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Iridoids and bis-iridoids from *Patrinia scabiosaefolia*†

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Ten new iridoids, patriscabioins A–J (**1–10**), and three unique bis-iridoids, patriscabiobisins A–C (**11–13**), together with seven known analogues, have been identified from whole plants of *Patrinia scabiosaefolia*. Compounds **1** to **8** are a series of 5,6-dihydrovaltrate hydrins with unique substituent groups in the Valerianaceae family such as isovaleryl and 3-methylcrotonyl. Furthermore, compounds **11** and **12** are the first reported bis-iridoids with two units connected by a 1,3-dioxane group, whereas compound **13** is linked by an ether bond between two units. The structures of all the compounds were established on the basis of extensive spectroscopic analysis as well as experimental and calculated ECD spectra. Compounds **1** and **3** showed moderate inhibitory activities on AChE with IC_{50} values of 37.6 and 10.5 μM, respectively. Moreover, compounds **1**, **3**, and **5** also showed moderate cytotoxic activity against HL-60, with IC_{50} values ranging from 1.2 to 27.6 μM.

1. Introduction

Iridoids, which derive their name from iridomyrmecin, iridolactone and iridodial, usually contain a bicyclic H-5/H-9β, *β*-cis-fused cyclopentan pyran ring. Iridoids are found in a large number of folk medicinal plants. Cleaving the cyclopentan or pyran ring results in derivatives called secoiridoids. Iridoids and secoiridoids have remarkable biological activities such as antiallergic, antiarthritic, anti-inflammatory, antispasmodic, antibacterial, antifungal, antiviral, antiprotozoal, anticancer, anticoagulant, antioxidant, neuroprotective, and nerve growth factor-potentiating; thus, they are currently attracting increasing attention.[1–4] Furthermore, iridoids are regarded as the bioactive compounds in some plants used in traditional medicines and are also considered to be chemotaxonomic markers in some cases such as the revision of *Asteridae* in 1959.[5]

Previous phytochemical investigations on genus *Patrinia* (Valerianaceae) showed that these plants contain many iridoids and their saponins.[6] *Patrinia scabiosaefolia* is a perennial herb that is distributed widely across China, except for the provinces of Ningxia, Qinghai, Xinjiang, Tibet and Hainan Island; it is used in sedation, antibacterial, and antiviral applications.[7] In order to enrich our knowledge of iridoids and further explore their bioactivities, the ethyl acetate extracts of whole plants of *P. scabiosaefolia* were investigated. This resulted in the isolation of ten new iridoids (**1–10**) and three unique bis-iridoids (**11–13**) along with seven known iridoids, confirmed to be stenopterin A (**14**),[8] jatamanvaltrate P (**15**),[9] (1*S*,3*R*,5*S*,7*S*,8*S*,9*S*)-3,8-epoxy-7-hydroxy-1-butoxy-4,11-dihyronepetane (**16**),[10] (1*S*,3*R*,5*S*,7*S*,8*S*,9*S*)-3,8-epoxy-7-hydroxy-1-methoxy-4,11-dihyronepetane (**17**),[10] jatamanin A (**18**),[11] 6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydro-cyclopenta[*c*]pyran-1 (3*H*)-one (**19**),[12] and villosol (**20**).[13] In addition, all the new compounds were evaluated for their inhibitory activities on acetylcholine esterase (AChE). Meanwhile, considering the cytotoxicity of iridoids,[14] we also tested the cytotoxicities of the new compounds against four human tumor cell lines (HL-60, SMMC-7721, MCF-7, and SW-480). Herein, we describe the isolation, structure elucidation,

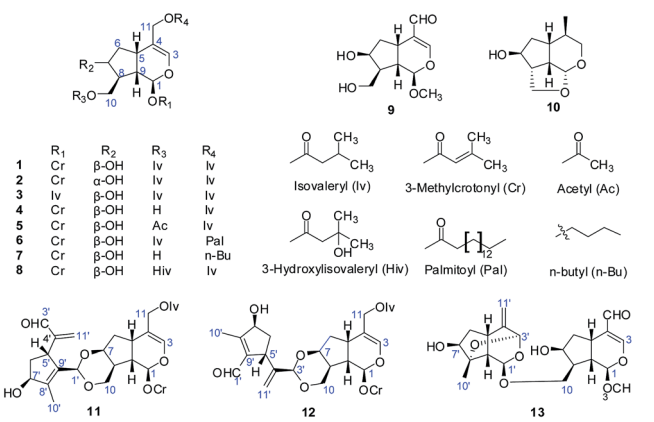

Fig. 1 The chemical structures of compounds **1–13**.

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and biological evaluation of these new iridoids and bis-iridoids (Fig. 1).

2. Results and discussion

2.1. Structure elucidation

The EtOH extract of *P. scabiosaefolia* was suspended in H_2O and partitioned with EtOAc. The EtOAc extract was repeatedly chromatographed to yield 20 iridoids, including ten new iridoids, patriscabioins A–J (**1–10**), and three new bis-iridoids, patriscabiobisins A–C (**11–13**).

Compound **1** was obtained as a light yellow oil, which was analyzed and determined to have the molecular formula $C_{25}H_{38}O_8$ based on HRESIMS at *m*/*z* 489.2454 $[M + Na]^+$ (calcd 489.2459) and its ^{13}C NMR spectrum. The ^1H and ^{13}C spectroscopic data (Tables 1 and 3) showed a hemiketal methine at δ_H 5.86 (1H, d, $J = 5.6$ Hz, H-1) and δ_C 91.5 (d, C-1); a trisubstituted olefinic bond at δ_H 6.38 (1H, s, H-3), δ_C 140.2 (d, C-3), and δ_C 113.7 (s, C-4); two oxygenated methylenes at δ_C 62.5 (t, C-10) and δ_C 63.6 (t, C-11); and an oxymethine at δ_C 71.1 (C-7). The above data clearly suggest **1** to be a 7,10,11-trihydroxy-3-ene iridoid with ten carbons in its skeleton.[8] Careful analysis of the ^{13}C NMR and 2D NMR spectra led to the discovery of two isovaleryl substituents at δ_C 174.1 (s), δ_C 43.4 (t), δ_C 25.7 (d), δ_C 22.4 (q), δ_C 22.4 (q) as well as δ_C 173.0 (s), δ_C 43.3 (t), δ_C 25.7 (d), δ_C 22.3 (q), δ_C 22.3 (q). In addition, a 3-methylcrotonyl group was discovered at δ_C 164.8 (s), δ_C 160.0 (s), δ_C 115.1 (d), δ_C 27.6 (q), δ_C 20.5 (q).[14] The 3-methylcrotonyl group should be attached to C-1 on the basis of HMBC correlation from H-1 (δ_H 5.86) to the ester carbonyl carbon (δ_C 164.8). The correlations from H-10 (δ_H 4.10, 4.52) to one isovaleryl substituent at δ_C 174.1 (s) and from H-11 (δ_H 4.57, 4.40) to the other isovaleryl at δ_C 173.0 (s) in HMBC suggested that the two isovaleroxy substituents were positioned at C-10 and C-11, respectively (Fig. 2).

The absolute configurations of C-1 and C-9 were both *S*, as found in all naturally occurring valepotriates.[15] Thus, the α-orientations of H-7 and H-8 were determined by the ROESY correlations of H-1 with H-7 and H-8. Additionally, the relative configuration of H-5 was β-orientation, as deduced by the correlations from H-9 to H-5 in the ROESY experiments. Thus, the structure of **1** was elucidated as (1*S*,5*S*,7*S*,8*S*,9*S*)-1-*O*-(3-methylcrotonyl)-7-hydroxy-10,11-diisovaleroxy-5,6-dihydrovaltrate hydrin, named patriscabioin A (Fig. 3).

Compound **2** possessed the same molecular formula as compound **1**, $C_{25}H_{38}O_8$, based on HRESIMS at *m*/*z* 489.2462 $[M + Na]^+$ (calcd 489.2459). The ^{13}C and ^1H NMR spectroscopic features were consistent with **1** but with a difference in the oxymethine signal at δ_C 75.4 (d, C-7), which was higher than that in compound **1** (δ_C 71.1, d, C-7). Meanwhile, there are some differences between compounds **1** and **2** at C-6, C-8, and C-10 in their ^{13}C NMR spectra. Because the positions of the two isovaleryl substituents and the 3-methylcrotonyl group were the same, it can be speculated that the configuration of C-7 is α-orientation; this was proved by the correlations of H-7 with H-6β and H-6β with H-5 and H-9 in the ROESY experiments. Hence, the structure of **2** was determined as (1*S*,5*S*,7*R*,8*S*,9*S*)-1-*O*-(3-methylcrotonyl)-7-hydroxy-10,11-diisovaleroxy-5,6-dihydrovaltrate hydrin; this compound was named patriscabioin B.

Compound **3** gave a molecular formula of $C_{25}H_{40}O_8$, deduced from HRESIMS at *m*/*z* 491.2607 $[M + Na]^+$ (calcd

Table 1 ^1H NMR data of compounds **1–5** (δ in ppm, *J* in Hz) in $CDCl_3$

No.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1	5.86 (d, 5.6)	5.86 (d, 4.8)	5.84 (d, 5.5)	5.86 (d, 5.6)	5.86 (d, 5.8)
3	6.38 (s)	6.40 (s)	6.37 (s)	6.39 (s)	6.39 (s)
5	2.99 (dd, 8.2)	3.00 (dd, 7.1)	2.98 (dd, 8.0)	2.92 (dd, 7.5)	2.99 (dd, 8.0)
6a	2.13 (m)	2.14 (m)	2.15 (m)	2.02 (m)	2.13 (m)
6b	1.67 (m)	1.87 (m)	1.68 (m)	1.81 (m)	1.68 (m)
7	4.15 (t, 3.5)	5.34 (br s)	4.16 (t, 3.4)	4.45 (m)	4.19 (t, 4.3)
8	2.06 (m)	2.07 (m)	2.04 (m)	2.01 (m)	2.08 (m)
9	2.18 (m)	2.19 (m)	2.18 (m)	2.40 (m)	2.20 (m)
10a	4.10 (dd, 11.4, 4.8)	4.10 (dd, 11.4, 4.6)	4.10 (dd, 11.4, 4.6)	3.97 (dd, 11.2, 3.5)	4.13 (dd, 11.4, 4.9)
10b	4.52 (dd, 11.4, 9.9)	4.54 (d, 10.7)	4.51 (dd, 11.4, 10.0)	3.81 (dd, 11.2, 6.8)	4.49 (dd, 11.4, 9.8)
11a	4.57 (d, 12.3)	4.58 (d, 12.2)	4.57 (d, 12.3)	4.60 (d, 12.2)	4.57 (d, 12.3)
11b	4.40 (d, 12.3)	4.42 (d, 12.2)	4.41 (d, 12.3)	4.38 (d, 12.2)	4.41 (d, 12.3)
R_1-2′	5.70 (s)	5.71 (s)	2.19 (m)	5.68 (s)	5.70 (s)
3′			2.09 (m)		
4′	1.92 (s)	1.94 (s)	0.94 (d, 2.6)	1.91 (s)	1.93 (s)
5′	2.18 (s)	2.20 (s)	0.94 (d, 2.6)	2.17 (s)	2.19 (s)
R_3-2″	2.21 (d, 7.2)	2.23 (d, 7.4)	2.23 (m)		2.09 (s)
3″	2.08 (m)	2.08 (m)	2.09 (m)		
4″	0.95 (d, 5.6)	0.96 (d, 5.5)	0.96 (d, 3.1)		
5″	0.95 (d, 5.6)	0.96 (d, 5.5)	0.96 (d, 3.1)		
R_4-2‴	2.17 (d, 8.9)	2.19 (d, 9.4)	2.22 (m)	2.16 (d, 8.3)	2.18 (d 8.3)
3‴	2.08 (m)	2.08 (m)	2.09 (m)	2.07 (m)	2.10 (m)
4‴	0.94 (d, 5.6)	0.96 (d, 5.5)	0.94 (d, 2.6)	0.95 (d, 6.7)	0.95 (d, 6.6)
5‴	0.94 (d, 5.6)	0.96 (d, 5.5)	0.94 (d, 2.6)	0.95 (d, 6.7)	0.95 (d, 6.6)

[a] ^1H NMR data recorded at 400 MHz. [b] ^1H NMR data recorded at 500 MHz.

Table 2 ^{1}H NMR data of compounds **6–10** (δ in ppm, J in Hz) in $CDCl_3$

No.	**6**[c]	**7**[b]	**8**[c]	**9**[b]	**10**[a]
1	5.87 (d, 5.5)	5.87 (d, 5.5)	5.88 (d, 5.6)	4.65 (d, 6.0)	4.80 (d, 2.0)
3a	6.40 (s)	6.29 (s)	6.41 (s)	7.21 (br s)	4.09 (d, 9.2)
3b					3.92 (d, 9.2)
4					2.05 (m)
5	3.00 (q, 7.8)	2.95 (q, 7.4)	2.99 (q, 6.8)	3.16 (q, 8.2)	1.73 (br s)
6a	2.16 (m)	2.03 (m)	2.13 (m)	2.30 (m)	1.90 (m)
6b	1.68 (m)	1.54 (m)	1.73 (m)	1.65 (m)	1.86 (m)
7	4.16 (br s)	4.45 (q, 5.5)	4.24 (t, 5.0)	4.44 (m)	4.19 (t, 3.3)
8	2.07 (m)	2.02 (m)	2.12 (m)	1.97 (m)	2.67 (m)
9	2.20 (m)	2.40 (m)	2.21 (m)	2.32 (m)	2.16 (m)
10a	4.53 (d, 10.9)	3.97 (dd, 11.2, 8.3)	4.52 (dd, 11.2, 9.8)	3.96 (dd, 11.2, 4.2)	4.15 (dd, 8.3, 2.6)
10b	4.11 (dd, 11.2, 4.0)	3.82 (dd, 11.2, 3.7)	4.41 (dd, 11.2, 4.9)	3.82 (dd, 11.2, 6.5)	3.87 (dd, 8.3, 3.5)
11a	4.58 (d, 12.2)	3.95 (d, 11.8)	4.59 (d, 12.3)	9.29 (s)	1.05 (d, 6.9)
11b	4.41 (d, 12.2)	3.74 (d, 11.8)	4.41 (d, 12.3)		
R_1-2′	5.70 (s)	5.69 (s)	5.71 (s)	3.56 (s)	
4′	1.94 (s)	1.92 (s)	1.94 (s)		
5′	2.20 (s)	2.17 (s)	2.20 (s)		
R_3-2″	2.19 (d, 10.7)		2.53 (s)		
3″	2.09 (m)				
4″	0.96 (d, 6.5)		1.25 (s)		
5″	0.96 (d, 6.5)		1.31 (s)		
R_4-2‴	2.33 (m)	3.37 (m)	2.19 (d, 9.3)		
		3.30 (m)			
3‴	1.58 (m)	1.53 (m)	2.10 (m)		
4‴	1.25 (m)	1.35 (m)	0.96 (d, 6.6)		
5‴	1.25 (m)	0.91 (t, 7.4)	0.96 (d, 6.6)		

[a] ^{1}H NMR data recorded at 400 MHz. [b] ^{1}H NMR data recorded at 500 MHz. [c] ^{1}H NMR data recorded at 600 MHz.

Table 3 ^{13}C NMR data of compounds **1–10** (δ in ppm, J in Hz) in $CDCl_3$

No.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[c]	**7**[b]	**8**[c]	**9**[b]	**10**[b]
1	91.5	91.3	91.8	91.2	91.5	91.5	91.4	91.3	103.5	91.8
3	140.2	141.0	140.1	140.8	140.4	140.3	138.9	140.4	161.3	63.0
4	113.7	112.8	114.0	112.9	113.7	113.8	114.6	113.4	123.6	41.1
5	33.0	33.4	32.9	33.1	33.1	33.0	32.9	33.0	30.3	30.1
6	39.5	37.7	39.5	40.4	39.7	39.5	40.4	39.8	41.4	36.3
7	71.1	75.4	71.1	73.9	71.2	71.1	74.2	71.3	74.3	76.9
8	46.7	47.8	46.8	46.4	46.5	46.8	46.4	45.9	47.3	36.1
9	40.7	40.7	40.7	39.9	40.8	40.6	40.1	40.9	40.4	49.4
10	62.5	60.9	62.4	61.8	62.9	62.6	61.9	63.1	62.0	69.0
11	63.6	63.7	63.6	63.7	63.7	63.7	70.0	63.6	190.7	12.9
R_1-1′	164.8	164.7	171.8	165.1	164.9	164.9	165.2	164.9	57.3	
2′	115.1	115.0	43.3	115.2	115.1	115.1	115.2	115.0		
3′	160.0	160.4	25.7	160.1	160.2	160.2	159.9	160.3		
4′	27.6	27.7	22.4	27.7	27.7	27.7	27.7	27.7		
5′	20.5	20.6	22.4	20.6	20.6	20.6	20.5	20.6		
R_3-1″	174.1	173.6	174.2		172.0	173.0		173.2		
2″	43.4	43.5	43.5		21.0	43.5		46.6		
3″	25.7	25.8	25.7			25.7		69.3		
4″	22.4	22.4	22.5			22.4		29.4		
5″	22.4	22.4	22.4			22.4		29.3		
R_4-1‴	173.0	173.0	173.0	173.2	173.1	175.0	69.2	173.0		
2‴	43.3	43.5	43.4	43.5	43.5	34.3	31.8	43.5		
3‴	25.7	25.8	25.7	25.7	25.7	25.0	19.4	25.7		
4‴	22.3	22.4	22.4	22.4	22.5	29.7	14.0	22.4		
5‴	22.3	22.4	22.4	22.4	22.4	29.7		22.4		

Palmitoyl of **6:** δ_C 29.7–29.1 (t, C-6-13), 31.9 (t, C-14), 22.7 (t, C-15), 14.2 (q, C-16)

[a] ^{13}C NMR data recorded at 100 MHz. [b] ^{13}C NMR data recorded at 125 MHz. [c] ^{13}C NMR data recorded at 150 MHz.

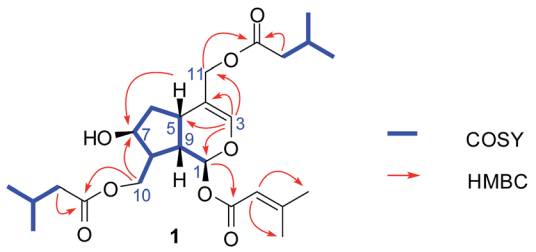

Fig. 2 Key $^{1}H-^{1}H$ COSY and HMBC correlations of compound **1**.

491.2602), which is two mass units higher than that of **1**. The NMR data were very similar to those of compound **1** except for the appearance of an isovaleryl substituent [δ_C 171.8.0 (s), δ_C 43.3 (t), δ_C 25.7 (d), δ_C 22.4 (q), δ_C 22.4 (q)] instead of the 3-methylcrotonyl group in compound **1**. Therefore, compound **3** has three isovaleryl substituents. These three isovaleryl substituents were assigned separately at C-1, C-10, and C-11 by means of HMBC spectra. Finally, the structure of **3** was characterized as (1*S*,5*S*,7*S*,8*S*,9*S*)-7-hydroxy-1,10,11-triisovaleroxy-5,6-dihydrovaltrate hydrin, named patriscabioin C.

Compound **4** had a molecular formula of $C_{20}H_{30}O_7$ as established from HRESIMS at *m*/*z* 405.1889 $[M + Na]^+$ (calcd 405.1884) and ^{13}C NMR spectroscopic data. Compared with compound **1**, compound **4** has one isovaleryl substituent and a 3-methylcrotonyl group according to careful analysis of the NMR data. The 3-methylcrotonyl group and isovaleryl group were attached at C-1 and C-11, respectively, according to correlations from H-1 to the ester carbonyl carbon at δ_C 165.1 (s) and from H-11 to the ester carbonyl carbon at δ_C 173.2 (s) in the HMBC spectra. Accordingly, the structure of compound **4** was characterized as (1*S*,5*S*,7*S*,8*S*,9*S*)-1-*O*-(3-methylcrotonyl)-7,10-dihydroxy-11-isovaleroxy-5,6-dihydrovaltrate hydrin, named patriscabioin D.

Compound **5** was analyzed to have a molecular formula of $C_{22}H_{32}O_8$ by a combination of HRESIMS at *m*/*z* 447.1983 $[M + Na]^+$ (calcd 447.1989) and ^{13}C NMR data. Compared with the ^{1}H NMR and ^{13}C NMR spectroscopic data of compound **4**, it could be determined that **5** has a similar structure to **4** except for the appearance of an acetyl group at δ_C 172.0 (s), δ_C 21.0 (q) in the ^{13}C NMR spectrum and δ_H 2.09 (s) in the ^{1}H NMR spectrum. This hinted that compound **5** is an acetyl derivative of **4** at C-10 on account of the correlation from H-10 to the acetyl carbonyl carbon at δ_C 172.0 (s) in the HMBC spectrum. Consequently, compound **5** was elucidated as (1*S*,5*S*,7*S*,8*S*,9*S*)-1-*O*-(3-methylcrotonyl)-7-dihydroxy-10-acetoxy-11-isovaleroxy-5,6-dihydrovaltrate hydrin, named patriscabioin E.

Compound **6** was formulated as $C_{36}H_{60}O_8$ from HRESIMS at *m*/*z* 659.3924 $[M + K]^+$ (calcd 659.3920). Comparison of the NMR data showed signals similar to those of **1** except for the presence of 14 methylenes and a methyl group in the high field region, which implied that a palmitoyl group replaced the original isovaleryl substituent. This finding agrees with the molecular weight exactly. Ultimately, the structure of **6**, interpreted from 2D NMR spectra, was confirmed as (1*S*,5*S*,7*S*,8*R*,9*S*)-1-*O*-(3-methylcrotonyl)-7-hydroxy-10-isovaleroxy-11-palmitoyl-oxy-5,6-dihydrovaltrate hydrin, named patriscabioin F.

The molecular formula of compound **7** was inferred to be $C_{19}H_{30}O_6$ by HRESIMS at *m*/*z* 377.1934 $[M + Na]^+$ (calcd 377.1935). The ^{1}H and ^{13}C NMR data revealed that compound **7** is an analogue of **4**; the only difference is the C-11 substituent group, which was defined as an *n*-butoxy unit by the ^{1}H and ^{13}C NMR data. By analysis of 2D NMR data, compound **7** was further identified as (1*S*,5*S*,7*S*,8*S*,9*S*)-1-*O*-(3-methylcrotonyl)-7-hydroxy-10-isovaleroxy-11-*n*-butoxy-5,6-dihydrovaltrate hydrin, named patriscabioin G.

Compound **8** possessed a molecular formula of $C_{25}H_{38}O_9$, deduced from HRESIMS at *m*/*z* 505.2407 $[M + Na]^+$ (calcd 505.2408). The ^{1}H and ^{13}C NMR spectroscopic data were almost identical to those of **1**; however, a methine (δ_C 25.7) was absent and an oxygenated tetrahedral carbon (δ_C 69.3) was present along with two methyl groups downfield at δ_C 29.3 (q), δ_H 1.31 (s); δ_C 29.4 (q), δ_H 1.25 (s). These spectroscopic differences suggested the presence of a 3-hydroxylisovaleryl group in **8** at C-10, in accordance with the HMBC correlation from H-10 to the carbonyl (δ_C 173.2) of the 3-hydroxylisovaleryl group. After assignment of the 1D and 2D NMR data of **8**, the structure was characterized as (1*S*,5*S*,7*S*,8*S*,9*S*)-1-*O*-(3-methylcrotonyl)-7-hydroxy-10-(3-hydroxylisovaleroxy)-11-isovaleroxy-5,6-dihydrovaltrate hydrin, named patriscabioin H.

Compound **9** was assigned a molecular formula of $C_{11}H_{16}O_5$ from HRESIMS at *m*/*z* 251.0885 $[M + Na]^+$ (calcd 251.0890) and ^{13}C NMR data. In addition to a methoxy group at δ_C 57.3, we observed a typical acetal group at δ_C 103.5 (d), δ_H 4.65 (d, $J = 6.0$ Hz); a conjugated aldehyde group at δ_C 190.7 (d), δ_H 9.29 (s); and an adjacent double bond at δ_C 161.3 (d), δ_C 123.6 (s). All these spectroscopic features are close to those of the known compound 1-*O*-methyl cachinol,[16] except for the absence of a methyl and the presence of an oxymethylene, suggesting that the methyl group at C-10 was oxygenated to an O-methylene group. After further confirmation of the structure by $^{1}H-^{1}H$ COSY, HSQC, HMBC, and ROESY data, it was assigned as (1*R*,5*S*,7*S*,8*S*,9*S*)-1-methoxy-7,10-dihydroxy-11-aldehyde-5,6-dihydrovaltrate hydrin, named patriscabioin I.

Compound **10** was found to have a molecular formula of $C_{10}H_{16}O_3$ by HRESIMS at *m*/*z* 207.0991 $[M + Na]^+$ (calcd 207.0992), with 3 degrees of unsaturation. The NMR spectra of **10** revealed a hemiketal methine at δ_C 91.8 (d, C-1) and δ_H 4.80 (d, $J = 2.0$ Hz); two oxygenated methylenes at δ_C 63.0 (t) and δ_C 69.0 (t); an oxymethine at δ_C 76.9 (t); and a methyl at δ_C 12.9 (q) and δ_H 1.05 (d, $J = 6.9$ Hz). These data suggested that the compound was a 7-hydroxy-3,4,5,6-tetrahydrovaltrate hydrin in which a methyl was changed to an oxymethylene. However, the cyclization of C-10 with OH-1 was confirmed by the correlations from H-1 to C-3 (δ_C 63.0, t) and C-10 (δ_C 69.0, t) in the HMBC spectrum. The β-configurations of H-1, Me-11, and H-8 and the α-configuration of H-7 were determined by the correlations of H-1/H-9, Me-11/H-1, H-8/H-9, and H-7/H-4 in the ROESY spectrum. Hence, the structure of compound **10** was established as (1*R*,4*R*,5*R*,7*S*,8*R*,9*S*)-1,10-epoxy-7-hydroxy-3,4,5,6-tetrahydrovaltrate hydrin, named patriscabioin J.

Compounds **11** and **12** possessed the same molecular formula, $C_{30}H_{40}O_9$, according to HRESIMS, with 11 degrees of unsaturation. The IR spectrum of compound **11** showed

absorption bands of hydroxy groups (3435 cm^{-1}), a conjugated group consisting of an aldehyde function (1731 cm^{-1}) and a double bond (1644 cm^{-1}), and two ethers (1126 and 1089 cm^{-1}). Furthermore, compound **12** showed similar absorptions to **11** in its IR spectrum. Careful analysis of the NMR data indicated that two compounds exhibited similar NMR spectroscopic features except for their data in the low magnetic field. Furthermore, their NMR spectroscopic data showed two distinct regions, indicating a dimer of two iridoid units. One was easily assigned to compound **4**; another was identified as 8,9-didehydro-7-hydroxydolichodial,[17] except for the absence of an aldehyde group and the presence of an acetal. This suggested that two units were linked through the aldehyde group. Also, the difference between compounds **11** and **12** was the position of the aldehyde group of 8,9-didehydro-7-hydroxydolichodial. In **11**, this group was linked at C-1′ based on the correlations of H-1′/C-10, C-7 in the HMBC spectrum, while compound **12** was placed at C-3′ by detailed analysis of the HMBC correlations from H-3′/C-10, C-7 (Fig. 5). In addition, detailed analysis of the ROESY data showed that the configurations of the two iridoid units are the same as in compound **4** and 8,9-didehydro-7-hydroxydolichodial. The α-configuration of H-1′ in **11** and the α-configuration of H-3′ in **12** were assigned by the correlations of H-1′/H-7 and H-3′/H-7 in the ROESY experiments, respectively (Fig. 6). The good agreement between the experimental and calculated ECD spectra of compounds **11** and **12** (Fig. 4) further proved the α-configurations of H-1′ of compound **11** and H-3′ of compound **12**. Hence, the structures of patriscabiobisin A (**11**) and patriscabiobisin B (**12**) were characterized as shown.

Compound **13** was isolated as a light yellow oil with a molecular formula of $C_{21}H_{28}O_8$ based on HRESIMS at *m*/*z* 447.1418 $[M + K]^+$ (calcd 447.1416). Its UV spectrum displayed absorptions of conjugated aldehyde and olefinic groups at 247 nm and 202 nm. The IR spectrum showed broad absorptions for a hydroxy group (3431 cm^{-1}), an α, β-unsaturated aldehyde group (1713, 1662 cm^{-1}), and an ether (1076 cm^{-1}). Detailed analysis of the ^1H and ^{13}C NMR spectroscopic data of **13** revealed that it exhibited two sets of C_{10}-iridoid signals, of which one unit was determined to be patriscabioin I (**9**) and the other was judged to be jatamanin D.[11] The difference was that the two sets of data shifted to a lower field [δ_C 62.0 (t) → δ_C 68.7 (t, C-10); δ_C 91.1 (d) → δ_C 97.5 (d, C-1′)]. This suggested that those two units were connected through C-10—*O*—C-1′, which was further verified by correlations from H-1′ (δ_H 5.15) to C-10

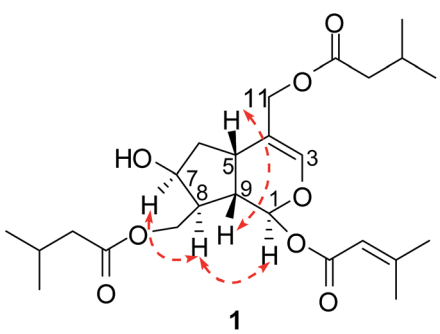

Fig. 3 Key ROESY correlations of compound **1**.

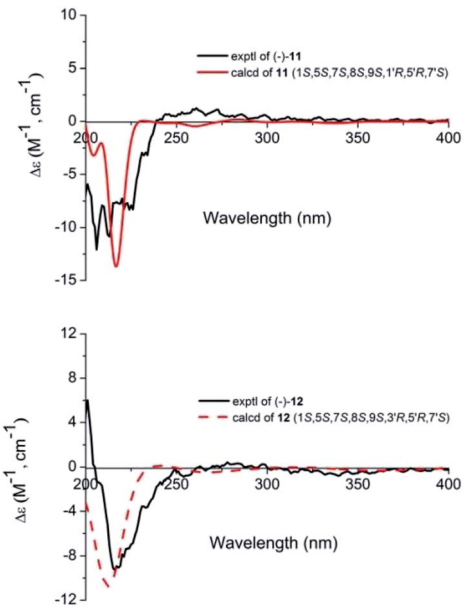

Fig. 4 Calculated and experimental ECD spectra of compounds **11** and **12** at the TDDFT/B3LYP/6-31G(d) level.

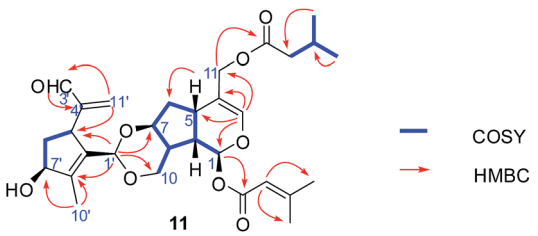

Fig. 5 Key ^1H–^1H COSY and HMBC correlations of compound **11**.

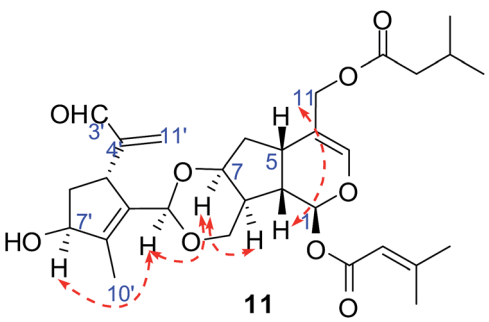

Fig. 6 Key ROESY correlations of compound **11**.

(δ_C 68.7) in the HMBC spectrum. The configurations of the two iridoid units were the same as in compound **9** and jatamanin D. Therefore, patriscabiobisin C (**13**) was characterized as shown.

Compounds **1** to **8** are a series of 5,6-dihydrovaltrate hydrins with substituent groups unique to the Valerianaceae family such as isovaleryl, 3-methylcrotonyl, 3-hydroxylisovaleryl, and palmitoyl groups. Compound **9** contains a conjugated aldehyde group with a double bond, and compound **10** contains a 6/5/5

Table 4 ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of compounds **11** and **12** ($CDCl_3$) and **13** (CD_3OD) (δ in ppm, J in Hz)

	11		**12**		**13**	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.89 (d, 5.2)	91.4	5.94 (d, 4.9)	91.1	5.06 (d, 3.8)	104.2
3	6.36 (s)	139.9	6.36 (s)	139.8	7.39 (s)	163.7
4		114.3		114.3		126.2
5	2.93 (q, 8.2)	33.2	3.04 (q, 8.0)	33.1	3.02 (q, 7.6)	30.3
6a	2.06 (m)	38.3	2.17 (m)	38.2	2.17 (m)	41.4
6b	1.58 (m)	77.8	1.68 (m)	78.0	1.74 (m)	73.5
7	4.15 (t, 3.7)		4.23 (t, 3.8)		4.21 (m)	
8	1.65 (m)	40.7	1.70 (m)	40.6	2.03 (m)	47.0
9	2.58 (m)	39.9	2.67 (m)	39.9	2.34 (m)	44.5
10a	4.07 (d, 12.1)	66.1	4.15 (d, 12.1)	66.2	3.91 (dd, 9.4, 7.6)	68.7
10b	3.98 (dd, 11.9, 2.8)		4.06 (dd, 12.1, 3.1)		3.74 (dd, 9.4, 6.3)	
11a	4.55 (d, 12.3)	63.7	4.59 (d, 12.3)	63.7	9.21 (s)	193.5
11b	4.41 (d, 12.3)		4.41 (d, 12.3)			
OMe					3.53 (s)	57.2
Cr-1		165.0		165.0		
2	5.70 (s)	115.2	5.69 (s)	115.2		
3		160.1		160.0		
4	1.94 (s)	27.7	1.94 (s)	27.7		
5	2.20 (s)	20.6	2.20 (s)	20.6		
Iv-1		173.0		173.1		
2	2.19 (d, 5.9)	43.5	2.19 (d, 4.4)	43.5		
3	2.07 (m)	25.7	2.10 (m)	25.8		
4	0.96 (d, 6.7)	22.5	0.96 (d, 6.6)	22.4		
5	0.96 (d, 6.7)	22.4	0.96 (d, 6.6)	22.4		
1′	5.11 (s)	97.2	9.99 (s)	188.6	5.15 (d, 3.0)	97.5
3′	9.52 (s)	194.0	4.95 (s)	101.6	5.04 (s)	95.0
4′		152.8		147.3		151.7
5′	3.98 (m)	41.8	3.84 (m)	42.5	3.12 (t, 5.9)	43.6
6a′	2.09 (m)	41.0	2.43 (m)	40.7	2.09 (m)	
6b′	1.96 (m)	41.0	1.90 (m)	40.7	1.87 (m)	
7′	4.74 (br s)	80.1	4.88 (br s)	80.0	3.81 (dd, 7.6, 3.1)	79.9
8′		143.2		161.5		83.6
9′		135.2		139.1	2.34 (m)	44.5
10′	1.88 (s)	11.7	2.23 (s)	11.5	1.38 (s)	19.4
11a′	6.18 (s)	133.2	5.18 (s)	112.8	4.92 (s)	107.4
11b′	5.90 (s)		4.76 (s)		4.82 (s)	

Table 5 Acetylcholinesterase (AChE) inhibitory activities of compounds **1** to **13**

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Inhibition (%)	68.53	29.73	75.23	−5.30	7.19	40.49	−42.42
SD	0.90	2.87	0.42	2.47	4.45	2.05	1.81
Inhibitiory activity	+++	++	+++	–	–	++	–
Compound	**8**	**9**	**10**	**11**	**12**	**13**	TA
Inhibition (%)	17.78	46.97	−14.54	36.03	21.91	37.87	51.01
SD	2.58	4.43	2.76	4.31	2.79	6.33	1.96
Inhibitiory activity	+	++	–	++	++	++	

ring system. Furthermore, compounds **11** to **13** are the first reported bis-iridoids; among these, compounds **11** and **12** are connected with a 1,3-dioxane group between two units, while compound **13** is linked by an ether bond.

2.2. Biological evaluation

P. scabiosaefolia has been used for sedation,[7] in which acetylcholine esterase inhibitors may be responsible for the sedation effects;[18] therefore, the inhibitory activities on AChE of all the new compounds were tested. The screening results (Table 5) showed that at concentrations of 50 μM, compounds **1** and **3** inhibited acetylcholine esterase activity over 60%, and compounds **2**, **6**, **9**, and **11** to **13** inhibited acetylcholine esterase activity from 20% to 50%; however, compounds **4**, **5**, **7**, **8**, and **10** basically had no inhibitory activities. Then, the IC_{50} values of compounds **1** and **3** were examined. Compared with the

reference compound tacrine (IC_{50} = 0.4 μM), these compounds showed moderate inhibitory activities on AChE, with IC_{50} values of 37.6 and 10.5 μM, respectively. These results suggested that the presence of 7β-OH and 10-Iv may be essential to the inhibitory activities of the compounds on acetylcholine esterase. Of course, the structure-activity relationships of the inhibitory activities of these compounds on acetylcholine esterase remain to be further explored.

The final concentration of tacrine (TA) was 0.333 μM, and the final concentrations of the compounds were 50 μM. "−": Inhibition (%) < 10%; "+": Inhibition (%) from 10% to 20%; "++": Inhibition (%) from 20% to 60%; "+++": Inhibition (%) > 60%.

Considering the cytotoxicity of iridoids,[14] all new compounds were evaluated for their cytotoxicities *in vitro* against four human cancer cell lines (HL-60, SMMC-7721, MCF-7, and SW-480) by MTT assay,[19] using cisplatin (DDP) and paclitaxel as positive controls (Table 6). As a result, compound **1** showed moderate cytotoxic activity, with IC_{50} values of 1.4, 7.2, and 7.1 μM against HL-60, SMMC-7721, and SW480, respectively, which is comparable to cisplatin (DDP). Meanwhile, compound **5** showed cytotoxicity against HL-60, with an IC_{50} value of 1.2 μM. Consequently, 7β-OH and the substituent at C-10 may be responsible for the cytotoxic activity of these iridoids. Furthermore, the Cr-group may improve the activity. Moreover, in order to know their selectivity, compounds **1–3**, **5** and **11** were tested for their cytotoxicities towards human normal epithelium cells (BEAS-2B). The results (Table 7) showed that human normal epithelium cells showed viable, even the concentration of these compounds was increased to 40 μM (Table 7).

Table 6 IC_{50} values (μM) of compounds **1** to **3**, **5** and **11** for human tumor cell lines[a]

Compound	HL-60	SMMC-7721	MCF-7	SW-480
1	1.4 ± 0.02	7.2 ± 0.29	27.6 ± 1.68	7.1 ± 0.35
2	—	—	—	24.3 ± 2.39
3	9.9 ± 1.52	13.8 ± 0.17	17.8 ± 0.54	10.0 ± 0.28
5	1.2 ± 0.05	7.1 ± 0.38	—	18.2 ± 0.19
11	17.9 ± 0.73	19.7 ± 0.62	23.9 ± 1.85	17.6 ± 0.26
Cisplatin	2.8 ± 0.12	5.9 ± 0.17	20.4 ± 1.07	7.6 ± 0.54
Paclitaxel	<0.008	<0.008	<0.008	<0.008

[a] "—": inactive for cell lines. Cisplatin and paclitaxel: positive controls.

Table 7 Cell viability of compounds **1** to **3**, **5** and **11** for human normal epithelium cells (BEAS-2B)

Concentration (μM)	Cell viability (%)						
	1	**2**	**3**	**5**	**11**	Cisplatin	Paclitaxel
0.064	95.9	96.3	97.9	97.9	97.7	93.0	93.1
0.32	98.8	98.0	99.1	98.1	97.6	94.2	93.5
1.6	98.9	98.8	97.4	97.1	94.9	92.1	89.1
8	99.1	99.5	96.9	97.6	95.8	82.9	83.3
40	97.5	97.2	92.9	94.4	86.9	61.3	53.9

3. Experimental section

3.1. General procedure

Optical rotations were obtained on a JASCO P-1020 digital polarimeter (Horiba, Tokyo, Japan). UV spectra were measured using a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Bruker Tensor 27 infrared spectrophotometer (Bruker Optics GmbH, Ettlingen, Germany) with KBr pellets. Mass spectra were performed on an API QSTAR time-of-flight spectrometer (MDS Sciqaszex, Concord, Ontario, Canada) and an LCMS-IT-TOF (Shimadzu, Kyoto, Japan) spectrometer. NMR spectra were recorded on Bruker AM-400, DRX-500 and Av III-600 instruments with TMS as the internal standard (Bruker, Bremerhaven, Germany). The chemical shifts were given in δ (ppm) with reference to the solvent signal. Column chromatography was performed on silica gel (200–300 and 300–400 mesh, Qingdao Marine Chemical Inc., Qingdao, China), Lichroprep Rp-18 gel (40 to 63 μm, Merck, Darmstadt, Germany), MCI gel CHP–20P (75 to 150 μm, Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 (20 to 150 μm, Amersham Biosciences, Uppsala, Sweden), and YMC*GEL ODS-A-HG (50 μm, YMC Co. Ltd. Japan). The fractions were monitored by TLC, and the spots were visualized by UV light and sprayed with 10% H_2SO_4 in EtOH, followed by heating.

3.2. Plant material

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

P. scabiosaefolia plants are abundant in local resources, and collection was permitted. Also, we ensured that the local population of *P. scabiosaefolia* was not destroyed by collecting specimens at different locations.

3.3. 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. Then, the residue (3 kg) was dissolved in water and partitioned successively with EtOAc to yield EtOAc extract (0.85 kg) after concentration. The EtOAc extract was subjected to silica gel column chromatography eluted with a gradient of petroleum ether-ethyl acetate (20 : 1 → 0 : 1, v/v) to obtain six fractions (1 to 6) by TLC plate analysis. Fraction 2 (64 g) was separated by 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.2-1 (5.9 g) was separated by Sephadex LH-20 column chromatography (MeOH–H_2O, 90 : 10, v/v), and Rp-18 column chromatography (MeOH–H_2O, 50 : 50 → 100 : 0, v/v) and was further purified by semi-prep. HPLC (MeOH–H_2O, 75 : 25, v/v) to afford **1** (14.1 mg, t_R = 30.9 min)

and **16** (15.0 mg, t_R = 23.2 min). Fr.2-2 (1.6 g) was chromatographed on a Sephadex LH-20 column (MeOH–H_2O, 90 : 10, v/v) and on a silica gel column with petroleum ether-ethyl acetate (10 : 1, v/v) to acquire **3** (5.7 mg). Fraction 3 (62 g) was separated by 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.3-1 (507.2 mg) was separated by Sephadex LH-20 column chromatography (MeOH–H_2O, 90 : 10, v/v) and silica gel column chromatography eluted with petroleum ether-ethyl acetate (6 : 1, v/v) and purified by semi-prep. HPLC (MeOH–H_2O, 95 : 5, v/v) to afford **6** (2.2 mg, t_R = 29.8 min). Fr.3-5 (10 g) and Fr.3-6 (5.7 g) were separated by Rp-18 column chromatography (MeOH–H_2O, 50 : 50 → 100 : 0, v/v), Sephadex LH-20 column chromatography (MeOH–H_2O, 90 : 10, v/v), and silica gel column chromatography eluted with petroleum ether-ethyl acetate (5 : 1, v/v) to obtain **2** (1.8 mg), **15** (2.0 mg), and **17** (23.0 mg). Fraction 4 (69 g) was separated by Rp-18 column chromatography (MeOH–H_2O, 50 : 50 → 100 : 0, v/v) to afford 7 subfractions (Fr.4-1 to Fr.4-6). Fr.4-1 (2.2 g) and Fr.4-2 (1.0 g) were all separated by Sephadex LH-20 column chromatography (MeOH–H_2O, 90 : 10, v/v) and silica gel column chromatography ($CHCl_3$–MeOH, 30 : 1, v/v) to obtain **10** (6.0 mg), **4** (8.0 mg), and **7** (3.5 mg). Fr.4-6 (1.7 g) was separated by successive silica gel column chromatography ($CHCl_3$–MeOH, 30 : 1, v/v and petroleum ether-ethyl acetate 1 : 1, v/v) to yield **5** (9.0 mg), **14** (20.0 mg), and **18** (5.0 mg); meanwhile, purification by semi-prep. HPLC (MeOH–H_2O, 72 : 28, v/v) afforded **8** (2.20 mg, t_R = 29.8 min). Fraction 6 (5.2 g) was isolated by the same method as Fr.4-6, and compounds **9** (3.0 mg), **19** (6.0 mg) and **20** (22.0 mg) were obtianed; in addition, compounds **11** (1.0 mg, t_R = 10.0 min) and **12** (1.0 mg, t_R = 12.5 min) were purified by semi-prep. HPLC (MeOH–H_2O, 50 : 50, v/v), and compound **13** (1.0 mg, t_R = 19.8 min) was obtained by semi-prep. HPLC (MeOH–H_2O, 35 : 65, v/v).

3.4. Spectral data of the new compounds

3.4.1 Patriscabioin A (1). Light yellow oil. $[\alpha]_D^{23}$ − 42.7 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 217 (3.94) nm; IR (KBr) ν_{max} 3432, 2960, 2930, 1731, 1645, 1126 cm^{-1}; positive ESIMS *m*/*z* 489 $[M + Na]^+$, HREIMS *m*/*z* 489.2454 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_8Na$, 489.2459); ^1H and ^{13}C NMR data, see Tables 1 and 3.

3.4.2 Patriscabioin B (2). Light yellow oil. $[\alpha]_D^{25}$ − 36.9 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 218 (3.87) nm; IR (KBr) ν_{max} 3453, 2960, 2930, 1732, 1646, 1125 cm^{-1}; positive ESIMS *m*/*z* 489 $[M + Na]^+$, HREIMS *m*/*z* 489.2462 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_8Na$, 489.2459); ^1H and ^{13}C NMR data, see Tables 1 and 3.

3.4.3 Patriscabioin C (3). Light yellow oil. $[\alpha]_D^{23}$ − 14.0 (*c* 0.39, MeOH); UV (MeOH) λ_{max} (log ε): 207 (3.41) nm; IR (KBr) ν_{max} 3428, 2961, 2931, 1734, 1665, 1191 cm^{-1}; positive ESIMS *m*/*z* 491 $[M + Na]^+$, HREIMS *m*/*z* 491.2607 $[M + Na]^+$ (calcd for $C_{25}H_{40}O_8Na$, 491.2602); ^1H and ^{13}C NMR data, see Tables 1 and 3.

3.4.4 Patriscabioin D (4). Light yellow oil. $[\alpha]_D^{24}$ − 39.3 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε): 217 (4.05) nm; IR (KBr) ν_{max} 3432, 2958, 2932, 1731, 1648, 1408, 1127 cm^{-1}; positive ESIMS *m*/*z* 405 $[M + Na]^+$, HREIMS *m*/*z* 405.1889 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_7Na$, 405.1884); ^1H and ^{13}C NMR data, see Tables 1 and 3.

3.4.5 Patriscabioin E (5). Light yellow oil. $[\alpha]_D^{24}$ − 70.4 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 218 (4.11) nm; IR (KBr) ν_{max} 3436, 2961, 2930, 1734, 1647, 1381, 1251, 1127 cm^{-1}; positive ESIMS *m*/*z* 447 $[M + Na]^+$, HREIMS *m*/*z* 447.1983 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_8Na$, 447.1989); ^1H and ^{13}C NMR data, see Tables 1 and 3.

3.4.6 Patriscabioin F (6). Light yellow oil. $[\alpha]_D^{25}$ − 6.6 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε): 223 (2.91) nm; IR (KBr) ν_{max} 3430, 2925, 2856, 1630, 1384, 1031 cm^{-1}; positive ESIMS *m*/*z* 620 $[M + K]^+$, HREIMS *m*/*z* 659.3924 $[M + K]^+$ (calcd for $C_{36}H_{60}O_8K$, 659.3920); ^1H and ^{13}C NMR data, see Tables 2 and 3.

3.4.7 Patriscabioin G (7). Light yellow oil. $[\alpha]_D^{24}$ − 20.7 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε): 220 (3.64) nm; IR (KBr) ν_{max} 3427, 2930, 1726, 1638, 1384, 1128, 1085 cm^{-1}; positive ESIMS *m*/*z* 377 $[M + Na]^+$, HREIMS *m*/*z* 377.1934 $[M + Na]^+$ (calcd for $C_{19}H_{30}O_6Na$, 377.1935); ^1H and ^{13}C NMR data, see Tables 2 and 3.

3.4.8 Patriscabioin H (8). Light yellow oil. $[\alpha]_D^{24}$ − 17.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 217 (3.41) nm; IR (KBr) ν_{max} 3431, 2963, 2929, 1729, 1633, 1382, 1125 cm^{-1}; positive ESIMS *m*/*z* 505 $[M + Na]^+$, HREIMS *m*/*z* 505.2407 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_9Na$, 505.2408); ^1H and ^{13}C NMR data, see Tables 2 and 3.

3.4.9 Patriscabioin I (9). Light yellow oil. $[\alpha]_D^{24}$ − 21.4 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 248 (3.56) nm; IR (KBr) ν_{max} 3421, 2928, 1670, 1627, 1385, 1188 cm^{-1}; positive ESIMS *m*/*z* 251 $[M + Na]^+$, HREIMS *m*/*z* 251.0885 $[M + Na]^+$ (calcd for $C_{11}H_{16}O_5Na$, 251.0890); ^1H and ^{13}C NMR data, see Tables 2 and 3.

3.4.10 Patriscabioin J (10). Light yellow oil. $[\alpha]_D^{24}$ + 11.7 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε): 216 (2.24) nm; IR (KBr) ν_{max} 3417, 2928, 1735, 1629, 1384, 1121 cm^{-1}; positive ESIMS *m*/*z* 207 $[M + Na]^+$, HREIMS *m*/*z* 207.0991 $[M + Na]^+$ (calcd for $C_{10}H_{16}O_3Na$, 207.0992); ^1H and ^{13}C NMR data, see Tables 2 and 3.

3.4.11 Patriscabiobisin A (11). Light yellow oil. $[\alpha]_D^{24}$ − 93.3 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε): 217 (4.24) nm; IR (KBr) ν_{max} 3435, 2960, 2928, 1731, 1644, 1127, 1090 cm^{-1}; positive ESIMS *m*/*z* 567 $[M + Na]^+$, HREIMS *m*/*z* 567.2567 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_9Na$, 567.2565); ^1H and ^{13}C NMR data, see Table 4.

3.4.12 Patriscabiobisin B (12). Light yellow oil. $[\alpha]_D^{24}$ − 45.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε): 219 (3.69) nm; IR (KBr) ν_{max} 3422, 2928, 1730, 1643, 1383, 1125, 1093 cm^{-1}; positive ESIMS *m*/*z* 567 $[M + Na]^+$, HREIMS *m*/*z* 567.2562 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_9Na$, 567.2565); ^1H and ^{13}C NMR data, see Table 4.

3.4.13 Patriscabiobisin C (13). Light yellow oil. $[\alpha]_D^{24}$ − 31.9 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε): 247 (3.82), 202 (3.66) nm; IR (KBr) ν_{max} 3431, 2929, 1625, 1384, 1121, 1077 cm^{-1}; positive ESIMS *m*/*z* 447 $[M + K]^+$, HREIMS *m*/*z* 447.1418 $[M + K]^+$ (calcd for $C_{21}H_{28}O_8K$, 447.1416); ^1H and ^{13}C NMR data, see Table 4.

3.5. Computational studies

CHARMM force field and DFT/TDDFT calculations were performed with Discovery Studio 4.0 and the Gaussian 09 program

package, respectively.[20] A conflex conformational search generated low-energy conformers within a 20 kcal mol^{-1} energy window that were subjected to geometry optimization using DFT without imposing any symmetry constraints at the B3LYP/6-31G(d) level. Frequency calculations were carried out at the same level to verify that the molecular structures were true minima. The calculated ECD spectra were generated by the program SpecDis2 using a Gaussian band shape with an exponential half-width of 0.3 eV from the dipole-length dipolar and rotational strengths.[21]

3.6. Acetylcholinesterase inhibitory activity

The acetylcholinesterase (AChE) inhibitory activities of the isolated compounds were assayed by the spectrophotometric method developed by Ellman *et al.*[22] with slight modifications. *S*-Acetylthiocholine iodide, *S*-butyrylthiocholine iodide, 5,5′-dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent), and acetylcholinesterase derived from human erythrocytes were purchased from Sigma Chemical. The 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^{-1}), 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 the AChE inhibitory activity assay, respectively. The hydrolysis of acetylthiocholine was monitored at 405 nm every 30 seconds for one hour. Tacrine was used as a positive control with a final concentration of 0.333 μM. All these actions were performed in triplicate. The percentage inhibition was calculated as follows: % inhibition $= (E - S)/E \times 100$ (E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound).

3.7. Cytotoxicity assays

The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7, and SW-480. The cells were obtained from ATCC (Manassas, VA, USA). All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-5 (3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Sigma, St. Louis, MO, USA).[23] Briefly, 100 μL of adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells per mL in 100 μL medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After the incubation, MTS (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS-50% DMF after removal of 100 μL medium. The optical density of the lysate was measured at 490 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.[24]

4. Conclusions

In conclusion, we have isolated and characterized 13 new and 7 known iridoids from the EtOAc extract of *P. scabiosaefolia*. Among these, compounds **11** to **13** are the first reported bis-iridoids. Several of the new compounds exhibited moderate inhibitory activities on AChE and moderate cytotoxic activities. This contribution enriches our knowledge of iridoids and their bioactivities.

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