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Studies on Triterpenoid Glycosides from Rhizomes of *Panacis majoris* and Their Antiplatelet Aggregation Activity

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Abstract A new triterpenoid glycoside(**1**) and seven known triterpenoid glycosides, pseudoginsenoside RT_2(**2**), yesanchinoside R_2(**3**), vinaginsenoside R13(**4**), vinaginsenoside R8(**5**), notoginsenoside E(**6**), 6‴-*O*-acetylginsenoside Re(**7**), 6″-*O*-acetylginsenoside Rb_1(**8**), were isolated from the rhizomes of *Panacis majoris*. The new triterpenoid glycoside was elucidated as 3-*O*-[*β*-*D*-glucopyranosyl-(1→2)-*β*-*D*-(6′-*O*-ethyl)-glucuronopyranosyl]-oleanolic acid-28-*O*-*β*-*D*-glucopyranoside by extensive spectroscopic and phytochemical methods. Compounds **2**—**8** were obtained from the plant for the first time. Compounds **3** and **4** displayed good activities against adenosine diphosphate (ADP)-induced platelet aggregation, and compounds **1**, **5**, **6** and **8** showed moderate activities. Compound **6** exhibited moderate antiplatelet aggregation activity induced by arachidonic acid(AA).

Keywords *Panacis majoris*; Rhizome; Triterpenoid glycoside; Antiplatelet aggregation

1 Introduction

The rhizomes of *Panacis majoris*, which are used as a kind of traditional Chinese herbal medicine for thousand years, belong to *Araliaceae* family and mainly grow in Yunnan, Shanxi and Sichuan provinces of China. This medicinal plant has been reported to exhibit various pharmacological activities, such as protecting heart and cardiovascular system[1], as well as anticancer[2], antiinflammatory[3], antihypertensive[4], antiallergic[5] and antidiabetic[6] effects. The triterpenoid glycosides are considerd to be the main sources of biological activities of the rhizomes of *Panacis majori*s and are mainly classified as dammarane-type, oleanane-type and ocotillol-type derivatives based on the structural differences in their aglycones. However, there have been relatively few studies on the chemical components in recent years except for a new triterpenoid saponin[7] and two new glycosides[8] obtained from the rhizomes of *Panacis majoris*. Hence, further investigations of the chemical components of this plant seem warranted.

In this paper, we described the isolation, structural elucidation and antiplatelet aggregation activities of a new compound(**1**) and seven known compounds(**2**—**8**).

2 Experimental

2.1 Instruments and Materials

Melting points were recorded on an XT-4 micro melting point apparatus and uncorrected. Optical rotation data were obained from a JASCO DIP 1000 polarimeter. An Applied Biosystems Q-Trap mass spectrometer and a Bruker micrOTOF Q II mass spectrometer were used to obtain the electrospray ionization mass spectrometry(ESIMS) and high resolution electrospray ionization mass spectrometry(HRESIMS) data. The infrared data were recorded on a Bruker Vertex 80V Fourier transform infrared(FTIR) spectrometer. The nucleus magnetic resonance[^{1}H NMR(600 MHz), ^{13}C NMR(150 MHz)] spectra were measured on a Bruker Avance-600 spectrometer in pyridine-d_5 using tetramethylsilane(TMS) as internal standard. Column chromatography was carried out on silica gel columns (200—300 mesh; Qingdao Haiyang Chemical Co., Ltd., China) and ODS(75—140 μm; Merk). Semipreparative high performance liquid chromatograply(HPLC) equipped with a Shimadzu LC-6A pump, a Shimadzu RID-10A refractive index detector and a cosmosil C_{18} column(250 mm×10 mm, 5 μm) was used for separating the samples. The maximal aggregation was recorded on an LBY-NJ2 platelet aggregation instrument (Beijing Precil Instrument Co., Ltd., China). Acetyl salicylic acid(purity≥98%, Sigma-Aldrich), ADP(purity≥98%, Sigma-Aldrich) and AA(purity≥98%, Aladdin) were used in the assay of antiplatelet aggregation activity. All the chemical reagents used in the experiment were of analytical grade.

2.2 Plant Material

The rhizomes of *Panacis majoris* were collected from Hebei Province of China(October, 2014). A voucher specimen (20141120) was deposited with the Herbarium from the School of Pharmaceutical Sciences of Jilin University, China.

2.3 Extraction and Isolation

Dried samples(10.0 kg) were crushed and extracted with 70% ethanol(3×100 L) at 93 °C for 3 h each time. After the removal of organic solvent, a residue(1.8 kg) was dissolved in distilled water at 50 °C(20 L) and extracted with ethyl acetate

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(10×10 L) and *n*-BuOH(10×10 L), successively.

The *n*-BuOH extract(2270.3 g) was subjected to column chromatography(CC) over silica gel[$V(CH_2Cl_2)$:V(MeOH):$V(H_2O)$= 15:1:0.5—0:1:0:1] to afford 25 fractions, F1—F25. F8(5.0 g) was performed on an open ODS column[V(MeOH):$V(H_2O)$= 1:2—3:1] to give a sub-fraction F8-2(80.1 mg) which was further separated by semi-preparative HPLC[$V(CH_3CN)$: $V(H_2O)$= 33.5:66.5] to afford compound **2**(t_R=20.3 min, 60.4 mg). F10 (3.1 g) was chromatographed on a column of ODS[V(MeOH): $V(H_2O)$=1:2—1:0] to yield 20 sub-fractions. A sub-fraction F10-7(150.9 mg) was purified by semi-preparative HPLC [$V(CH_3CN)$:$V(H_2O)$=20:80] to give compounds **5**(t_R= 12.8 min, 30.2 mg) and **6**(t_R=14.5 min, 50.6 mg). Compound **7**(t_R=22.7 min, 50.3 mg) was obtained from F18 using semi-preparative HPLC[$V(CH_3CN)$:$V(H_2O)$=25:75]. F20 was chromatographed on silica gel CC[$V(CH_2Cl_2)$:V(MeOH): $V(H_2O)$=12:1:0.1—1:1:0.1] to obtain a sub-fraction F20-10 (100.0 mg), which was further purified by semi-preparative HPLC[$V(CH_3CN)$:$V(H_2O)$=40:60] to obtain compounds **3**(t_R= 18.0 min, 25.9 mg), **4**(t_R=30.1 min, 34.6 mg) and **8**(t_R=40.5 min, 30.9 mg). Fraction F24(5.3 g) was performed repeatly on a silica gel column chromatography[$V(CH_2Cl_2)$:V(MeOH): $V(H_2O)$=8:1:0.1—1:1:0.1] to give a sub-fraction F24-4(30.6 mg) with purification by semi-preparative HPLC[$V(CH_3CN)$: $V(H_2O)$=45:55] to afford compound **1**(t_R=21.3 min, 18.3 mg).

2.4 Acid Hydrolysis of Compound 1

A solution of compound **1**(2.5 mg) in MeOH-HCl(1:1, volume ratio) was placed in a sealed capillary. After heating at 80 °C for 5 h, the solution together with the standard samples was analyzed by means of silica gel thin layer chromatography(TLC) with *n*-BuOH-AcOH-H_2O(5:1:4, volume ratio, upper layer) and $CHCl_3$-MeOH-H_2O(16:8:1, volume ratio) as developing solvents, and *O*-phthalic acid-aniline as the detection reagent. From compound **1**, a glucuronic acid and two glucoses were detected. After the analysis of preparative TLC of the sugar mixture, the purified glucuronic acid and glucose were obtained. The *D* configurations of glucuronic acid and glucose were confirmed by the optical rotation values; *D*-glucuronic acid, $[\alpha]_D^{20}$=+7; *D*-glucose, $[\alpha]_D^{20}$=+22.

2.5 Antiplatelet Aggregation Assay

The assays of antiplatelet aggregation activities of compounds **1—8** induced by ADP and AA were done according to the turbidimetric method[9] with acetyl salicylic acid as the positive control. Blood samples were obtained from male New Zealand white rabbits and anticoagulated with 3.8% sodium citrate(9:1, volume ratio). Platelet-rich plasma(PRP) was obtained by centrifugating the citrated blood at 1500 r/min for 5 min, and centrifugating the remaining blood samples at 3000 r/min for 10 min yielded platelet-poor plasma(PPP), which was used as the test blank. The tested compounds(10 μL) were added to PRP(180 μL), then the mixture was incubated in the test cuvettes for 5 min at 37 °C before the addition of the inducer(ADP or AA). An LBY-NJ2 platelet aggregation instrument was used to record the maximal aggregation within 5 min. Platelet aggregation inhibition(%) was calculated according to the formula:

$$\text{Inhibition}(\%)=(1-A/B)\times 100\%$$

where A is the maximal aggregation in the presence of compounds and B is the maximal aggregation in the presence of solvent. The results were expressed as the values of 50% inhibitive concentration(IC_{50}).

3 Results and Discussion

3.1 Identification

From the *n*-BuOH extract of the rhizomes of *Panacis majoris*, eight triterpenoid glycosides, including a new compound(**1**) and seven known compounds(**2—8**) were obtained by means of silica gel, ODS column chromatography and semi-preparative HPLC. The structures of compounds **1—8** are shown in Fig.1, and the ^{1}H NMR and ^{13}C NMR data of compound **1** are listed in Table 1.

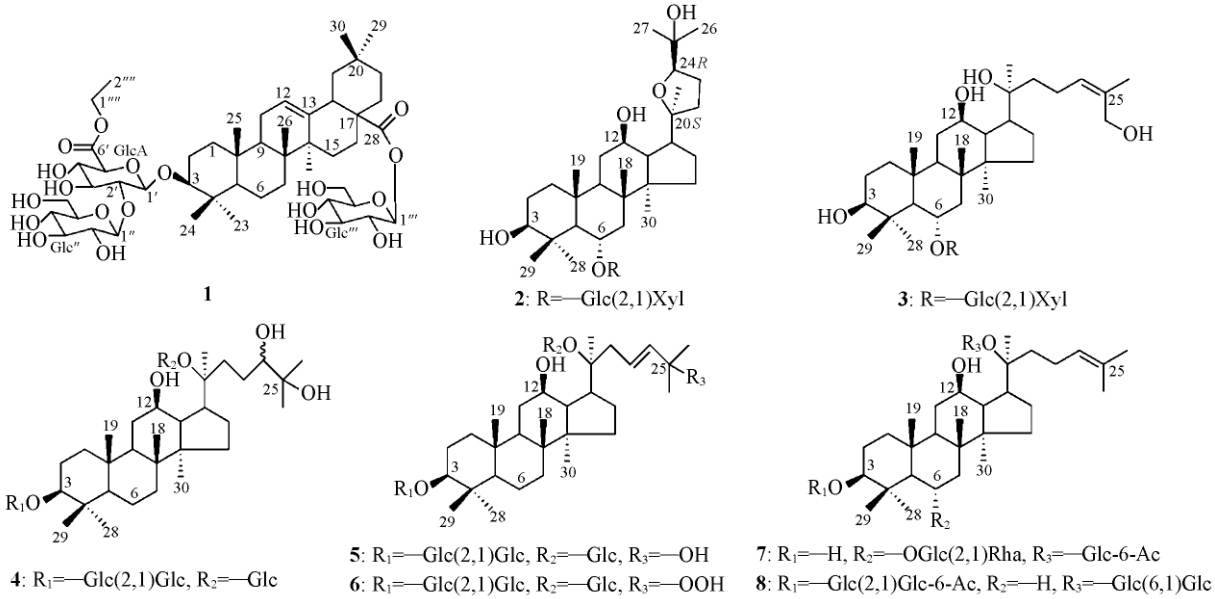

Fig.1 Chemical structures of compounds 1—8

Table 1 NMR Data for compound 1(in C_5D_5N)*

Position	δ_H	δ_C	Position	δ_H	δ_C
1	1.30(m); 0.69(m)	38.7	28		176.5
2	1.99(m); 1.71(m)	26.7	29	0.79(s, 3H)	33.3
3	3.13(dd, *J*=4.2, 11.4 Hz)	89.4	30	0.76(s, 3H)	23.8
4		39.7	3-*O*-GlcA		
5	0.59(d, *J*=12.0 Hz)	55.9	1′	4.85(d, 1H, *J*=7.2 Hz)	105.5
6	1.18(m); 1.13(m)	18.6	2′	4.20(m)	82.7
7	1.71(m); 1.66(m)	32.7	3′	4.21(m)	77.7
8		40.0	4′	4.33(m)	73.0
9	1.47(m)	48.1	5′	4.39(m)	77.0
10		37.0	6′		170.1
11	1.96(m); 1.85(m)	23.5	-Glc″		
12	5.29(m)	123.0	1″	5.28(d, 1H, *J*=7.2 Hz)	106.1
13		144.3	2″	4.00(m)	77.2
14		42.3	3″	4.13(m)	78.1
15	2.22(m); 1.05(m)	28.4	4″	4.23(m)	71.8
16	1.73(m); 1.62(m)	23.9	5″	3.81(m)	78.4
17		47.1	6″	4.37(m); 4.33(m)	62.8
18	3.08(dd, *J*=6.0, 12.0 Hz)	41.9	28-*O*-Glc‴		
19	1.64(m); 1.18(m)	46.3	1‴	6.23(d, 1H, *J*=7.8 Hz)	95.8
20		30.9	2‴	4.09(m)	74.3
21	1.03(m); 0.99(m)	34.1	3‴	3.91(m)	79.5
22	1.27(m); 1.23(m)	33.2	4‴	4.25(m)	71.2
23	1.14(s, 3H)	28.2	5‴	4.15(m)	79.1
24	0.96(s, 3H)	16.8	6‴	4.36(m); 4.32(m)	62.3
25	0.71(s, 3H)	15.6	6′-*O*-ethyl		
26	0.97(s, 3H)	17.6	1⁗	4.17(m); 4.14(m)	61.4
27	1.13(s, 3H)	26.2	2⁗	1.06(t, *J*=12.0 Hz)	14.3

* ^{13}C NMR: 150 MHz, ^{1}H NMR: 600 MHz; *J* in Hz.

Compound **1** was obtained as a white powder. Its molecular formula is $C_{50}H_{80}O_{19}$ on the basis of HRESIMS measured at *m/z* 985.5381$[M+H]^+$. The IR spectrum displayed absorption bands for hydroxyl, carbonyl and olefinic groups at 3424.4, 1745.3 and 1631.6 cm^{-1}, respectively.

The ^{1}H NMR spectrum showed signals of seven methyl groups at δ_H 0.71(s, 3H), 0.76(s, 3H), 0.79(s, 3H), 0.96(s, 3H), 0.97(s, 3H), 1.13(s, 3H) and 1.14(s, 3H) and an olefinic proton at δ_H 5.29(s, 1H). The ^{13}C NMR and DEPT spectra displayed fifty carbon signals, including one carbonyl carbon(δ_C 176.5), two olefinic carbons(δ_C 123.0 and 144.3), ten methylenes(δ_C 46.3, 38.7, 34.1, 33.2, 32.7, 28.4, 26.7, 23.9, 23.5 and 18.6), three methines(δ_C 55.9, 48.1 and 41.9) and an oxygenated methine(δ_C 89.4). The above NMR data suggested that compound **1** was an oleanane-type triterpenoid derivative. Moreover, comparison of the NMR data of compound **1** with those of chikusetsusaponin V[10] indicated that their signals were similar, except for the presence of an additional ethyl group in compound **1**. HMBC spectrum(Fig.2) displays the correlations between H2⁗(δ_H 1.06) of the ethyl group and C1⁗(δ_C 61.4) of the ethyl group, and between H1⁗(δ_H 4.14) of the ethyl group and C6′(δ_C 170.1) of the GlcA, indicating that the ethyl group could be located at C6′ of the glucuronic acid. Moreover, the *β*-configuration of 3-OH was confirmed by the NOESY correlation between H3(δ_H 3.13) and H23(δ_H 1.14).

^{1}H NMR spectrum also exhibited three anomeric protons at δ_H 4.85(d, *J*=7.2 Hz, 1 H), 5.28(d, *J*=7.2 Hz, 1 H) and 6.23 (d, *J*=7.8 Hz, 1 H), which correlated with the anomeric carbons

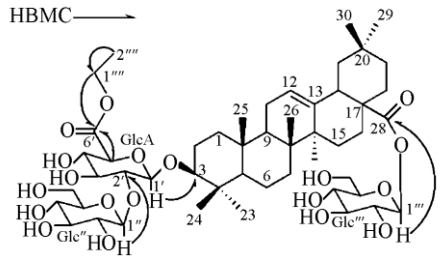

Fig.2 Key HMBC correlations of compound 1

at δ_C 105.5(C1′), 106.1(C1″) and 95.8(C1‴) in the HSQC spectrum, respectively. The carbonyl carbon signal(δ_C 170.1) and two carbon signals(δ_C 62.3, 62.8) implied the presence of a glucuronic acid and two glucoses. The conclusion is also in agreement with the results of acid hydrolysis. The anomeric configurations of sugar units were determined to be *β* for *D*-glucuronic acid and *D*-glucopyranose on the basis of the coupling constants(*J*=7.2, 7.2 and 7.8 Hz). In addition, the HMBC spectrum(Fig.2) displays the correlations between H1″ (δ_H 5.28) of Glc″ and C2′(δ_C 82.7) of GlcA, and between H1′ (δ_H 4.85) of GlcA and C3(δ_C 89.4) of aglycone, and between H1‴(δ_H 6.23) of Glc‴ and C28(δ_C 176.5) of aglycone. Based on the foregoing findings, the structure of compound **1** was determined as 3-*O*-[*β*-*D*-glucopyranosyl-(1→2)-*β*-*D*-(6′-*O*-ethyl)-glucuronopyranosyl]-oleanolic acid 28-*O*-*β*-*D*-glucopyranoside.

Seven known compounds were identified as pseudoginsenoside RT_2(**2**)[11], yesanchinoside R_2(**3**)[12], vinaginsenoside R13(**4**)[13], vinaginsenoside R8(**5**)[14], notoginsenoside E(**6**)[15],

6‴-*O*-acetylginsenoside Re(**7**)[12], 6″-acetylginsenoside Rb_1(**8**)[16] by comparing their spectral data with the literature values.

3.2 Characterization of Compound 1

Compound **1** is a white power. m. p. 241—243 °C; IR(KBr), $\tilde{\nu}$/cm^{-1}: 3424.4, 2923.4, 1745.3, 1631.6, 1386.3, 1083.9. ESIMS: *m/z* 1007.5[M+Na]$^+$. HRESIMS: *m/z* 985.5381 [M+H]$^+$(calcd. for $C_{50}H_{80}O_{19}H^+$, *m/z* 985.5390).

3.3 Antiplatelet Aggregation Activities

As shown in Table **2**, in the case of ADP as the inducer, compounds **3** and **4** have good activities with IC_{50} values of 18.27 and 11.34 μmol/L, respectively, compounds **1**, **5**, **6** and **8** show moderate activities with IC_{50} values of 40.54, 25.18, 29.45 and 36.90 μmol/L, respectively, while compounds **2** and **7** display weak activities. Similarly, in the experiment of arachidonic acid(AA)-induced platelet aggregation, compound **6** exhibits moderate activity with an IC_{50} value of 17.43 μmol/L, compounds **1** and **3** show relatively weak activities with IC_{50} values of 67.85 and 34.75 μmol/L, respectively, while compounds **2**, **4**, **5**, **7** and **8** are almost inactive.

Table 2 Antiplatelet aggregation activities of compounds 1—8

Compd.	IC_{50}/(μmol·L^{-1})[a]	
	ADP	AA
1	40.54±0.31	67.85±1.19
2	90.35±1.05	>100
3	18.27±0.20	34.75±0.31
4	11.34±0.99	80.93±1.56
5	25.18±1.27	>100
6	29.45±2.01	17.43±0.79
7	>100	>100
8	36.90±0.86	91.90±2.12
Acetyl salicylic acid[b]	38.79±1.73	10.67±0.42

a. The results were exhibited as the mean±SD; *b*. acetyl salicylic acid was used as the positive control.

4 Conclusions

In this study, eight triterpenoid glycosides(**1—8**) were obtained from the rhizomes of *Panacis majoris* and their structures were confirmed by NMR experiments in conjunction with MS and IR data, as well as the results of acid hydrolysis. Moreover, compound **1** was a new triterpenoid glycoside, and compounds **2—8** were obtained from the plant for the first time. The antiplatelet aggregation activities induced by ADP and AA of compounds **1—8** were also evaluated. The results of the antiplatelet aggregation activities showed that the isolated compounds from the rhizomes of *Panacis majoris* exhibited diverse platelet aggregation activities induced by ADP and AA.

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