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NOTE

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

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The cytotoxicity of five diterpenoids (Glaucocalyxins A-E) from the whole plant of *Rabdosia japonica* var. *glaucocalyx* against four human tumor cell lines (HL-60,6T-CEM, LOVO and A549) was tested, respectively. The results suggested that Glaucocalyxins A, B and D showed an apparent activity with IC_{50} values from 0.0490 to 2.65 μg/mL. Glaucocalyxin A showed a strong activity (IC_{50} = 0.0490 μg/mL) against 6T-CEM cells, which is stronger than that of doxorubicin (IC_{50} = 0.0809 μg/mL). However, Glaucocalyxins C and E exhibited no cytotoxic activities against the four cell lines with IC_{50} values higher than 100 μg/mL. Furthermore, Glaucocalyxins A, B, D and X exhibited the cytotoxicity in the order: A > B > D > X. Therefore, the structure-activity relationship of the diterpenoids can be concluded that the α,β-unsaturated ketone moiety in ring D is the leading active sites; OH-7 and OH-14 play an important role in keeping the cytotoxicity.

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

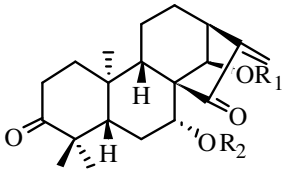
The genus *Rabdosia japonica* (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara is a member of the family Labiatae, subfamily Ocimoideae, tribe Plectrantheae and is mainly distributed in northeast Asia. It is a widely growing plant species in northern part of China and has been used as folk medicine for the treatment of hepatitis, gastricism, mastitis and coughing in China[1]. The cytotoxic activities of the extracts and compounds from tribe Plectrantheae against various human tumour cell lines have been reported and diterpenoids were regarded as the cytotoxically active constituents in several literatures[2-5]. However, up to date, reports on cytotoxic activity of the diterpenoids from *R. japonica* var. *glaucocalyx* were very scarce and only cytotoxic activities of cyclohexane fraction of ethanol extract on kB and BGC cell lines[6], Glaucocalyxins A-C on ECA cells of mice[7] and Glaucocalyxin A on BEL-7402 and HO-8910 cell lines[8] have been investigated. In the course of our early investigation, cytotoxic activities of two new diterpenoids (Glaucocalyxin F and Glaucocalyxin X) on HL-60,6T-CEM, LOVO and A549 cells have been investigated[9]. The objective of the investigation reported here, therefore, was to discover the diterpenoids from the whole plant, evaluate their cytotoxic activities and infer the structure activity relationship of cytotoxicity.

General experimental procedures: 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.

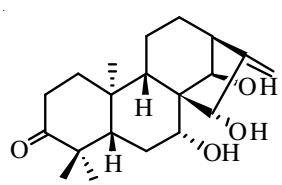
The plant materials were collected in June 2009 from Antu, Jilin province and identified as *R. japonica* var. *glaucocalyx* by Prof. Hanming Zhang, College of Pharmacy, Second Military Medical University. A voucher specimen (No. 20090603) has been deposited in the herbarium of College of Bio-information,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

Extraction and isolation: The dried whole plants (10 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 ethyl acetate fraction (200 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 Glaucocalyxin B[10] (2.1 g) and the filter liquor, concentrated under vacuum, was further chromatographed over Sephadex LH-20 using $CHCl_3$-MeOH (1:1) to yield Glaucocalyxin C[10] (100 mg). The fraction C-2

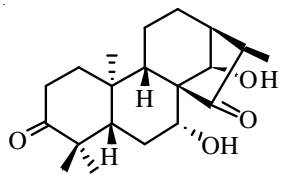
was subjected to column chromatography over silica gel using a gradient solvent system petroleum ether-acetone (10:1; 1:1; 0:1) to afford three fractions (C2-1, C2-2, C2-3). The fraction C2-2 was recrystallized in acetone to yield Glaucocalyxin A[10] (2.8 g) and the filter liquor, concentrated under vacuum, was subjected to column chromatography over silica gel using a gradient solvent system petroleum ether-acetone (3:1) to obtain Glaucocalyxin D[10] (1.1 g). The fraction C2-3 was further chromatographed over silica gel using a gradient solvent system petroleum ether-acetone (2:1) to obtain Glaucocalyxin E[10] (45 mg). The five isolated compounds had purities of 98.7-99.4 % by the external-standard HPLC method in our laboratory and their chemical structures (Fig. 1) were confirmed by spectral analysis.



Glaucocalyxin A R_1=H; R_2=H
Glaucocalyxin B R_1=$COCH_3$; R_2=H
Glaucocalyxin D R_1=H; R_2=$COCH_3$



Glaucocalyxin C



Glaucocalyxin E

Fig. 1. Formulas of the diterpenoids

Cell lines and cell cultures: The human tumor cell lines, HL-60,6T-CEM, LOVO and A549, were provided by experimental Center of Tumor Pharmacology, Shanghai Institute of Pharmaceutical Industry, China and mainmined in RPMI 1640 supplemented with l5 % NBS and 100 units/mL penicillin and 100 mg/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.

The cytotoxicity of the five diterpenoids from *R. japonica* var. *glaucocalyx* against four cultured human tumor cell lines, *i.e.* HL-60,6T-CEM, LOVO and A549 was tested (Table-1). Doxorubicin, which is one of the most effective and widely used chemotherapeutic drugs employed in the treatment of human cancers, was used as a control.

Although the cytotoxicity of many diterpenoids from tribe Plectrantheae against various human tumor cell lines have been reported[2-5], the activity of the five diterpenoids from *R. japonica* var. *glaucocalyx* against four human tumor cell lines, *i.e.* HL-60,6T-CEM, LOVO and A549 should be the first report. It is noteworthy that Glaucocalyxins A, B and D showed an apparent activity (IC_{50} = 0.0490-2.65 μg/mL) against the four cell lines. Glaucocalyxin A showed an especially strong activity against 6T-CEM cells (IC_{50} = 0.0490 μg/mL) which was higher than that of doxorubicin (IC_{50} = 0.0809 μg/mL). Glaucocalyxins C and E exhibited no cytotoxic activities against the four human tumor cells with IC_{50} values higher than 100 μg/mL, which may be due to the presence of the α,β-unsaturated ketone moiety in ring D in the case of Glaucocalyxins A, B and D, while Glaucocalyxins C and E have no this group. Our early investigation also indicated that Glaucocalyxin X exhibited high cytotoxicity on HL-60,6T-CEM, LOVO and A549 and Glaucocalyxin F showed no activity against these cell lines[9]. Furthermore, Glaucocalyxins A, B, D and X exhibited the cytotoxicity in the order: A > B > D > X, according to the data listed in Table-1 and our previous investigation[9]. Consequently, it is concluded that OH-7 and OH-14 play an important role in keeping the cytotoxicity. which might originate from the hydrogen bonding between the OH and the carbonyl group at C-15. Further modifications of Glaucocalyxins A are in progress to infer the structure-activity relationship.

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

Sample	Median inhibitory concentration (IC_{50}) (μg/mL)			
	A549	LOVO	HL-60	6T-CEM
Glaucocalyxin A	1.06	0.837	0.118	0.0490
Glaucocalyxin B	1.72	1.15	0.572	0.105
Glaucocalyxin C	> 100	> 100	> 100	> 100
Glaucocalyxin D	2.65	1.43	1.28	0.172
Glaucocalyxin E	> 100	> 100	> 100	> 100
Doxorubicin	0.0228	0.375	0.00352	0.0809

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