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Triterpenoids and iridoids from *Patrinia scabiosaefolia*

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ABSTRACT

Three new triterpenoids, patrinolides B-D (**1**–**3**), and two new iridoids, patriscabioins K–L (**9**–**10**), together with five known compounds (**4**–**8**) were isolated from the extract of the whole plants of *Patrinia scabiosaefolia*. Compounds **1**, **9**, and **10** contained the unique substituents in Valerianaceae family, such as isovalery and 3-methylcrotonyl. Compound **2** was a 24-nor-ursane triterpenoid. Their structures were established on the basis of extensive spectroscopic analysis (UV, IR, MS, 1D and 2D NMR). The inhibitory activities against nitric oxide synthase (NOS) of all triterpenoids were tested. The results showed that compound **4** had moderate inhibitory activity with IC_{50} of 10.1 μM. Furthermore, it also showed strongest inhibitory activities on AChE with IC_{50} values of 10.0 μM.

1. Introduction

Valerianaceae family comprises 13 genus, about 400 species, mostly distributed in the north temperate regions. In China, there are 3 genus (*Patrinia*, *Valeriana*, and *Nardostachys*), approximately 30 species all over the country. Some species used as medicines or spices raw materials, and only a few species' tender leaves are edible [1]. Some of these species were included in Pharmacopoeia, such as *V. officinalis* L. in the European Pharmacopoeia in 1983, *P. scabiosaefolia* Fisch in Chinese pharmacopoeia in 1995, and *V. jatamansi* Jones in Chinese pharmacopoeia in 2010, which attracted increasing attentions at home and abroad. Our groups have studied some species of genus of Valeriana and Nardostachys [2–9]. In order to perfect our research and find more bioactive compounds, we selected the plant of *P. scabiosaefolia*.

Patrinia scabiosaefolia (Family Valerianaceae), a perennial herb, is mostly distributed in Mainland China, which traditionally has been used for the treatment of initial stages of edema, appendicitis, endometriosis and inflammation [10–11]. Previous phytochemical investigations on this genus showed the existence of triterpenes, iridoids, saponins, sesquiterpenes, flavonoids, coumarins, and lignans [11]. In the continuing searching for more compounds and better bioactivities, we investigated the ethyl acetate extract of the whole plants of *P. scabiosaefolia* and acquired 3 new triterpenoids (**1**–**3**) and 2 new iridoids (**9**–**10**), together with 5 known triterpenoids (**4**–**8**) (Fig. 1). The known compounds were identified as 3*β*-hydroxy-24-nor-urs-4 (23), 12-dien-28-oic acid (**4**) [12], 3*β*-hydroxy-urs-20 (30)-en-28-oic acid (**5**) [13], tormentic acid (**6**) [14], oleanolic acid (**7**) [15], 23-hydroxy-3-oxo-urs-12-en-28-oic acid (**8**) [16] by comparison with the MS and NMR spectra data reported in the literature. Considering that *P. scabiosaefolia* is used for the treatment of inflammation in Chinese folk medicine, the inhibitory activity of all isolated triterpenoids against nitric oxide synthase (NOS) were evaluated. Their inhibitory effects on acetylcholine esterase (AChE) were also tested. Herein, we report the isolation, structure elucidation, and bioactivities of these isolates.

2. Experimental

2.1. General methods

Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were measured using a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained on Bruker Tensor-27 infrared spectrophotometer with KBr pellets. ESI-MS spectra were recorded on a Bruker HTC/Esquire spectrometer, HRESIMS spectra were recorded on an API Qstar Pulsar instrument. NMR spectra were recorded on Bruker AM-400, DRX-500 or Av III-600 instruments with TMS as the internal standard (Bruker, Bremerhaven, Germany). The chemical shifts were given in δ (ppm) scale with reference to the solvent

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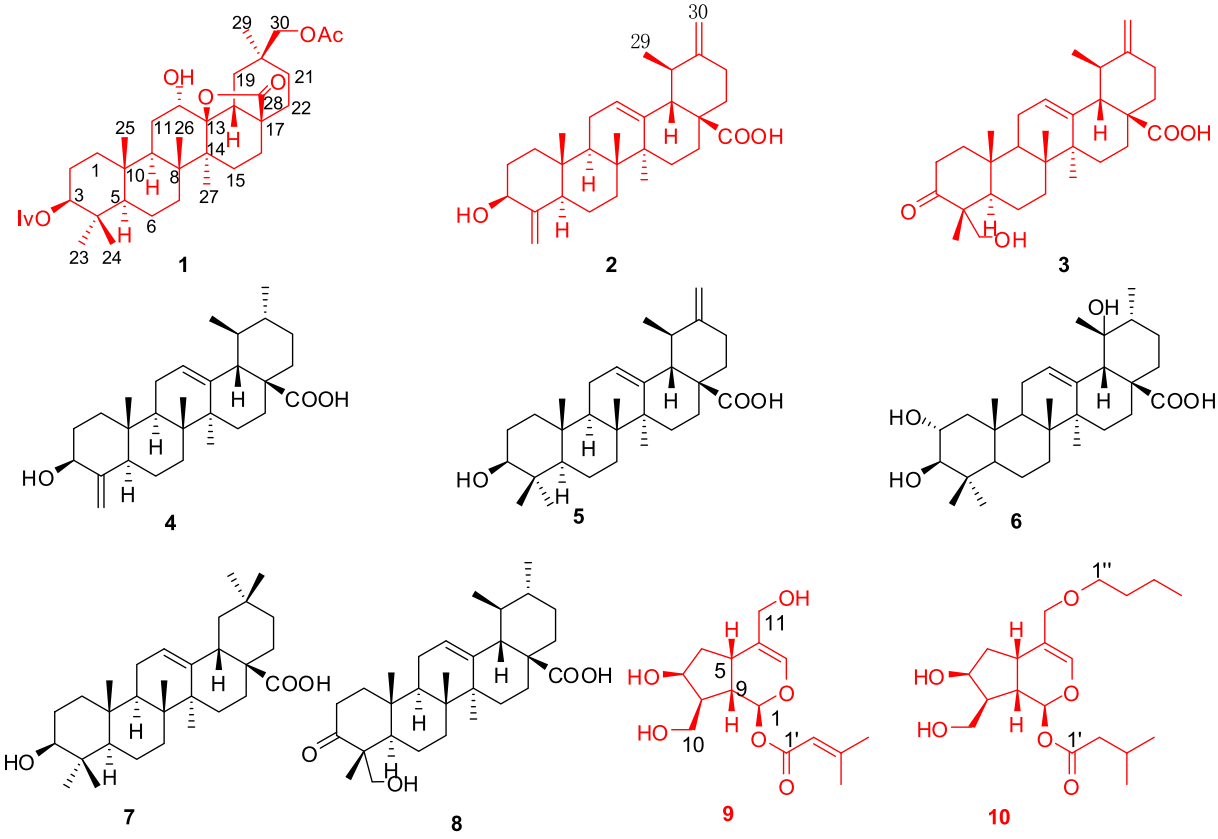

Fig. 1. Chemical structures of compounds **1–10**.

signal. Mass spectra were recorded on an API QSTAR time-of-flight spectrometer (MDS Sciqaszex, Concord, Ontario, Canada) or LCMS-IT-TOF (Shimadzu, Kyoto, Japan) spectrometer. Semi-preparative HPLC was run on Agilent 1100 liquid chromatography with diode array detector (DAD), Zorbax-SB-C18 column (5 μm; 25 cm × 9.4 mm i.d.) at a flow rate of 2.0 mL/min. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Co., China), Rp-18 (40–63 μm, Merck), MCI gel CHP-20P (75–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan), and Sephadex LH-20 (20–150 μm, Amersham Biosciences, Uppsala, Sweden). TLC was performed on $HSGF_{254}$ (0.2 mm, Qingdao Marine Chemical Co., China) or Rp-18 F_{254} (0.25 mm, Merck). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH, followed by heating.

2.2. Plant material

The whole plants of *P. scabiosaefolia* were collected in October 2001 from Shucheng county, Anhui Province, People's Republic of China, which were stored in a cool and dry place at room temperature. The material was identified by Prof. Shou-Jin Liu at Anhui University of Chinese Medicine and a voucher specimen (Wan1295) was deposited at Anhui University of Chinese Medicine.

2.3. Extraction and isolation

The air-dried and powdered whole plants (29 kg) of *P. scabiosaefolia* were extracted with 95% ethanol (3 × 75 L) under room temperature and concentrated under reduced pressure. The extract (3 kg) was suspended in water and partitioned with ethyl acetate (EtOAc). The EtOAc extract (0.85 kg) was divided into six parts (Fr.1-Fr.6) by silica gel column chromatography eluted with a gradient of petroleum ether–ethyl acetate (20:1 → 0:1, v/v).

Fr.2 (64 g) was subjected to silica gel column chromatography eluted with a gradient of petroleum ether–acetone (5:1 → 1:1, v/v) to afford 4 subfractions (Fr.2-1 to Fr.2-4). Fr.2-3 (11.8 g) was separated by Rp-18 column chromatography (MeOH-H_2O, 50:50 → 100:0, v/v) and Sephadex LH-20 column chromatography (MeOH-H_2O, 90:10, v/v), and then compound **1** (68 mg) was acquired through recrystallization in methanol.

Fr.3 (62 g) was submitted to silica gel column chromatography eluted with a gradient of petroleum ether–acetone (5:1 → 1:1, v/v) to afford 6 subfractions (Fr.3-1 to Fr.3-6). Fr.3-3 (11 g) was applied to Rp-18 column chromatography (MeOH-H_2O, 50:50 → 100:0, v/v) and further Sephadex LH-20 column chromatography (MeOH-H_2O, 90:10, v/v), then purified by silica gel column chromatography eluted with petroleum ether–ethyl acetate (4:1, v/v) to obtain **7** (56 mg), as well as purified by semi-prep. HPLC (MeCN-H_2O, 75:25, v/v) to afford **2** (26 mg, t_R = 20.0 min) and **4** (16 mg, t_R = 26.9 min). Fr.3-6 (5.7 g) was separated by the same methods and purified by semi-prep. HPLC (MeOH-H_2O, 60:40, v/v) to afford **3** (4 mg, t_R = 23.5 min), and compound **8** (11 mg) was obtained by recrystallization in methanol.

Fraction 5 (56 g) was applied to Rp-18 column chromatography (MeOH-H_2O, 50:50 → 100:0, v/v) and further Sephadex LH-20 column chromatography (MeOH-H_2O, 90:10, v/v) to afford 7 subfractions (Fr.5-1 to Fr.5-7). Fr.5-1 (230 mg) was performed by silica gel column chromatography eluted with petroleum ether–ethyl acetate (2:1, v/v), and purified by semi-prep. HPLC (MeOH-H_2O, 68:32, v/v) to afford **5** (4.0 mg, t_R = 21.8 min) and **6** (3.0 mg, t_R = 25.6 min). Fr.5-6 (3.4 g) was performed by silica gel column chromatography eluted with chloroform–methanol (20:1, v/v), and purified by semi-prep. HPLC (MeOH-H_2O, 45:55, v/v) to afford **9** (1.0 mg, t_R = 11.1 min) and **10** (5.0 mg, t_R = 22.2 min).

Table 1
The ^{1}H and ^{13}C NMR data of **1–3** (500 MHz, **1** and **2** in $CDCl_3$, **3** in CD_3OD, δ in ppm, J in Hz).

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.71 (m) 1.05 (m)	38.5 t	1.73 (m) 1.16 (m)	38.7 t	1.92 (m) 1.51 (m)	38.8 t
2	1.64 (m) 1.57 (m)	23.6 t	1.96 (m) 1.43 (m)	32.4 t	2.47 (m) 2.40 (m)	36.7 t
3	4.49 (m)	80.4 d	4.00 (dd,11.3, 5.6)	73.2 d		219.9 s
4		37.8 s		153.0 s		53.5 s
5	0.83 (s)	55.2 d	1.56 (m)	50.3 d	1.97 (m)	48.2 d
6	1.52 (m) 1.44 (m)	17.6 t	1.56 (m) 1.46 (m)	21.0 t	1.45 (m) 1.45 (m)	20.5 t
7	1.46 (m) 1.42 (m)	31.1 t	1.49 (m) 1.34 (m)	31.5 t	2.21 (m) 1.39 (m)	33.4 t
8		42.3 s		39.7 s		40.7 s
9	1.58 (m)	44.5 d	1.66 (m)	45.1 d	1.75 (m)	47.5 d
10		36.3 s		38.7 s		37.4 s
11	1.49 (m) 1.49 (m)	28.3 t	2.18 (m) 1.94 (m)	24.1 t	2.01 (m) 2.01 (m)	24.6 t
12	3.91 (s)	75.7 d	5.28 (t, 2.8)	126.3 d	5.29 (t, 3.5)	127.0 d
13		90.9 s		137.8 s		140.0 s
14		41.8 s		42.2 s		43.6 s
15	1.86 (m) 1.16 (m)	27.8 t	1.88 (m) 1.16 (m)	27.9 t	1.97 (m) 1.18 (m)	29.2 t
16	2.13 (m) 1.30 (m)	21.2 t	2.01 (m) 1.76 (m)	24.3 t	2.23 (m) 1.75 (m)	25.4 t
17		44.4 s		48.2 s		49.2 s
18	2.22 (m)	49.7 s	2.28 (m)	54.6 d	2.28 (m)	56.8 d
19	1.57 (m) 1.24 (m)	33.8 t	2.35 (m)	37.3 d	2.41 (m)	38.6 d
20		35.5 s		152.6 s		154.6 s
21	2.02 (m) 1.92 (m)	34.1 t	2.28 (m) 2.23 (m)	32.2 t	2.33 (m) 1.67 (m)	33.4 t
22	1.65 (m) 1.57 (m)	27.3 t	1.84 (m) 1.68 (m)	38.8 t	1.84 (m) 1.62 (m)	40.5 t
23	0.86 (s)	28.1 q	5.00 (s) 4.63 (s)	102.1 t	3.59 (s) 3.34 (s)	68.1 t
24	0.85 (s)	16.6 q			0.91 (s)	18.0 q
25	0.89 (s)	16.2 q	0.74 (s)	13.6 q	1.04 (s)	15.9 q
26	1.13 (s)	18.6 q	0.81 (s)	17.0 q	0.92 (s)	17.8 q
27	1.29 (s)	18.6 q	1.14 (s)	23.5 q	1.21 (s)	23.9 q
28		179.5 s		183.0 s		181.4 s
29	1.00 (s)	28.0 q	1.00 (d, 6.3)	16.2 q	1.01 (d, 6.5)	16.8 q
30	4.51 (m) 3.54 (m)	67.8 t	4.69 (s) 4.63 (s)	105.3 t	4.67 (br s) 4.62 (br s)	105.2 t
3-Iv-1′		173.1 s				
2′	2.18 (d, 4.5)	44.0 t				
3′	2.10 (m)	25.8 d				
4′	0.95 (d, 6.3)	22.5 q				
5′	0.95 (d, 6.3)	22.4 q				
30-Ac		172.2 s				
	2.07 (s)	21.0 q				

Table 2
The ^{1}H and ^{13}C NMR data of **9** (600 MHz) and **10** (500 MHz) (δ in ppm, J in Hz, MeOH).

Position	**9**		**10**	
	δ_H	δ_C	δ_H	δ_C
1	5.95 (d, 4.9)	93.0 d	6.01 (d, 4.4)	93.2 d
3	6.28 (s)	138.5 d	6.27 (s)	139.4 d
4		119.6 s		116.9 s
5	2.99 (q, 7.8)	33.3 d	2.89 (q, 7.4)	33.1 d
6	2.04 (m) 1.80 (m)	40.8 t	2.00 (m) 1.83 (m)	40.5 t
7	4.31 (m)	73.4 d	4.30 (m)	73.3 d
8	1.96 (m)	48.9 d	1.95 (m)	48.7 d
9	2.22 (m)	42.9 d	2.25 (m)	42.9 d
10	3.81 (dd, 10.9, 7.4) 3.73 (dd, 10.9, 5.6)	62.2 t	3.82 (dd, 11.0, 7.2) 3.72 (dd, 11.0, 5.0)	62.2 t
11	4.04 (d, 12.4) 3.90 (d, 12.4)	62.3 t	4.01 (d, 11.8) 3.73 (dd, 11.8, 5.0)	70.7 t
1′		166.5 s		173.2 s
2′	5.70 (s)	116.3 d	2.22 (m)	44.3 t
3′		160.8 s	2.08 (m)	26.9 d
4′	1.93 (s)	27.5 q	0.96 (d, 6.7)	22.7 q
5′	2.17 (s)	20.5 q	0.96 (d, 6.7)	22.6 q
1″			3.43 (m) 3.33 (m)	70.0 t
2″			1.54 (m) 1.38 (m)	32.9 t
3″			1.38 (m)	20.5 t
4″			0.93 (t, 7.4)	14.3 q

2.4. Spectroscopic data

Patrinolide B (**1**): White, amorphous powder; [α]22 D: + 9.1 (c 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.17), 216 (2.99) nm; IR (KBr) ν_{max} 3435, 2958, 2932, 1770, 1736, 1631, 1467, 1250, 1040 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; negative-ESI-MS: m/z 659 [M + HCOO]$^-$; HR-ESI-MS m/z 659.4161 [M + HCOO]$^-$ (calcd for $C_{37}H_{58}O_7$, 659.4165).

Patrinolide C (**2**): White, amorphous powder; [α]22 D: + 260.6 (c 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.19) nm; IR (KBr) ν_{max} 3429, 2934, 1700, 1647, 1454, 1384, 1050, 893 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; negative-ESI-MS: m/z 437 [M - H]$^-$; HR-ESI-MS m/z 437.3063 [M - H]$^-$ (calcd for $C_{29}H_{42}O_3$, 437.3061).

Patrinolide D (**3**): White, amorphous powder; [α]22 D: + 32.2 (c 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.12) nm; IR (KBr) ν_{max} 3425, 2928, 1696, 1635, 1384, 1050 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; positive-ESI-MS: m/z 491 [M + Na]$^+$; HR-ESI-MS m/z 491.3135 [M + Na]$^+$ (calcd for $C_{30}H_{44}O_4$, 491.3132).

Patriscabioin K (**9**): Light yellow oil; [α]22 D: − 223.3 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.55) nm; IR (KBr) ν_{max} 3415, 2934, 1727, 1644, 1447, 1384, 1225, 1117, 1017 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; positive-ESI-MS: m/z 321 [M + Na]$^+$; HR-ESI-MS m/z 321.1310 [M + Na]$^+$ (calcd for $C_{15}H_{22}O_6$, 321.1309).

Patriscabioin L (**10**): Light yellow oil; [α]22 D: − 24.13 (c 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.55) nm; IR (KBr) ν_{max} 3420, 2960, 2933, 1749, 1667, 1630, 1087 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; positive-ESI-MS: m/z 379 [M + Na]$^+$; HR-ESI-MS m/z 379.2092 [M + Na]$^+$ (calcd for $C_{19}H_{32}O_6$, 379.2091).

2.5. Acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE) inhibitory activity of the compounds isolated was assayed by the spectrophotometric method developed by Ellman et al. with slightly modification [17]. *S*-Acetylthiocholine iodide, *S*-butyrylthiocholine iodide, 5,5′-dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent), acetylcholinesterase derived from human erythrocytes were purchased from Sigma Chemical. Compounds were dissolved in DMSO. The reaction mixture (totally 200 μL) containing phosphate buffer (pH 8.0), test compound (50 μM), and acetyl cholinesterase (0.02 U/mL), was incubated for 20 min (37 °C). Then, the reaction was initiated by the addition of 40 μL of solution containing DTNB (0.625 mM) and acetylthiocholine iodide (0.625 mM) for AChE inhibitory activity assay, respectively. The hydrolysis of acetylthiocholine was monitored at 405 nm every 30 s for 1 h. Tacrine was used as positive control with final concentration of 0.333 μM. All these actions were performed in triplicate. The percentage inhibition was calculated as follows: % inhibition = (E − S)/E × 100 (E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound).

2.6. Nitric oxide production in RAW264.7 macrophages

Murine macrophage cell line RAW264.7 was obtained from Cell Bank of Chinese Academy of Sciences. RAW264.7 cells were seeded in 96-well cell culture plates (1.5×10^5 cells/well) and treated with serial dilutions of the compounds with a maximum concentration of 25 μM in triplicate, followed by stimulation with 1 μg/mL LPS (Sigma, St. Louis, MO, USA) for 18 h. NO production in the supernatant was assessed by Griess reagents (Reagent A & Reagent B, respectively, Sigma). The absorbance at 570 nm was measured with a microplate reader (Thermo, Waltham, MA, USA), N^G-Methyl-L-arginine acetate salt (L-NMMA, Sigma), a well-known nitric oxide synthase (NOS) inhibitor, was used as a positive control [18]. The viability of RAW264.7 cells was evaluated by the MTS assay simultaneously to exclude the interference of the cytotoxicity of the test compounds.

3. Results and discussion

Patrinolide B (**1**) was obtained as white amorphous power with a molecular formula of $C_{37}H_{58}O_7$ determined by negative-ion HR–ESI–MS (*m/z* 659.4161 $[M + HCOO]^-$, calcd for 659.4165) and ^{13}C NMR data (Table 1). IR spectrum (KBr) showed characteristic absorptions for hydroxyl (3435 cm^{-1}), ester carbonyl (1736 cm^{-1}), and lactone functionalities (1040 cm^{-1}). The ^{13}C NMR displayed exactly 37 carbon signals. Carefully analysis of the ^{13}C NMR and HMBC spectroscopics data found an isovaleryl substituent at δ_C 173.1 (s), δ_C 44.0 (t), δ_C 25.8 (d), δ_C 22.5 (q), δ_C 22.4 (q), and an acetyl group at δ_C 172.2 (s), δ_C 21.0 (q). Thus, there were 30 carbon signals remained. The ^1H NMR spectroscopic data showed six methyl signals at δ_H 0.86 s, 0.85 s, 0.89 s, 1.13 s, 1.29 s, 1.00 s, and two protons at δ_H 4.51 m, 3.54 m, coupled with information of the ^{13}C NMR spectroscopic at δ_C 28.1 q, 16.6 q, 16.2 q, 18.6 q, 18.6 q, 28.0 q and 67.8 t. Also, the carbonyl carbon signal at δ_C 179.5 together with the quaternary carbon at δ_C 90.9, as well as the oxygen-methine (δ_H 4.49 m; δ_C 80.4) suggested compound **1** was a triterpenoid with a 3-hydroxyl-28,13*β*-lactone-oleanane skeleton [19], which Me-30 was oxygenated to an oxygenated methylene through the upfield shift of C-29 (33.3 → 28.0) and downfield shift of C-20 (31.6 → 35.5) [20]. The isovaleryl substituent and acetyl group were assigned at C-3 and C-30 respectively, through the correlations from H-3 to the isovaleryl at δ_C 173.1 (s), and H-30 to the acetyl group at δ_C 172.2 (s). A hydroxyl group at C-12 was determined by the long-range correlations in the HMBC from H-12 (δ_H 3.91) to C-13 (δ_C 90.9), C-14 (δ_C 41.8), and C-9 (δ_C 44.5).

The relative configurations of **1** were acquired by the ROESY experiment and comparison with structurally similar compounds [19–21]. The *α*-orientation of H-3 was determined by the ROESY correlations of H-3 with H-23. Additionally, the relative configuration of H-12 was *β*-orientation established by the correlations from H-12 to H-18 in the ROESY experiments. Thus, the structure of **1** was identified as 3*β*-isovaleroxy-12*α*-hydroxy-30-acetoxy-olean-28,13*β*-olide.

Patrinolide C (**2**) was isolated as white amorphous powder. Its molecular formula $C_{29}H_{42}O_3$ was determined by its HR-ESI-MS at *m/z* 437.3063 $[M-H]^-$ (calcd for 437.3061) and ^{13}C NMR data (Table 1), which indicates 9 degrees of unsaturation. Judging from the 1D NMR and HSQC spectroscopic data (Table 1) found four methyl signals at δ_H 0.74 (s, Me-25), δ_H 0.81 (s, Me-26), δ_H 1.14 (s, Me-27), δ_H 1.00 (d, J = 6.3 Hz, Me-29); a carbonyl at δ_C 183.0 (s, C-28); and three groups of double bonds at δ_C 126.3 (d, C-12), δ_C 137.8 (s, C-13); δ_C 153.0 (s, C-4), δ_C 102.1 (t, C-23); δ_C 152.6 (s, C-20), δ_C 105.3 (t, C-30); as well as hydroxylated methine at δ_H 4.00 (dd, J = 11.3, 5.6 Hz, H-3), δ_C 73.2 (d). All these data were very similar to those of compound **4** except the lack of a methyl and methine and appearance of two olefinic carbons at δ_C 152.6 (s, C-20), δ_C 105.3 (t, C-30). This suggested that **2** was a dehydrogenation product of **4** at C-20/C-30, as deduced from the HMBC correlations of H-30 with C-19 and C-21 (Fig. 2). Therefore, the structure of **2** was formulated as 3*β*-hydroxy-24-nor-urs-4 (23), 12 (13), 20 (30)-trien-28-oic acid.

Patrinolide D (**3**), white amorphous powder, had a molecular formula of $C_{30}H_{44}O_4$ based on the positive-ion HR-ESI-MS at *m/z* 491.3135 $[M + Na]^+$ (calcd for 491.3132) and ^{13}C NMR data (Table 1). The ^1H NMR exhibited four methyl signals at δ_H 0.91 (s, Me-24); δ_H 1.04 (s, Me-25); δ_H 0.92 (s, Me-26); δ_H 1.21 (s, Me-27); δ_H 1.01 (d, J = 6.5 Hz, Me-29). The ^{13}C NMR data showed two carbonyls [δ_C 219.9 (s, C-3); δ_C 181.4 (s, C-28)], two double bonds [δ_C 127.0 (d, C-12), δ_C 140.0 (s, C-13); δ_C 154.6 (s, C-20), δ_C 105.2 (t, C-30)], and an oxy-methylene [δ_C 68.1 (t)]. All these data suggested **3** was an analogue of the known compound **8**, while the double bond formed between C-30 and C-20 through the correlations from H-30 to C-19 and C-21. So, the structure of **3** was elucidated as 23-hydroxy-3-oxo-urs-12 (13), 20 (30)-dien-28-oic acid.

Patriscabioin K (**9**) was isolated as light yellow oil. The molecular formula, $C_{15}H_{22}O_6$, was established by HR-ESI-MS *m/z* 321.1310 $[M + Na]^+$ (calcd for 321.1309) and ^{13}C NMR data (Table 2). Its UV spectrum displayed absorption at 218 nm. The IR spectrum showed absorptions of hydroxy (3415 cm^{-1}), ester carbonyl (1727 cm^{-1}), olefinic groups (1644 cm^{-1}). The ^1H and ^{13}C NMR spectra showed a methine proton signal at δ_H 5.95 (d, J = 4.9 Hz, H-1), δ_C 93.0 (d, C-1) typical of an iridoid. Careful analysis of the ^{13}C NMR found the presence of an olefinic bond at δ_C 138.5 (d, C-3), and δ_C 119.6 (s, C-4), an oxy-methine at δ_C 73.4 (d, C-7), two oxygenated methylene at δ_C 62.2 (t, C-10) and δ_C 63.2 (t, C-11) and three methines at δ_C 33.3 (d, C-5), 48.9 (d, C-8), 42.9 (d, C-9). This implied that **9** is a 7,10,11-trihydroxy-3-en iridoid with a ten carbon skeleton [22]. Thus, there are carbon signals remaining at δ_C 166.5 (s), 116.3 (d), 160.8 (s), 27.5 (q), 20.5 (q), which was determined as a 3-methylcrotonyl group [23]. The 3-methylcrotonyl group was assigned at C-1 through the correlation from H-1 to the ester carbonyl carbon (δ_C 166.5) in HMBC spectrum (Fig. 2).

The *S*-configurations of C-1 and C-9 were determined by the natural occurring valepotriates [23]. The *α*-orientations of H-7 and H-8 were acquired from the correlations from H-8 to H-1, and H-7 to H-8 in the ROESY experiment. It could be determined the configuration of H-5 was *β* by the correlation from H-5 to H-9 in the ROESY spectrum. Therefore, the structure of **9** was elucidated as (1*S*,5*S*,7*S*,8*S*,9*S*)-1-*O*-(3-methyl-crotonyl)-7,10,11-trihydroxy-5,6-dihydrovaltrate hydrin.

Patriscabioin L (**10**) was obtained as light yellow oil. A molecular formula of $C_{19}H_{32}O_6$ deduced from HR-ESI-MS at *m/z* 379.2092 $[M + Na]^+$ (calcd for 379.2091) and ^{13}C NMR data (Table 2). Careful analysis of the NMR data found it showed signals similar to those of **9** except the presence of an isovaleryl group instead of the 3-methylcrotonyl group at δ_C 173.2 (s), δ_C 44.3 (t), δ_C 26.9 (d), δ_C 22.7 (q), δ_C 22.6 (q), and a *n*-butyl at δ_C 70.0 (t), δ_C 32.9 (t), δ_C 20.5 (t), δ_C 14.3 (q). The isovaleryl group and *n*-butyl were assigned at C-1 and C-11 respectively, based on the HMBC correlations from H-1 to the ester carbonyl carbon (δ_C 173.2) and from H-11 to the oxy-methylene carbon (δ_C 70.0). The configurations were acquired by the ROESY experiment. Thus, the structure of **10** was elucidated as (1*S*,5*S*,7*S*,8*S*,9*S*)-1-isovaleroxy-7,10-dihydroxy-11-*n*-butoxy-5,6-dihydrovaltrate hydrin.

Compounds from *P. scabiosaefolia* contain not only triterpenoids (**1–8**) but iridoids (**9–10**). Among them, iridoids are unique constituents only existing in few family, such as Valerianaceae, Scrophulariaceae, Gentianaceae. Moreover, this study found that the major component of *P. scabiosaefolia* is triterpenoid, which is difference from the other genus of Valerianaceae family according to our groups' continuous study. In conclusion, triterpenoid could be taken as a chemotaxonomic marker in *Patrinia* genus.

Among the triterpenoids, the skeleton of compounds **1** and **7** is oleanane-type, while the skeleton of compounds **2–6**, **8** is ursane-type. Compound **1** is a 28,13*β*-lactone-oleanane with an isovalery at C-3, a unique substituent group in the triterpenoids from Valerianaceae family. Compound **2** is a 24-nor-ursane, however compound **3** contains a ketone carbonyl at C-3, a hydroxyl at C-23, two double bonds at C-12

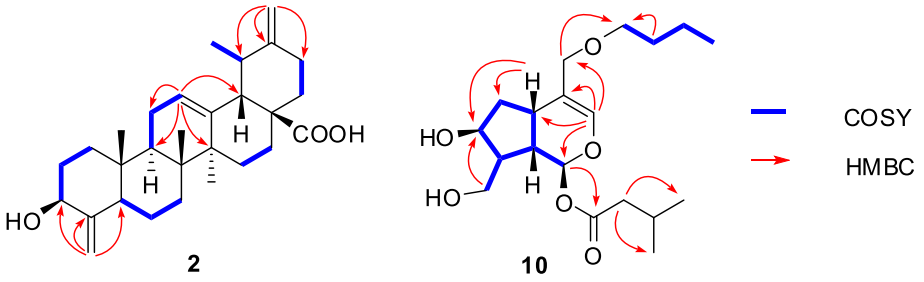

Fig. 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compounds **2** and **10**.

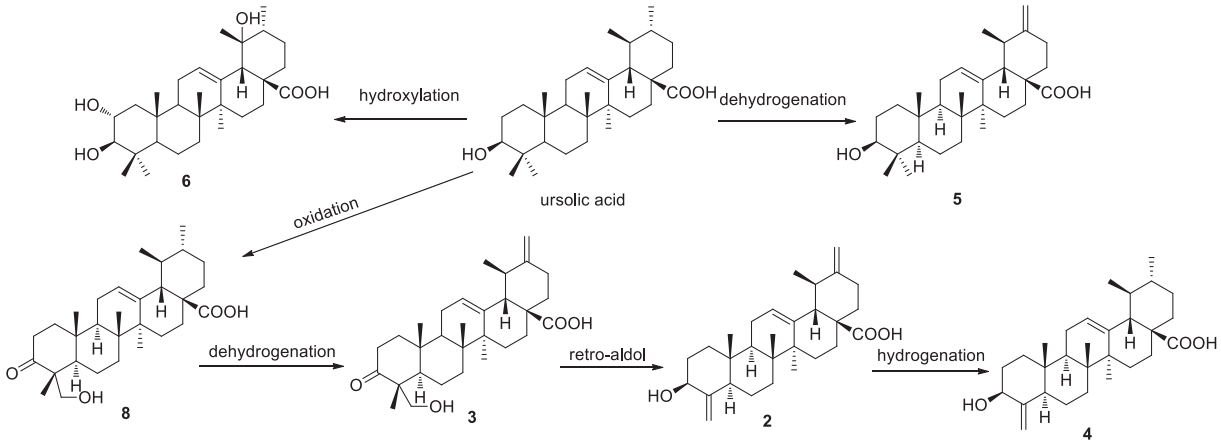

Fig. 3. The possible pathway for the transformation of compounds **2–6**, **8**.

(13) and C-20 (30), and a carboxyl at C-28. All these ursane triterpenoids were derived from ursolic acid. The possible pathway for the transformation of these isolated ursane triterpenoids were elaborated as shown in Fig. 3. Coumpounds **9–10** were two 5,6-dihydrovaltrate hydrin with hydroxyls at C-7, C-10 and C-11.

Because of the folk application of *P. scabiosaefolia* for inflammatory, the inhibitory activities against nitric oxide synthase (NOS) of all isolated triterpenoids were tested. The results showed that the inhibition rate of compound **4** was 89.51 ± 0.94%, while other compounds' inhibitory rates were less than 50%. Then, the IC_{50} value of compound **4** was examined as 10.1 μM, better than the positive control L-NMMA (32.9 μM). In addition, compound **4** showed moderate inhibitory activities on AChE with IC_{50} values of 10.0 μM.

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Supplementary data

1D and 2D NMR, HRESIMS of compounds **1-3** and **9-10**, are available as Supporting Information. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.fitote.2017.04.011.

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