

New triterpenoid saponins from Patrinia scabiosifolia

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Phytochemical investigation of methanol extract from the whole plants of *Patrinia scabiosifolia* Fisch. resulted in the isolation of three new triterpenoid saponins (1–3) along with twelve known triterpenoids (4–15). The structures of the new compounds were established as 11 α , 12 α -epoxy-3-*O*- β -D-xylopyranosyl-olean-28, 13 β -olide (1), 11 α , 12 α -epoxy-3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)- β -D-xylopyranosyl-olean-28, 13 β -olide (2), and 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-xylopyranosyl oleanolic acid 28-*O*- β -D-glucopyranoside (3) on the basis of various spectroscopic analyses (including different 1D and 2D NMR spectroscopies and high-resolution electrospray ionization mass spectrometry) and chemical evidences.

Keywords: Patrinia scabiosifolia; Valerianaceae; triterpenoid saponin

1. Introduction

The *Patrinia* genus (Valerianaceae family) includes about 20 species around the world, which are widely distributed in Asia and North America. Of these, more than 10 species grow in Mainland China, many of which have applications in either traditional Chinese medicine or folklore herbs to treat fever, stasis, and inflammation along with detoxication and mobilization of blood circulation [1]. Previous investigations on the chemical constituents of Patrinia species have led to the characterization of several compound classes including triterpenoids, iridoids, flavonoids, and sterols. Pentacyclic triterpenoids are the dominant constituents within the genus Patrinia and exhibit oleanane, hederagenin, or ursane skeletons [2]. As part of our ongoing search for new natural compounds with interesting biological activities, we carried out

phytochemical investigations on the whole plants of Patrinia scabiosifolia Fisch. collected in China. We have reported four new saponins along with six known compounds isolated from the water part of methanolic extract [3]. Further isolation of ethanol acetate part of the extract resulted in three new saponins (1-3, Figure 1), together with 12 known compounds (4-15)identified by comparison of their NMR spectral data with those reported in the literature [4]. Among them, compounds 1 and 2 possessed an olean-28, 13β -olide skeleton with 11α , 12α -epoxy, which has been rarely found in natural sources. The present study reports the isolation and structural elucidation of these triterpenoids, and the cytotoxic activities of several compounds against HepG-2 cell were also evaluated by 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay in vitro.

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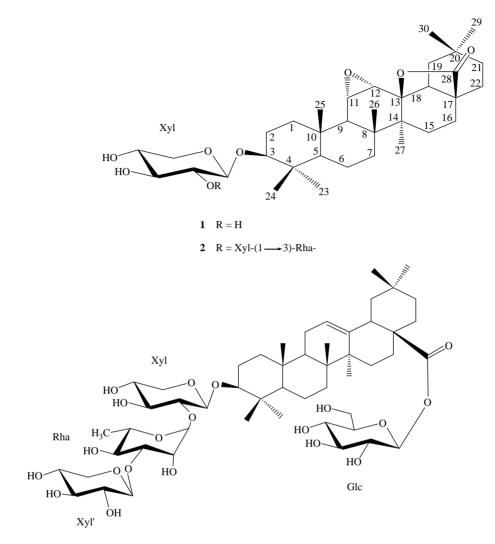


Figure 1. Chemical structures of compounds 1-3.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder. The positive HR-ESI-MS exhibited a pseudomolecular ion peak at m/z 625.3716 [M + Na]⁺, corresponding to the molecular formula of C₃₅H₅₄O₈. The IR spectrum of 1 exhibited absorptions at 3423 cm⁻¹ (-OH), 1769 cm⁻¹ (ester carbonyl), and 871 cm⁻¹ (epoxy). The ¹H NMR spectrum of 1 showed signals for seven tertiary methyl groups at $\delta_{\rm H}$ 1.37, 1.32, 1.22, 1.06, 1.01, 0.98, and 0.88 (each 3H, s), coupled with information from the ¹³C NMR spectrum (seven sp³ carbons at $\delta_{\rm C}$: 16.5, 17.4, 19.0, 20.5, 23.6, 28.0, and 33.2) (Table 1). The carbonyl carbon signal at $\delta_{\rm C}$ 179.0 together with the quaternary carbon at $\delta_{\rm C}$ 87.7 indicated that the aglycon possessed a 28, 13β-lactone [5]. Two oxygen-bearing methylenic protons at $\delta_{\rm H}$ 3.17 (1H, d, J = 4.0 Hz) and 3.32 (1H, d, J = 4.0 Hz), and two corresponding oxygen-bearing methine carbons at $\delta_{\rm C}$ 52.9 and 57.4 indicated the presence of a ternary oxygen ring. After extensive 2D NMR analysis, the aglycon of **1** was

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87.7- 87.7 - 144.3 41.0 - 41.0 - 42.3 27.2 $1.09m, 1.78m$ 23.9 42.3 21.7 $1.39m, 2.29$ br dd $(13.1, 5.5)$ 21.8 $1.42m, 2.27m$ 23.9 21.7 $1.39m, 2.29$ br dd $(13.1, 5.5)$ 21.8 $1.42m, 2.27m$ 23.9 24.2 - 44.2 - 47.1 23.9 21.7 $1.39m, 2.29$ br dd (13.5) 20.61 dd $(2.5, 13.5)$ 24.9 2.61 dd $(2.5, 13.5)$ 24.2 38.1 $1.82m, 2.06$ d (13.5) 38.1 $1.86m, 2.06$ d (13.5) 30.9 31.6 27.8 $1.74m, 1.85$ br dd $(14.0), 1.45$ br dd $(14.0), 1.42$ br d (14.0) 34.2 27.8 $1.77m$ $1.74m, 1.86$ br dd $(14.0), 34.2$ 28.3 16.9 1.27 28.0 $1.37s$ 1.69 1.29 s $1.74m, 1.86$ br dd $(14.0), 33.2$ 28.3 16.5 $1.06s$ $1.74m, 1.86$ br dd $(14.0), 4.2$ 28.3 17.5 17.4 1.01 s $1.74m, 1.86$ br dd $(14.0, 4.2)$ 28.3 16.5 $1.06s$ 1.75 1.278 1.75 17.4 $1.01s$ 1.75 1.75 1.278 17.6 $1.22s$ 10.18 1.76 1.76 17.6 $1.32s$ 10.90 $1.33s$ 20.9 23.2 $0.98s$ 23.2 $0.98s$ 23.3 23.6 $0.89s$ 23.6 $0.89s$ 23.3 23.6 $0.98s$ 23.6 $0.98s$	12	57.4	3.32 d (4.0)	57.4	3.31 d (4.0)	123.0	5.50 br s
41.0 $-$ 41.0 $-$ 42.327.21.09 m, 1.78 m23.9 br dd (13.1, 5.5)21.2 br dd (13.1, 5.5)27.2 1.12 m, 1.78 m28.321.71.39 m, 2.29 br dd (13.1, 5.5)21.8 1.42 m, 2.27 m23.921.71.39 m, 2.29 br dd (13.1, 5.5)21.8 1.42 m, 2.27 m23.944.2 $-$ 44.2 $-$ 44.2 m23.949.92.61 dd (2.5, 13.5)38.1 1.86 m, 2.06 d (13.5)30.938.11.82 m, 2.06 d (13.5)38.1 1.86 m, 2.06 d (13.5)30.931.6 $-$ 31.6 $-$ 27.8 1.74 m, 1.86 br dd (14.0), 1.42 br d (14.0)34.227.81.74 m, 1.85 br dd (14.0, 4.2)27.8 1.77 d (14.0), 1.42 br d (14.0)34.228.01.37 s1.75 1.01 s1.77 d (14.0), 1.42 br d (14.0)34.228.11.37 s1.69 1.29 s1.01 s1.75 1.01 s1.75 1.01 s20.51.20 s1.01 s1.01 s1.01 s1.75 1.01 s1.76 s17.41.01 s1.75 1.01 s1.01 s1.75 1.01 s1.76 s17.9.0-1.75 1.01 s1.01 s1.75 1.01 s1.76 s20.51.20 s1.03 s20.5 1.21 s26.3 3.3 3.3 3.3 3.3 3.3 3.3 3.3 3.3 3.3	13	87.7	1	87.7	1	144.3	1
27.2 $1.09m$, $1.78m$ 27.2 $1.12m$, $1.78m$ 28.3 21.7 $1.39m$, 2.29 br dd $(13.1, 5.5)$ 21.8 $1.42m$, $2.27m$ 23.9 21.7 $1.39m$, 2.29 br dd $(13.1, 5.5)$ 21.8 $1.42m$, $2.27m$ 23.9 21.7 $1.39m$, 2.29 br dd $(13.1, 5.5)$ 21.8 $1.42m$, $2.27m$ 23.9 44.2 $ 44.2$ $ 47.1$ 23.9 49.9 2.61 dd $(2.5, 13.5)$ 38.1 $1.86m$, 2.06 d (13.5) 46.3 31.6 $ 38.1$ $1.86m$, 2.06 d (13.5) 34.6 34.6 31.6 $ 31.6$ $ 2.74$ d (14.0) , 1.42 br d (14.0) 34.2 31.6 $ 2.78$ 1.27 d (14.0) , 1.42 br d (14.0) 34.2 27.8 $1.74m$, 1.85 br dd $(14.0, 4.2)$ 27.9 1.27 d $(14.0), 1.42$ br d (14.0) 34.2 27.8 $1.74m$, 1.86 br dd $(14.0, 4.2)$ 33.3 27.9 1.45 $1.74m$, 1.86 br dd $(14.0, 4.2)$ 28.0 1.37 s 1.06 s 1.27 s 1.75 1.01 s 1.75 177.4 1.01 s $1.74m$, 1.86 br dd $(14.0, 4.2)$ 28.3 177.6 1.28 1.75 1.75 1.75 20.5 1.20 s 1.23 s 1.25 s 1.75 177.6 1.29 s 1.29 s 1.29 s 17.6 179.0 1.33 s 20.5 1.28 17.6 20.5 1.29 s 1.29 s 1.29 s 17.6 179.0 $ 179.7$ <	14	41.0	1	41.0	1	42.3	1
$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	15	27.2	1.09 m, 1.78 m	27.2	1.12 m, 1.78 m	28.3	1.26 m, 2.42 m
44.2 - 44.2 - 47.1 49.9 $2.61 \mathrm{dd} (2.5, 13.5)$ 50.5 $2.61 \mathrm{dd} (2.5, 13.5)$ 41.9 38.1 $1.82 \mathrm{m}, 2.06 \mathrm{d} (13.5)$ 38.1 $1.86 \mathrm{m}, 2.06 \mathrm{d} (13.5)$ 46.3 31.6 - 31.6 - 31.6 - 30.9 34.6 $1.27 \mathrm{br} \mathrm{d} (14.0), 1.45 \mathrm{br} \mathrm{d} (14.0), 1.42 \mathrm{br} \mathrm{d} (14.0), 34.6$ $34.2 \mathrm{br} \mathrm{d} (14.0), 34.2$ $34.2 \mathrm{br} \mathrm{d} (14.0), 34.2$ 27.8 $1.74 \mathrm{m}, 1.85 \mathrm{br} \mathrm{dd} (14.0, 4.2)$ $27.8 \mathrm{l}, 1.74 \mathrm{m}, 1.86 \mathrm{br} \mathrm{dd} (14.0, 4.2)$ $33.3 \mathrm{cos} \mathrm{d} (14.0, 4.2)$ 28.0 $1.37 \mathrm{s}$ $1.74 \mathrm{m}, 1.85 \mathrm{br} \mathrm{dd} (14.0, 4.2)$ $27.9 \mathrm{l}, 145 \mathrm{s}$ $1.74 \mathrm{m}, 1.86 \mathrm{br} \mathrm{dd} (14.0, 4.2)$ 28.0 $1.37 \mathrm{s}$ $1.06 \mathrm{s}$ $1.75 \mathrm{m}, 1.86 \mathrm{br} \mathrm{dd} (14.0, 4.2)$ $38.3 \mathrm{cos} 17.5 c$	16	21.7	1.39 m, 2.29 br dd (13.1, 5.5)	21.8	1.42 m, 2.27 m	23.9	2.05 d (13.5), 2.17 br d (13.5)
49.92.61 dd $(2.5, 13.5)$ 50.52.61 dd $(2.5, 13.5)$ 41.938.11.82 m, 2.06 d (13.5) 38.11.86 m, 2.06 d (13.5) 46.331.6-31.6-30.934.61.27 br d (14.0) , 1.45 br d (14.0) , 1.45 br d (14.0) , 1.42 br d (14.0) 34.227.81.74 m, 1.85 br dd $(14.0, 4.2)$ 27.81.74 m, 1.86 br dd $(14.0, 4.2)$ 28.01.37 s1.06 s1.45 s1.27 d $(14.0, 4.2)$ 28.11.37 s27.91.45 s1.74 m, 1.86 br dd $(14.0, 4.2)$ 28.01.37 s1.01 s1.75 1.01 s1.75 1.01 s17.41.01 s1.75 1.01 s1.29 s17.6 1.75 1.01 s17.41.01 s1.75 1.01 s1.01 s17.6 1.76 1.01 s20.51.22 s1.9.01.33 s20.5 1.21 s17.6 1.76 1.01 s17.90-1.75 1.01 s1.01 s1.75 1.01 s17.6 1.01 s20.51.22 s1.9.01.33 s20.5 1.21 s17.6 1.76 s20.60.88 s20.50.98 s23.3 2.0.98 s23.3 2.0.98 s23.60.88 s23.60.89 s23.60.89 s23.8 2.3 8	17	44.2	I	44.2	1	47.1	1
$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	18	49.9	2.61 dd (2.5, 13.5)	50.5	2.61 dd (2.5, 13.5)	41.9	3.28 br d (13.5)
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	19	38.1	1.82 m, 2.06 d (13.5)	38.1	1.86 m, 2.06 d (13.5)	46.3	$1.32 \mathrm{m}, 1.84 \mathrm{m}$
34.6 1.27 br d (14.0), 1.45 br d (14.0) 34.6 1.27 d (14.0), 1.42 br d (14.0) 34.2 $1.18m$,27.8 $1.74m$, 1.85 br dd (14.0, 4.2) 33.3 $1.38m$,28.0 1.37 s $1.74m$, 1.86 br dd (14.0, 4.2) 33.3 $1.37m$,28.0 1.37 s $1.74m$, 1.85 br dd (14.0, 4.2) 33.3 1.47 s16.5 1.06 s 1.69 1.29 s 1.75 1.32 s17.4 1.01 s 1.75 1.21 s 1.75 1.32 s17.4 1.01 s 1.75 1.21 s 1.75 1.32 s17.4 1.01 s 1.75 1.21 s 1.76 1.17 s17.4 1.01 s 1.75 1.21 s 1.76 1.17 s17.4 1.01 s 1.75 1.21 s 1.76 1.17 s17.9 1.33 s 20.5 1.21 s 1.76 1.17 s179.0 $ 1.75$ 1.21 s 1.76 1.35 s20.5 1.32 s 1.90 1.33 s 26.3 1.35 s2179.0 $ 179.7$ $ 179.7$ $-$ 23.6 0.88 s 23.6 0.89 s 23.8 0.99 s	20	31.6	1	31.6	I	30.9	I
27.8 $1.74m$, 1.85 br dd (14.0, 4.2) 27.8 $1.74m$, 1.86 br dd (14.0, 4.2) 33.3 $1.38m$ 28.0 $1.37s$ 27.9 $1.45s$ 1.76 $1.32s$ 16.5 $1.06s$ 1.69 $1.29s$ 1.75 $1.32s$ 17.4 $1.01s$ $1.29s$ 1.75 $1.32s$ 17.4 $1.01s$ $1.21s$ 1.75 $1.32s$ 17.4 $1.01s$ 1.75 $1.21s$ 1.76 17.6 $1.21s$ $1.01s$ 1.76 $1.17s$ 19.0 $1.33s$ 20.5 $1.21s$ 17.6 179.0 $ 179.7$ $ 176.6$ $ 179.7$ $ 177.6$ $1.37s$ 23.6 $0.98s$ 33.2 $0.98s$ 33.3 $0.99s$ 23.6 $0.88s$ 23.6 $0.89s$ 23.8 $0.97s$	21	34.6	1.27 br d (14.0), 1.45 br d (14.0)	34.6	1.27 d (14.0), 1.42 br d (14.0)	34.2	1.18 m, 1.43 m
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	22	27.8	1.74 m, 1.85 br dd (14.0, 4.2)	27.8	1.74 m, 1.86 br dd (14.0, 4.2)	33.3	£
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	23	28.0	$1.37\mathrm{s}$	27.9	1.45 s	28.3	$1.47 \mathrm{s}$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	24	16.5	1.06 s	16.9	1.29 s	17.5	1.32 s
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	25	17.4	1.01 s	17.5	1.01 s	15.8	0.96 s
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	26	20.5	1.22 s	20.5	1.21 s	17.6	1.17 s
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	27	19.0	1.32 s	19.0	1.33 s	26.3	1.35 s
33.2 0.98 s 33.2 0.98 s 33.3 23.6 0.88 s 23.6 0.89 s 23.8	28	179.0	1	179.7	1	176.6	1
23.6 0.88 s 23.6 0.89 s 23.8	29	33.2	0.98 s	33.2	0.98 s	33.3	0.99 s
	30	23.6	0.88 s	23.6	0.89 s	23.8	0.97 s

Table 1. The ¹H and ¹³C NMR spectral data for a solvcon moieties of compounds 1-3 in noridine d_s (J in Hz).

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characterized as 11α , 12α -epoxy-olean-28, 13 β -olide, referring to the reported data of 11α , 12α -epoxy-3-*O*-β-D-glucuronopyranosyl-olean-28, 13_β-olide [6]. In HMBC spectrum of 1 (Figure 2), the correlations of 11, 12-epoxy ring could be observed from H-11 ($\delta_{\rm H}$ 3.17) to C-10 ($\delta_{\rm C}$ 36.6) and C-12 $(\delta_{\rm C} 57.4)$, from H-12 $(\delta_{\rm H} 3.32)$ to C-11 $(\delta_{\rm C} 52.9)$ and C-14 $(\delta_{\rm C} 41.0)$, from H-9 $(\delta_{\rm H} 1.79)$ to C-11 ($\delta_{\rm C} 52.9$), and from H-18 $(\delta_{\rm H} 2.61)$ with C-12 $(\delta_{\rm C} 57.4)$; the correlations of 28, 13-lactone ring could be observed from H-15 ($\delta_{\rm H}$ 1.09), H-18 $(\delta_{\rm H}~2.61)$ and H-27 $(\delta_{\rm H}~1.32)$ to C-13 (δ_C 87.7), from H-18 (δ_H 2.61) to C-28 ($\delta_{\rm C}$ 179.0), respectively, thus confirming the moiety connection of the aglycon. Further, NOE relationships (Figure 2) between H-11 at $\delta_{\rm H}$ 3.17 and Me-25 at $\delta_{\rm H}$ 1.01/Me-26 at $\delta_{\rm H}$ 1.22 (Me-26), and between H-12 at δ_{H} 3.32 and Me-26 at δ_{H} 1.22 convinced the α -configuration of the epoxy ring. The presence of one sugar could be deduced from its anomeric proton signal at $\delta_{\rm H}$ 4.90 (1H, d, $J = 7.5 \,\rm{Hz}$) and anomeric carbon signal at $\delta_{\rm C}$ 107.8 (Table 2). The β -anomeric configuration of D-xylose unit was determined from its ${}^{3}J_{\text{H1,H2}}$ coupling constants (7.5 Hz) [7]. The linkage position of β -D-xylose was shown to be at C-3 of the aglycon by detecting a correlation from the anomeric proton at $\delta_{\rm H}$ 4.90 (Xyl-1) to C-3 at $\delta_{\rm C}$ 88.5 in the HMBC spectrum (Figure 2). On acid hydrolysis with 2 M CF₃COOH, 1 afforded sugar moiety that was identified as D-xylose based on the gas chromatography (GC) analysis of its chiral derivative [8]. The structure of 1 was finally established as 11α, 12α-epoxy-3-O-β-D-xylopyranosylolean-28, 13_β-olide.

Compound **2** was obtained as a white amorphous powder. The positive FTICR-HR-ESI-MS exhibited a pseudomolecular ion peak at m/z 903.4716 [M + Na]⁺, corresponding to the molecular formula of C₄₆H₇₂O₁₆. The IR spectrum of **2** exhibited absorptions at 3418 cm⁻¹ (-OH), 1770 cm⁻¹ (ester carbonyl), and 871 cm^{-1} (epoxy). A detailed comparison of the ¹H and ¹³C NMR chemical shifts of 2 with those of **1** revealed the same aglycon moiety for both compounds, with differences of sugar moieties (Tables 1 and 2). On acid hydrolysis with 2 M CF₃COOH, 2 afforded sugar moieties as L-rhamnose and D-xylose in the ratio of 1:2 based on the GC analysis of its chiral derivative [8]. The ¹H and ¹³C NMR spectra of **2** exhibited three sugar anomeric protons at $\delta_{\rm H}$ 6.67 (1H, br s), 5.46 (1H, d, J = 7.5 Hz), 4.91 (1H, d, $J = 7.0 \,\mathrm{Hz}$), with three anomeric carbon signals at $\delta_{\rm C}$ 101.7, 107.8, and 106.3 correspondingly. The methyl signal at $\delta_{\rm H}$ 1.74 (3H, d, J = 6.0 Hz) and $\delta_{\rm C}$ 18.7 also indicated the presence of a rhamnose. The glycosylation shift of C-3 ($\delta_{\rm C}$ 88.4) revealed that 2 was a 3-monodesmosidic glycoside. The identities of the oligosaccharide sequence were determined by a combination of HSQC, HMBC, and NOESY experiments (Table 2). Following HMBC correlations could be observed: from H-1 of the xylose ($\delta_{\rm H}$ 4.91, inner) to C-3 ($\delta_{\rm C}$ 88.4) of the aglycon, H-1 ($\delta_{\rm H}$ 6.67) of rhamnose to C-2 (δ_C 77.2) of the xylose (inner), and H-1 of another xylose ($\delta_{\rm H}$ 5.46) (terminal) to C-3 ($\delta_{\rm C}$ 83.3) of rhamnose (Figure 2). The β -configuration at the anomeric positions of the xylose units was determined from its ${}^{3}J_{H1,H2}$ coupling constants (7.0-8.0 Hz) [7], and the chemical shift of C-5 ($\delta_{\rm C}$ 69.8) indicated the usual α -configuration for the rhamnose unit [9]. These configurations were also confirmed from the NOE relationships between H-1 and H-3 and between H-1 and H-5 of monosaccharide moieties (Figure 2) [10]. The structure of 2 was thus established as 11α, 12α-epoxy-3-O-β-D-xylopyranosyl- $(1 \rightarrow 3)$ - α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - β -D-xylopyranosyl-olean-28, 13β-olide.

Compound **3** was obtained as a white amorphous powder. The positive HR-ESI-MS exhibited a pseudomolecular ion peak at m/z 1051.5448 [M + Na]⁺, corresponding to the molecular formula of C₅₂H₈₄O₂₀. The IR spectrum of **3**

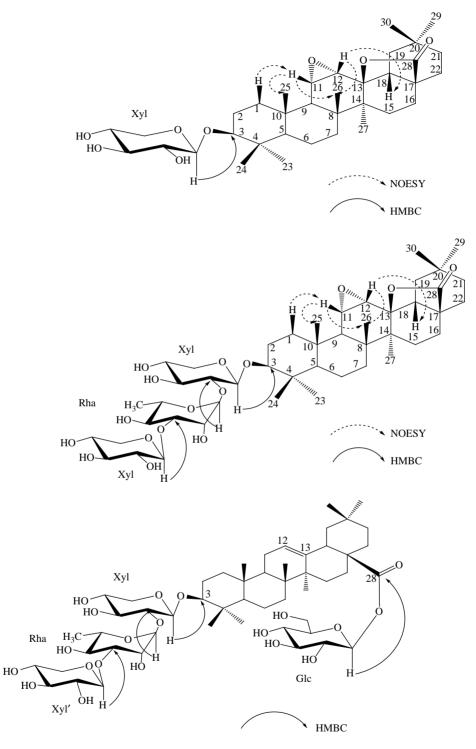


Figure 2. Key HMBC and NOESY correlations for compounds 1-3.

	1		2		3	
No.	¹³ C	¹ H	¹³ C	$^{1}\mathrm{H}$	¹³ C	$^{1}\mathrm{H}$
3-Xyl						
1	107.8	4.90 d (7.5)	106.3	4.91 d (7.0)	106.3	4.91 d (7.5)
2	75.6	4.11 br d (7.5)	77.2	4.36 m	77.0	4.35 m
3	78.8	4.24 m	79.9	4.28 m	80.0	4.27 m
4	71.4	4.32 m	71.6	4.26 m	71.7	4.25 m
5	67.2	4.46 m	67.2	4.41 m	67.2	4.40 m
		3.85 d (10.5)		3.78 d (10.0)		3.78 d (10.5)
Rha						
1			101.7	6.67 br s	101.6	6.67 br s
2			72.1	5.09 br s	72.1	5.08 br s
3			83.3	4.89 m	83.2	4.89 m
			73.2	4.62 m	73.2	4.61 br d (9.5)
4 5			69.8	4.87 m	69.8	4.86 dd (6.0, 9.5)
6			18.7	1.74 d (6.0)	18.7	1.72 d (6.0)
Xyl′						
1			107.8	5.46 d (7.5)	107.8	5.46 d (7.5)
2 3			75.8	4.17 br d (7.5)	75.8	4.16 br d (7.5)
			78.7	4.30 m	78.7	4.28 m
4			71.3	4.34 m	71.3	4.31 m
5			67.6	4.43 br d (9.0)	67.7	4.42 m
				3.82 d (9.0)		3.82 d (11.0)
28-Glc						
1					95.9	6.42 d (8.0)
2					74.3	4.28 m
3					79.1	4.35 m
4					71.3	4.43 m
5					79.5	4.12 m
6					62.4	4.54 m, 4.49 m

Table 2. The ¹H and ¹³C NMR spectral data for sugar moieties of compounds 1-3 in pyridine- d_5 (*J* in Hz).

exhibited absorptions at $3416 \,\mathrm{cm}^{-1}$ (-OH), 1750 cm⁻¹ (ester carbonyl), and 1641 cm^{-1} (double bond). The NMR spectra of compound 3 showed characteristic signals of a triterpenoid saponin. Seven tertiary methyl groups at $\delta_{\rm H}$ 0.96, 0.97, 0.99, 1.17, 1.32, 1.35, and 1.47, and corresponding seven sp³ carbons at $\delta_{\rm C}$ 15.8, 23.8, 33.3, 17.6, 17.5, 26.3, and 28.3 could be observed, together with one trisubstituted olefinic proton at $\delta_{\rm H}$ 5.50 (1H, br s) and two sp² olefinic carbons at $\delta_{\rm C}$ 123.0 and 144.3, which indicated the typical olean-12-ene aglycon signals (Table 1). The chemical shifts of C-3 (δ_C 88.7) and C-28 $(\delta_{\rm C} 176.6)$ revealed that **3** was a bisdesmosidic glycoside. The ¹H and ¹³C NMR spectra of 3 exhibited four sugar anomeric protons at $\delta_{\rm H}$ 6.67 (br s), 6.42 (d, J = 8.0 Hz), 5.46 (d, J = 7.5 Hz), and 4.91 (d, J = 7.5 Hz), and carbons at $\delta_{\rm C}$ 95.9, 101.6, 106.3, and 107.8, which ascribed to remaining 22 oligosaccharide carbon signals of 3 (Table 2). The methyl carbon signal at $\delta_{\rm C}$ 18.7 and the doublet methyl proton signal at δ_H 1.72 (3H, d, $J = 6.0 \,\mathrm{Hz}$) indicated the presence of a 6-deoxy sugar. The identities of the oligosaccharide sequence were determined by a combination of COSY, HSQC, HMBC, and NOESY experiments. The oligosaccharide connected to C-3 of the aglycon was elucidated as C-3-Xyl- $(1 \rightarrow 3)$ -Rha- $(1 \rightarrow 2)$ -Xyl, same as that of 2. A disaccharide part at C-28 of the aglycon was established by the HMBC correlation from the H-1 ($\delta_{\rm H}$ 6.42) of glucose to C-28 ($\delta_{\rm C}$ 176.6) of the aglycon (Figure 2). On acid hydrolysis with 2 M CF₃COOH, **3** afforded sugar moieties that were identified as L-rhamnose, D-xylose, and D-glucose in the ratio of 1:2:1 based on the GC analysis of its chiral derivative [8]. On the basis of the above results, the structure of **3** was finally established as 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-xylopyranosyl oleanolic acid 28-*O*- β -D-glucopyranoside.

Moreover, 12 known triterpenoid compounds were also isolated and identified by comparing their NMR and MS spectral data with those reported in the literature. They are assigned to be oleanoic acid (4) [3], 2α -hydroxyoleanoic acid (5) [3], 3α -ursoloic acid (6) [4], 3-hydroxyolean-11-oxo-12-en-28-oic acid (7) [4], 3, 11-dioxo-olean-12-en-28-oic acid (8) [4], 29-hydroxy-3-oxo-olean-12-en-28-oic acid (9) [4], 3 β , 12 α -dihydroxy-oleanan-13β, 28-olide (10) [4], oleanolic acid 28-O- β -D-glucopyranosyl- $(1 \rightarrow 6)$ - β -D-glucopyranosyl ester (11) [4], $3-O-\beta-D$ xylopyranosyl oleanolic acid 28-O-B-Dglucopyranosyl ester (12) [4], $3-O-\alpha-L$ rhamnopyranosyl- $(1 \rightarrow 2)$ - β -D-xylopyranosyl-oleanolic acid 28-O-B-D-glucopyranosyl ester (13) [4], oleanolic acid 3-O- β -D-xylopyranoside (14) [4], and oleanolic acid 3-*O*- α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - β -D-xylopyranoside (15) [4]. Among them, seven compounds 6-10, 12, and 13 were isolated from this genus for the first time.

Selected compounds **2**, **3**, and **8–11** were evaluated for their cytotoxic activities against human hepatoma cell lines (HepG-2) by the MTT assay *in vitro*, while compound **1** was not tested due to limited amount. Compound **2** exhibited moderate cytotoxicity to HepG-2 cells with IC₅₀ value of 28.5 μ M, while other compounds did not show cytotoxicity at a high sample concentration of 100 μ M.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer PL341 polarimeter, and IR spectra were recorded on a Perkin-Elmer 938G instrument (Perkin-Elmer, Inc., Waltham, Massachusetts, USA). The NMR spectra were recorded on a Varian 500 MHz NMR System (Varian, Inc., Palo Alto, California, USA). HR-ESI mass spectra were recorded with Finnigan LCQ-Deca (for compounds 1-3; Thermo-Finnigan, Inc., San Jose, California, USA) and Varian 7.0 T FTICR (for compound 2; Varian, Inc., Palo Alto, California, USA) mass spectrometers. GC analysis was carried out on a Shimadzu GC-14C gas chromatograph (Shimadzu Corporation, Kyoto, Japan) using a Rtx-1 capillary column $(30 \text{ m} \times 0.25 \text{ mm}, \text{ i.d.})$; flame ionization detector; detector temperature, 250°C; injection temperature, 250°C; initial temperature was at 100°C then raised to 180°C at the rate of 10°C/min; and from 180°C to 230°C at the rate of 3°C/min; carrier gas, N2 (0.8 ml/min). HPLC separations were performed on a Shimadzu LC-20A series apparatus with an SPD-20A UV (Shimadzu detector Corporation), equipped with a 250×20 mm i.d. preparative Cosmosil AR-II C18 column (Nacalai Tesque, Inc., Kyoto, Japan); Medium pressure liquid chromatography was performed on a Lisure HP Purifier apparatus with a UV detector (Lisure science corporation, Suzhou, China). Silica gel (200-300 mesh); (Qingdao Haiyang Chemical, Inc., Qingdao, China) and Cosmosil C₁₈ reversed-phase silica gel (75 µm, Nacalai tesque corporation) were used for column chromatography. The spray reagent for saponins was ethanolic H_2SO_4 (10%).

3.2 Plant material

The whole plants of *P. scabiosifolia* were collected from Guangnan County, Yunnan

Province, China, in August 2008, and the botanical origin of the material was identified by Prof. Lin Zhang, College of Biomedical Engineering & Instrument Science, Zhejiang University, Hangzhou, China. The voucher specimens (No. PS080726) are deposited at the Department of Natural Medicinal Chemistry, College of Pharmaceutical Science, Soochow University, Suzhou, China.

3.3 Extraction and isolation

The air-dried plants (2.5 kg) were extracted with 95% aqueous methanol (v/v) for three times (8 liters, 1.5 h each) at room temperature. After evaporation, the residue was suspended in H₂O and partitioned using EtOAc $(6 \times 4 \text{ liters})$. The EtOAc fraction (81.3 g) was subjected to vacuum liquid chromatography on silica gel $(30 \times 25 \text{ cm})$ using a stepwise gradient elution of CHCl₃–MeOH (100:1 – 70:30) to afford 11 subfractions (PE-1-11). PE-6 (5.32 g) was subjected to silica gel column with gradient elution of CHCl3-MeOH (30:1-25:1), followed by medium pressure liquid chromatography over ODS-C₁₈ column eluted with MeOH-H₂O (75:25-95:5), and further purified by Sephadex LH-20 with CHCl₃-MeOH (1:1) to yield 1 (10.2 mg). PE-10 (5.46 g) was further chromatographed on silica gel column, followed by preparative HPLC (MeOH-H₂O 78:12; flow rate 2 ml/min; UV 210 nm detection) to yield 13 (29.7 mg), 2 (18.7 mg), and 11 (17.5 mg), respectively. PE-11 (1.02 g) was further chromatographed on silica gel column eluted with $CHCl_3$ -MeOH (9:1 – 8:2) to yield **3** (21.4 mg). By similar separation procedures, compound 8 (22.3 mg) from PE-1 (12.17 g), 5 (16.7 mg), 6 (13.1 mg), 9 (18.4 mg) and **10** (16.6 mg) from PE-2 (3.15 g), 4 (240 mg) and 7 (12.5 mg) from PE-3 (4.23 g), 14 (24.7 mg) from PE-7 (11.49 g), **12** (33.6 mg) and **15** (20.3 mg) from PE-9 (5.03 g) were also obtained, respectively.

3.3.1 Compound 1

White amorphous powder; $[\alpha]_{D}^{20} - 2.1$ (*c* = 0.22, MeOH); IR (KBr) ν_{max} 3423 (-OH), 2936, 2871 (CH), 1769 (C=O), 871 (epoxy) cm⁻¹; ¹H NMR (pyridine-*d*₅, 500 MHz) and ¹³C NMR (pyridine-*d*₅, 125 MHz) spectral data: see Tables 1 and 2; HR-ESI-MS: *m/z* 625.3716 [M + Na]⁺ (calcd for C₃₅H₅₄O₈Na, 625.3711).

3.3.2 Compound 2

White amorphous powder; $[\alpha]_D^{20} - 8.2$ (*c* = 0.19, MeOH); IR (KBr) ν_{max} 3418 (-OH), 2933, 2866 (CH), 1770 (C=O), 871 (epoxy) cm⁻¹; ¹H NMR (pyridine-*d*₅, 500 MHz) and ¹³C NMR (pyridine-*d*₅, 125 MHz) spectral data: see Tables 1 and 2; HR-ESI-MS: *m*/*z* 903.4593 [M + Na]⁺, FTICR-HR-ESI-MS: *m*/*z* 903.4716 [M + Na]⁺ (calcd for C₄₆H₇₂O₁₆Na, 903.4713).

3.3.3 Compound 3

White amorphous powder; $[\alpha]_D^{20} - 7.7$ (*c* = 0.20, MeOH); IR (KBr) ν_{max} 3416, 2943, 1750, 1641, 1073 cm⁻¹; ¹H NMR (pyridine-*d*₅, 500 MHz) and ¹³C NMR (pyridine-*d*₅, 125 MHz) spectral data: see Tables 1 and 2; HR-ESI-MS: *m/z* 1051.5448 [M + Na]⁺ (calcd for C₅₂H₈₄O₂₀Na, 1051.5488).

3.3.4 Acid hydrolysis and GC analysis of 1–3

Each compound (3 mg) was dissolved in 2 M CF₃COOH (2 ml) and heated at 120°C for 4 h in a sealed tube. The reaction mixture was extracted with EtOAc (2 ml × 3). Each remaining aqueous layer was concentrated to dryness to give a residue. The residue was dissolved in pyridine (1 ml), and then L-cysteine methyl ester hydrochloride (2 mg) was added to the solution. The mixture was heated at 60°C for 1 h, and trimethylchlorosilane (0.5 ml) was added, followed by heating at 60°C for 30 min. Then, the solution was concentrated to dryness and dissolved in water $(1 \text{ ml} \times 3)$, followed by extraction with *n*-hexane $(1 \text{ ml} \times 3)$. The hexane extract was subjected to GC analysis [8]. The absolute configurations of the monosaccharides were confirmed to be L-rhamnose, D-xylose, and D-glucose by comparison of the retention times of monosaccharide derivatives with those of standard samples: L-rhamnose (8.32 min), D-xylose (10.55 min), and D-glucose (15.23 min), respectively.

3.4 MTT cytotoxicity assay

The bioassay was carried out according to the method described by Chen et al. [11]. Compounds **2**, **3**, and **8–11** were evaluated against the HepG-2 human hepatoma cell lines, and compound **2** was cytotoxic to HepG-2 cells with IC₅₀ value of 28.5 μ M.

Supporting information

Supporting information of compounds 1-3 can be found in the online version of this article.

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