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NOTE

Cytotoxic Diterpenoids from *Rabdosia japonica* var. *glaucocalyx*

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The structures of two new diterpenoids [glaucocalyxin X(**1**) and glaucocalyxin F(**2**)] from the whole plant of *Rabdosia japonica* (Burm. f.) Hara var. glaucocalyx (Maxim.) Hara. were confirmed furthermore on the basis of ^{1}H-^{1}H COSY and NOESY spectrum, respectively. Compounds **1** and **2** were tested *in vitro* for their cytotoxicity against human tumour cell lines HL-60,6T-CEM, LOVO and A549. Compound **1** exhibited good cytotoxicity with IC_{50} values of 3.16, 1.57,1.73 and 3.31 μg/mL, respectively. but compound **2** showed no activity against the above cancer cell lines with IC_{50} values all higher than 100 μg/mL.

Key Words: *Rabdosia japonica* var. glaucocalyx, Diterpenoid, Cytotoxicity, Human tumour cell lines.

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. It is widely growing plant species in Northern part of China and has been used as folk medicine for the treatment of hepatitis, gastricism, mastitis and coughing in China[1]. Previous investigations on the secondary metabolites of *Rabdosia japonica* var. *glaucocalyx* have revealed the presence of diterpenoids[2-4], flavonoids[5-8], triterpenoids[8,9]. In the course of our early investigation, two new diterpenoids have been isolated from the whole plant and elucidated and named glaucocalyxin X(**1**) and glaucocalyxin F(**2**)[10], but the stereochemistry should be confirmed further. The cytotoxic activities of diterpenoids from tribe Plectrantheae against various human tumor cell lines have been reported in literature[11-13]. The objective of the investigation reported here, is to confirm the structure and evaluate the cytotoxic activities of the two new diterpenoids.

General experimental procedures: 2D NMR spectra were recorded in $CDCl_3$ on a Brucker-APX-600 spectrometer. the optical density at 570 nm was taken using a Labsystems-WellscanMK-2 automated immunoanalyzer.

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The plant material was collected in October 2001 from Anshan, Liaoning province and identified as *R. japonica* var. glaucocalyx by Professor 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, China.

Cell lines and cell cultures: The human tumor cell lines, HL-60,6T-CEM, LOVO and A549, were provided by experimental Center of tumour Pharmacology, Shanghai institute of Pharmaceutical industry, China. and mainmined in RPMI 1640 supplemented with 15 % NBS and 100 units/mL penicillin and 100 μg/mL streptomycin. After incubation in 5 % CO_2 at 37 ºC for 3-6 generations.

Cytotoxicity test: The compound was screened for *in vitro* cytotoxicity by using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay method.

^{1}H-^{1}H correlations from H-4' at δ 0.82 (q, 3H) to H-3' at δ 1.28 (m, 2H), H-3' to H-2' at δ 1.39 (m, 2H) and H-2' to H-1′ at δ 4.84 (t, 1H, *J* = 5.4 Hz) of compound **1** were observed from the ^{1}H-^{1}H COSY spectrum (Fig. 1) which confirmed the connectivity of C-4' at δ 13.9 (q) to C-3' at δ 17.0 (t), C-3' to C-2' at δ 36.8(t), C-2' to C-1' at δ 93.8(d)[10].

The stereochemistry of **2** was deduced from its NOESY spectrum. Spatial connectivities were observed between H-3 and H-5 as well as H3-18, H-7 and H-5 as well as H-9 and H-14 and H3-20 (Fig. 2). Therefore compound **2** was denominated to 3α,7α,14β-trihydroxy-16β-methyl-*en t*-kauren-15-one.

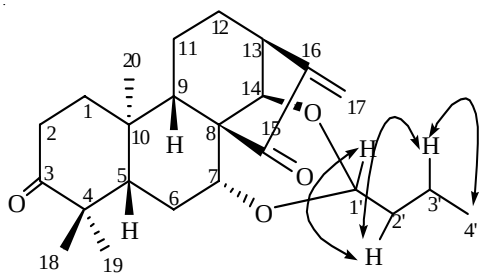

Fig. 1. Key COSY Correlations of **1**

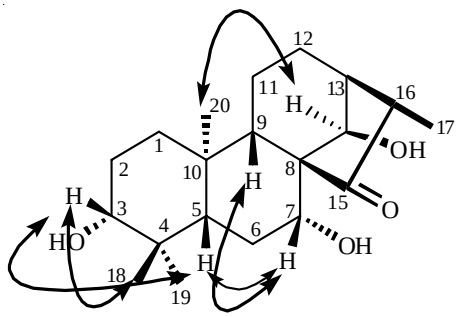

Fig. 2. Key NOESY Correlations of **2**

The cytotoxicity of the two compounds against four cultured human tumor cell lines, HL-60, 6T-CEM, LOVO and A549 was test and the results are shown in Table-1.

The activity of doxorubicin (DOX), which is one of the most effective and widely used chemotherapeutic drugs employed in the treatment of human cancers, is added for comparison (Table-1). Compound **2** didn't show cytotoxic activities against the four human tumour cells with IC_{50} values because of its concentration higher than 100 μg/mL. However, compound **1** exhibited good cytotoxic activities against the four human tumor cells with IC_{50} values within a concentration range of

1.57-3.31 μg/mL, meanwhile the inhibitory ratio at 100 μg/mL was all 100 %. The compound **1** was more selective against LOVO and 6T-CEM cell lines of colon and lung cancer.

TABLE-1
CYTOTOXICITY OF THE TWO NEW
DITERPENOIDS FROM *R. japonica* var. *glaucocalyx*

Compound		**1**	**2**	DOX
A549	IC_{50} (μg/mL)	3.31	>100	0.0228
	IC% (100 μg/mL)	100	26.24	99.96
LOVO	IC_{50} (μg/mL)	1.73	>100	0.375
	IC % (100 μg/mL)	100	32.90	99.59
HL-60	IC_{50} (μg/mL)	3.16	>100	0.00352
	IC % (100 μg/mL)	100	0	100
6T-CEM	IC_{50} (μg/mL)	1.57	>100	0.0809
	IC % (100 μg/mL)	100	32.65	80.77

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