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Dammarane-type saponins from *Panax quinquefolium* and their inhibition activity on human breast cancer MCF-7 cells

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ABSTRACT

A new compound, named quinquefoloside-L_c (**1**), together with nine known compounds, was isolated from leaves of *Panax quinquefolium*, and its structure was elucidated as 3β,12β,20S-trihydroxy-25-methoxydammar-23-ene 3-*O*-β-D-glucopyranosyl (1→2) β-D-glucopyranosyl-20-*O*-β-D-xylopyanosyl (1→6) β-D-glucopyranoside (**1**), on the basis of MS, 1D-and 2D-NMR experiments as well as by chemical degradation. The cytotoxicity of these compounds against human breast cancer MCF-7 cell line was also tested by MTT method.

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1. Introduction

Panax quinquefolium, known as American ginseng, has been used for stress and fatigue characterized by insomnia, poor appetite, nervousness, and restlessness. It has been used for conditions of weakness, convalescence, low resistance, poor immunity or debility due to chronic disease. It is also used in the regulation of various metabolic disturbances including blood sugar and lipid levels. Previously the characteristic constituents of ginseng and American ginseng and bioactive ginsenosides on anti-atherosclerosis and immunostimulating actions were studied [1–4]. In continuation of our research in the hemolytic interaction of ginsenosides, the chemical constituents of leaves of *P. quinquefolium* were studied [5,6]. This paper reports the isolation and structural determination of a new saponin, compound **1** (Fig. 1). The extensive application of ^{1}D (^{1}H, ^{13}C-NMR and DEPT) and ^{2}D (COSY, HSQC, HMBC, TOCSY and ROESY) NMR techniques was performed to characterize the structures and to establish the ^{1}H and ^{13}C resonance assignments of the new saponin. On the other hand, the cytotoxicities of isolated saponins against human breast cancer MCF-7 cell line were also tested by MTT method.

2. Experimental

2.1. General

HPLC was performed using a Hitachi 7100 (Hitachi Corp, Japan) with UV Detector at 203 nm; Column: ODS (5 μm, 250×4.6 mm, YMC, Japan); HR ESI-MS: 9.4 T Q-FT-MS Apex Qe (Bruker), ESI-MS: Agilent 1100 LC/MSD Trap-SL spectrometer(USA). Macroporous resin D101 (Nankai University Resin, China) and ODS-A silica gel (120 Å, 50 μm, YMC, Japan) were used for column chromatography. TLC was performed on a precoated kieselgel GF254 plate (0.2–0.25 mm, 200×100 mm, Qingdao Haiyang Chemical Group Co., Qingdao, China) using $CHCl_3$–MeOH–H_2O, and detection

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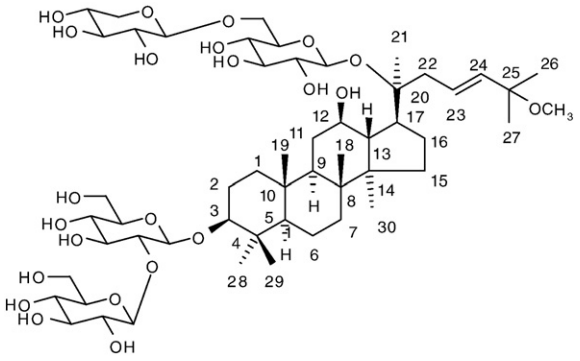

Fig. 1. Structure of compound 1.

was achieved by spraying 10% H_2SO_4/EtOH solution followed by heating.

2.2. Plant

Leaf of *P. quinquefolium* L, was collected from Liaoning Province, China, in June 2004 and was identified by Prof. Tingguo Kang, College of Pharmacy, Liaoning University of Traditional Chinese Medicine. A voucher specimen (20040802) has been deposited at the College of Pharmacy, Liaoning University of Traditional Chinese Medicine.

2.3. Extraction and isolation

The leaves of *P. quinquefolium* (6 kg) were extracted with 60% alcohol. The alcoholic extract was concentrated under reduced pressure, then subjected to macroporous resin D101 and eluted with water, 70% alcohol and 95% alcohol. The 70% alcohol extract (100 g) was chromatographed over silical gel eluting with $CHCl_3$-MeOH in a step-wise manner to furnish 6 fractions, which were further chromatographed repeatedly over ODS column with mixture of CH_3OH–H_2O to yield compounds **1** (30 mg), **2** (25 mg), **3** (20 mg), **4** (15 mg), **5** (20 mg), **6** (15 mg), **7** (20 mg), **8** (150 mg), **9** (30 mg) and **10** (22 mg).

Quinquefoloside-L_c (**1**, Fig. 1). White powder mp 193–195 °C; $[\alpha]+5.3$ (*c* 0.1, MeOH); UV_{max} (MeOH): 203.5 nm; IR bands (KBr) 3367, 2918, 2841, 1583, 1462 cm^{-1}; ^1H NMR (600 MHz, C_5D_5N) and ^{13}C NMR (150 MHz, C_5D_5N) see Table 1; Important HMBC, ROESY and TOCSY correlations of compound **1**: see Figs. 2 and 3. HRESIMS *m/z*: 1131.5921 $[M+Na]^+$(Calcd. for $C_{54}H_{92}O_{23}Na$); ESI-MS (positive mode) *m/z*: 1131 $[M+Na]^+$; ESI-MS (negative mode): 1108 $[M-H]^-$.

2.4. Acid hydrolysis of compound 1

Compound **1** (3 mg) in 10% HCl-dioxane (1:1, 1 mL) were each heated at 80 °C for 4 h in a water bath. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then

Table 1
^1H NMR and ^{13}C NMR data for **1** (600 and 150 MHz, C_5D_5N, *J* in Hz, δ in ppm).

C	δ_H	δ_C		C	δ_H	δ_C	
		1	quinquefoloside-L_a			1	quinquefoloside-L_a
1	1.53 t (11.9),0.72 t (11.2)	39.2	39.2	3-Glc			
2	2.18 d (10.2),1.82 m	26.8	26.8	1	4.92 d (7.5)	105.2	105.2
3	3.26 dd (11.8,4.2)	89.0	89.0	2	4.25 m	83.5	83.5
4		39.7	39.7	3	4.25 t (9.1)	78.0	78.0
5	0.66 d (11.6)	56.4	56.4	4	4.33 m	71.7	71.7
6	1.47 t (13.1),1.36 m	18.5	18.5	5	3.93 m	78.2	78.2
7	1.42 t (14.6),1.20 d (11.3)	35.2	35.1	6	4.50 dd (11.2,2.4), 4.46 dd (11.6,3.8)	62.7	62.7
8		40.1	40.1	-Glc′			
9	1.35 m	50.2	50.2	1′	4.92 d (7.5)	106.1	106.1
10		36.9	36.9	2′	4.13 m	77.2	77.2
11	1.55 m	30.6	30.6	3′	3.92 m	78.3	78.3
12	4.11 m	70.4	70.4	4′	4.33 m	71.7	71.7
13	2.00 t (10.6)	49.8	49.7	5′	4.32 m	78.4	78.4
14		51.5	51.5	6′	4.56 d (10.0), 4.34 o	62.9	62.9
15	1.98 m,0.98 t (9.3)	31.0	30.9	20-Glc″			
16	1.78 m	26.5	26.5	1″	5.14 d (7.8)	98.3	98.3
17	2.52 m	51.7	51.8	2″	3.92 m	75.0	75.1
18	1.00 s (3H)	16.0	16.0	3″	4.16 m	79.1	79.0
19	0.83 s (3H)	16.3	16.6	4″	4.19 m	71.1	72.1
20		83.2	83.3	5″	4.03 m	76.8	76.7
21	1.58 s (3H)	23.1	23.2	6″	4.69 d (9.6),4.29 o	69.9	69.1
22	3.17 dd (14.0,5.4), 2.68 dd (14.1,8.7)	39.8	39.8	-Xyl (-Ara)			
23	6.06 m	127.0	126.8	1	5.38 d (7.6)	105.8	104.5
24	5.69 d (15.8)	138.4	138.6	2	4.00 t (7.6)	74.9	71.7
25		75.1	75.1	3	4.12 m	78.1	74.1
26	1.36 s (3H)	26.4	26.5	4	4.13 m	71.4	68.5
27	1.33 s (3H)	26.3	26.1	5	4.31 m,3.68 t (10.3)	67.1	65.5
28	1.28 s (3H)	28.2	28.1				
29	1.10 s (3H)	16.6	16.6				
30	0.93 s (3H)	17.4	17.3				
25-OCH_3	3.23 s (3H)	50.4	50.4				

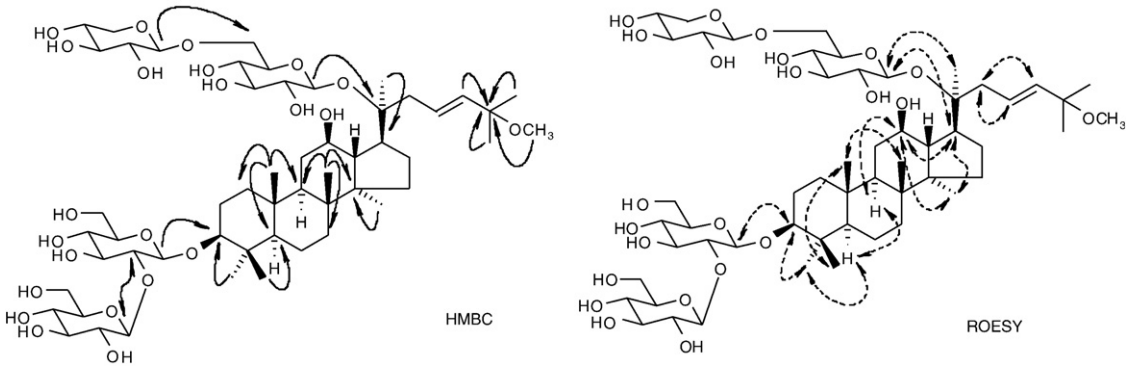

Fig. 2. Key HMBC and NOESY correlations of compound 1.

extracted with $CHCl_3$ (1 mL×3). After concentration, H_2O layer (monosaccharide portion) was examined by TLC with *n*-BuOH-AcOH-H_2O (4:1:5, upper layer) compared with authentic samples.

2.5. Determination of sugar components

The monosaccharide subunits were obtained by hydrochloric acid hydrolysis as described above. The sugar residue was then dissolved in 2 mL of H_2O and analyzed by HPLC under the following conditions as described in literature [10]: column, Kaseisorb LC NH_2-60-5, 250×4.6 mm i.d. (Tokyo Kasei Kogyo Co. Ltd); solvent, MeCN–H_2O (3:1); flow rate, 0.8 ml/min; detection, optical rotation. Determination of D-glucose and D-xylose present in the sugar fraction was carried out by comparison of its retention times and optical rotation (positive optical rotation) with those of authentic sample.

2.6. Cytotoxicity assay

The cytotoxicity bioassay was performed against human breast tumor (MCF-7) cell lines. MCF-7 were maintained in RPMI 1640 (Gibco) containing 10% FBS (Gibco), 2 mg/ml sodium bicarbonate, 100 μg/ml penicillin sodium salt and 100 μg/ml streptomycin sulfate. Cells were grown to 70% confluence, trypsinized with 0.05% trypsin–2 mM EDTA, and plated for experimental use. In all experiments, cells were grown in RPMI-1640 medium with 10% FBS for 24 h prior to treatment. All compounds were dissolved in DMSO at a concentration of 100 mM, then diluted in a tissue culture medium and filtered before use. 1.5×10^4 MCF-7 cells were seeded in 96 well tissue culture plates and treated with the tested compounds or vehicle (0.1% DMSO) at various concentrations and incubated for 48 h followed by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay at 570 nm. Briefly, IC_{50} values of the tested compounds on MCF-7 cell lines were obtained from the concentration effect curves.

3. Results and discussion

A new compound, named Quinquefoloside-L_c (**1**), together with nine known saponins **2**–**10,** was isolated from leaves of *P. quinquefolium*, and its structure was elucidated as 3*β*, 12*β*, 20*S*-trihydroxy-25-methoxydammar-23-ene 3-*O*-*β*-D-glucopyranosyl (1→2) *β*-D-glucopyranosyl-20-*O*-*β*-D-xylopyanosyl (1→6) *β*-D-glucopyranoside (**1**). Compounds **2**–***5***, which were isolated for the first time from the title plant, were clarified as

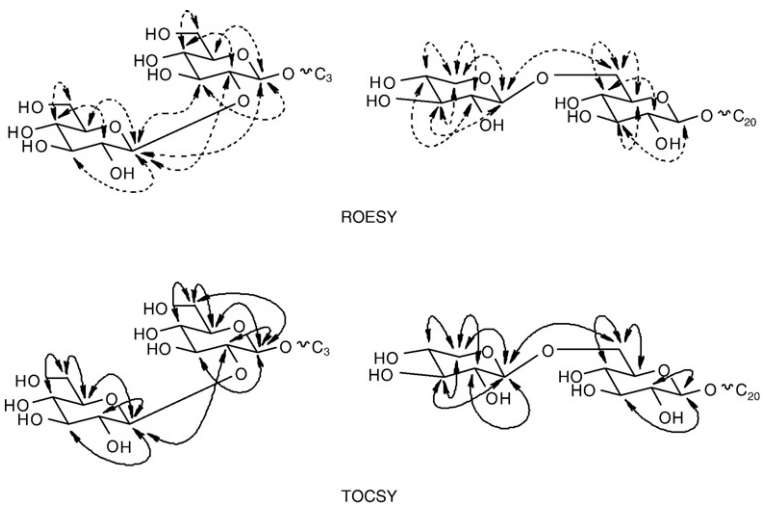

Fig. 3. ROESY and TOCSY correlations of the sugar subunits in compound 1.

gypenoside LXXI (**2**), gypenoside-LXIX (**3**), viaginsenoside-R_8 (**4**) [7] and (3β,12β,24*S*)-12,24-dihydroxy-20-*O*-α-L-arabinopyranosyl (1→6) β-D-glucopyranosyl)]dammar-25-en-3-*O*-β-D-glucopyranosyl (1→2) β-D-glucopyranoside (**5**) [8]. Known saponins, majoroside-F_1 (**6**), 24(α)-majoroside-F_1 (**7**) [9], ginsenoside-Re (**8**), ginsenoside-Rb_1 (**9**) and quinquefoloside-La (**10**) [6], were identified by comparison of their spectral data with the reported values.

Compound **1** was obtained as an amorphous powder. The molecular weight was determined from the positive HRESIMS at *m/z* 1131.5921 for the $[M+Na]^+$ion (calcd. 1131.5922 for $C_{54}H_{92}O_{23}Na$). It was positive to the Liebermann–Burchard reagents, suggesting **1** is a saponin. The ^{13}C-NMR and DEPT spectra furnished 54 carbon signals, of which 23 were assigned to the sugar moieties, 31 to a triterpene moiety containing a methoxyl at δ50.4. The triterpene moiety was identified as 3β, 12β, 20S-trihydroxy-25-methoxydammar-23-ene, whose ^{1}H, ^{13}C-NMR data were assigned by comparing with those of quinquefoloside-L_a, a new saponin we isolated previously from the title plant [6]. The difference between quinquefoloside-L_a and compound **1** was the sugar moieties. On acid hydrolysis, glucose and xylose, instead of glucose and arabinose in quinquefoloside-L_a, were detected by TLC comparison with authentic samples. HPLC analysis of hydrolysates of **1** indicated that the glucosyl and xylosyl are D-configuration, with a ratio of 3:1. The inspection of anomeric carbons disclosed the presence of four glycosyl units in **1**. Moreover, the chemical shifts, signal multiplicities, the coupling constants, and their magnitude in the ^{1}H-NMR spectrum, as well as the ^{13}C-NMR data, indicated β-configurations for the three glucosyl and a xylosyl, which were further attributed by 2D-NMR and comparison of their ^{1}H, ^{13}C-NMR data with those of quinquefoloside-L_b, another new saponin we isolated [6]. The positions and sequences of the four glycosyl linkages were deduced from the HMBC experiment. Correlations were observed between H-1 of one 3-inner glucose (3-Glc-1) and C-3 of the aglycone, H-1 of the 3-outer glucose (3-Glc′-1) and C-2 of 3-Glc-1, H-1 of the xylose and C-6 of 20-Glc, H-1 of 20-Glc and C-20 of the aglycone. All ^{1}H and ^{13}C-NMR signals in *1* were completely assigned using ^{1}H-^{1}H COSY, HSQC, ROESY and TOSCY spectra, whose correlations were shown in Figs. 2 and 3.

Compound **1**, together with other known compounds, was assayed for cytotoxicity against MCF-7 cell line using MTT method. Ginsenoside-Rh_2, a well-known active ingredient possessing anti-cancer activity, was used as a positive control. The result revealed that only compound *5* and ginsenoside Rh_2 showed inhibition effect with IC_{50} values of 93.8 and 20.0 μmol/l, respectively, indicating ginsenosides bearing three or four glycosyls showed weaker anti-cancer activity than ginsenosides with one glycosyl.

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