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Two triterpenoids and other constituents from *Petasites tricholobus*

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Abstract

Two migrated ursane skeleton triterpenoids, one is D:B-friedoursane-3α,16α-dihydroxy-7α,8α-epoxy-5(10)-ene, named petatrichol A (**1**) and the other one represents a novel triterpenoid carbon framework, named petatrichol B (**2**), along with 10 known compounds were isolated from the rhizome of *Petasites tricholobus*. Their structures were elucidated on the basis of spectroscopic methods (IR, EIMS, HRMS, 1D and 2D NMR). The triterpenoids were assayed against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. Compounds **1** and **2** exhibited significant antibacterial activity against *B. subtilis*.

Keywords: *Petasites tricholobus*; Compositae; Triterpenoid; Antibacterial activity

1. Introduction

Petasites species (Compositae) are mainly distributed in the northern parts of the Eurasian and North American continents. The characteristic components of it are sesquiterpenoids, especially eremophilane (Yaoita and Kikuchi, 1995), bakkenane (Wu et al., 1999) and oplopane (Hayashi, 1989). The flower buds of *Petasites tricholobus* were usually used as that of *Tussilago farfara* in northwest China to treat many diseases, including coughs, bronchitis and asthmatic disorders. However, no report has been found about the constituents of this plant to date. In order to find the biological active compounds, we studied the chemical constituents of *P. tricholobus*, and isolated two new migrated ursane-type triterpenoids with $\Delta^{5,10}$ double bond and epoxy group in the molecules (**1** and **2**) and 10 known compounds (**3**–**12**) from the rhizome of this plant. The antibacterial activity of triterpenoids is also described. It was found that compounds **1** and **2** possess strong antibacterial activity against *Bacillus subtilis*.

2. Results and discussion

Compound **1** was isolated as white powder. Its EIMS spectrum showed the molecular ion peak at *m/z* 456. The molecular formula $C_{30}H_{48}O_3$ was determined by HRESIMS (*m/z* 479.3511 $[M + Na]^+$, calc. 479.3501). The ^1H and ^{13}C NMR (DEPT) spectrum of **1** displayed the signals of six tertiary methyls, two secondary methyls, eight methylenes, six methines (three of them were connected with oxygen) and eight quarternary carbons, which indicated that compound **1** was a pentacyclic triterpenoid possessing a tetrasubstituted double bond (δ_C 126.4, 134.9, C), two methines connected with hydroxy (δ_H 3.50, 3.75 and δ_C 75.2, 77.2) and an epoxide group (δ_H 3.16 and δ_C 67.1 C, 51.3 CH) (Table 1). The IR spectrum also revealed the presence of hydroxy (3380 cm^{-1}) and double bond (1665 cm^{-1}).

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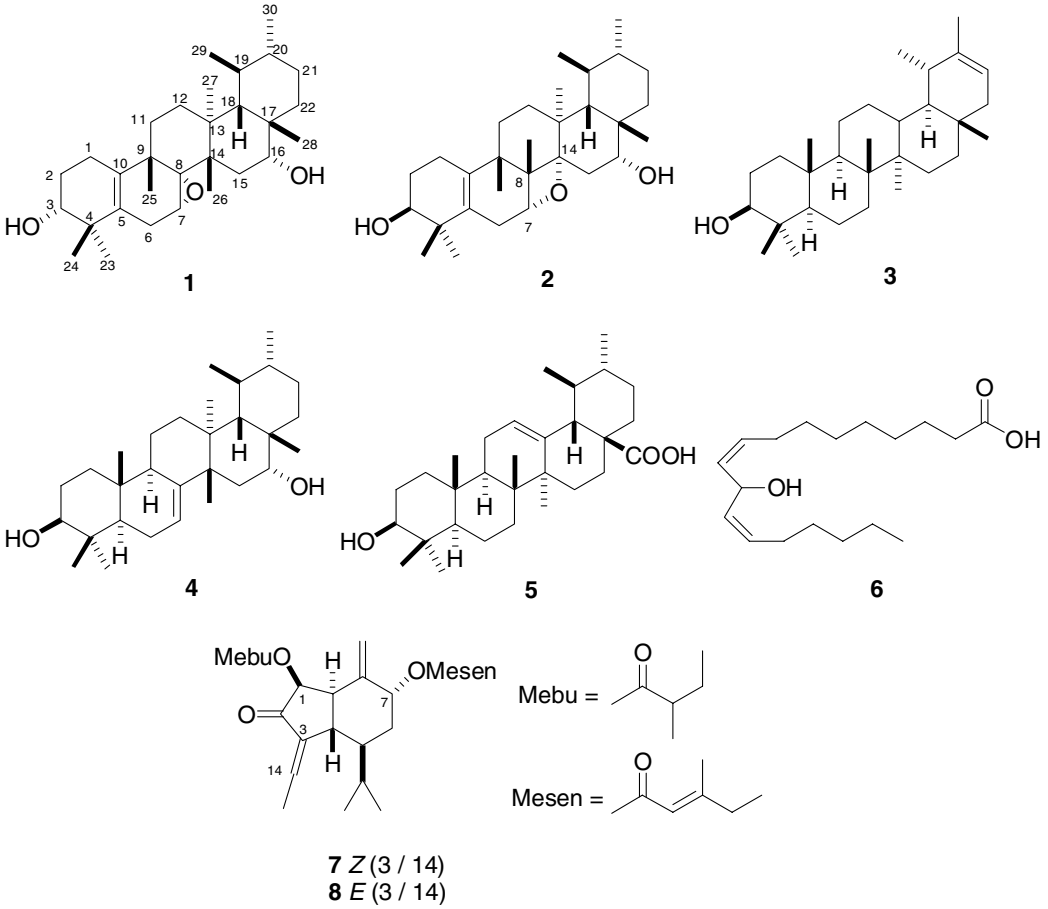


The partial structure (shown by heavy lines in Fig. 1) of **1** was solved by the HMBC correlations of the eight methyl groups. Detailed analysis of other correlations in HMBC spectrum (Table 1) could confirm the locations of substituents. The correlations of H-1 (δ_H 2.02, 1.96) with C-10 (δ_C 134.9), H-2 (δ_H 1.64, 1.60) with C-10 (δ_C 134.9), C-1 (δ_C 20.9), C-3 (δ_C 75.2), C-4 (δ_C 39.0), CH_3-23 (δ_H 1.05) with C-3 (δ_C 75.2), CH_3-24 (δ_H 0.94) with C-3 (δ_C 75.2) and H-6 (δ_H 2.62, 2.24) with C-5 (δ_C 126.4) indicated that a double bond was between C-5 and C-10 and a hydroxyl was located at C-3. The correlations of H-7 (δ_H 3.16) with C-8 (δ_C 67.1), and H-6 (δ_H 2.62, 2.24) with C-7 (δ_C 51.3), C-8 (δ_C 67.1) confirmed that the epoxide group was between C-7 and C-8, and the correlations of CH_3-28 (δ_H 1.23) with C-16 (δ_C 77.2), H-15 (δ_H 1.13, 1.59) with C-16 (δ_C 77.2) indicated that a hydroxyl was at C-16. The coupling constants of H-3 (*dd*, $J = 6.4$, 4.4 Hz), H-16 (*dd*, $J = 12.8$, 5.2 Hz) and H-7 (*brd*, $J = 3.2$ Hz) showed the presence of α-hydroxy at C-3, C-16 and 7α,8α-epoxy. The relative configurations of **1** were further established by the NOESY spectrum (Fig. 1). The NOE interactions between H_3-24 (δ_H 0.94) – H-6β (δ_H 2.24) – H_3-25 (δ_H 1.20) – H_3-26 (δ_H 1.24) – H-16 (δ_H 3.75) – H_3-28 (δ_H 1.23) – H_3-29 (δ_H 1.02) – H-18 (δ_H 1.25), suggested the 25, 26, 28, 29 methyls and H-18 were on β orientation. The correlations between H-19 and 27, 30 methyls, at the same time, not observed correlation between 26 and 27 methyls indicated that 27 and 30 methyls were on α orientation. The cross peaks of H-7 (δ_H 3.16) with H_3-26 (δ_H 1.24) and H-15β (δ_H 1.13) in NOESY spectrum also supported the α-configuration of epoxy. The skeleton of **1** and relative configurations of eight methyls were same as that of rhoiptelenol (Kitajima et al., 1994; Jiang et al., 1995). From above evidence, compound **1** was established as D:B-friedoursane-3α,16α-dihydroxy-7α,8α-epoxy-5(10)-ene, and named as petatrichol A.

The EIMS of compound **2** revealed a molecular ion peak at *m/z* 456. The ^{13}C NMR (DEPT) spectra (Table 2) exhibited 30 carbon signals ($8 \times CH_3$, $8 \times CH_2$, $6 \times CH$, $8 \times C$), and the molecular composition was deduced to be $C_{30}H_{48}O_3$, which was confirmed by positive HRSIMS (*m/z* 439.3576 $[M - H_2O + 1]^+$, calc. 439.3576). Its IR spectrum showed the absorption for hydroxy (3306 cm^{-1}) and double bond (1654 cm^{-1}). Comparison of the ^1H and ^{13}C NMR (DEPT) data of **2** (Table 2) with those of **1** (Table 1) revealed that **2** also has a migrated ursane skeleton similar to that of **1**, but without three-membered epoxy group. The framework of **2** was also constructed by the correlations in HMBC spectrum (Table 2 and Fig. 2). The correlations of CH_3-26 (δ_H 1.12) with C-9 (δ_C 39.7), C-7 (δ_C 78.7), C-14 (δ_C

Table 1
^1H, ^{13}C NMR (DEPT) spectral data and HMBC correlations of **1** ($CDCl_3$, δ ppm)[a,b]

Position	δ_H (*J* in Hz)	δ_C (DEPT)	HMBC
1α	1.96 *m*		
1β	2.02 *m*	20.9 *t*	C-10
2α	1.64 *m*		
2β	1.60 *m*	26.7 *t*	C-1, C-3, C-4, C-10
3	3.50 *dd* (6.4, 4.4)	75.2 *d*	
4	–	39.0 *s*	
5	–	126.4 *s*	
6α	2.62 *brd* (17.4)		
6β	2.24 *brd* (17.4)	26.1 *t*	C-5, C-7, C-8
7	3.16 *brd* (3.2)	51.3 *d*	C-5, C-8
8	–	67.1 *s*	
9	–	40.1 *s*	
10	–	134.9 *s*	
11α	1.39 *m*		
11β	1.35 *m*	28.7 *t*	
12α	1.43 *m*		
12β	1.49 *m*	28.5 *t*	
13	–	40.4 *s*	
14	–	42.3 *s*	
15α	1.59 *m*	32.8 *t*	
15β	1.13 *m*		C-14, C-16, C-26
16	3.75 *dd* (12.8, 5.2)	77.2 *d*	
17	–	37.9 *s*	
18	1.25 *m*	53.5 *d*	C-29
19	1.10 *m*	35.7 *d*	C-27, C-30
20	1.61 *m*	31.9 *d*	
21α	1.16 *m*		
21β	1.58 *m*	28.9 *t*	
22α	1.64 *m*		
22β	1.86 *m*	26.7 *t*	
23	1.05 *s*	26.0 *q*	C-3, C-4, C-5, C-24
24	0.94 *s*	22.2 *q*	C-3, C-4, C-5, C-23
25	1.20 *s*	26.0 *q*	C-8, C-9, C-10, C-11
26	1.24 *s*	20.9 *q*	C-8, C-13, C-14, C-15
27	1.01 *s*	16.9 *q*	C-12, C-13, C-14, C-18
28	1.23 *s*	35.2 *q*	C-16. C-17, C-18, C-22
29	1.02 *d* (6.0)	25.1 *q*	C-18, C-19, C-20
30	0.93 *d* (6.0)	22.6 *q*	C-19, C-20, C-21

[a] Assigned by HSQC and HMBC spectrum.
[b] Some ^1H peaks overlapped in the range of 1.1–2.2 ppm, hence the couple constants could not be measured.

Table 2
^1H, ^{13}C NMR (DEPT) spectral data and HMBC correlations of **2** ($CDCl_3$, δ ppm)[a,b]

Position	δ_H (*J* in Hz)	δ_C (DEPT)	HMBC
1α	1.86 *m*		
1β	2.05 *m*	23.3 *t*	
2α	1.65 *m*		
2β	1.72 *m*	27.4 *t*	
3	3.52 *dd* (9.2, 2.8)	75.7 *d*	
4	–	39.3 *s*	
5	–	130.7 *s*	
6α	1.74 *m*		
6β	2.18 *m*	27.2 *t*	C-4, C-5
7	4.51 *dd* (4.4, 4.0)	78.7	C-5, C-9
8	–	47.6 *s*	
9	–	39.7 *s*	
10	–	134.0 *s*	
11α	1.26 *m*		
11β	1.43 *m*	28.8 *t*	
12α	1.46 *m*		
12β	1.58 *m*	31.7 *t*	
13	–	40.4 *s*	
14	–	89.8 *s*	
15α	1.90 *m*		
15β	2.54 *dd* (13.8, 3.6)	35.7 *t*	C-8, C-13, C-14, C-16
16	3.35 *dd* (13.2, 3.6)	75.5 *d*	
17	–	37.5 *s*	
18	1.26 *m*	52.0 *d*	
19	1.12 *m*	36.0 *d*	
20	1.83 *m*	32.0 *d*	
21α	1.26 *m*		
21β	1.48 *m*	27.8 *t*	
22α	1.58 *m*		
22β	1.88 *m*	23.5 *t*	
23	1.05 *s*	25.7 *q*	C-3, C-4, C-5, C-24
24	0.94 *s*	20.8 *q*	C-3, C-4, C-5, C-23
25	0.98 *s*	24.8 *q*	C-8, C-9, C-10, C-11
26	1.12 *s*	17.7 *q*	C-7, C-8, C-9, C-14
27	0.83 *s*	20.1 *q*	C-12, C-13, C-14, C-18
28	1.21 *s*	32.7 *q*	C-16. C-17, C-18, C-22
29	1.06 *d* (6.0)	23.7 *q*	C-18, C-19, C-20
30	0.93 *d* (6.0)	22.3 *q*	C-19, C-20, C-21

[a] Assigned by ^1H–^1H COSY and HMBC spectrum.
[b] Some ^1H peaks overlapped in the range of 1.1–2.2 ppm, hence the couple constants could not be measured.

89.8), CH_3-27 (δ_H 0.83) with C-14 (δ_C 89.8) and CH_3-25 (δ_H 0.98) with C-8 (δ_C 47.6) indicated that CH_3-26 located at C-8 and the epoxy group at C-7, C-8 changed to the epoxy group at C-7, C-14 in comparison with **1**, in accordance with its unsaturation degree (seven). The correlations of CH_3-23 (δ_H 1.05) with C-3 (δ_C 75.7), C-5 (δ_C 130.7), CH_3-24 (δ_H 0.94) with C-3 (δ_C 75.7), C-5 (δ_C 130.7), CH_3-25 (δ_H 0.98) with C-10 (δ_C 134.0), H-6 (δ_H 2.18, 1.74) with C-5 (δ_C 130.7) suggested the presence of a hydroxy at C-3 and a double bond between C-5 and C-10. The correlations of H-15 (δ_H 2.54, 1.90) with C-16 (δ_C 75.5) and CH_3-28 (δ_H 1.21) with C-16 (δ_C 75.5) indicated a hydroxy was at C-16. The locations of the substituents were further supported by the mass spectral fragments observed in the EIMS of **2** (Scheme 1). The relative configurations of 3β-OH and 16α-OH were confirmed by the coupling constants of H-3 (*dd*, $J = 9.2$, 2.8 Hz) and H-16 (*dd*, $J = 13.2$, 3.6 Hz).

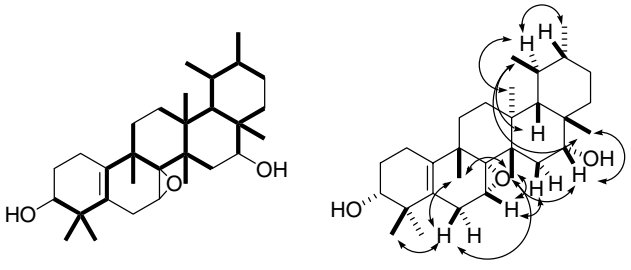


Fig. 1. The partial structure solved by the HMBC correlations of methyls and the key correlations in the NOESY spectra in **1**.

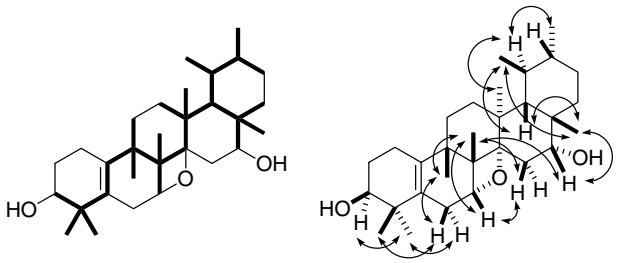


Fig. 2. The partial structure solved by the HMBC correlations of methyls and key correlations in the NOESY spectra in **2**.

In the NOESY spectrum, the correlations of CH_3-26 with CH_3-25, H-7, H-16 and CH_3-28 with H-16, H-18, CH_3-29 suggested that 25, 26, 28, 29 methyls and H-18 situated on β orientation. The correlations between H-19 and CH_3-27, CH_3-30 indicated α orientation of 27 and 30 methyls. The coupling constant of H-7 (*dd*, $J = 4.4$, 4.0 Hz) and the correlation of H-7 (δ_H 4.51) with CH_3-26 (δ_H 1.12) showed that the epoxy group at C-7 and C-14 was α orientation. On the above conclusions, compound **2** was finally established as 26(14 → 8)abeo–D:B-friedoursane-3β, 16α-dihydroxy-7α, 14α-epoxy-5(10)-ene, and named as petatrichol B. This kind of triterpenoid carbon framework has not been found to be reported before.

By comparing the spectral data with those reported in the literatures, compound **3**–**11** were identified, respectively, as pseudotaraxasterol (Reynolds et al., 1986), Bauer-7-ene-3β,16α-diol (Yaoita and Kikuchi, 1998), ursolic acid (Seo et al., 1975), 11-hydroxyoctadeca-9, 12-dienoic acid (Kadyrov et al., 1976), 7α-(4-methylsenecioyloxy)-1β-(2- methybutyrylxy)oplopa-3(14)*Z*, 8(10)-dien-2-one (Bohlmann et al., 1981; Kikuchi and Suzuki, 1992), 7α-(4-methylsenecioyloxy)-1β-(2-methybutyrylxy)oplopa-3(14)*E*, 8(10)-dien-2-one (Bohlmann et al., 1981), 3,4-dihydroxycinnamaldehyde (Woo and Kang, 1979), 4-hydroxy-3-methoxycinnamaldehyde (Liptai et al., 1980), and glycerol 1-octadecanoate (Bus et al., 1976). Compound **12** were identified as β-sitosterol on the basis of mp and TLC comparison with an authentic sample.

Table 3
Antibacterial activity of compound **1**–**5**[a]

Compound	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*
1	++	+	+++
2	++	+	+++
3	++	–	+
4	++	–	+
5	++	+	++
Chloramphenicol	+++	+++	+++
DMSO (4%)	–	–	–

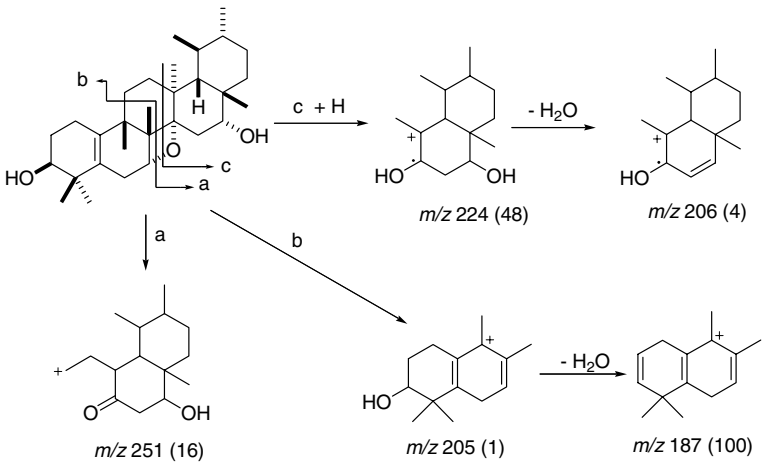
[a] Zone diameter of growth inhibition: <10 mm (–), 10–12 mm (+), 13–15 mm (++) and 16–20 mm (+++).

The antibacterial activities of **1**–**5** were determined against *Escherichia coli*, *Staphylococcus aureus* and *B. subtilis*, and compared with chloramphenicol (Table 3). The results indicate that **1**–**5** possess medium antibacterial activity against *E. coli*, **1** and **2** exhibit strong antibacterial activity against *B. subtilis*.

3. Experimental

3.1. General

Melting points were determined with a Kofler melting point apparatus and are uncorrected. IR spectra were recorded with a Nicolet NEXUS 670 FT-IR spectrometer. Optical rotations were measured using a Perkin–Elmer 341 polarimeter. ^1H, ^{13}C NMR (DEPT) and 2D NMR were recorded on Varian Mercury plus-400 spectrometer using TMS as internal standard. HRSIMS spectra were acquired on Bruker APEX II spectrometer. HRESIMS spectra were acquired on Oster TOF API



Scheme 1. Mass fragmentation pattern of compound **2**.

Pular I spectrometer. EIMS on HP-5988A GC/MS instrument. Silica gel (200–300 and 300–400 mesh) used for CC and silica GF_{254} for TLC were supplied by the Qingdao Marine Chemical Factory in China. Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.2. Plant material

The rhizome of *P. tricholobus* Franch was collected in Zhang county, Gansu province of China in August 2002. It was identified by Prof. Guo-Liang Zhang, Department of Biology, Lanzhou University. A voucher specimen (No. 020816) was deposited in the Institute of Organic Chemistry, Lanzhou University.

3.3. Extraction and isolation

The air-dried rhizome of *P. tricholobus* (2.4 kg) were pulverized and extracted with methanol three times (7 days each time) at room temperature. The solvent was evaporated under reduced pressure to give an extracts (206 g), and then suspended in hot water (60 °C, 900 ml). This suspension was extracted successively with $CHCl_3$ and *n*-BuOH. The $CHCl_3$ soluble fraction was concentrated under reduced pressure to afford a residue (24 g). This residue was subjected to a silica gel column chromatography (200–300 mesh, 240 g) with a gradient of petroleum ether–acetone (40:1, 20:1, 10:1, 5:1, 2:1) as eluent. Five fractions were collected according to TLC analysis. The fraction of petroleum ether–acetone (40:1, 6.8 g) was separated on a silica gel column chromatography (200–300 mesh, 80 g) with petroleum ether–$CHCl_3$ (3:1, 1:1), a mixture was obtained from the part of petroleum ether–$CHCl_3$ (1:1), the mixture (90 mg) was separated by preparative TLC with petroleum ether–acetone (20:1) to afford **7** (35 mg, $Rf = 0.30$) and **8** (6 mg, $Rf = 0.21$). From the fraction of petroleum ether–acetone (20 : 1), crude crystals of **3** and **12** were obtained successively, then recrystallized from $CHCl_3$ to give **3** (126 mg) and **12** (264 mg). The fraction of petroleum ether–acetone (10:1, 1.8 g) was subjected to a silica gel column chromatography (300–400 mesh, 20 g) and eluted with petroleum ether–acetone (10:1, 5:1) to give **4** (168 mg) and the mixture of **1** and **2**, the mixture (15 mg) was separated by a silica gel column chromatography (300–400 mesh, 1 g) with CH_2Cl_2:CH_3OH (150:1), obtained **1** (4 mg) and **2** (1 mg). The fraction of petroleum ether–acetone (5:1, 1.6 g) was subjected to a silica gel column chromatography (300–400 mesh, 32 g) and eluted with petroleum ether–acetone (10:1, 5:1, 3:1) to give three fractions (A, B, C). Fraction A (10:1, 182 mg) was separated on a silica gel column chromatography (300–400 mesh, 200 mg) eluting with benzene–AcOEt (3:1) and purified by preparative TLC with petroleum ether–AcOEt (3:1) to afford **10** (1 mg, $Rf = 0.28$) and **6** (12 mg, $Rf = 0.18$). Fraction B (5:1, 78 mg) was recrystallized from $CHCl_3$ to give **11** (6 mg). Fraction C (3:1, 60 mg) was purified by preparative TLC with petroleum ether–acetone (5:1) to give **9** (2 mg, $Rf = 0.24$). The fraction of petroleum ether–acetone (2:1, 860 mg) was separated by a silica gel column chromatography (300–400 mesh, 9 g) and eluted with $CHCl_3CH_3OH$ (20:1) to afford **5** (25 mg).

3.4. Petatrichol A (*1*)

White powder, mp 186–187 °C; $[\alpha]_D^{17} + 43$ ($CHCl_3$, *c* 0.24,); IR υ_{max}^{KBr} cm^{-1}: 3380 (OH), 1665 (C = C); HRESIMS: *m/z* = 479.3511 $[M + Na]^+$ ($C_{30}H_{48}O_3Na$, calc. 479.3501); EIMS 70eV, *m/z* (rel. int.): 456 $[M]^+$ (5), 438 $[M - H_2O]^+$ (4), 423 $[M - H_2O–CH_3]^+$ (4), 236 (8), 203 (11), 187 (11), 177 (14), 161 (16), 135 (25), 109 (37), 95 (43), 43 (100); For ^1H (400 MHz) and ^{13}C NMR (DEPT) (100 MHz) data see Table 1; For 2D NMR (HMBC and NOESY) spectral correlations see Table 1 and Fig. 1.

3.5. Petatrichol B (*2*)

White powder, mp 191–192 °C; $[\alpha]_D^{22} - 20$ ($CHCl_3$, *c* 0.12); IR υ_{max}^{KBr} cm^{-1}: 3306 (OH), 1654 (C=C); HRSIMS: *m/z* = 439.3571 $[M - H_2O + 1]^+$ ($C_{30}H_{47}O_2$, calc. 439.3576); EIMS 70 eV, *m/z* (rel. int.): 456 $[M]^+$ (1), 438 $[M - H_2O]^+$ (1), 423 $[M - H_2O - CH_3]^+$ (1), 271 (1), 251 (16), 224 (48), 206 (4), 205 (1), 187 (100), 119 (46); For ^1H (100 MHz) and ^{13}C NMR (DEPT) (100 MHz) data see Table 2; For 2D NMR (HMBC and NOESY) spectral correlations see Table 2 and Fig. 2.

3.6. Antibacterial assay

The antibacterial assay was carried employing the cup–plate method. Chloramphenicol was used as a positive control. Three strains bacteria *E. coli*, *S. aureus* and *B. subtilis* were cultured in beef broth and incubated at 37 °C for 24 h. After dilution of beef broth, the three bacteria were cultured in agar medium dishes, respectively, six cups (8 × 10 mm) were put onto the dishes, and each tested compound (0.2 ml of 100 μg/ml) was, respectively, added in to the cups under aseptic conditions. The dishes were cultured at 37 °C for 24 h. The zone of inhibition of the growth of bacteria, produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity.

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