FULL PAPER

New *ent*-Pimarane Diterpenes from the Roots of *Aralia dumetorum*

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Four new *ent*-pimarane diterpenes were isolated from the EtOH extract of *Aralia dumetorum*, together with three known compounds involving *ent*-pimar-8(14),15-dien-19-oic acid (**5**), *ent*-pimar-8(14),15-dien-19-ol (**6**), and *ent*-kaur-16-en-19-oic acid (**7**). By detailed analyses of the MS, IR, and NMR data, the structures of four new diterpenes were characterized as (5*β*,9*β*,10α,13α)-pimara-6,8(14),15-trien-18-oic acid (**1**), (5*β*,7*β*,9*β*,10α,13α)-7-methoxypimara-8(14),15-dien-18-oic acid (**2**), (5*β*,9*β*,10α,13α,14*β*)-14-methoxypimara-7,15-dien-18-oic acid (**3**), and (5*β*,10α,13α,14α)-14-hydroxypimara-7,9(11),15-trien-18-oic acid (**4**). The cytotoxic activities of compounds **1** – **7** were assayed *in vitro* through MTT method.

Keywords: *Aralia dumetorum*, *ent*-Pimarane diterpenes, Cytotoxic activity.

Introduction

Aralia dumetorum Hand.-Mazz., a perennial herb belonged to the Araliaceae family, was mainly distributed in Yunnan and Guizhou provinces in China. Its roots had been used for the treatment of tinnitus and dizziness. Previous investigation [1 – 12] suggested the *ent*-pimarane, *ent*-kaurane diterpenes and triterpenes were the main constituents in the *Aralia* genus and possessed antitumor [13][14], anti-inflammatory [15], and antidiabetic [16] activities. Hitherto, no investigation on *A. dumetorum* was reported. During our search for antitumor active constituents from natural source, the EtOH extract of *A. dumetorum* was found to exhibit significant cytotoxicity in four tested cell lines. Subsequent phytochemical investigation on the folk medicine resulted in the isolation of four new *ent*-pimarane diterpenes, namely (5*β*,9*β*,10α,13α)-pimara-6,8(14),15-trien-18-oic acid (**1**), (5*β*,7*β*,9*β*,10α,13α)-7-methoxypimara-8(14),15-dien-18-oic acid (**2**), (5*β*,9*β*,10α,13α,14*β*)-14-methoxypimara-7,15-dien-18-oic acid (**3**), and (5*β*,10α,13α,14α)-14-hydroxypimara-7,9(11),15-trien-18-oic acid (**4**), as well as three known compounds involving *ent*-pimar-8(14),15-dien-19-oic acid (continentalic acid, **5**) [3], *ent*-pimar-8(14),15-dien-19-ol (**6**) [17], *ent*-kaur-16-en-19-oic acid (kaurenoic acid, **7**) [3] (*Fig. 1*). By *in vitro* MTT methods, the cytotoxicities of compounds **1** – **7** were assayed. This paper presented the isolation and structural determination of compounds **1** – **4**, as well as the cytotoxicities of all isolates.

Results and Discussion

Compound **1** was isolated as a white amorphous powder. The molecular formula was deduced as $C_{20}H_{28}O_2$ based on the HR-ESI-MS at *m/z* 299.2020 $[M - H]^-$ (calc. for $C_{20}H_{27}O_2^-$ 299.2011). The IR spectrum showed absorptions at 3245 – 2500, 1708, 1636, 910, 863 cm^{-1}, suggesting the presence of COOH and olefinic functions. In the ^{1}H-NMR spectrum (*Table 1*), a CH_2=CH group at *δ*(H) 5.71 (1 H, *dd*, *J* = 17.6, 10.4 Hz), 4.96 (1 H, *dd*, *J* = 10.4, 2.0 Hz), 4.86 (1 H, *dd*, *J* = 17.6, 2.0 Hz); a trisubstituted double bond at *δ*(H) 5.23 (1 H, *s*); and a *cis*-disubstituted double bond at *δ*(H) 6.07 (1 H, *dd*, *J* = 11.2, 2.4 Hz), 6.03 (1 H, br. *d*, *J* = 11.2 Hz) were observed, together with three tertiary Me signals at *δ* (H) 1.32, 1.07, and 0.61 (each 3 H, *s*). The ^{13}C-NMR spectrum (*Table 1*) displayed 20 C-signals, of which five quaternary C-atoms (involving one COOH and one olefinic ones), six CH (involving four olefinic ones), six CH_2 (involving one olefinic one), and three Me groups were exhibited. The above NMR data suggested compound **1** possessed a pimarane diterpene skeleton. Analyses of the 1D- and 2D-NMR spectra suggested compound **1** possessed a similar structure to that of *ent*-pimar-8(14),15-dien-19-oic acid [17]. However, compound **1** contained one more double bond than *ent*-pimar-8(14),15-dien-19-oic acid [17], which was verified by the HMBCs (*Fig. 2*) from H–C(6) (*δ*(H) 6.07) to C(5) (*δ*(C) 55.5), and from H–C(7) (*δ*(H) 6.03) to C(8) (*δ*(C) 135.9). To characterize the relative configuration of compound **1**, an

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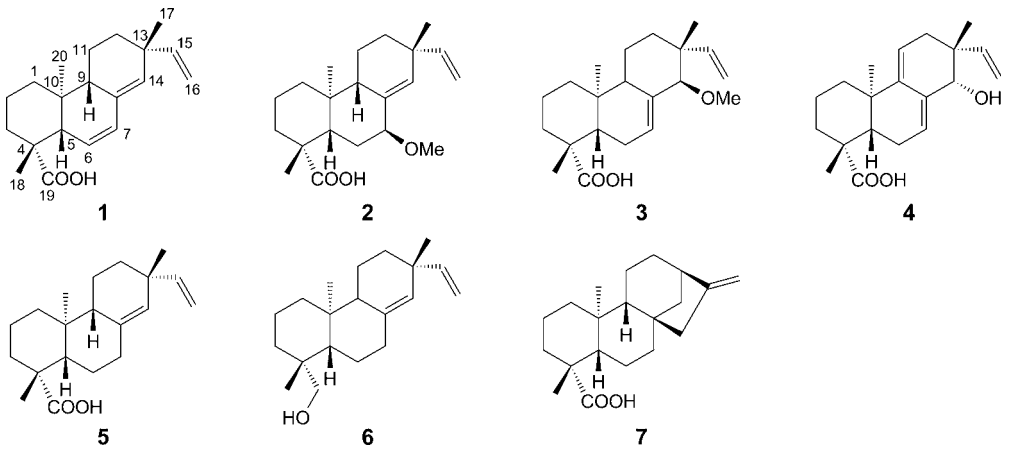

Fig. 1. The structures of compounds **1** – **7**.

ROESY experiment was performed. The ROESY correlations between H–C(5)/H–C(9), H–C(9)/Me(17), H–C(5)/Me(18), in combination with the none correlations between Me(20)/Me(18), H–C(9)/Me(20) suggested H–C(5), H–C(9), Me(17), and Me(18) were spatially non-antarafacial. Biogenetically, the Me(17) and Me(18) in *ent*-pimarane diterpenes [1][2] isolated from the *Aralia* genus were generally in β-configuration. This allowed a biogenetic assumption that the H–C(5), H–C(9), Me(17), and Me(18) were β-oriented. Finally, the structure of compound **1** was deduced as (5β,9β,10α,13α)-pimara-6,8(14),15-trien-18-oic acid (**1**) (*Fig. 1*).

Compound **2** was isolated as a white amorphous powder and assigned the molecular formula $C_{21}H_{32}O_3$ by the HR-ESI-MS at *m/z* 331.2286 $[M - H]^-$ (calc. for $C_{21}H_{31}O_3$ 331.2273). The IR spectrum showed similar absorptions to compound **1**. The ^{1}H-NMR spectrum (*Table 1*) revealed the presence of a CH_2=CH at δ(H) 5.72 (1 H, *dd*, *J* = 17.2, 10.4 Hz), 4.97 (1 H, *dd*, *J* = 10.4, 2.0 Hz), 4.87 (1 H, *dd*, *J* = 17.6, 1.6 Hz); a trisubstituted double bond at δ(H) 5.44 (1 H, *s*), three tertiary Me signals at δ(H) 1.19, 1.08, and 0.66 (each 3 H, *s*), besides a methoxyl at δ(H) 3.16 (3 H, *s*). The ^{13}C-NMR data (*Table 1*) were also essentially identical with compound **1**, implying that the compound **2** was structurally similar to **1**. The main difference between compounds **2** and **1** was that there was no double bond at C(6) in compound **2**. In addition, compound **2** possessed a MeO group at C (7) deduced by the long-range correlation between δ(H) 3.16 (3 H, *s*, MeO) and C(7) (δ(C) 83.3) in the HMBC spectrum (*Fig. 2*). The MeO at C(7) was determined as β-configuration by the ROESY correlations (*Fig. 3*) between δ(H) 3.16 (MeO) and H–C(5), H–C(9), and Me (17). The other HMBC, ^{1}H,^{1}H-COSY (*Fig. 2*), and ROESY (*Fig. 3*) correlations allowed the full H-atom and carbon assignments of compound **2**. Lastly, the structure of compound **2** was deduced as (5β,7β,9β,10α,13α)-7-methoxypimara-8(14),15-dien-18-oic acid (**2**) (*Fig. 1*).

Compound **3** was obtained as a white amorphous powder and had the molecular formula $C_{21}H_{32}O_3$ revealed by the HR-ESI-MS at *m/z* 331.2281 $[M - H]^-$ (calc. for $C_{21}H_{31}O_3$ 331.2273). A comparison of the ^{1}H- and ^{13}C-NMR data (*Table 1*) with those of compound **2** showed high similarity, suggesting compound **3** was also an *ent*-pimarane diterpene. The structure of compound **3** differed from compound **2** mainly in the location of olefinic and MeO groups. As indicated in the 1D- and 2D-spectra (*Table 1* and *Fig. 2*), both compounds possessed two double bonds and both compounds contained a CH_2=CH group at C(13). Nevertheless, there was a double bond locating at C(7) in compound **3**, instead of a double bond at C(8)(14) in compound **2**. This was confirmed by the HMBC correlations (*Fig. 2*) between H–C(7) and C(5), C(6), C(9), and C(14). The HMBC cross-peak for MeO at δ(H) 3.16 (3 H, *s*) and C(14) (δ(C) 88.9) established the linkage of MeO at C(14). The α-configuration of H(14) was inferred by the ROESY correlations (*Fig. 3*) between δ(H) 3.05 (1 H, *s*) and H–C(15), H–C(16), as well as the correlations due to δ(H) 3.16 (MeO) and Me(17), and H–C(9). The other correlations from HMBC, ^{1}H,^{1}H-COSY (*Fig. 2*), and ROESY (*Fig. 3*) further confirmed the structure of compound **3**. Consequently, compound **3** was elucidated as (5β,9β,10α,13α,14β-14-methoxypimara-7,15-dien-18-oic acid (**3**) (*Fig. 1*).

Compound **4** was obtained as a white amorphous powder and had a similar *ent*-pimarane skeleton as **3**. It was assigned the molecular formula $C_{20}H_{28}O_3$ by analysis of the HR-ESI-MS at *m/z* 315.1953 $[M - H]^-$ (calc. for $C_{20}H_{27}O_3^-$ 315.1960). Comparing the ^{1}H- and ^{13}C-NMR spectra (*Table 1*) data of compound **4** with those of compound **3** suggested there was one more double bond in compound **4**. Additionally, compound **4** contained a OH at C(14), instead of a MeO at C(14) in compound **3**. The extra double bond was deduced to be located at C(9) =(11) by the HMBCs (*Fig. 2*) from H–C(7), Me(20) to C(9), and from H–C(12) to C(9), C(11). The linkage of OH at C(14) was determined by the HMBC correlations (*Fig. 2*) from H–C(14) to C(7), C(8); and from H–C(15), Me(17) to C(14). The OH group at C(14) was proposed as α-configuration by the ROESY correlations (*Fig. 3*) of H–C(14)/H–C(17) and H–C(14)/H–C(5). Therefore, the struc-

Table 1. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) data of compounds **1** – **4**. δ in ppm, J in Hz

Position	**1**[a])		**2**[b])		**3**[a])		**4**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.68 – 1.72 (*m*), 1.03 – 1.07 (*m*)	37.2 (*t*)	1.69 – 1.71 (*m*), 1.08 – 1.13 (*m*)	40.2 (*t*)	1.84 – 1.88 (*m*) 1.01 – 1.09 (*m*)	39.7 (*t*)	1.91 – 1.94 (*m*), 1.37 – 1.41 (*m*)	37.2 (*t*)
2	1.50 – 1.51 (*m*), 1.25 – 1.27 (*m*)	18.6 (*t*)	1.81 – 1.84 (*m*), 1.44 – 1.46 (*m*)	20.8 (*t*)	1.81 – 1.87 (*m*) 1.45 – 1.49 (*m*)	19.5 (*t*)	1.94 – 1.97 (*m*), 1.57 – 1.61 (*m*)	19.7 (*t*)
3	2.20 – 2.23 (*m*), 1.07 – 1.09 (*m*)	37.4 (*t*)	2.14 – 2.16 (*m*), 1.02 – 1.08 (*m*)	39.4 (*t*)	2.17 (*dd*, *J* = 13.6, 2.0), 1.01 – 1.09 (*m*)	38.0 (*t*)	2.13 – 2.21 (*m*), 1.06 (*ddd*, *J* = 13.2, 4.0, 3.6)	37.9 (*t*)
4	–	43.3 (*s*)	–	44.6 (*s*)	–	43.7 (*s*)	–	44.0 (*s*)
5	2.18 – 2.20 (*m*)	55.5 (*d*)	1.72 – 1.74 (*m*)	49.9 (*d*)	1.35 – 1.41 (*m*)	51.6 (*d*)	1.50 (*dd*, *J* = 12.0, 4.4)	50.0 (*d*)
6	6.07 (*dd*, *J* = 11.2, 2.4)	127.4 (*d*)	2.07 – 2.16 (*m*)	31.3 (*t*)	2.44 – 2.50 (*m*), 2.25 – 2.33 (*m*)	24.2 (*t*)	2.71 – 2.75 (*m*), 2.41 – 2.48 (*m*)	24.5 (*t*)
7	6.03 (br. *d*, *J* = 11.2)	128.2 (*d*)	3.62 (*t*, *J* = 2.8)	83.3 (*d*)	5.57 (*dd*, *J* = 2.4, 4.0)	126.7 (*d*)	5.92 (br. *d*, *J* = 4.8)	123.3 (*d*)
8	–	135.9 (*s*)	–	137.0 (*s*)	–	133.3 (*s*)	–	132.6 (*s*)
9	1.94 – 1.99 (*m*)	49.2 (*d*)	1.88 – 1.92 (*m*)	47.4 (*d*)	1.84 – 1.88 (*m*)	46.4 (*d*)	–	143.8 (*s*)
10	–	37.9 (*s*)	–	40.4 (*s*)	–	35.6 (*s*)	–	37.0 (*s*)
11	1.81 – 1.85 (*m*), 1.53 – 1.54 (*m*)	19.4 (*t*)	1.56 – 1.64 (*m*), 1.37 – 1.44 (*m*)	19.9 (*t*)	1.43 – 1.49 (*m*), 1.31 – 1.40 (*m*)	20.8 (*t*)	5.42 (br. *s*)	115.7 (*d*)
12	1.62 – 1.64 (*m*), 1.28 – 1.32 (*m*)	35.3 (t)	1.60 – 1.64 (*m*), 1.27 – 1.30 (*m*)	36.7 (*t*)	1.72 (*ddd*, *J* = 4.8, 13.6, 14.0) 1.31 – 1.40 (*m*)	32.2 (*t*)	2.13 – 2.21 (*m*)	36.5 (*t*)
13	–	38.9 (*s*)	–	40.0 (*s*)	–	41.0 (*s*)	–	41.3 (*s*)
14	5.23 (*s*)	131.6 (*d*)	5.44 (*s*)	135.9 (*d*)	3.05 (*s*)	88.9 (*d*)	3.94 (br. *s*)	75.1 (*d*)
15	5.71 (*dd*, *J* = 17.6, 10.4)	147.4 (*d*)	5.72 (*dd*, *J* = 17.2, 10.4)	147.7 (*d*)	5.73 (*dd*, *J* = 17.6, 11.2)	144.6 (*d*)	5.78 (*dd*, *J* = 17.6, 10.8)	145.4 (*d*)
16	4.96 (*dd*, *J* = 10.4, 2.0) 4.86 (*dd*, *J* = 17.6, 2.0)	113.2 (*t*)	4.97 (*dd*, *J* = 10.4, 2.0) 4.87 (*dd*, *J* = 17.2, 1.6)	114.0 (*t*)	4.99 (*dd*, *J* = 11.2, 1.2) 4.96 (*dd*, *J* = 17.6, 1.2)	112.9 (*t*)	5.10 (*d*, *J* = 11.6) 5.09 (*d*, *J* = 17.2)	113.9 (*t*)
17	1.07 (*s*)	29.0 (*q*)	1.08 (*s*)	30.3 (*q*)	1.05 (*s*)	24.7 (*q*)	0.97 (*s*)	17.1 (*q*)
18	1.32 (*s*)	28.2 (*q*)	1.19 (*s*)	29.4 (*q*)	1.27 (*s*)	29.2 (*q*)		182.4 (*s*)
19	–	181.8 (*s*)	–	181.5 (*s*)	–	183.5 (*s*)	1.26 (*s*)	28.7 (*q*)
20	0.61 (*s*)	11.8 (*q*)	0.66 (*s*)	13.9 (*q*)	0.72 (*s*)	14.4 (*q*)	0.88 (*s*)	20.1 (*q*)
MeO	–	–	3.16 (*s*)	55.0 (*q*)	3.16 (*s*)	55.4 (*q*)	–	–

[a]) Measured in $CDCl_3$. [b]) Measured in CD_3OD. – = No signal.

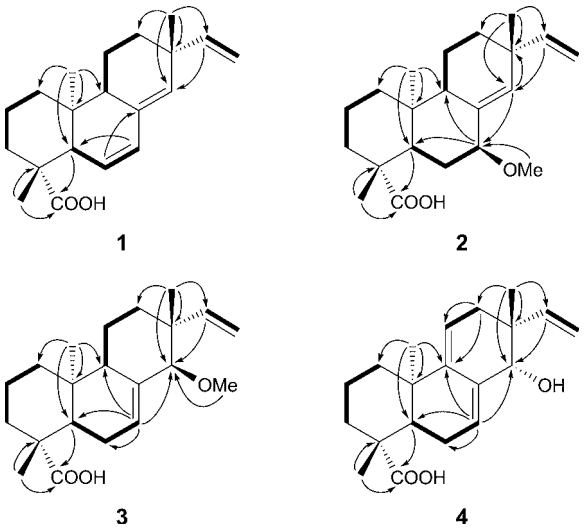

Fig. 2. Selected HMBC and $^1H,^1H$-COSY correlations of compounds **1** – **4**.

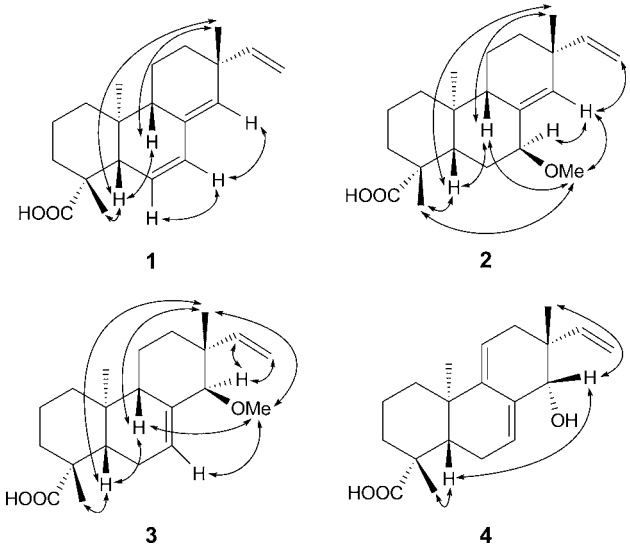

Fig. 3. Key ROESY correlations of compounds **1** – **4**.

ture of compound **4** characterized as (5β,10α,13α,14α)-14-hydroxypimara-7,9(11),15-trien-18-oic acid (**4**) (*Fig. 1*).

The known compounds **5** – **7** were identified by comparing the NMR data with literatures [3][17].

All the isolates were tested for their cytotoxicities in NB4 (human acute promyelocytic leukemia cell), A549 (human lung adenocarcinoma epithelial cell), PC3 (human prostate cancer cell), and MCF7 (human breast adenocarcinoma cell) cell lines. Results were summarized in *Table 2*. Compounds **4**, **6**, **7** showed notable cytotoxic activity in the tested NB4 cells, and compounds **1** – **4**, **6** exhibited well cytotoxicities in A549 cells. For PC3 cell line, only compounds **1** and **7** showed cytotoxicity, and all the isolates could significantly suppress the MCF7 cells except compound **1**.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary data (the 1D- and 2D-NMR data of compounds (**1** – **4**) associated with this article can be found.

Experimental Part

General

Column chromatography (CC): silica gel (SiO_2; 200 – 300 mesh; *Qingdao Meigao Chemical Company*, Qingdao, China); *Sephadex LH-20* (*Pharmacia Fine Chemical Co. Ltd.*, Uppsala, Sweden). HPLC: *Agilent 1260* (Palo Alto, CA, USA) liquid chromatograph equipped with a *Venusil XBP C18*; *Bonna-Agela Technologies Inc.*, Tianjin, China (10 × 250 mm, 5 μm) column. Optical rotations: *Horiba SEPA-300* polarimeter (*Horiba Seisakusho*, Tokyo, Japan). IR Spectra: *Bio-Rad FTS-135* spectrometer (*Bio-Rad*, Berkeley, CA, USA); $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AM-400* NMR (*Bruker Corporation*, Zurich, Switzerland); δ in ppm rel. to Me_4Si as internal standard, J in Hz. MS: *VG Auto Spec-3000* (VG, Manchester, UK) spectrometer; in m/z.

Plant Material

The roots of *A. dumetorum* were collected in Chuxiong, Yunnan province, P. R. China, in August 2011, and identified as *A. dumetorum* Hand.-Mazz. by Dr. *QingsongYang* from Yunnan Minzu University. A voucher specimen (YNMZ-20110815) was deposited with the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University.

Table 2. The cytotoxic activities of compounds **1** – **7**[a])

Compound	IC_{50} [μM]			
	NB4	A549	PC3	MCF7
1	> 10	5.6 ± 0.7	5.1 ± 0.9	> 10
2	> 10	4.4 ± 0.6	> 10	5.8 ± 0.7
3	> 10	9.3 ± 0.8	> 10	8.0 ± 0.5
4	9.4 ± 0.7	8.6 ± 0.9	> 10	4.2 ± 0.4
5	> 10	> 10	> 10	5.0 ± 0.7
6	9.1 ± 0.9	8.3 ± 0.5	> 10	7.6 ± 0.6
7	8.5 ± 0.4	> 10	5.8 ± 0.6	8.4 ± 0.3
Paclitaxel	0.02 ± 0.005	0.03 ± 0.007	0.2 ± 0.06	0.1 ± 0.002

[a]) Values were the means of three independent experiments.

Extraction and Isolation

The dried and powdered roots of *A. dumetorum* (10 kg) was extracted with 95% EtOH (80 l) under reflux for three times. After being concentrated *in vacuo*, the extract (1 kg) was partitioned between H_2O, petroleum ether (PE), and AcOEt, respectively, to provide a PE fraction (400 g) and AcOEt (130 g) fraction. The PE fraction was subjected to a silica gel CC with gradient elution (PE/AcOEt 80:1, 40:1, 20:1, 10:1, 5:1, 2:1, 1:1 (*v/v*)) to yield eight fractions (*Frs. 1 – 8*). *Fr. 6* (60 g) was subjected to a silica gel CC (500 g) and eluted with PE/acetone (40:1, 20:1) to afford five fractions (*Frs. 6a – 6e*). *Fr. 6b* (710 mg) was further purified by HPLC (MeOH/H_2O 80:20) give compound **1** (50 mg), **3** (32 mg) and **5** (85 mg). *Fr. 6c* (570 mg) was performed on a silica gel CC (15 g, PE/AcOEt, 40:1) to offer three fractions (*Frs. 6c1 – 6c3*). The *Fr. 6c2* (103 mg) was purified by a *Sephadex LH-20* CC ($CHCl_3$/MeOH 1:1) to obtain compound **2** (70 mg). Compounds **4** (20 mg) and **6** (43 mg) were obtained from *Fr. 6c3* (210 mg) by HPLC (MeOH/H_2O 75:25). *Fr. 6d* (320 mg) was subjected to a silica gel CC (10 g, PE/AcOEt, 30:1) and further purified by HPLC (MeOH/H_2O 70:30) to yield compound **7** (61 mg).

(5*β*,9*β*,10α,13α)-Pimara-6,8(14),15-trien-18-oic Acid (= (1*R*,4a*S*,4b*S*,7*R*,10a*S*)-7-Ethenyl-1,2,3,4,4a,4b,5,6,7,10a-decahydro-1,4a,7-trimethyl-1-phenanthrenecarboxylic Acid; **1**). White amorphous powder. $[\alpha]_D^{21.6} = -48.0$ ($c = 0.11$, $CHCl_3$). IR (KBr): 3245 – 2500, 1708, 1636, 910, 863. NMR: *Table 1*. ESI-MS (neg.): 299 ($[M - H]^-$). HR-ESI-MS (neg.): 299.2020 ($[M - H]^-$, $C_{20}H_{27}O_2^-$; calc. 299.2011).

(5*β*,7*β*,9*β*,10α,13α)-7-Methoxypimara-8(14),15-dien-18-oic Acid (= (1*R*,4a*S*,4b*S*,7*R*,9*S*,10a*S*)-7-Ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-9-methoxy-1,4a,7-trimethyl-1-phenanthrenecarboxylic Acid; **2**). White amorphous powder. $[\alpha]_D^{20.5} = -60.0$ ($c = 0.05$, $CHCl_3$). IR (KBr): 3243 – 2500, 1707, 1630, 1068, 993, 864. NMR: *Table 1*. ESI-MS (neg.): 331 ($[M - H]^-$). HR-ESI-MS (neg.): 331.2286 ($[M - H]^-$, $C_{21}H_{31}O_3^-$; calc. 331.2273).

(5*β*,9*β*,10α,13α,14*β*)-14-Methoxypimara-7,15-dien-18-oic Acid (= (1*R*,4a*S*,4b*S*,7*R*,8*R*,10a*S*)-7-Ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-8-methoxy-1,4a,7-trimethyl-1-phenanthrenecarboxylic Acid; **3**). White amorphous powder. $[\alpha]_D^{20.9} = -67.0$ ($c = 0.10$, $CHCl_3$). IR (KBr): 3245 – 2500, 1707, 1630, 1069, 997, 863. NMR: *Table 1*. ESI-MS (neg.): 331 ($[M - H]^-$). HR-ESI-MS (neg.): 331.2281 ($[M - H]^-$, $C_{21}H_{31}O_3^-$; calc. 331.2273).

(5*β*,10α,13α,14α)-14-Hydroxypimara-7,9(11),15-trien-18-oic Acid (= (1*R*,4a*R*,7*R*,8*S*,10a*S*)-7-Ethenyl-1,2,3,4,4a,6,7,8,10,10a-decahydro-8-hydroxy-1,4a,7-trimethyl-1-phenanthrenecarboxylic Acid; **4**). White amorphous powder. $[\alpha]_D^{25.4} = -24.5$ ($c = 0.50$, $CHCl_3$). IR (KBr): 3246 – 2500, 1710, 1654, 1127, 1068, 954. NMR: *Table 1*. ESI-MS (neg.): 315 ($[M - H]^-$). HR-ESI-MS (neg.): 315.1953 ($[M - H]^-$, $C_{20}H_{27}O_3^-$; calc. 315.1960).

Cytotoxicitic Assay

Cytotoxic activities were evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method in NB4, A549, PC3, and MCF7 cell lines. Briefly, the cell suspensions (200 ml) at a density of 5×10^4 cells/ml were plated in 96-well microtiter plates and incubated for 24 h at 37 °C in a humidified incubator at 5% CO_2. The test compound (2 ml in DMSO) solution at different concentrations was added to each well and further incubated for 72 h under the same conditions. Then, 20 ml of the MTT solution was added to each well and incubated for 4 h. The old medium (150 ml) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a *Spectra Max Plus* plate reader at 540 nm. Dose–response curves were generated and the IC_{50} values were defined as the concentration of compound required to inhibit cell proliferation by 50%. Paclitaxel (purity >98%, purchased from *Sigma-Aldrich Trading Co, Ltd*., Shanghai, China), an approved agent for the treatment of many tumors, was used as the positive control.

REFERENCES

[1] J. R. Hanson, A. F. White, *Phytochemistry* **1970**, *9*, 1369.
[2] O. H. Kang, H. S. Chae, J. G. Choi, Y. C. Oh, Y. S. Lee, J. H. Kim, M. J. Seung, H. J. Jang, J. H. Lee, D. H. Shin, D. Y. Kwon, *Eur. J. Pharmacol.* **2008**, *601*, 179.
[3] M. K. Lee, T. M. Hung, B. S. Min, I. S. Lee, M. K. Na, M. H. Woo, J. K. Son, Y. H. Kim, J. S. Choi, K. H. Bae, *Nat. Prod. Sci.* **2009**, *15*, 50.
[4] R. J. Choi, E. M. Shin, H. A. Jung, J. S. Choi, Y. S. Kim, *Phytomedicine* **2011**, *18*, 677.
[5] J. H. Cho, J. Y. Lee, S. S. Sim, W. K. Whang, C. J. Kim, *Pulm. Pharmcol. Ther.* **2010**, *23*, 190.
[6] J. H. Lyu, G. S. Lee, K. H. Kim, H. W. Kim, S. I. Cho, S. I. Jeong, H. J. Kim, Y. S. Ju, H. K. Kim, R. T. Sadikot, J. W. Christman, S. R. Oh, H. K. Lee, K. S. Ahn, M. Joo, *J. Ethnopharm.* **2011**, *137*, 1442.
[7] Q. H. Wang, J. Zhang, X. Ma, X. Y. Ye, B. Y. Yang, Y. G. Xia, H. X. Kuang, *Chin. J. Nat. Med.* **2011**, *9*, 17.
[8] J. J. Hsiao, H. C. Chiang, *Phytochemistry* **1995**, *39*, 899.
[9] T. Miyase, N. Sutoh, D. M. Zhang, A. Ueno, *Phytochemistry* **1996**, *42*, 1123.
[10] N. X. Nhiem, H. Y. Lim, P. V. Kiem, C. V. Minh, V. K. Thu, B. H. Tai, T. H. Quang, S. B. Song, Y. H. Kim, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6143.
[11] Y. Satoh, S. Sakai, M. Katsumata, M. Nagasao, M. Miyakoshi, Y. Ida, J. Shoji, *Phytochemistry* **1994**, *36*, 147.
[12] M. Hu, K. Ogawa, Y. Sashida, P. G. Xiao, *Phytochemistry* **1995**, *39*, 179.
[13] H. L. Oh, H. Y. Lim, Y. Park, Y. Lim, H. C. Koh, Y. H. Cho, C. H. Lee, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 797.
[14] H. L. Oh, C. H. Lee, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1347.
[15] J. H. Lee, C. S. Jeong, J. H. Lee, C. Jeong, *Environ. Toxicol. Pharmacol.* **2009**, *28*, 333.
[16] Y. S. Chung, Y. H. Choi, S. J. Lee, S. Choi, J. Lee, H. Kim, E. K. Hong, *J. Ethnopharmcol.* **2005**, *101*, 49.
[17] A. Matsuo, S. Uto, M. Nakayama, S. Hayashi, *Tetrahedron Lett.* **1976**, *28*, 2451.

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