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Cytotoxic *ent*-kaurene diterpenoids from *Isodon phyllostachys*

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Abstract

ent-Kaurene diterpenoids, phyllostachysins D–H (**1**–**5**), together with nine known compounds, rabdoloxins A–B (**6**–**7**), rabdoinflexin B (**8**), amethystoidin A (**9**), rabdokunmin D (**10**), macrocalyxin E (**11**), 5,7-dihydroxy-4′-hydroxylflavone (**12**), oleanolic acid (**13**) and daucosterol (**14**), were isolated from aerial parts of *Isodon phyllostachys*. Structures were elucidated on the basis of spectroscopic methods, especially using 2D-NMR spectroscopic analyses. All *ent*-kaurenoids were tested for their cytotoxic effects against K562 cells. Compound **9** was the most potent with an IC_{50} value of 0.69 μg/ml.

Keywords: *Isodon phyllostachys*; Labiatae; *ent*-Kaurenoids; Phyllostachysins D–H; Cytotoxic effect

1. Introduction

The genus *Isodon* (Labiatae) is well-known as a rich source of *ent*-kaurane diterpenoids, of which about 500 compounds have been reported (Sun et al., 2001). *I. phyllostachys*, which is distributed throughout the northwest district of the Yunnan Province of China, has been used as an antiphlogistic or antibiotic agent in folk medicine. In previous investigations, some *ent*-kaurenoids including phyllostachysins A–B (Fujita et al., 1985; Chen et al., 1991), phyllostachysin C and sculponeatins B–C (Hou et al., 2000) were reported. In our ongoing search for bioactive diterpenoids from *Isodon* genus plants, the reinvestigation on the chemical constituents of *I. Phyllostachys* collected in Zhongdian County of Yunnan Province, China, led to the isolation of 14 compounds including five new *ent*-kaurene diterpenoids, phyllostachysins D–H (**1**–**5**), along with six known *ent*-kaurenoids, rabdoloxins A–B (**6**–**7**) (Sun et al., 1991), rabdoinflexin B (**8**) (Wang et al., 1989), amethystoidin A (**9**) (Wang et al., 1984), rabdokunmin D (**10**) (Zhang and Sun, 1989), and macrocalyxin E (**11**) (Wang et al., 1986), 5,7-dihydroxy-4′-hydroxylflavone (**12**) (Ternai and Markham, 1976), oleanolic acid (**13**) and daucosterol (**14**). In addition, all *ent*-kaurenoids were evaluated for their inhibitory effect against K562 cells. In this paper, we wish to report the isolation, structure elucidation of these new compounds and the bioassay results.

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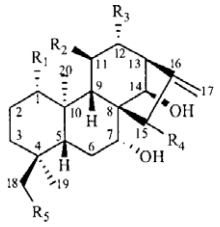

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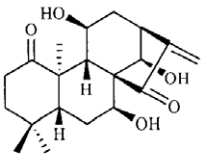
2	H	OH	OH	β-OH	H
3	H	OH	H	=O	H
6	H	=O	OH	=O	OH
7	H	OH	OH	=O	H
8	OH	OH	H	=O	H
9	H	=O	H	=O	OH
10	H	OH	H	=O	OH



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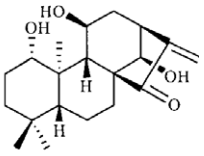

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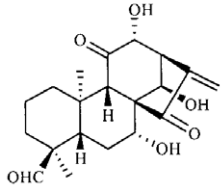

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2. Results and discussion

The acetone extract prepared from aerial parts of *I. phyllostachys* was partitioned between EtOAc and water. The EtOAc layer was repeatedly subjected to column chromatography on silica gel, semipreparative and preparative HPLC to afford five new compounds, phyllostachysins D–H (**1**–**5**), along with nine known compounds (**6**–**14**).

Compound **1**, isolated as a white amorphous powder, exhibited a pseudo molecular ion peak $[M+Na]^+$ at *m/z* 371 in the positive ESI-MS, consistent with the molecular formula $C_{20}H_{28}O_5$ deduced from positive HR-ESI-MS (*m/z* 371.1827 $[M+Na]^+$; calcd. 371.1834). The IR spectrum showed absorption bands at 1643 and 3448 cm^{-1}, revealing the presence of a double bond and hydroxyl groups, respectively. The above evidence, together with the carbon signals typical for *ent*-kaurene skeleton including three methyls (δ 33.6, 21.8 and 18.3 attributable to C-18, 19 and 20), three methines (δ 69.9, 53.3 and 52.8 due to C-9, 13 and 5), three quaternary carbons (δ 60.2, 41.2 and 33.5 assignable to C-8, 10 and 4) and an α,β-unsaturated ketone group (δ 206.6, 144.9 and 122.5 assigned as C-15, 16 and 17) in the ^{13}C NMR spectrum. From this, along with the structure of the known diterpenoids isolated from this plant, we assumed that compound **1** is an analogue of *ent*-kaur-16-en-15-one. Comparison of the 1D- and 2D-NMR spectroscopic data of **1** with those of rabdoloxin B (**7**) revealed that their structures were very similar to each other. The only difference between **1** and **7** was that the hydroxyl group at C-11 (δ 70.9, *d*) in **7** (Sun et al., 1991) was replaced by a carbonyl group (δ 208.6, *s*) in **1,** which was supported from the chemical shifts C-9 (δ 67.6, *d*) in **7** downfield to C-9 (δ 69.9, *d*) in **1** for the deshielding effect of the carbonyl group at C-11. This was further confirmed by the HMBC cross-peaks of H-12 (δ 4.32, *d*, $J = 3.3$ Hz) and H-13 (δ 3.78, *d*, $J = 2.0$ Hz) with C-11 (Fig. 1).

The relative configuration of **1** was determined by the analysis of a ROESY experiment. The hydroxyl groups at C-7, C-12 and C-14 were shown to be in the α, α and β orientations, respectively, as deduced from the ROESY correlations of H-7 (δ 4.86, *dd*, $J = 12.0$, 4.6 Hz) with H-5 (δ 1.05, *dd*, $J = 12.4$, 4.6 Hz) and H-9 (δ 2.24, *s*), and of H-12 (δ 4.32, *d*) with H-17b (δ 5.62, *br s*) and H-13 (δ 3.78, *d*), as well as of H-14 (δ 6.26, *br s*) with H-20 (δ 1.63, *s*) (Fig. 1). So, compound **1** was identified as 7α,12α,14β-trihydroxy-*ent*-kaur-16-en-11,15-dione, named phyllostachysin D.

Phyllostachysin E (**2**), a white amorphous powder, gave rise to a quasi-molecular ion peak $[M+Na]^+$ at *m/z* 375 in its positive ESI-MS, corresponding to a molecular formula of $C_{20}H_{32}O_5$, which was verified by HR-ESI-MS and NMR spectroscopic data. General analysis of the NMR data of **2** showed that its spectrum resembled that of rabdoloxin B (**7**) except for the resonance at C-15. The difference can be rationalized as the carbonyl group at C-15 in **7** being replaced by an oxygenated methine (δ 80.2, *d*) in **2**, which was confirmed by the HMBC correlations from H-7 (δ 4.45, *d*, $J = 11.3$ Hz), H-9 (δ 2.32, *br s*) and H-14 (δ 5.63, *br s*) to C-15 (δ 80.2, *d*). The β-orientation of the OH-15 was suggested from the chemical shift of C-9 from δ 67.6 (*d*) in **7** to the abnormal upfield shift δ 59.3 (*d*) in **2**, due to the γ-gauche steric compression effect between OH-15β and H-9 (Wu et al., 1993; Han et al., 2003). Thus, **2** was determined to be 7α,11β,12α,14β,15β-pentahydroxy-*ent*-kaur-16-ene.

Phyllostachysin F (**3**), obtained as an amorphous powder, had the molecular formula of $C_{20}H_{30}O_4$ established by HR-ESI-MS (*m/z* 357.2050 $[M+Na]^+$, calcd. 357.2041) and ^{13}C NMR spectroscopic data (Table 1). Comparison of the ^{13}C NMR and DEPT data of **3** with those of the known compound rabdoloxin B (**7**) showed that the only difference was the oxygenated methine at C-12 in **7** being replaced by a methylene in **3**. The ^1H NMR and HMQC spectra of **3** showed the presence of two protons assigned to C-12 at δ 2.35 (1H, *m*, H-12α) and δ 2.25 (1H, *m*, H-12β). The HMBC correlations of H-9 (δ 1.91, *d*, $J = 4.9$ Hz) and H-14 (δ 5.19, *br s*) with C-12 (δ 40.8, *t*)

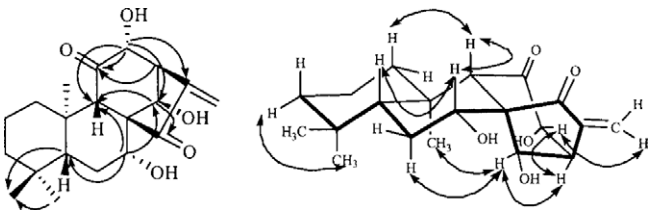

Fig. 1. Selected HMBC and ROESY correlations of compound **1**.

Table 1
^{13}C NMR spectroscopic data data for compounds **1**–**5** (C_5D_5N, 100 MHz, δ in ppm)

Carbon	1	2	3	4	5
1	39.8 (*t*)	40.3 (*t*)	39.6 (*t*)	215.2 (*s*)	80.5 (*d*)
2	18.6 (*t*)	18.9 (*t*)	18.7 (*t*)	35.5 (*t*)	30.3 (*t*)
3	41.4 (*t*)	41.8 (*t*)	41.7 (*t*)	41.9 (*t*)	39.8 (*t*)
4	33.5 (*s*)	33.4 (*s*)	33.3 (*s*)	33.1 (*s*)	33.3 (*s*)
5	52.8 (*d*)	53.4 (*d*)	53.4 (*d*)	53.9 (*d*)	54.5 (*d*)
6	29.8 (*t*)	30.8 (*t*)	29.8 (*t*)	29.9 (*t*)	19.0 (*t*)
7	73.2 (*d*)	76.2 (*d*)	74.7 (*d*)	73.8 (*d*)	27.4 (*t*)
8	60.2 (*s*)	53.9 (*s*)	60.2 (*s*)	59.5 (*s*)	58.0 (*s*)
9	69.9 (*d*)	59.3 (*d*)	66.4 (*d*)	59.1 (*d*)	67.8 (*d*)
10	41.2 (*s*)	38.7 (*s*)	38.8 (*s*)	52.7 (*s*)	45.1 (*s*)
11	208.6 (*s*)	71. 4(*d*)	64.4 (*d*)	67.3 (*d*)	66.7 (*d*)
12	78.8 (*d*)	75.2 (*d*)	40.8 (*t*)	39.9 (*t*)	41.7 (*t*)
13	53.3 (*d*)	58.0 (*d*)	46.2 (*d*)	46.2 (*d*)	46.7 (*d*)
14	71.1 (*d*)	73.2 (*d*)	76.1 (*d*)	76.3 (*d*)	73.6 (*d*)
15	206.6 (*s*)	80.2 (*d*)	207.1 (*s*)	206.9 (*s*)	209.3 (*s*)
16	144.9 (*s*)	155.6 (*s*)	151.1 (*s*)	150.9 (*s*)	151.3 (*s*)
17	122.5 (*t*)	110.3 (*t*)	113.8 (*t*)	114.2 (*t*)	111.9 (*t*)
18	33.6 (*q*)	33.5 (*q*)	33.5 (*q*)	31.9 (*q*)	33.3 (*q*)
19	21.8 (*q*)	21.7 (*q*)	21.8 (*q*)	22.6 (*q*)	21.6 (*q*)
20	18.3 (*q*)	17.3 (*q*)	18.1 (*q*)	17.6 (*q*)	15.0 (*q*)

further confirmed the above deductions. Accordingly, compound **3** was assigned as 7α,11β,14β-trihydroxy-*ent*-kaur-16-en-15-one.

Phyllostachysin H (**4**), an amorphous powder, had the molecular formula $C_{20}H_{28}O_5$, as determined by HR-ESI-MS, ^{13}C NMR (Table 1) and DEPT data. Examination of ^1H and ^{13}C NMR spectroscopic data revealed that **4** should also be an analogue of *ent*-kaurane-16-en-15-one. Careful spectroscopic data analysis showed that it was similar to those of **3**, with the methylene at C-1 in **3** being replaced by a carbonyl group (δ 215.2, *s*) in **4**. The latter was confirmed by HMBC correlations of H-5 (δ 1.30, *dd*, $J = 11.3$, 3.9 Hz) and H-9 (δ 2.60, *br s*) with C-1 (δ 215.2, *s*). The other difference was that the α-OH at C-7 in **3** was changed to a β-OH in **4**, which can be explained by ROESY correlation between H-7 (δ 4.64, *dd*, $J = 11.2$, 4.8 Hz) and H_2-6 (δ 2.13), and the abnormal upfield shift of C-9 (δ 59.1, *d*) due to the γ-gauche steric compression effect between OH-7β and H-9 in **4**. So, compound **4** was established to be 7β,11β,14β-trihydroxy-*ent*-kaur-16-en-1,15-dione.

Phyllostachysin H (**5**) was obtained as white powder. Pseudo molecular ion peak $[M+Na]^+$ at *m*/*z* 357 in ESI-MS, together with NMR spectroscopic data, revealed a molecular formula $C_{20}H_{30}O_4$, which was further confirmed by positive HR-ESI-MS ($[M+Na]^+$ at *m*/*z* 357.2047, calcd. 357.2041). Analysis of the spectroscopic data of **5** with those of **3** established that there were many similarities (e.g. same molecular weight) between them except for different substituted position of hydroxyl groups. Three oxygenated methines in **5** were assigned to C-1 (δ 80.5, *d*), C-11 (δ 66.7, *d*) and C-14 (δ 73.6, *d*), respectively, by the related HMBC correlations (Fig. 2). The above evidence, together with analysis of the ROESY spectrum (Fig. 2), determined compound **5** to be 1α,11β,14β-trihydroxy-*ent*-kaur-16-en-15-one.

Table 2
Cytotoxic effects against K562

Compounds	K562 IC_{50} (μg/ml)
Cisplatin	1.11
1	2.92
2	>200
3	1.15
4	4.58
5	6.71
7	2.74
9	0.69

All *ent*-kaurenoids were tested for their cytotoxicity against human-tumor K562 cells, with cisplatin as a positive control (see Table 2). Compared with cisplatin ($IC_{50} = 1.11$ μg/ml), compound **9** showed the most potent effect against K562 cells with an $IC_{50} = 0.69$ μg/ml. Compounds **1**, **3**, **4**, and **7** displayed modest activities (Table 2) and compounds **5**, **6**, **8**, **10**, and **11** showed weak activities against K562 cells. Compound **2** was completely inactive, which suggests that the α,β-unsaturated ketone moiety present in each of this type diterpenoids was necessary for inhibitory activity (Node, 1984; Han et al., 2004). Recently, Leung and Sun reported a novel mechanism for inhibition of nuclear factor-κB DNA-binding activity with *ent*-kaurenoids isolated from *Isodon rubescens* (Leung et al., 2005). In this regard, further investigation of their anti-cancer activities, structure–activity relationships and mechanisms of action are in progress.

3. Concluding remarks

In conclusion, we have isolated and structurally characterized five new *ent*-kaurenoid derivatives from *I.*

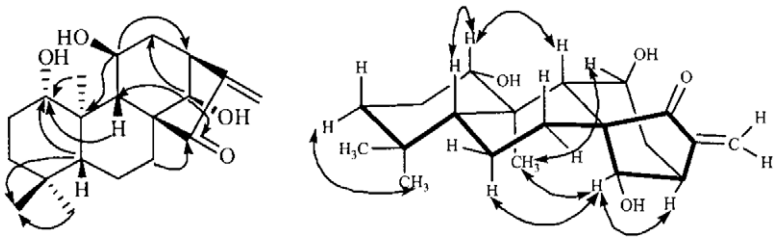

Fig. 2. Selected HMBC and ROESY correlations of compound **5**.

phyllostachys, which were identified to be cytotoxic to K562 cells. Interestingly, previous phytochemical investigations on this plant reported three C-20-oxygenated *ent*-kaurenoid, phyllostachysins A–C, but in this study, we isolated other type of *ent*-kaurenoids (C-20-non-oxygenated) phyllostachysins D–H from *I. phyllostachys* collected from different region. This difference may perhaps be attributed to the different ecological environment. So, continued investigation of this species, especially those grown in different climatic environments, may represent a promising strategy for the discovery of other new diterpenoids.

4. Experimental part

4.1. General

^{13}C NMR spectra were performed on a Bruker AM-400, and ^{1}H NMR, ROESY, HMQC, HMBC experiments were on DRX-500 spectrometer with pyridine-d_5 as solvent and TMS as internal standard. MS spectra were taken on a VG Auto Spec-3000 magnetic sector instrument. Optical rotations were measured on a SEPA-300 polarimeter, whereas UV-210A spectrometer. IR spectra were recorded on a BIO-RAD FTS-135 spectrometer with KBr pellets. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Factory, PR China). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} column. Preparative HPLC was performed on a Shimadazu LC-8A preparative liquid chromatography with a Shimadazu PRC-ODS (K) column.

4.2. Plant material

The plant material was collected in Zhongdian County of Yunnan Province, PR China, in August 2004 and identified by Prof. Zhong–Wen Lin. A voucher specimen (KIB-2004-085 Lin) was deposited in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, PR China.

4.3. Extraction and isolation

The powdered air-dried aerial parts of *I. phyllostachys* (4.0 kg) were extracted three times with Me_2CO at room temperature for 24 h and filtered. The filtrate was evaporated and the resulting residue partitioned between H_2O and EtOAc. The EtOAc fraction (102 g) was subjected to silica gel CC (100–200 mesh, 1.5 kg), eluting with $CHCl_3$/Me_2CO (1:0–0:1) to give 5 fractions L_1–L_5. Fraction L_1 was subjected to silica gel CC, eluting with petroleum ether/acetone (from 9:1 to 6:4) to yield 5,7-dihydroxy-4′-hydroxylflavone (**12**) (16 mg), oleanolic acid (**13**) (36 g), and daucosterol (**14**) (25 mg). Fractions L_2 and L_3 were submitted to repeated silica gel CC with cyclohexane/2-propanol (9:1) and followed by semipreparative and preparative HPLC to yield phyllostachysin D (**1**) (25 mg), phyllostachysin G (**4**) (6 mg) rabdoloxin A (**6**) (5 mg), rabdoloxin B (**7**) (10 mg), phyllostachysin F (**3**) (15 mg) and phyllostachysin H (**5**) (11 mg). In the same way, fraction L_4 yielded phyllostachysin E (**2**) (12 mg) rabdoinflexin B (**8**) (11 mg), amethystoidin A (**9**) (10 mg), rabdokunmin D (**10**) (6 mg) and macrocalyxin E (**11**) (8 mg).

4.4. Phyllostachysin D (*1*)

White amorphous powder: $[\alpha]_D^{19.1} + 106.9$ (*c* 0.184, C_5H_5N); UV (MeOH) $\lambda_{max}(\log \epsilon)$: 225 (3.92) nm; IR (KBr) ν_{max} cm^{-1}: 3448, 2932, 1722, 1643, 1464, 1395, 966, 932; EIMS (70 ev) *m*/*z* (rel. int.): 348 $[M]^+$ (2), 192 (12), 124 (60), 123 (100), 109 (61), 95 (29), 69 (51); HR-ESI-MS *m*/*z*: 371.1827 $[M+Na]^+$ (calcd. for $C_{20}H_{28}O_5Na$, 371.1834); ^{1}H NMR (pyridine-d_5, 500 MHz) δ 1.84 (1H, *m*, H-1α), 2.21 (1H, *m*, H-1β), 1.46 (1H, *m*, H-2α), 1.21 (1H, *m*, H-2β), 1.04 (2H, *m*, H-3), 1.05 (1H, *dd*, $J = 12.4$, 4.6 Hz, H-5β), 2.12 (2H, *m*, H-6), 4.86 (1H, *dd*, $J = 12.0$, 4.6 Hz, H-7β), 2.24 (1H, *s*, H-9β), 4.32 (1H, *d*, $J = 3.3$ Hz, H-12β), 3.78 (1H, *d*, $J = 2.0$ Hz, H-13α), 6.26 (1H, *br s*, H-14α), 6.43 (1H, *br s*, H-17a), 5.62 (1H, *br s*, H-17b), 0.81 (3H, *s*, Me-18), 0.80 (3H, *s*, Me-19), 1.63 (3H, *s*, Me-20); for ^{13}C NMR spectroscopic data, see Table 1.

4.5. Phyllostachysin E (*2*)

White amorphous powder: $[\alpha]_D^{19.4} - 6.9$ (*c* 0.304, C_5H_5N); UV (MeOH) $\lambda_{max}(\log \epsilon)$: 206 (3.82) nm; IR (KBr) ν_{max} cm^{-1}: 3383, 2926, 2869, 1462, 1442, 1083, 1007; EIMS (70 ev) *m*/*z* (rel. int.): 352 $[M]^+$ (2), 334 (3), 174 (55), 124 (35), 123 (100), 109 (54); HR-ESI-MS *m*/*z*: 375.2155 $[M+Na]^+$ (calcd. for $C_{20}H_{32}O_5Na$, 375.2147); ^{1}H NMR (pyridine-d_5, 500 MHz) δ 2.06 (1H, *m*, H-1α), 1.20 (1H, *m*, H-1β), 1.32 (2H, overlap, H-2), 1.32 (2H, overlap, H-3), 1.09 (1H, *d*, $J = 12.2$ Hz, H-5β), 2.06 (2H, *m*, H-6), 4.45 (1H, *br d*, $J = 11.3$ Hz, H-7β), 2.32 (1H, *br s*, H-9β), 4.38 (1H, *br s*, H-11α), 5.98 (1H, *d*, $J = 4.6$ Hz, H-12β), 3.41 (1H, *br s*, H-13α), 5.63 (1H, *br s*, H-14α), 4.62 (1H, *d*, $J = 2.7$ Hz, H-15α), 5.74 (1H, *br s*, H-17a), 5.44 (1H, *br s*, H-17b), 0.79 (3H, *s*, Me-18), 0.82 (3H, *s*, Me-19), 1.60 (3H, *s*, Me-20); for ^{13}C NMR spectroscopic data, see Table 1.

4.6. Phyllostachysin F (*3*)

White amorphous powder: $[\alpha]_D^{19.3} - 50.2$ (*c* 0.630, C_5H_5N); UV (MeOH) $\lambda_{max}(\log \epsilon)$: 233 (3.72) nm; IR (KBr) ν_{max} cm^{-1}: 3393, 2930, 2870, 2848, 1719, 1651, 1451, 1367, 1092, 1062; EIMS (70 ev) *m*/*z* (rel. int.): 334 $[M]^+$ (1), 316 (10), 192 (7), 124 (53), 123 (100), 109 (71); HR-ESI-MS *m*/*z*: 357.2050 $[M+Na]^+$ (calcd. for $C_{20}H_{30}O_4Na$, 357.2041); ^{1}H NMR (pyridine-d_5, 500 MHz) δ 1.79 (1H, *m*, H-1α), 1.51 (1H, *m*, H-1β), 1.64 (1H, *m*, H-2α), 2.00 (1H, *m*, H-2β), 1.29 (2H, *m*, H-3), 0.95 (1H, *br*

d, $J = 12.3$ Hz, H-5β), 2.15 (2H, *m*, H-6), 4.82 (1H, *dd*, $J = 11.9$, 3.5 Hz, H-7β), 1.91 (1H, *d*, $J = 4.9$ Hz, H-9β), 4.20 (1H, *br d*, $J = 3.98$ Hz, H-11α), 2.35 (1H, *m*, H-12α), 2.25 (1H, *m*, H-12β), 3.29 (1H, *br s*, H-13α), 5.19 (1H, *br s*, H-14α), 6.27 (1H, *br s*, H-17a), 5.40 (1H, *br s*, H-17b), 0.79 (3H, *s*, Me-18), 0.90 (3H, *s*, Me-19), 1.00 (3H, *s*, Me-20); for ^{13}C NMR spectroscopic data, see Table 1.

4.7. Phyllostachysin G (*4*)

White amorphous powder: $[\alpha]_D^{19.1} - 85.8$ (*c* 0.202, C_5H_5N); UV (MeOH) λ_{max}(log ϵ): 235 (3.78) nm; IR (KBr) ν_{max} cm^{-1}: 3411, 2934, 1720, 1703, 1650, 1448, 1113, 1060, 977, 917; EIMS (70 ev) *m*/*z* (rel. int.): 348 $[M]^+$ (5), 330 (11), 196 (100), 153 (51), 152 (70), 139 (92), 105 (34), 91 (50), 69 (40); HR-ESI-MS *m*/*z*: 371.1846 $[M+Na]^+$ (calcd. for $C_{20}H_{28}O_5Na$, 371.1834); ^{1}H NMR (pyridine-d_5, 500 MHz) δ 2.13 (2H, overlap, H-2), 1.62 (2H, *m*, H-3), 1.30 (1H, *dd*, $J = 11.3$, 3.9 Hz, H-5β), 2.13 (2H, overlap, H-6), 4.64 (1H, *dd*, $J = 11.2$, 4.8 Hz, H-7α), 2.60 (1H, *br s*, H-9β), 4.34 (1H, *br d*, $J = 4.5$ Hz, H-11α), 2.30 (2H, *m*, H-12), 3.30 (1H, *br s*, H-13α), 5.12 (1H, *br s*, H-14α), 6.29 (1H, *br s*, H-17a), 5.41 (1H, *br s*, H-17b), 0.73 (3H, *s*, Me-18), 0.96 (3H, *s*, Me-19), 1.38 (3H, *s*, Me-20); for ^{13}C NMR spectroscopic data, see Table 1.

4.8. Phyllostachysin H (*5*)

White amorphous powder: $[\alpha]_D^{19.1} - 57.2$ (C_5H_5N, *c* 0.510,); UV (MeOH) λ_{max}(log ϵ): 243.8 (3.79) nm; IR (KBr) ν_{max} cm^{-1}: 3443, 2948, 2873, 1717, 1647, 1260, 1081, 1063, 994; EIMS (70 ev) *m*/*z* (rel. int.): 334 $[M]^+$ (4), 316 (10), 174 (52), 162 (74), 137 (79), 121 (65), 109 (100), 91 (80), 81 (86), 69 (57); HR-ESI-MS *m*/*z*: 357.2047 $[M+Na]^+$ (calcd. for $C_{20}H_{30}O_4Na$, 357.2041); ^{1}H NMR (pyridine-d_5, 500 MHz) δ 3.72 (1H, *dd*, $J = 10.0$, 5.0 Hz, H-1β), 1.92 (1H, *ddd*, $J = 10.0$, 7.2, 3.5 Hz, H-2α), 1.75 (1H, *ddd*, $J = 13.5$, 5.0, 3.9 Hz, H-2β), 1.32 (1H, *ddd*, $J = 10.0$, 7.2, 3.9 Hz, H-3α), 1.20 (1H, *ddd*, $J = 13.5$, 10.0, 3.5 Hz, H-3β), 0.97 (1H, *br d*, $J = 10.9$ Hz, H-5β), 1.59 (2H, *m*, H-6), 2.65 (1H, *br d*, $J = 13.5$ Hz, H-7α), 2.05 (1H, *ddd*, $J = 13.5$, 9.3, 4.1 Hz, H-7β), 2.44 (1H, *br s*, H-9β), 6.01 (1H, *br s*, H-11α), 2.40 (2H, *m*, H-12), 3.35 (1H, *br s*, H-13α), 4.97 (1H, *br s*, H-14α), 6.21 (1H, *br s*, H-17a), 5.33 (1H, *br s*, H-17b), 0.78 (3H, *s*, Me-18), 0.78 (3H, *s*, Me-19), 1.35 (3H, *s*, Me-20); for ^{13}C NMR spectroscopic data, see Table 1.

4.9. Cytotoxicity experiments

Cytotoxicity evaluations were performed using the MTT method for the human tumor K562 cells. Briefly, 4×10^4/ml cells were added to each well (90 μl/well), and incubated with various concentrations of drugs (100, 30, 10, 3, 1, 0.3 μg/ml) or without drugs in three replicates for 48 h at 37 °C in a humidified atmosphere of 5% CO_2. After 48 h, 10 μl of methyl thiazol tetrazalium (MTT) solution (5 mg/ml) were added to each well, which were incubated for another 4 h. Then 10% SDS–5% isobutanol–0.012 M HCl was added to each well (100 μl/well). After 12 h at room temperature, the OD value of each well was recorded on Model680 reader at 570 nm.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2006.05.002.

References

Chen, Y.P., Sun, L.P., Sun, H.D., 1991. Diterpene constituents of *Rabdosia phyllostachys*. Acta Bot. Yunnan 13, 331–334.

Fujita, T., Sun, H.D., Takeda, Y., Minami, Y., Marunaka, T., Lin, Z.W., Xu, Y.L., 1985. Structure of phyllostachysin A: novel antineoplastic diterpenoid from *Rabdosia phyllostahys*. J. Chem. Soc., Chem. Commun., 1738–1739.

Han, Q.B., Jiang, B., Zhang, J.X., Niu, X.M., Sun, H.D., 2003. Two novel *ent*-kaurene diterpenoids from *Isodon rubescens*. Helv. Chim. Acta 86, 773–777.

Han, Q.B., Xiang, W., Li, R.T., Li, M.L., Li, S.W., Sun, H.D., 2004. Cytotoxic *ent*-kaurane diterpenoids from *Isodon rubescens* var. rubescens. Planta Med. 70, 269–272.

Hou, A.J., Yang, H., Jiang, B., Zhao, Q.S., Lin, Z.W., Sun, H.D., 2000. A new *ent*-kaurane diterpenoid from *Isodon phyllostachys*. Fitoterapia 71, 417–419.

Node, M., 1984. Research and development of new anticancer agent from *Isodon japonica* and *I. trichocarpa*. Suzuken Memorial Foundation 3, 112–118.

Sun, H.D., Xu, Y.L., Jiang, B., 2001. Diterpenoids from *Isodon* species. Sciences Press, Beijing, pp. 4–5.

Sun, H.D., Lin, Z.W., Shen, X.Y., Takeda, Y., Fujita, T., 1991. *ent*-Kaurene diterpenoids from *Rabdosia loxothyrsa*. Phytochemistry 30, 603–606.

Ternai, B., Markham, K.R., 1976. Carbon-13 NMR studies of flavonoids–I: flavones and flavonols. Tetrahedron 32, 565–569.

Wang, X.R., Wang, Z.Q., Dong, J.G., 1986. Chemical structure of macrocalyxin E. Acta Bot. Sin. 28, 415–418.

Wang, Z.Q., Node, M., Xu, F.M., Hu, H.P., Fuji, K., 1989. Terpenoids. LIV. The structures of rabdoinflexins A and B, new diterpenoids from *Rabdosia inflexa* (Thunb.). Hara. Chem. Pharm. Bull. 37, 2683–2686.

Wang, Z.Q., Wang, X.R., Dong, J.G., Xue, Z.W., Wang, X.W., 1984. Chemical structure of henryine A. Acta Bot. Sin. 26, 634–638.

Wu, S.H., Zhang, H.J., Lin, Z.W., Sun, H.D., 1993. Terpenoids from *Isodon grandifolia* var . *atuntzensis*. Phytochemistry 34, 1176–1178.

Zhang, H.J., Sun, H.D., 1989. Diterpenoids from *Rabdosia kunmingensis*. Phytochemistry 28, 3405–3409.

Leung, C.H., Grill, S.P., Lam, W., Han, Q.B., Sun, H.D., Cheng, Y.C., 2005. Novel mechanism of inhibition of nuclear factor-κB DNA-binding activity by diterpenoids isolated from *Isodon rubescens*. Mol. Pharmacol. 68, 286–297.