



ent-Kaurene diterpenoids from *Isodon phyllostachys*



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ABSTRACT

Four new ent-kaurene diterpenoids, phyllostachysins M–P (**1–4**) have been isolated from the aerial parts of *Isodon phyllostachys*. Compound **1** features a novel dihydro-2H-pyran ring motif, which was formed by an unusual cleavage in ring-A. **2** and **3** bear a rare 20-nor-ent-kaurene structure, but **4** has a rare 7,20-cyclo-ent-kaurene skeleton. Their structures were elucidated on the basis of extensive spectroscopic analyses. Compounds **1**, **3**, and **4** were evaluated for their cytotoxic activity against five human tumor cell lines.

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The genus *Isodon*, consisting of about 150 species, is distributed throughout the world, but mainly in tropical and subtropical Asia.¹ Previous phytochemical investigation on this genus resulted in the isolation of a large number of diterpenoids with various novel carbon skeletons, such as scopariusic acid,² scopariusins A–C,³ ternifolide A,⁴ neolaxiflorins A and B,⁵ nervonin A,⁶ maoecrystal Z,⁷ and maoecrystal V.⁸ Some diterpenoids from the *Isodon* species have attracted considerable attention for their potential antibacterial, anti-inflammatory, and antitumor applications, such as eriocalyxin B,⁹ adenanthin,¹⁰ and xerophilus B.¹¹

As part of a systematical research for more new diterpenoids with novel structure or/and antitumor activity, the aerial parts of *I. phyllostachys*, collected in Muli County and Yinyuan County of the Sichuan Province, People's Republic of China, have been investigated. As a result, four new diterpenoids (**1–4**) with rare ent-kaurene structure were obtained. Compound **1** features a novel dihydro-2H-pyran ring motif from an unusual cleavage in ring-A. **2** and **3** bear a rare 20-nor-ent-kaurene structure, and **4** has a rare 7,20-cyclo-ent-kaurene skeleton. Herein, we describe the isolation, structure elucidation of these new diterpenoids.

Phyllostachysin M (**1**)¹² was isolated as a white amorphous powder and exhibited a negative ion peak at m/z 347.1855 ($[M-H]^-$, calcd 347.1858), corresponding to $C_{20}H_{28}O_5$ with seven

degrees of unsaturation. The IR spectrum showed absorption bands for hydroxyl (3439 cm^{-1}), carbonyl (1703 cm^{-1}), and olefinic (1659 and 1631 cm^{-1}) groups. There were two obvious tertiary methyl singlets (δ_H 1.05, s; δ_H 0.96, s) and three signals of olefinic group (δ_H 6.32, s; δ_H 5.04, s; δ_H 4.92, s) in ^1H NMR spectrum (Table 1). Only 19 carbon signals (including 6 weak signals: C-2, C-5, C-6, C-8, C-18, and C-20) were recorded in the ^{13}C NMR spectrum of compound **1**. There was one obvious ^1H -signal (δ_H 3.68) in ^1H NMR spectrum. But in ^{13}C NMR spectrum, the corresponding ^{13}C -signal could not be detected. The HSQC correlation of ^1H -signal (δ_H 3.68) with ^{13}C -signal (hidden, δ_C 79.0) testified that a carbon (C-3) did not resonate in its appropriate frequency. Finally, the ^{13}C NMR, DEPT and HSQC spectra of compound **1** (Table 1) indicated the presence of one carbonyl group, one exomethylene, two tertiary methyls, five methylenes (one oxygenated), seven methines (three oxygenated and one olefinic) and four quaternary carbons (two olefinic). Except for two carbon-carbon double bonds and the carbonyl group, seven degrees of unsaturation suggested that compound **1** should possess 4 rings in its structure. Based on the data above mentioned and the structures of ent-kauranoids previously isolated from *Isodon* species,² compound **1** was tentatively assigned as an ent-kauranoid.

The ^1H - ^1H COSY (Fig. 2) revealed three spin systems: H₂-1/H₂-2/H-3, H-5/H-6, and H-9/H₂-11/H₂-12/H-13/H₂-14. The HMBC correlations (Fig. 2) from H-3 (δ_H 3.68, dd, $J = 8.8, 4.0\text{ Hz}$) to C-20 (δ_C 137.8) and H-20 (δ_H 6.32, s) to C-3 (δ_C 79.0) and C-10 (δ_C 109.8) disclosed the fact that an epoxy link was present between

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Table 1
¹H and ¹³C NMR Spectroscopic Data for phyllostachysins M–P (**1–4**) (δ in ppm, *J* in Hz).

Position	1 ^a		2 ^b		3 ^b		4 ^b	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.46, m	57.9 (t)	5.60, m	118.8 (d)	5.77, d (2.8)	120.9 (d)	4.11, m	72.9 (d)
2 α	1.53, m	30.1 (t)	2.54, m	32.1 (t)	2.45 ^c , m	32.5 (t)	1.84 ^c , m	30.7 (t)
2 β			2.45 ^c , m		2.75 ^c , m		1.99 ^c , m	
3	3.68, dd (8.8, 4.0)	79.0 (d)	3.75, dd (10.1, 5.1)	74.6 (d)	3.87, s	74.9 (d)	1.27, m	38.8 (t)
4		34.5 (s)		37.9 (s)		38.5 (s)		33.1 (s)
5 β	1.86 ^c , m	48.8 (d)	2.77, d (10.2)	49.3 (d)	2.69 ^c , m	53.3 (d)	2.28, s	63.3 (d)
6 α	4.00, d (11.0)	74.2 (d)	3.98, d (9.7)	72.7 (d)	4.51, d (11.1)	76.3 (d)		212.1 (s)
7		210.7 (s)	3.94, d (2.1)	81.6 (d)		213.3 (s)		88.4 (s)
8		57.6 (s)		48.8 (s)		59.0 (s)		57.4 (s)
9 β	2.61, d (6.4)	37.2 (d)	3.19, s	36.3 (d)	3.14, s	41.8 (d)	1.84 ^c , m	57.0 (d)
10		109.8 (s)		138.4 (s)		135.9 (s)		48.6 (s)
11 α	1.64, dd (14.4, 4.1)	18.6 (t)	1.84 ^c , m	20.2 (t)	1.82 ^c , m	20.6 (t)	2.80, m	22.7 (t)
11 β	1.91 ^c , m		2.40 ^c , m		2.41 ^c , m		1.99 ^c , m	
12 α	1.74, m	31.1 (t)	1.49 ^c , m	32.4 (t)	1.50, dd (9.6, 5.3)	32.3 (t)	1.38, m	31.4 (t)
12 β	1.35, m		1.74, s		1.82 ^c , m		2.21, m	
13 α	2.64, m	41.0 (d)	2.64, s	42.4 (d)	2.72 ^c , m	42.6 (d)	2.89, dd (9.4, 4.3)	35.1 (d)
14 α	1.79, d (11.3)	34.0 (t)	1.53, d (11.3)	33.6 (t)	1.82 ^c , m	35.4 (t)	3.16, d (12.2)	26.7 (t)
14 β	1.23, dd (11.2, 4.9)		1.05, dd (11.3, 5.1)		1.37, dd (11.2, 5.0)		2.14, dd (12.2, 4.5)	
15 α	4.83, s	73.0 (d)	4.75, s	82.4 (d)	5.58, s	75.1 (d)		205.6 (s)
16		156.9 (s)		157.9 (s)		158.3 (s)		153.4 (s)
17a	4.92, m	105.8 (t)	5.10, s	105.1 (t)	5.16, s	106.7 (t)	5.18, s	114.3 (t)
17b	5.04, s		5.45, s		5.54, s		5.89, s	
18	0.96, s	25.5 (q)	1.81, s	28.6 (q)	1.66, s	27.8 (q)	1.33, s	33.9 (q)
19	1.05, s	23.0 (q)	1.49, s	14.4 (q)	1.33, s	23.3 (q)	0.94, s	22.7 (q)
20	6.32, s	137.8 (d)					4.74, s	82.4 (d)

^a Data were recorded in DMSO on Bruker DRX-600 spectrometer.

^b Data were recorded in C₅D₅N on Bruker DRX-600 spectrometer.

^c Overlapping signals.

C-3 and C-20, and a carbon-carbon double bond existed between C-20 and C-10. The key HMBC correlations from H-3 to C-1, C-4, C-5, and C-19; from H₃-18 (δ_H 0.96, s) to C-3, C-4, C-5, and C-19; from H-5 (δ_H 1.86, m) to C-6, C-10, C-18, C-19, and C-20; and from H-20 to C-5 and C-9, revealed that a dihydro-2*H*-pyran ring motif (C-3, C-4, C-5, C-10, C-20, and an oxygen atom) formed through a cleavage between C-1 and C-10, and an epoxy formation between C-3 and C-20. The HMBC correlations from H-6 (δ_H 4.00, d, *J* = 11.0 Hz) to C-4, C-5, and C-7; from H-9 (δ_H 2.61, d, *J* = 6.4 Hz) to C-5, C-7, C-8, C-10, C-11, C-12, C-14, C-15, and C-20; and from H₂-17 (δ_H 5.04, s; δ_H 4.92, s) to C-13, C-15, and C-16, indicated that **1** was a 1,10-*sec*-3,20-epoxy *ent*-kauranoid, possessing a unusual dihydro-2*H*-pyran ring motif (Fig. 2). The HMBC correlations from H-9, H-14 α (δ_H 1.79, d, *J* = 11.3 Hz), and H₂-17 to C-15 (δ_C 73.0), implied that C-15 (δ_C 73.0) was substituted by an OH group. The ROESY correlations of H₂-2/H₃-18/H-5/H-9, H-6/H₃-19, H-6/H-14 α , and H-15/H-14 β demonstrated that C-2, H-5, and H-9 are β -orientation, H-6 and H-15 are α -orientation (Fig. 1). Thus,

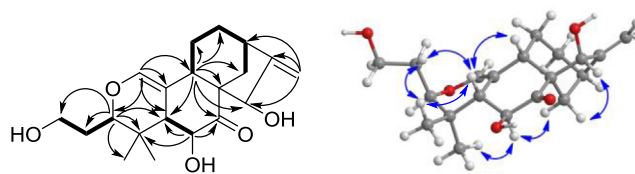


Fig. 2. ¹H-¹H COSY (bold), selected HMBC (arrow) and key ROESY correlations of **1**.

compound **1** was determined as 1,6 β ,15 β -trihydroxy-3,20-epoxy-1,10-*seco-ent*-kaur-10,16-dien-7-one.

Phyllostachysin N (**2**)¹³ was obtained as a white amorphous powder and showed a sodium adduct ion at *m/z* 343.1882 ([M + Na]⁺, calcd 343.1880) corresponding to a molecular formula C₁₉H₂₈O₄ with six degrees of unsaturation, which suggested that **2** should be a rare nor-diterpenoid. The IR spectrum showed absorption bands for hydroxyl (3441 cm⁻¹) and olefinic (1641 cm⁻¹) groups. There were two obvious tertiary methyl singlets (δ_H 1.81, s; δ_H 1.49, s) and three signals of olefinic group (δ_H 5.60, m; δ_H 5.45, s; δ_H 5.10, s) in ¹H NMR spectrum (Table 1). The ¹³C NMR and DEPT data of compound **2** (Table 1) showed the presence of one exomethylene, two methyls, four methylenes, eight methines (four oxygenated and one olefinic) and four quaternary carbons (two olefinic). Comparisons of the ¹H and ¹³C NMR data of **2** with those of rubescensin N¹⁴ indicated that both compounds had same skeleton and similar structure. The ¹H-¹H COSY revealed three spin systems, H-1/H₂-2/H-3, H-5/H-6/H-7, and H-9/H₂-11/H₂-12/H-13/H₂-14 (Fig. 3). The HMBC correlations from H-1, H₂-2, Me-18, and Me-19 to C-3 (δ_C 74.6), from H-5 and H-7 to C-6 (δ_C 72.7), from H-5, H-14 β , and H-15 to C-7 (δ_C 81.6), and from H-7, H-14 α , and H₂-17 to C-15 (δ_C 82.4), implied that all of C-3, C-6, C-7, and C-15 were substituted by OH group, respectively (Fig. 3). The ROESY correlations of H-3/H₃-18 β , H-3/H-5 β , H-6/H₃-19 α , H-7/H-15/H-14 β demonstrated that H-3, H-6, H-7, and H-15 were β , α , α , α -oriented, respectively. Therefore, the structure of compound **2**

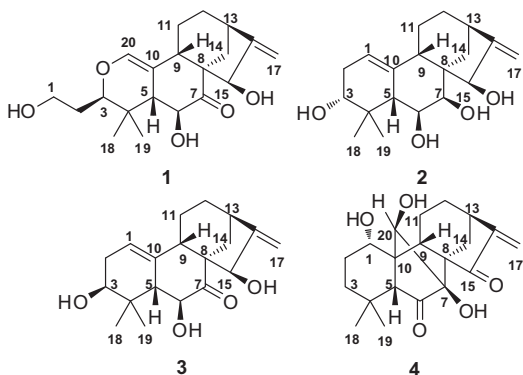


Fig. 1. Structures of compounds **1–4**.

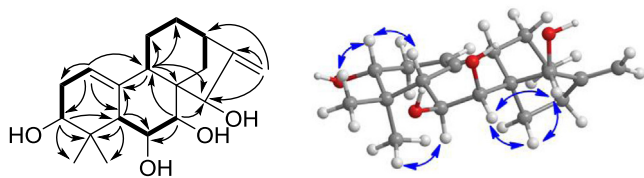


Fig. 3. ^1H - ^1H COSY (bold), selected HMBC (arrow) and key ROESY correlations of **2**.

was identified as $3\alpha,6\beta,7\beta,15\beta$ -tetrahydroxy-20-nor-*ent*-kaur-16-ene.

Phyllostachysin O (**3**)¹⁵ had the molecular formula $\text{C}_{19}\text{H}_{26}\text{O}_4$ by HRESIMS and ^{13}C NMR data, which suggested that **3** would be also a rare nor-diterpenoid. The IR absorption bands at 3433, 1704, 1662, and 1629 cm^{-1} indicated the existence of hydroxyl, carbonyl, and olefinic groups. The ^1H NMR data (Table 1) showed characteristic resonances of two tertiary methyl group (δ_{H} 1.66, s; δ_{H} 1.33, s) and olefinic groups (δ_{H} 5.77, d, $J = 2.8$ Hz; δ_{H} 5.54, s; δ_{H} 5.16, m) as **2**. The ^{13}C NMR and DEPT data (Table 1) of **3** showed the presence of one carbonyl group, one exomethylene, two tertiary methyls, four methylenes, seven methines (three oxygenated and one olefinic) and four quaternary carbons (two olefinic). Comparisons of ^{13}C NMR data of **3** with those of **2** indicated that both compounds had similar structure. The differences were the resonance of carbonyl (C-7, δ_{C} 213.3) in **3** instead of an oxygenated methine (C-7, δ_{C} 81.6) in **2**, along with the orientation of OH-3 (β in **3**, α in **2**). The ^1H - ^1H COSY revealed three spin systems, H-1/ H_2 -2/H-3, H-5/H-6, and H-9/ H_2 -11/ H_2 -12/H-13/ H_2 -14. The HMBC correlations from H-1, H-2, Me-18, and Me-19 to C-3 (δ_{C} 74.9), and from H_2 -17 and H-14 β to C-15 (δ_{C} 75.1) implied an OH group at C-3 and C-15, respectively. The HMBC correlations from H-5, H-9, H-15 to C-7 (δ_{C} 213.3), showed a ketone group at C-7. The ROESY correlations of H-6/ H_3 -19 α and H-15/H-14 β demonstrated that H-6 and H-15 were α -oriented. The HO-3 was assigned to be β -oriented through the ROESY correlation of H-3/H-6 α and the observation of shielding of C-18 β (δ_{C} 27.8, Δ 6.1 ppm) due to space compression effect between HO-3 β and CH₃-18 β , compared with C-18 β (δ_{C} 33.9) in **4** (without OH-3 β). Thus, compound **3** was established as $3\beta,6\beta,15\beta$ -trihydroxy-20-nor-*ent*-kaur-16-en-7-one.

Phyllostachysin P (**4**)¹⁶ had the molecular formula $\text{C}_{20}\text{H}_{26}\text{O}_5$ by HRESIMS and ^{13}C NMR data, corresponding to eight degrees of unsaturation. The ^1H NMR data (Table 1) showed characteristic resonances of two tertiary methyl group (δ_{H} 1.33, s; δ_{H} 0.94, s) and exomethylene group (δ_{H} 5.89, s; δ_{H} 5.18, s). The ^{13}C NMR and DEPT data (Table 1) of **4** exhibited 20 carbon signals, including two carbonyls, one exomethylene, two tertiary methyls, five methylenes, five methines (two oxygenated), four quaternary carbons (one olefinic) and an oxygenated tertiary carbon. Deducting three degrees of unsaturation accounted for one carbon-carbon double bond and two carbonyls, the remaining five degrees of unsaturation suggested that compound **1** should possess 5 rings in its structure. Careful comparison of ^{13}C NMR spectroscopic data for compound **4** and rubescensin D¹⁷ indicated that both compounds had same skeleton and similar structure. The only difference was the resonance of methylene (C-14, δ_{C} 26.7) in **4** instead of an oxygenated methine (C-14, δ_{C} 73.8) in rubescensin D. The ^1H - ^1H COSY revealed two spin systems, H-1/ H_2 -2/ H_2 -3 and H-9/ H_2 -11/ H_2 -12/H-13/ H_2 -14 (Fig. 4). The ROESY correlations of H-1 with H-3 β , H-5, and H-9 showed that H-1 was β -oriented. The ROESY correlations of H-20 with H-2 α and H_3 -19 α demonstrated that H-20 was α -oriented. Accordingly, compound **4** was identified as $1\alpha,7,20\beta$ -trihydroxy-7,20-cyclo-*ent*-kaur-16-en-6,15-dione.

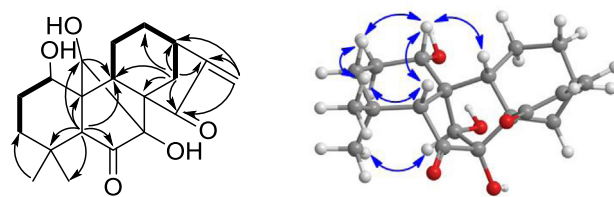


Fig. 4. ^1H - ^1H COSY (bold) and selected HMBC (arrow) and key ROESY correlations of **4**.

Compounds **1**, **3**, and **4** were tested for their cytotoxic activity¹⁸ against acute leukemia (HL-60), hepatic cancer (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW-480), but none of them exhibited cytotoxic activity ($\text{IC}_{50} > 10 \mu\text{M}$).

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.tetlet.2016.12.034>. These data include MOL files and InChIKeys of the most important compounds described in this article.

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- Phyllostachysin M (**1**): white amorphous powder; $[\alpha]_{\text{D}}^{25} +131$ (c 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 240 (2.4), 204 (3.4) nm; IR (KBr) ν_{max} 3439, 3432, 2956, 2929, 2871, 1703, 1659, 1631, 1466, 1390, 1371, 1246, 1175, 1160, 1117, 1087, 1079, 1052, 1033 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1; negative HRESIMS $[\text{M}-\text{H}]^-$ m/z 347.1855 (calcd for $\text{C}_{20}\text{H}_{27}\text{O}_5$, 347.1858).
- Phyllostachysin N (**2**): white amorphous powder; $[\alpha]_{\text{D}}^{25} -38$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.0) nm; IR (KBr) ν_{max} 3441, 2927, 2864, 1750, 1641, 1456, 1386, 1367, 1278, 1264, 1136, 1113, 1049, 1020 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1; positive HRESIMS $[\text{M}+\text{Na}]^+$ m/z 343.1882 (calcd for $\text{C}_{19}\text{H}_{28}\text{O}_4\text{Na}$, 343.1880).
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- Phyllostachysin O (**3**): white amorphous powder; $[\alpha]_{\text{D}}^{25} +13$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 317 (1.9), 245 (2.6), 204 (3.0) nm; IR (KBr) ν_{max} 3433, 2932, 2873, 1704, 1662, 1629, 1592, 1456, 1385, 1366, 1307, 1249, 1126, 1106, 1092, 1077, 1052 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1; positive HRESIMS $[\text{M}+\text{Na}]^+$ m/z 341.1725 (calcd for $\text{C}_{19}\text{H}_{26}\text{O}_4\text{Na}$, 341.1723).
- Phyllostachysin P (**4**): white amorphous powder; $[\alpha]_{\text{D}}^{25} -68$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (3.2), 196 (2.8) nm; IR (KBr) ν_{max} 3440, 3431, 2925, 2857, 1631, 1384, 1361, 1135, 1077, 1055, 1039 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1; positive HRESIMS $[\text{M}+\text{Na}]^+$ m/z 369.1677 (calcd for $\text{C}_{20}\text{H}_{26}\text{O}_5\text{Na}$, 369.1672).
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