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Petasins from the Rhizomes of *Ligularia fischeri* and Its Derivatives

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Abstract: Five eremophilane sesquiterpenoids were isolated from the rhizomes of *Ligularia fischeri* grown in Henan province of China and identified as 3*α*-Tigloyloxyeremophila-9,11-dien-8-one (**3**), Isopetasan (**4**), Neopetasan (**5**), Isopetasol (**6)** and Petasol (**7**), by spectroscopic methods. Petasin (**1**) previously isolated was analyzed to determine its absolute configuration by X-ray single crystal diffraction. Five petasin derivatives including three new compounds (**2a, 6a** and **6b**) were synthesized. 12 eremophilane sesquiterpenoids including the derivatives except for **4** (minor amount) were assessed against human neuroblastoma (SKN-SH) by the SRB method. **1** was also tested on inhibiting prostate carcinoma (Bc3) cell lines. The results showed **1** has the strong inhibitory activity against SKN-SH and Bc3 cell lines and other compounds with *α*-isopropylidene group also have the strong activity against the human neuroblastoma (SKN-SH). The above results indicated the *α*-isopropylidene group might be the active center against pulmonary carcinoma (SKN-SH) in the same eremophilane-type skeleton. Petasins may be interesting for their good bioactivities.

Keywords: *Ligularia fischeri*; sesquiterpenoids; isolation and structural modification; single crystal X-ray diffraction.

1. Introduction

The herbal plant *Ligularia fischeri*, a species of *Ligularia* genus (Compositae), distributed in China, was known as kidney, horseshoe leaves, gourd seven, etc. It is one of the most popular species used as a chinese medicinal herb in the folk. Its roots and rhizomes, named shanziwan, can moisturize the lung and has antitussive and expectorant effects. Its leaves was edible as a potherb in northeast China. Previous chemical studies on *L. fischeri* reported on eremophilane-type sesquiterpenoids and some other compounds [1-18]. Focusing on potentially active compounds, we also studied the roots of *Ligularia fischeri* distributed in Taihang mountains, Henan province, and isolated 11 compounds from the roots of *L. fischeri* including petasin and isopetasin[19]. Petasin showed inhibitory effects on human glioma cell U251, human hepatoma cell SMMC7721, human gastric carcinoma SGC7901,

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human neuroblastoma SK-N-BE (2), especially potent on human neuroblastoma SK-N-SH cell line[20]. Petasin was one of the most important sesquiterpene ester with the highest spasmolytic activity [21]. It also exhibited antileukotriene and antihistamine activity [22]. Thomet O. A. R and his coworkers reported that petasin might inhibit inflammatory effector functions in human eosinophils by disrupting signalling events at the level of proximal to phospholipase C (PLC), besides its potential inhibitory activity within mitogen-activated protein kinase (MAPK) and LT pathways [23]. Petasin also appeared to be one major active compound of *Petasites hybridus* extract, since it demonstrated the same inhibitory activities on calcium fluxes and subsequent LT generation in both eosinophils and neutrophils as the plant extract of *Petasites hybridus* does [24].

This paper further reports the isolation of other five petasin derivatives from *L. fischeri*. and their identification by NMR spectral method. The X-ray single diffraction experiment was carried out for further determination of petasin's absolute configuaration. Petasin as the main sesquiterpene in this plant was also quantitative by HPLC method. Five petasin derivatives were synthesized including three new compounds (**2a**, **6a**, **6b**). The above 12 eremophilane-type sesquiterpenoids except for compound **4** (for a small amount) were tested on cytotoxic activity against the human neuroblastoma (SKN-SH) to study their structure–activity relationships. **1** was also tested on inhibiting prostate carcinoma Bc3 cell lines. For comparing difference of NMR data with each other among the isolated sesquiterpenoids, The NMR data of petasin and isopetasin were also shown in Table 1.

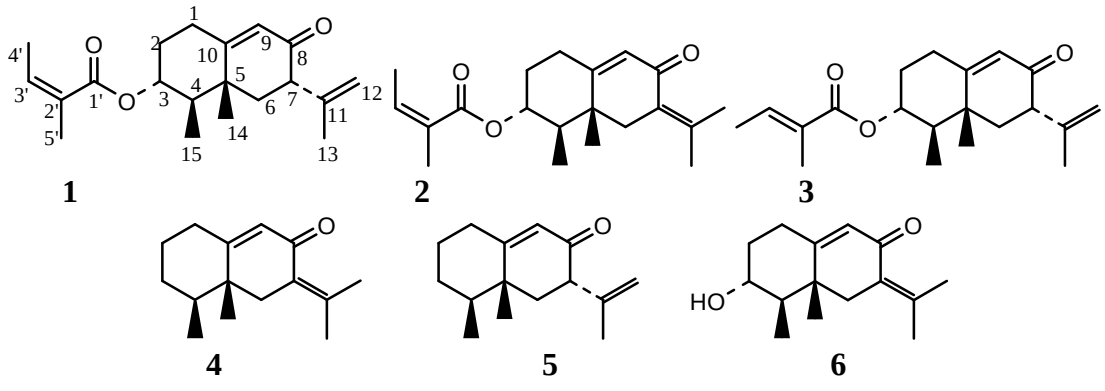
2. Materials and Methods

2.1. Plants Material

The roots of *Ligularia fischeri* (10 kg) were collected at Taihang mountains of Henan province, China, in October 2008 and identified by Prof. Ruoyong Liu of Zhengzhou University. The voucher specimen (No.200810) has been deposited at the Department of Traditional Chinese Medicine, School of Pharmaceutical Sciences, Zhengzhou University. Other reagent solutions were analytical grade (Tianjin Siyou Co.Ltd, China).

2.2 Extraction and isolation.

The air-dried materials were smashed and extracted by 95% (v/v) EtOH with flash extractor in two times. The extract (720 g) was obtained after the removal of EtOH under reduced pressure by rotatory evaporation. The above residue of *L. vellerea* was suspended in hot H_2O and then extracted with EtOAc. The EtOAc portion afforded, on concentration, a residue (320 g). The residue was subjected to silica gel (200-300 mesh) column chromatography with petroleum ether/EtOAc (10:1-1:1) to give nine crude fractions (Fr.P1-9). Fr.P2 afforded **4** (4 mg) and **5** (13 mg) after silica gel column chromatography repeatedly. Fr.P3 was subjected to silica gel column chromatography and recrystallization and afforded **1** (28 g), **2** (200 mg) and **3** (300 mg). Fr.P6 was dealt with MCI gel CHP-40P, Sephadex LH-20 and Silica gel column chromatography to afford **6** (300 mg) and **7** (20 mg)



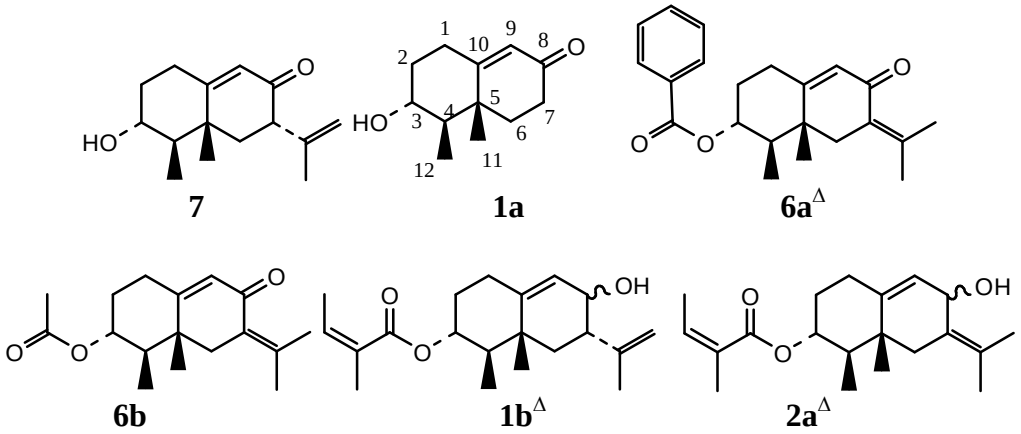


Figure 1. Chemical structures of compounds **1**-**7**, **1a**, **1b**, **2a**, **6a** and **6b**; $^{\Delta}$means new compound

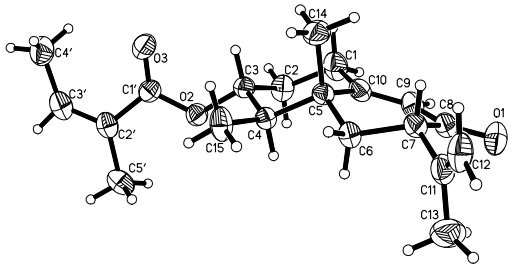


Figure 2. ORTEP drawing of compound **1**

2.3 Preparation of petasin derivatives.

*Preparation of **1a***. Petasin (0.105 g) was put into round bottom flask (50 mL), which was resolved with methanol (5 mL), and then NaOH solution(1 mol/L, 2 mL) was dropped into the container with stirring. The reaction was in progress in temperature 75℃. After 24 hours, the above solution was added with the deluted HCl solution(2 mol/L) and neutralized, and then the methanol removed. The remnant was extracted with EtOAc and the was obtained, The EtOAc extract was concentrated and purified by silica gel column chromatography, isopetasol **(6)** and **1a** was obtained with the outcome 85% and 15%, respectively.

*Preparation of **6a***. Isopetasol (0.152 g) was put into round bottom flask (50 mL), which was resolved with CH_2Cl_2 (2.5 mL), and then pyridine 10 d ("d" means "drop". After this, "d" with the meaning), benzoyl chloride 18 d, 4 grain DMAP was dropped into the container with stirring. The reaction was in progress in room temperature. After one hour, the above solution was added with 15 ml CH_2Cl_2, and then pyridine washed by saturated Na_2CO_3 solution and removed. The remnant was purified by silica gel column chromatography (PE: EA, 5:1, v/v), **6a** was obtained with the outcome 90%. This is a new compound.

*Preparation of **6b***. Isopetasol (0.050 g) was put into round bottom flask (50 mL), which was resolved with CH_2Cl_2 (2.5mL), and then pyridine 8 drops, Ac_2O 6d, 4 grain DMAP was dropped into the container with stirring. The reaction was in progress in room temperature. After 30 minutes, pyridine in the above solution was washed by saturated Na_2CO_3 solution and removed, then the remnant was extracted with CH_2Cl_2 and purified by silica gel column chromatography (PE: EA, 5:1, v/v), **6b** was obtained with the outcome 95%.

*Preparation of **1b***. Petasin (0.302 g) was put into round bottom flask (50 mL), which was resolved with methanol (10 mL), and then $NaBH_4$ (0.201 g) was put into the container with ice bath. The reaction was in progress in room temperature for 30 minutes, then the methanol was removed.

The remnant was purified by silica gel column chromatography (PE: EA, 5:1, v/v), **1b** was obtained with the outcome 95%.

*Preparation of **2a***. Isopetasin (0.301 g) was put into round bottom flask (50 mL), which was resolved with THF (5 mL), and then $LiAlH_4$ (0.101 g) was put into the container with ice bath. The reaction was in progress in room temperature for 60 minutes, then the NaOH solution was added for ending the reaction. The reaction system was extracted by H_2O/EA (1:3, v/v). EA fraction was dried by waterless Na_2SO_4, then EA removed.The remnant was purified by silica gel column chromatography (PE: EA, 3:1, v/v), **2a** was obtained with the outcome 40%.

2.4 Biological activity studies.

The cytotoxicities of compounds **1**-**3**, **5**-**7**, **1a**, **1b**, **2a**, **6a** and **6b** were investigated by means of the SRB (sulfonyl rhodamine B staining method) assay with human neuroblastoma(SK-N-SH) cell line, **1** also with Bc3 cell line. Cancer cell lines was obtained from the Shanghai Institute of Life Science of the Chinese Academy of Science and cultured according to the supplier's instruction. The cells were seeded in 96-well plates, incubated with 10% bovine serum at 37ºC and with 5% CO_2 for 24 h, and treated with the compounds at different concentrations for 72 h. Cisplatin was used as a positive control. The absorbance of the extracted SRB was measured at 510 nm. The experiments were carried out in triplicate. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%.

2.5 Quantitative analysis of petasin in L. fischeri by RP-HPLC.

The analysis was performed on Agilent 1200 series HPLC with VWD detector and Kromasil C_{18} (250 mm×4.6 mm, 5 μm) column, under the eluting conditions: the moble phase: methanol-acetonitrile-water (31: 38: 31, v/v/v); the detected wavelength: 240 nm; the flow rate: 1.2 mL/min; column temperature: 25 ℃. As the main active compound, petasin was analyzed quantitatively.

3. Results and Discussion

3.1. Structure elucidation

Petasin (= *[1R-[1α, 2α(Z), 7β, 8aα]-1, 2, 3, 4, 6, 7, 8, 8a-octahydro-1, 8a-dimethyl-7- (1-methylethenyl)-6-oxo-2-naphthalenyl -2-methyl-2-butenoic acid ester; 3α-hydroxy-eremophila-9, 11-dien-8-one- (Z)-2-methylcrotonate*; **1**). The NMR data seen in Table 1 in accordance with petasin previously isolated from Petasites hybridus [25]. The absolute configuaration was determined by X-ray single crystal diffraction (Table 2-4 and Figure 2). HSQC and HMBC experiments permitted the assignment of the remaining H- and C-atoms and the detection of ^{1}H, ^{13}C long-range coupling, respectively.

Isopetasin (= *(2Z)- (1R, 2R, 8aR)-1, 2, 3, 4, 6, 7, 8, 8a-octahydro-1, 8a-dimethyl-7-(1-methylethylidene)-6-oxo-2-naphthalenyl-2-methyl-2-butenoic acid ester*; *3α-hydroxy-eremophila-7(11), 9-dien-8-one-(Z)-2-methylcrotonate*; **2**). The NMR data seen in Table 1 in accordance with Isopetasin previously reported from *Ligularia sagitta* [26].

3α-Tigloyloxyeremophil- 9, 11-dien-8- one (= *(1R, 2R, 7R, 8aR)-1, 2, 3, 4, 6, 7, 8, 8a-octahydro-1, 8a-dimethyl-7-(1-methylethenyl)-6-oxo-2-naphthalenyl-(E)-2-methyl-2-butenoic acid ester*; **3**). White crystal (Petroleum ether-EtOAc), mp: 93-95ºC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.95 (1H, td, J = 10.8, 4.4 Hz, H-3), 5.80 (1H, d, J =1.7 Hz, H-9), 4.83, 5.00 (1H each, s, H_2-12), 1.75(3H, s, Me-13), 1.25 (3H, s, H-14) , 0.96 (3H, d, J = 6.9 Hz, H-15), 6.88 (1H, m, H-3'), 1.80 (3H, d, J = 6.4 Hz, H-4'), 1.90 (3H, s, H-5'). The NMR data seen in Table 1 in accordance with *3α-Tigloyloxyeremophil- 9, 11-dien-8- one* previously reported from *Ligularia kanaitzensis* [27].

Isopetasan (= *(4aR-cis)-3, 4, 4a, 5, 6, 7-hexahydro-4a, 5-dimethyl-3- (1-methylethylidene)-2(1H)-Naphthalenone; Eremophila-7(11), 9-dien-8-one; Fukinone*; **4**). Colorless oil; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.74 (1H, d, J =1.2 Hz, H-9), 2.09 (3H, d, J = 1.6 Hz, H-12), 1.75 (3H, s, H-13), 0.97 (3H,

s, H-14), 0.95 (3H, d, *J* = 6.4 Hz, H-15). The ^{1}H-NMR data of **4** revealed that the H-atom to OH group was missing to compare with that of **6** and that was identified with isopetasan (dehydrofukinone) previously reported [28].

Neopetasan (= *(3S, 4aR, 5S)-4, 4a, 5, 6, 7, 8-hexahydro-4a, 5-dimethyl-3-(1-methylethenyl)-2(3H)-Naphthalenone*; *Eremophila-9, 11-dien-8-one; Alloeremophilone;* **5).** Colorless oil; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.75 (1H, d, *J* =1.6 Hz, H-9), 4.82, 4.98 (1H each, s, H_2-12), 1.75 (3H, s, H -13), 1.17 (3H, s, H -14), 0.93 (3H, d, *J* =6.4 Hz, H -15). The ^{1}H-NMR data of **5** revealed that the H-atom to OH group was disapeared to compare with that of **7** and that was neopetasan (fukinone) previously isolated from *Petasites japonicus* [28].

Isopetasol (*= (4aR, 5R, 6R)-, 4, 4a, 5, 6, 7, 8-hexahydro-6-hydroxy-4a, 5-dimethyl-3-(1-methylethylidene)- 2(3H)-Naphthalenone; 3α-hydroxy-eremophila-7(11), 9-dien-8-one*; **6**). white crystal (EtOAc), mp: 120-122 ºC; $[\alpha]_D^{17}$ +73 (c=0.5, MeOH); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.58 (1H, td, *J* = 11.2, 4.1 Hz, H-3), 5.77(1H, d, *J* =1.6 Hz, H-9), 2.09(3H, d, *J* =1.6 Hz, H-12), 1.86(3H, s, H-13), 0.98 (3H, s, H-14), 1.11 (3H, d, *J* =6.4 Hz, H-15). The NMR data of **6** revealed that the angeloxy group was missing to compare with that of **2** and that was in accord with isopetasol previously isolated from *Petasites Fragrans* [29].

Petasol (*= (3S, 4aR, 5R, 6R)- 4, 4a, 5, 6, 7, 8-hexahydro-6-hydroxy-4a, 5-dimethyl-3-(1-methylethenyl)-, 2(3H)-Naphthalenone; Sencathenone*; **7**). Colorless oil; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.60 (1H, dt, *J* = 10.8, 4.4 Hz, H-3), 5.75 (1H, s, H-9), 4.80, 4.96 (1H each, s, H_2-12), 1.72 (3H, s, H-13), 1.16 (3H, s, H-14), 1.06 (3H, d, *J* =6.4 Hz, H-15). The NMR data of **7** revealed that the angeloxy group was disapeared to compare with that of **1** and that was in accord with petasol previously isolated from *Petasites Fragrans* [29].

Deisopropylideneisopetasol (*= [4aR-(4a**α**, 5**α**, 6β)]- 4, 4a, 5, 6, 7, 8-hexahydro-6-hydroxy-4a, 5-dimethyl-2(3H)-Naphthalenone,* ***1a***), ^{1}H-NMR (400 MHz,$CDCl_3$) δ: 5.77 (1H, s, H-9), 3.63 (1H, td, *J* =4.4, 10.8 Hz, H-3), 1.13 (3H, s, H-11) and 1.08 (3H, d, *J* = 6.4 Hz, H-12). ^{13}C-NMR data shown in Table 1. To compare with **1**, The NMR data of **1a** showed the isopropylidene group (δ (H) 4.80 (1H, s), 4.96 (1H, s), 1.72 (3H, s) and δ (C) 143.3 (s), 114.5 (t), 20.0 (q)) and the angeloxy group (δ (H) 6.08 (1H, m), 1.99 (3H, d, *J* = 6.4Hz), 1.90 (3H, s) and δ (C) 167.6, 127.9, 138.2, 15.8, 20.6) disappeared and confirmed the structure of **1a** as deisopropylideneisopetasol. ^{1}H- and ^{13}C-NMR data were assigned by HSQC and HMBC

3-O-Benzoyl-eremophila-7, 9-diene-8-one (*=Isopetasol benzoate,* ***6a*****)**. White crystal. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.07 (2H, t, *J* = 7.2, 8.4 Hz, phenyl H), 7.58 (1H, m, phenyl H), 7.45 (2H, t, *J* =7.6 Hz, phenyl H), 5.80 (1H, s, H-9), 5.10 (1H, td, *J* =11.2, 4.0 Hz, H-3), 2.96 (1H, d, *J* = 14.0 Hz, H-6), 2.11 (3H, s, H-12), 1.87 (3H, s, H-13), 1.07 (3H, s, H-14), 1.05 (3H, d, *J* = 6.8 Hz, H-15). To compare with **6**, ^{1}H NMR data of **6a** showed the existence of the benzoyl group (δ (H) 8.07 (2H), 7.58 (1H), 7.45 (2H) at C (3) and the chemical shift at H-3 (5.10 (1H, td, *J* =11.2, 4.0 Hz) to low field confirmed the structure as 3α-benzoyleremophila-9, 11-dien-8-one (new compound).

3-O-Acetyl-eremophila-7, 9-diene-8-one ([4aR-(4aα, 5α, 6β)]-6-(acetyloxy)-4, 4a, 5, 6, 7, 8-hexahydro-4a, 5-dimethyl-3-(1-methylethylidene)- 2(3H)-Naphthalenone, ***6b***). White crystal. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 5.78 (1H, s, H-9), 4.83 (1H, td, *J* = 4.4, 11.6 Hz, H-3), 2.92 (1H, d, *J* =14.0 Hz, H-6), 2.18 (3H, s, OAc), 2.10 (3H, s, H-12), 1.86 (3H, s, H-13), 1.03 (3H, s, H-14), 0.98 (3H, d, *J* = 6.8 Hz, H-15). To compare with **6**, ^{1}H NMR data of **6b** showed the existence of the acetyl group (δ (H) 2.18 (3H, s) at C (3) and the chemical shift at H-3 (δ (H) 4.83, 1H, td) to low field confirmed the structure as 3α-acetyleremophila-9, 11-dien-8-one.

3-O-Ang-eremophila-9, 11-diene-8-ol (***1b***). ^{1}H-NMR (400MHz, $CDCl_3$) δ: 6.03 (1H, m, H-3'), 5.40(1H, s, H-9), 4.89 (2H, s, H-12), 4.88 (1H, m, H-3), 4.08 (1H, d, *J* = 9.6 Hz, H-8), 1.98, 1.96 (3H in all, s, H-4'), 1.87 (3H, s, H-5'), 1.72 (3H, s, H-13), 1.09 (3H, s, H-14), 0.89, 0.88 (3H in all, overlapped, H-15). To compare with **1**, ^{1}H NMR data of **1b** showed one germinal H-atom to a OH group (δ (H) 4.08(1H, d, *J* = 9.6 Hz, H-8), which confirmed the structure as 3-O-ang-eremophi-9, 11-diene-8-ol (new compound).

3-O-Ang-eremophila-7, 9-diene-8-ol (***2a***). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.05 (1H, m, H-3'), 5.81 (1H, m, H-9), 5.60 (1H, m, H-3), 2.35 (1H, m, H-6), 2.06 (3H, d, *J* = 1.6 Hz, H-4'), 2.00 (3H, s, H-5'), 1.36 (3H, s, H-13), 1.34 (3H, s, H-12), 0.96 (3H, d, *J* = 6.8 Hz, H-15), 0.87 (3H, s, H-14). To compare with **2**, ^{1}H NMR data of **2a** showed one germinal H-atom to a OH group (δ (H) 5.60 (1H, d, H-8), which confirmed the structure as 3-O-ang-eremophi-7, 9-diene-8-ol (new compound).

Table 1. ^{13}C NMR data of compounds **1-7, 1a** (at 100 MHz in $CDCl_3$, δ in ppm).

Position (C)	1	2	3	4	5	6	7	1a
1	30.6	31.7	30.6	30.5	30.4	30.5	40.0	31.3
2	31.6	30.1	31.5	26.5	26.4	35.3	35.1	35.3
3	73.0	73.3	73.2	32.6	33.0	71.3	70.9	71.1
4	47.3	46.2	47.4	42.5	43.7	49.1	50.2	49.8
5	40.0	42.2	40.0	41.9	39.5	42.0	39.8	39.2
6	41.6	41.2	41.7	41.0	41.6	41.2	41.7	35.7
7	50.3	128.0	50.3	128.2	51.0	127.3	50.3	33.4
8	198.5	191.6	198.5	192.4	199.0	191.9	198.9	199.5
9	124.6	126.7	124.6	126.1	124.1	126.4	124.3	124.3
10	166.8	165.1	166.9	168.9	170.2	166.4	168.2	169.2
11	143.3	143.4	143.3	142.3	143.8	143.3	143.4	17.2
12	114.5	22.1	114.4	22.0	114.1	22.1	114.4	10.5
13	20.0	22.6	20.0	22.6	20.0	22.6	20.0	
14	17.2	17.2	17.2	16.0	16.0	17.3	17.2	
15	10.5	10.8	10.5	15.5	15.1	10.8	10.4	
1’	167.6	167.7	167.6					
2’	127.9	127.2	128.7					
3’	138.2	137.9	137.3					
4’	15.8	15.7	14.4					
5’	20.6	20.6	12.1					

Table 2. Crystal data of compound **1** (Petasin)

Crystal data	**1**
Empirical formula	$C_{20}H_{28}O_3$
Formula weight	316.42
Temperature / K	291.15
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a / Å, b / Å, c / Å	7.7281(6), 9.9204(8), 24.568(2)
α/°, β/°, γ/°	90.00, 90.00, 90.00
Volume / $Å^3$	1883.5(3)
Z	4
ρ_{calc} / mg mm^{-3}	1.116
μ / mm^{-1}	0.579
F(000)	688
Crystal size / mm^3	0.35 × 0.30 × 0.28
2θ range for data collection	7.2 to 134.1°
Index ranges	$-9 \le h \le 6$, $-11 \le k \le 11$, $-29 \le l \le 29$
Reflections collected	7323
Independence reflections	3330[R(int) = 0.0187]
Data/restraints/parameters	3330/1/222
Goodness-of-fit on F^2	1.049
Final R indexes [I>2σ (I)]	R_1 = 0.0425, wR_2 = 0.1195
Final R indexes [all data]	R_1 = 0.0495, wR_2 = 0.1275
Largest diff. peak/hole / e $Å^{-3}$	0.114/-0.098

Table 3. Selected Bond Lengths for compound **1** (Petasin)

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O1	C8	1.213 (3)	C4	C5	1.559 (3)
O2	C1’	1.330 (2)	C4	C15	1.524 (3)
O2	C3	1.462 (2)	C5	C6	1.527 (3)
O3	C1’	1.191 (3)	C5	C10	1.510 (3)
C1	C2	1.516 (4)	C5	C14	1.543 (3)
C1	C10	1.500 (3)	C6	C7	1.528 (3)
C1’	C2’	1.496 (3)	C7	C8	1.516 (4)
C2	C3	1.508 (3)	C7	C11	1.507 (3)
C2’	C3’	1.318 (3)	C8	C9	1.454 (4)
C2’	C5’	1.525 (4)	C9	C10	1.331 (3)
C3	C4	1.514 (3)	C11	C12	1.319 (5)
C3’	C4’	1.480 (4)	C11	C13	1.498 (4)

Table 4. Selected Bond Angels for compound **1** (Petasin)

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
C1’	O2	C3	119.04 (16)	C10	C5	C4	108.99 (16)
C10	C1	C2	113.6 (2)	C10	C5	C6	109.62 (16)
O2	C1’	C2’	111.86 (19)	C10	C5	C14	107.8 (2)
O3	C1’	O2	122.1 (2)	C14	C5	C4	111.60 (16)
O3	C1’	C2	126.1 (2)	C5	C6	C7	115.00 (18)
C3	C2	C1	110.6 (2)	C8	C7	C6	110.27 (18)
C1’	C2’	C5’	117.1 (2)	C11	C7	C6	112.02 (19)
C3’	C2’	C1’	120.7 (2)	C11	C7	C8	113.56 (19)
C3’	C2’	C5’	122.2 (2)	O1	C8	C7	122.2 (2)
O2	C3	C2	106.22 (18)	O1	C8	C9	121.8 (2)
O2	C3	C4	108.25 (16)	C9	C8	C7	115.97 (18)
C2	C3	C4	111.84 (16)	C10	C9	C8	125.3 (2)
C2’	C3’	C4’	128.9 (3)	C1	C10	C5	116.52 (18)
C3	C4	C5	111.93 (18)	C9	C10	C1	121.2 (2)
C3	C4	C15	111.63 (18)	C9	C10	C5	122.3 (2)
C15	C4	C5	112.95 (19)	C12	C11	C7	120.7 (3)
C6	C5	C4	108.07 (16)	C12	C11	C13	121.8 (3)
C6	C5	C14	110.8 (2)	C13	C11	C7	117.5 (3)

3.2 Inhibitory effects on tumor cells.

The inhibitory effects of natural compounds **1**-**3** and **5**-**7** and preparative petasin derivatives **1a**, **1b**, **2a**, **6a** and **6b** were investigated by means of the SRB (sulfonyl rhodamine B staining method) assay with human neuroblastoma(SK-N-SH) cell line, **1** also with Bc3 cell line. The results showed compounds **1**, **3**, **5** and **7** with potent inhibitory effects on human neuroblastoma(SK-N-SH) cell line with IC_{50}<1 μM. Compound **1b** showed moderate inhibitory effect with 1 μM <IC_{50} <10 μM. Compound **2**, **6**, **1a**, **2a**, **6a** and **6b** showed no inhibitory effect with IC_{50} >10 μM. Compound **1** also have potent activity against Bc3 cell with IC_{50} 4.83 μM. The IC_{50} values of Cisplatin (positive control) was 2.30 and 1.90 μM on SK-N-SH and Bc3 cell lines, respectively.

Compounds **1**, **3**, **5**, **7** and **1b** have the same structural characteristic with 7β-H, 9 (10), 11 (12)-dien and 8-oxo groups except compound **1b** which has no 8-oxo group, whereas **1b** has weaker activity than that has 8-oxo group. So we could make a supposal that 7β-H, 9 (10), 11 (12)-dien group, especially α- isopropylidene (7β-H, 11 (12)-en) group would be the active center in the eremophilane skeleton.

3.3 HPLC analysis

The isolated process of chemical constituent of *L. fischeri* showed petasin was abundant, which was used as muscle relaxant clinically, therefore petasin was quantified in the root part of this plant and isolated petasin was used as standard material.

The equation of standard curve was Y= 69.223X - 95.153 (r=0.9998) (Y as peak's areas and X as concentrations of standard solution) and the linearity was good in the range of 10-100μg/ml. The precision of the established method was determined by preparing the *Ligularia fischeri* sample of the same batch in six replicated determinations and the RSD value was 1.40% (n=6). The same sample solution was analyzed successively for a period of 24 hours and the retention time and peak area of petasin were almost unchanged with RSD 0.92% (n=6). The result showed the solution was stable during the test. Recovery test was made by adding petasin with three different mass to the roots of *Ligularia fisheri* and then extracting. The extracts with different concentrations were analyzed. The average recovery was 93.1% and the RSD was 1.19% (n=3). The limit of detection (LOD) and the limit of quantification (LOQ) were 0.052μg/ml and 0.17μg/ml, respectively. The mean content of petasin with the retention time t_R 16.49 min was up to 1.52% in the roots of *L. fischeri* under the previously described conditions. The high amount of petasin with important activity such as antitumor effect indicated the plant *L. fischeri* could be evaluated as a natural resource of petasin.

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