to column chromatography over silica gel using a gradient solvent system petroleum ether-acetone (10:1; 1:1; 0:1) to afford three subfractions (C 2-1, C 2-2, C 2-3). The sub-fraction C 2-1 was further chromatographed over Sephadex LH-20 using $CHCl_3$-MeOH (1:1) to yield compound **2** (12 mg). The subfraction C 2-2 was recrystallized in acetone to yield compound **3** (1.5 g) and the filter liquor, concentrated under vacuum, was subjected to column chromatography over silica gel using a gradient solvent system petroleum ether-acetone (3:1) to obtain compound **6** (15 mg). The sub-fraction C 2-3 was further chromatographed over silica gel using a gradient solvent system petroleum ether-acetone (2:1) to obtain compound **7** (20 mg) and the filter liquor was further chromatographed over Sephadex LH-20 using $CHCl_3$-MeOH (1:1) to obtain compound **1** (32 mg).

RESULTS AND DISCUSSION

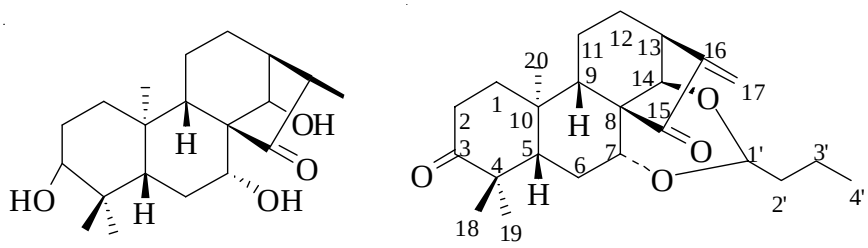
The dried whole plant were chopped and extracted with 80 % EtOH three times under reflux. The EtOH extract was suspended in water and extracted with petroleum ether, ethyl acetate and *n*-butanol in order. The ethyl acetate residue was chromatographed over silica gel column and Sephadex LH-20 column to obtain 7 diterpenoids (**1~7**). Compound **3~7** were identified as glaucocalyxin A (**3**), glaucocalyxin B (**4**), glaucocalyxin C (**6**), glaucocalyxin D (**5**) and glaucocalyxin E (**7**)[2-4] on the basis of their NMR spectral data and by comparison of their physical properties with those reported in the literature.

Compound **1**, a white powder, $[\alpha]^{20}_D$ -158 (c 0.8, $CHCl_3$), exhibited a $[M+Na]^+$ ion peak at m/z 359 in ESI-MS and the molecular formula $C_{20}H_{32}O_4$ was determined by HRESI-MS (m/z 359.2197 $[M+Na]^+$, calcd. 359.2198 for $C_{20}H_{32}O_4Na$). The ^{13}C NMR and DEPT spectra of **1** gave 20 carbon signals for 4 methyls, 5 methylenes, 7 methines and 4 quaternary carbons, including 1 carbonyl group (δ_C 222.31). The NMR data of **1** were similar to those of glaucocalyxin C which has been reported from *Rabdosia japonica* (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara.[2,3], except for the disappearance of a carbonyl group and a double bond group. In HMQC spectra, the correlation of each carbon except quaternary carbon and its

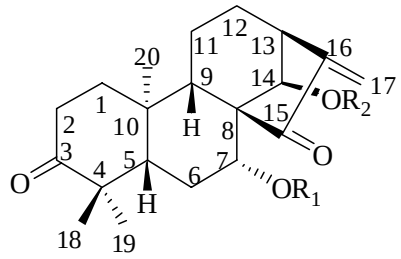
direct coterminous hydrogen was confirmed. In HMBC spectra, the long range correlations were observed (Table-1), indicating that the carbonyl group was at C-15 and there was a hydroxyl group at C-3. meanwhile the new methyl came from C-17 which was a methylene with double bond. Thus, the structure of **1** was elucidated as shown Fig. 1 and named glaucocalyxin F.

TABLE-1
^{1}H (600 MHz) AND ^{13}C (150 MHz) NMR SPECTRAL DATA AND HMBC CORRELATIONS FOR COMPOUND **1** AND **2** ($CDCl_3$, δ, ppm)

Positions	**1**			**2**		
	δ_C	δ_H	HMBC (H-C)	δ_C	δ_H	HMBC (H-C)
1	37.61t	0.85 (m, 1H) 1.71 (m, 1H)	C-2, C-3, C-5 C-3, C-5, C-10	37.85t	1.25 (m, 1H) 1.90 (m, 1H)	C-2, C-8, C-20 C-2, C-5, C-10
2	28.19t	1.69 (m, 1H) 1.99 (m, 1H)	C-1, C-5 C-5, C-10	33.88t	2.37 (m, 1H) 2.45 (m, 1H)	C-1, C-3 C-1, C-3
3	78.42d	3.21 (dd, 1H, *J* = 4.8, 12.0 Hz)	C-2	215.60s		
4	39.24s			47.15s		
5	52.63d	0.91 (dd, 1H, *J* = 2.4, 12.0 Hz)	C-6, C-7, C-10, C-18	50.87d	1.41 (m, 1H)	C-1, C-6, C-7, C-9, C-11
6	24.61t	1.73 (m, 1H) 1.75 (m, 1H)	C-4, C-5, C-7, C-8, C-5, C-7, C-10	23.01t	1.71 (m, 1H) 2.07 (m, 1H)	C-5, C-7, C-8, C-10 C-5, C-7, C-10
7	75.56d	4.23 (dd, 1H, *J* = 4.2, 12.0 Hz)	C-8, C-14	71.51d	4.17 (m, 1H)	C-6, C-8, C-14, C-15, C-1’
8	60.99s			55.24s		
9	54.33d	1.11 (d, 1H, *J* = 8.4Hz)		51.47d	1.42 (m, 1H)	C-1, C-5, C-8, C-20
10	38.72s			38.11s		
11	17.45t	1.53 (m, 1H) 1.55 (m, 1H)	C-5, C-7 C-8, C-10	18.07t	1.45 (m, 1H) 1.49 (m, 1H)	C-9, C-12, C-13 C-8, C-10, C-12, C-13
12	27.15t	1.59 (m, 1H) 1.62 (m, 1H)	C-10 C-13	30.79t	1.79 (m, 1H) 1.89 (m, 1H)	C-9, C-13, C-14 C-9, C-14, C-16
13	42.12d	2.46 (m, 1H)		43.13d	3.06 (s, 1H)	C-11, C-14
14	75.18d	4.96 (s, 1H)	C-16	77.07d	4.43 (s 1H)	C-8, C-12, C-15, C-16
15	222.31s			205.62s		
16	42.88d	2.91 (t, 1H, J= 7.2 Hz)	C-11, C-13, C-17	146.47s		
17	9.21q	1.14 (d, 3H, J= 7.2 Hz)	C-16	117.44t	5.32 (s, 1H) 6.10 (s, 1H)	C-13, C-15, C-16 C-13, C-15, C-16
18	28.34q	1.03 (s, 3H)	C-5, C-10	26.38q	1.10 (s, 3H)	C-4, C-5
19	15.60q	0.84 (s, 3H)	C-4, C-5	21.34q	1.03 (s, 3H)	C-3, C-4, C-5, C-18
20	18.03q	1.09 (s, 3H)	C-1, C-5, C-10	16.93q	1.06 (s, 3H)	C-1, C-5, C-10
1′				93.85d	4.84 (t, 1H, *J* = 5.4 Hz)	C-7, C-14, C-2’, C-3’
2′				36.80t	1.39 (m, 2H)	C-1’, C-3’, C-4’
3′				17.04t	1.28 (m, 2H)	C-1’, C-2’, C-4’
4′				13.87q	0.82 (t, 3H, *J* = 7.2 Hz)	C-2’, C-3’



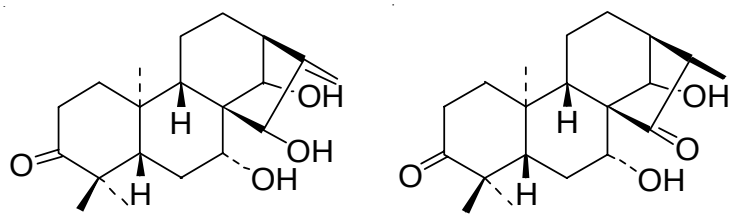
1 **2**



3 $R_1 = R_2 = H$

4 $R_1 = H$, $R_2 = COCH_3$

5 $R_1 = COCH_3$, $R_2 = H$



6 **7**

Fig. 1. Structures of compounds **1-7**

Compound **2**, a light yellow powder, $[\alpha]^{20}_D$ -137° (c 0.5, $CHCl_3$). ESI-MS m/z 409 $[M+Na]^+$ and the HRESI-MS spectrum gave its quasimolecular ion at m/z 409.2354 $[M+Na]^+$ (calcd. 409.2355) consistent with a molecular formula of $C_{24}H_{34}O_4$. The ^{13}C NMR and DEPT spectra of **2** gave 24 carbon signals for 4 methyls, 8 methylenes, 6 methines and 6 quaternary carbons, including 2 carbonyl group (δ_C 215.60 and 205.62) and a double bond (δ_C 146.47 and 117.44). The NMR data of **2** were similar to those of ent-7β,14α-dihydroxy-16-kauren-3,15-dione (glaucocalyxin A) which has been reported from *Rabdosia japonica* (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara[2,3], except for the appearance of a group additional signals for 1 methyl, 2 methylenes and 1 methine (δ_C 93.85). In HMQC spectra, the correlation of each carbon except quaternary carbon and its direct coterminous hydrogen was confirmed. The ^1H NMR spectrum of **2** exhibited the peak of H-1', H-2', H-3' and H-4' to be d, m, m and q respectively. In HMBC spectra, the long range correlations

from H-7 at δ 4.17 (m, 1H), H-2' at δ 1.39 (m, 2H) and H-3' at δ 1.28 (m, 2H) to C-1′ at δ 93.85, H-4' at δ 0.82 (q, 3H) and H-3' at δ 1.28 (m, 2H) to C-2′ at δ 36.80 were observed (Table-1), indicating that the additional butyl group was connected to the oxygen of C-7 and C-14. Thus, the structure of **2** was elucidated (Fig. 1) and named glaucocalyxin X.

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