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ent-Abietane diterpenoids from *Isodon rubescens* var. *rubescens*

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Abstract

ent-Abietane diterpenoids, hebeiabinins A–F (**1**–**5**), together with seven known diterpenoids were isolated from leaves of *Isodon rubescens* var. *rubescens*. The structures of **1**–**5** were established on the basis of spectroscopic analyses, including application of 2D NMR spectroscopic techniques. The diterpenoids isolated were evaluated for the cytotoxicity against A549, HT-29, and K562 tumor cells. Compound **5** was the most active with IC_{50} value of 0.91 μM against A549 cells.

Keywords: *Isodon rubescens* var. *rubescens*; Labiatae; *ent*-Abietane; Hebeiabinin; Diterpenoid; Cytotoxicity

1. Introduction

The primary and ultimate attention of our group to *Isodon* (Labiatae) species is the various bioactivities, structural complexities, and interesting chemical diversity of their diterpenoids. Over the past 30 years, as part of a research for novel natural products as useful leads for discovery of therapeutic agents to treat cancer, more than 50 *Isodon* species of China were phytochemically investigated by our group with about 500 new diterpenoids (mainly *ent*-kauranoids) isolated and characterized (Sun et al., 2001). The leaves of *Isodon rubescens* var. *rubescens* (which is the most studied species and known in China by the name, "*donglingcao*") are still used by local people in Henan province for treatment of respiratory and gastrointestinal bacterial infections, inflammation, and cancer. In 1977, the standard extract (mainly containing oridonin, ponicidin, and rosemarinic acid) of this plant was successfully developed into a drug product used for treating sore throats and inflammation in the People's Republic of China (People's Health Press, 1977). Our previous phytochemical investigations on this plant showed that the structural types of the secondary metabolites changed with different ecological environments of habitat for this plant (Han et al., 2003, 2004a). In our ongoing search for bioactive diterpenoids from genus *Isodon*, a reinvestigation of the chemical constituents of *I. rubescens* var. *rubescens*, which was collected in different regions of China, led to isolation of five new *ent*-abietanoids, hebeiabinins A–F (**1**–**5**), along with other known compounds. The structural elucidation of these new *ent*-abietanoids and their cytotoxic evaluation are the subjects of this article.

2. Results and discussion

Analysis of the neutral part of the acetone extract from leaves of *I. rubescens* var. *rubescens* led to isolation of five

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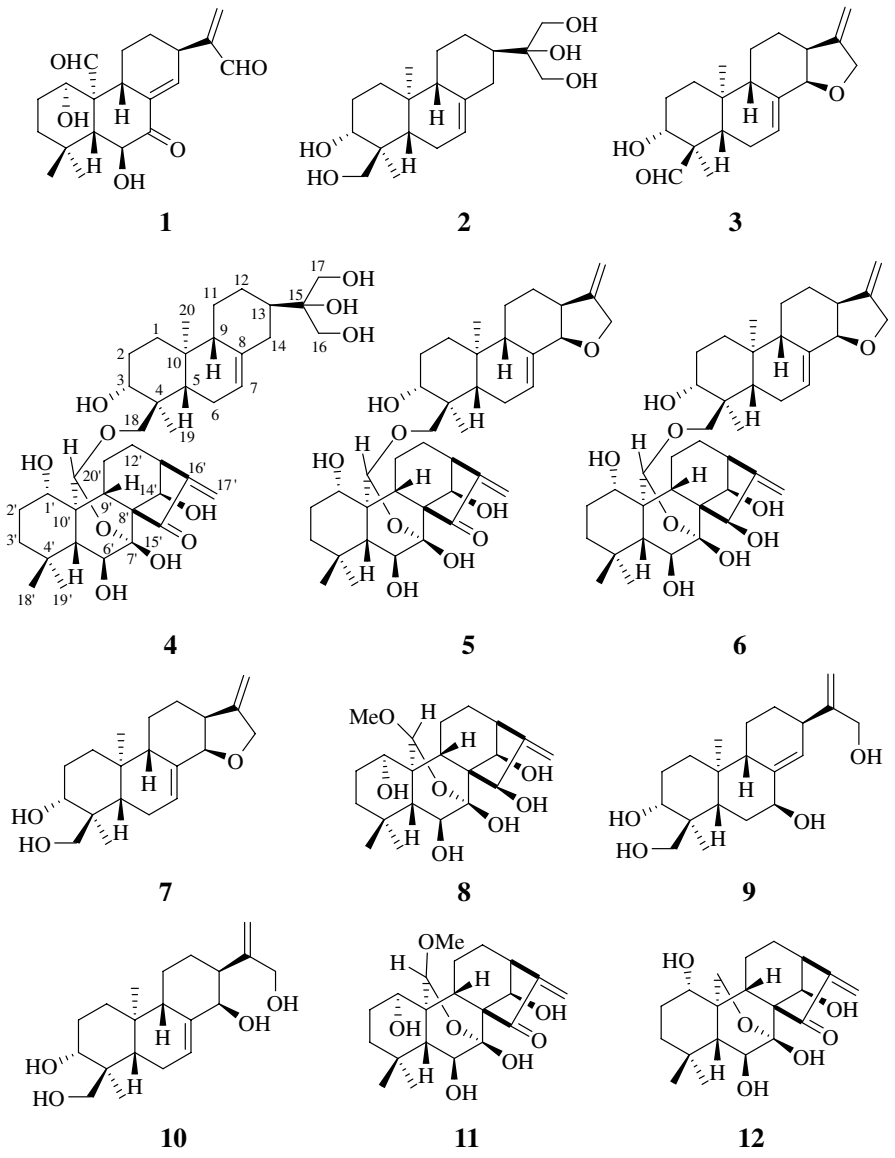
new abietanoids (**1**–**5**), with two of them being the dimeric diterpenoids (**4** and **5**). Together with these new natural products, the following known substances, rubescensin M (**6**) (Han et al., 2004b), rubescensin J (**7**) (Han et al., 2004b), rubescensin O (**8**) (Han et al., 2003a), rubescensin P (**9**) (Han et al., 2004b), rubescensin I (**10**) (Han et al., 2004b), rabdoternin F (**11**) (Takeda et al., 1994), and oridonin (**12**) (Fujita et al., 1970; Zhao et al., 1984) were also obtained.



Hebeiabinin A (**1**) was isolated as amorphous powder, and yielded a pseudomolecular ion peak in the positive HRESIMS spectrum at *m/z* 369.1685 $[M+Na]^+$, indicative of the molecular formula $C_{20}H_{26}O_5$ and equating to eight double bond equivalents. Immediately identifiable from the NMR spectroscopic data for **1** (Table 1) were resonances consistent with one trisubstituted double bond (δ_C 135.9, *s* and δ_C 140.4, *d*), as well as one 1,1-disubstituted double bond (δ_C 150.2, *s* and δ_C 134.5, *t*), two aldehyde carbons (δ_C 193.4, *d* and δ_C 206.4, *d*), and a ketone carbon (δ_C 200.2, *s*). In the absence of any other sp or sp^2 carbon, the gross structure of **1** must be tricyclic. Interpretation of the ^1H–^1H COSY and HMBC data readily suggested that **1** was an abietane diterpenoid. Observation of the HMBC correlations from H-1 (δ_H 3.65, *dd*, $J = 11.1$, 5.0 Hz) to C-5, C-9, C-20, and C-3, and from H-6 (δ_H 4.34, *d*, $J = 12.0$ Hz) to C-4, C-5, C-7, and C-10 permitted the assignment of hydroxyl groups at C-1 and C-6, respectively, while the HMBC correlations from H-20 (δ_H 10.05, *s*) to C-1, C-9, and C-10 led to the location of an aldehyde at C-20. The ketonic carbon was assigned as C-7 by the correlations from H-5, H-6, and H-14 to C-7 in the HMBC spectrum. Based on the analysis of the HMBC correlations of **1**, the olefinic bond conjugated with aldehydic group was located at C-13 (Fig. 1).

Table 1
^{1}H and ^{13}C NMR spectroscopic data δ (ppm) for compounds **1**–**3** (J in Hz)

No.	**1**		**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1α	–	77.1	1.79 overlapped	38.1	1.79 *br d* (13.5)	37.6
1β	3.65 *dd* (11.1, 5.0)	–	1.18 overlapped	–	1.09–1.15 *m*	–
2α	1.95–2.00 *m*	30.5	1.85 −1.95 *m*	27.9	1.84–1.89 *m*	27.3
2β	1.87–1.92 *m*	–	1.85–1.95 *m*	–	1.84–1.89 *m*	–
3α	1.54 overlapped	40.2	–	73.6	–	72.3
3β	1.45 *dt* (13.6, 3.5)	–	4.22 overlapped	–	4.20 *dd* (9.7, 5.6)	–
4	–	34.1	–	43.0	–	55.7
5	1.71 *d* (12.0)	56.0	1.90 overlapped	43.0	1.72 *dd* (12.1, 3.9)	41.5
6α	4.34 *d* (12.0)	73.1	2.02 *m*	23.4	1.50–1.54 *m*	25.0
6β	–	–	2.02 *m*	–	1.96–2.00 *m*	–
7	–	200.2	5.38 *br s*	120.3	5.75 *br d* (3.0)	128.6
8	–	135.9	–	138.1	–	135.6
9	2.61–2.65 *m*	48.6	1.76 overlapped	53.0	2.04 *br d* (11.2)	49.2
10	–	56.6	–	35.3	–	34.2
11α	2.65–2.70 *m*	27.5	1.78 overlapped	26.0	1.62–1.66 *m*	24.0
11β	1.56 overlapped	–	1.17 overlapped	–	0.99–1.04 *m*	–
12α	2.02–2.08 *m*	29.1	2.26 *br d* (12.1)	26.8	1.62 overlapped	29.0
12β	1.20–1.28 *m*	–	1.55 *q* like (12.0)	–	1.41 overlapped	–
13	3.43–3.47 *m*	35.0	2.12 *t* like (12.1)	42.3	2.38–2.42 *m*	46.0
14α	6.77 *br s*	140.4	2.80 *br d* (13.6)	35.9	4.14 *d* (3.8)	83.5
14β	–	–	2.43 *t* like (13.0)	–	–	–
15	–	152.0	–	75.5	–	154.7
16	9.53 *s*	193.4	4.23 overlapped	65.0	4.53, 4.24 2 *d* (13.3)	69.7
17a	6.25 *br s*	134.5	4.23 overlapped	65.0	5.00 *br s*	103.3
17 b	6.20 *br s*	–	4.23 overlapped	–	4.54 *br s*	–
18	1.16 *s*	33.2	4.11, 3.65 2 *d* (13.3)	67.6	9.46 *s*	206.5
19	0.95 *s*	21.1	1.14 *s*	13.1	1.40 *s*	9.5
20	10.05 *s*	206.4	0.89 *s*	15.9	0.78 *s*	15.0

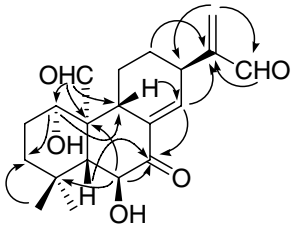


Fig. 1. Selected HMBC correlations for **1**.

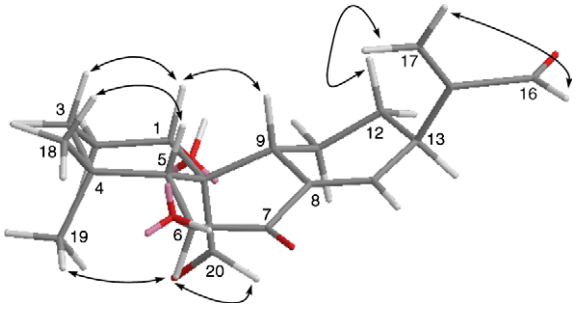
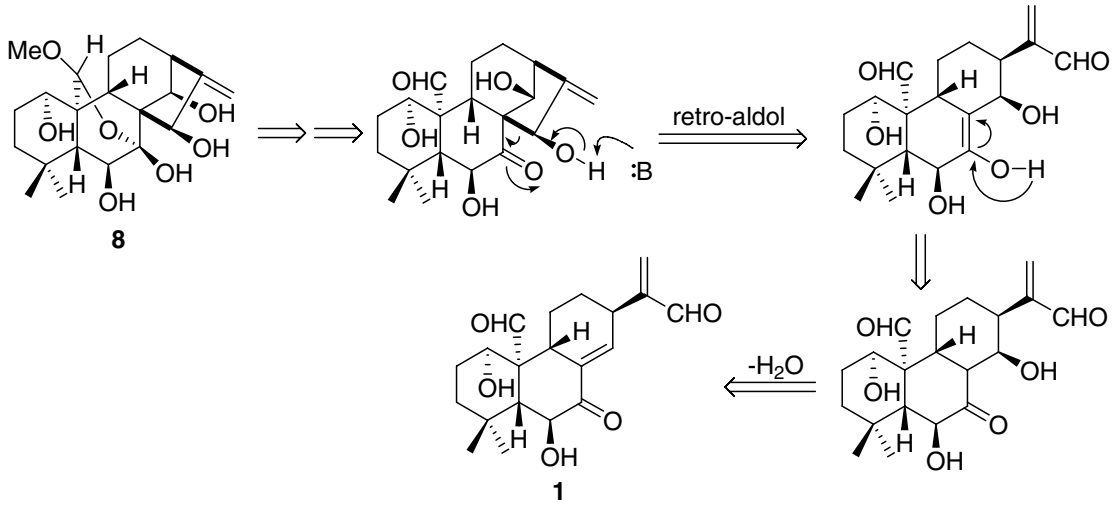


Fig. 2. Selected ROESY correlations for **1**.

The relative stereochemistry of **1** was determined through analysis of proton coupling constants and correlations observed in ROESY spectrum as shown in a computer-generated 3D drawing (Fig. 2). The large coupling constant between H-5 and H-6 ($J = 12.0$ Hz) suggested that both protons were axial, which was further confirmed by the intense ROESY correlations from H-6 to H_3-19 and H-20. In addition, the observation of ROESY correlations from H-1 to H-3β and H-9β placed them on the same face of the molecule. As depicted in Fig. 2, a ROESY correlation between H-17b and H-12β suggested that H-13 had an α-orientation. Although the absolute stereochemistry of **1** remained unassigned, the close biogenetic relationship of **1**–**8**, as shown in Scheme 1, might indicate that the absolute stereochemistry of **1** was the same as that of **8**. Considering that all the *ent*-kauranoids isolated from genus *Isodon* possess an *ent*-configuration, hebeiabinin A (**1**) was presumed to be an *ent*-abietanoid. Accordingly, the structure of **1** is proposed as 1α,6β-dihydroxy-7,17,20-trioxo-*ent*-abieta-8(14),15(17)-diene.

Hebeiabinin B (**2**) was obtained as amorphous solid and had the molecular formula, $C_{20}H_{34}O_5$, as deduced from its HRESIMS ($[M+Na]^+$ m/z 377.2299). Its IR, MS, and NMR spectroscopic data suggested **2** to be an *ent*-abietanoid, with one trisubstituted double bond and five oxygenated carbons. A careful analysis of the 2D NMR spectroscopic data and comparison with that of rubescensin I (**10**) led to the conclusion that C-3, C-15, C-16, C-17, and C-18 were each substituted by a hydroxyl group, and that the double bond was assigned to C-7 and C-8 on the basis of the HMBC correlations from H-7 to C-6,



Scheme 1. Proposed biogenetic pathway to account for formation of **1**.

C-5, and C-9. Moreover, because of the ROESY correlations from H-3 to H_2-18, the hydroxyl group at C-3 was determined to be in an α-orientation. The large coupling constant between H-13 and H-12β ($J = 13.0$ Hz) indicated that both protons were axial, and thus the C-15 carbon was placed at the β-position of ring C. Accordingly, the structure of **2** was assigned to be 3α,15,16,17,18-pentahydroxy-*ent*-abieta-7-ene.

Hebeiabinin C (**3**) was assigned the molecular formula, $C_{20}H_{28}O_3$, as deduced from the positive HRESIMS (*m*/*z* 339.1937 $[M+Na]^+$). Comparison of the spectroscopic data of **3** with those of **7** revealed that they were quite similar, except for the moiety at C-18. Observation of the presence of an aldehyde group (δ_C 206.5, *d*), and the absence of an oxymethylene carbon in the ^{13}C NMR spectrum of **7**, showed that an aldehyde group at the C-18 position was evident for **3** instead of a hydroxyl group at the same position in **7**. Thus, reduction of **3** with $NaBH_4$ led to the corresponding derivative, whose spectroscopic data were coincident with those of **7**. Therefore, compound **3** was unambiguously determined as 3α-hydroxy-14,16-epoxy-*ent*-abieta-7,15(17)-diene-18-al.

Hebeiabinin D (**4**) was obtained as amorphous solid, the IR spectrum of which showed a hydroxyl absorption (3418 cm^{-1}) and a ketone conjugated with an *exo*-methylene functionality (1709 and 1642 cm^{-1}). Its molecular formula was established to be $C_{40}H_{60}O_{11}$ by HRESIMS analysis (*m*/*z* 739.4025 $[M+Na]^+$). Its ^{13}C NMR spectrum showed 40 carbon signals, which indicated the presence of two diterpene units. The gross structure of **4** was elucidated by careful comparison with the NMR spectroscopic data of **2** and **11**. Each pair of these ^1H and ^{13}C NMR spectroscopic signals of **1** (Table 1) seemed to be due to those halves (parts **4a** and **4b**, Fig. 3). In part **4a**, the close similarity of the NMR spectroscopic data to those of known compound **11** suggested a similar substructure. On the other hand, comparison of the remaining carbon and proton NMR resonances of **4** with those of **2** indicated the

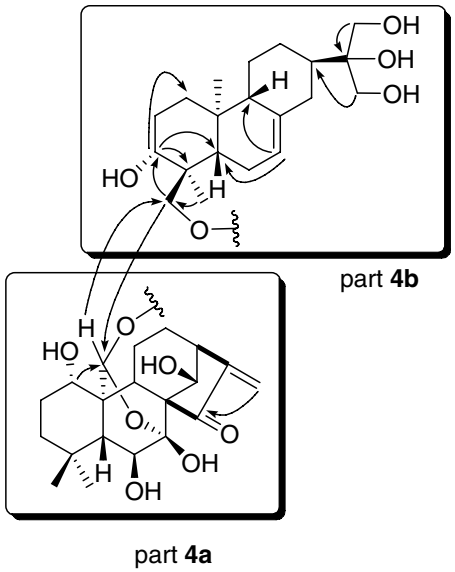


Fig. 3. Selected HMBC correlations for **4**.

gross structure of part **4b** (Fig. 3). The connection between parts **4a** and **4b** was provided by the downfield chemical shift of C-18 from δ_C 67.6 in hebeiabinin B (**2**) to δ_C 71.9 in **4**, and HMBC correlations from H_2-18 to C-20′, and from H-20′ to C-18 (Fig. 3), giving rise to the connectivity of C-18 to C-20′ through an ether bond. The relative configuration of the molecule was deduced from its analogy with **2** and **11**, and the correlations observed in the ROESY spectrum. The configuration of C-20′ was deduced to be *S* on the basis of the ROESY correlations from H-20′ to H-6′ and H_3-19′. All of the other fragments of **4** were in complete agreement with the stereochemistry reported for the same regions in **2** and **11**, respectively.

Compound **5** exhibited the molecular formula $C_{40}H_{56}O_9$, as deduced from its HRESIMS ($[M+Na]^+$, *m*/*z* 681.3980). The IR spectrum showed absorption bands at ν_{max} 3420 cm^{-1}, indicative of hydroxyl groups, and at

1705 and 1641 cm^{-1}, which could be assigned to an α,β-unsaturated carbonyl group. This assignment was supported by the ^{13}C NMR resonance at δ_C 210.2 (*s*), δ_C 153.4 (*s*) and δ_C 119.0 (*t*). Compared with those of **5** and **6**, the ^1H NMR spectrum of **5** revealed one major difference, the lack of the signal due to the H-15′. Furthermore, the chemical shift observed for C-15′ appeared in **5** at δ_C 210.2 (*s*), which together with the HMBC correlations observed from H_2-17′, H-14′, and H-13′ to C-15′, confirmed the presence of an α,β-unsaturated carbonyl group. Therefore, structure **5** is proposed to be the dimeric diterpenoid, named hebeiabinin E.

All diterpenoids were evaluated for their cytotoxicity against the human A549, HT-29, and K562 cells. As determined by a sulforhodamine B (SRB) assay (Monks et al., 1991), **5** demonstrated significant inhibitory activity against A549 and HT-29 cells with IC_{50} values of 0.91 and 1.81 μM, respectively. Compounds **1**, **4**, **11**, and **12** were less active (Table 4), whereas compounds **2**, **3**, and **6**–**10** were completely inactive with IC_{50} values of >100 μM.

3. Conclusion

The genus *Isodon* of the family Labiatae has been known to be rich in diterpenoids (mainly *ent*-kauranoids), with more than 600 structures being identified from 75 plant species (Sun et al., 2001). *I. rubescens* var. *rubescens* is the most studied species and known in China by the name, "*donglingcao*". Up to the present, more than 50 diterpenoids have been identified from it. Interesting, we found that the second metabolites of this plant are mainly *ent*-kauranoids and *ent*-abietanoids. These two types of diterpenoids are clearly biosynthetically related, in which *ent*-abietanoids may be biosynthesized from *ent*-kauranoids. For example, compound **1** is obvious biosynthesised from a 7,20-epoxy-*ent*-kauranoid by a retro-aldol reaction leading to the cleavage of the C-8,15 bond and loss of H_2O to give the C-8,14 double bond. Recently, the C-8,15 bond cleavage of *ent*-kauranoids under Mitsunobu (Aoyagi et al., 2004) or basic conditions (Xu and Wang, 2005) to form *ent*-abietanoids has been investigated.

4. Experimental

4.1. General

Melting points were obtained on an XRC-1 apparatus and were uncorrected. Optical rotations were carried out on a Perkin-Elmer model 241 polarimeter. IR spectrometer were measured in a Bio-Rad FTS-135 spectrometer with KBr pellets. MS were recorded on a VG Auto spec-3000 spectrometer or on a Finnigan MAT 90 instrument. 1D and 2D NMR spectra were measured on either a Bruker AM-400 or a Bruker DRX-500 instrument with TMS as internal standard. Semipreparative HPLC were performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} (9.4 mm × 25 cm column). CC was performed either on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), silica gel H (10–40μm; Qingdao Marine Chemical Inc), or Lichroprep RP-18 gel (40–63μm; Merck, Darmstadt, Germany). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

4.2. Plant material

The leaves of *I. rubescens* var. *rubescens* were collected in Hebei Province, People's Republic of China, in September 2004. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB04101811) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences.

4.3. Extraction and isolation

Air-dried and powdered leaves of *I. rubescens* var. *rubescens* (3.0 kg) were extracted with acetone/H_2O (7:3, 20 L × 3, each 2 days) at room temperature. After evaporating the solvents in vacuo at 45 °C, a residue (320 g) was obtained, which was dissolved in H_2O (2.5 L) and extracted successively with petroleum ether (1 L × 2) and EtOAc (2 L × 2). The EtOAc extract (120 g) was applied to a MCI-gel CHP 20 P column (eluted with CH_3OH–H_2O/9:1, then CH_3OH). The CH_3OH–H_2O (9:1) fraction (105 g) was subjected to silica gel CC (200–300 mesh, 1.0 kg), eluting with $CHCl_3$–Me_2CO (from 1:0 to 0:1) to afford fractions A–G. Fraction C (6.5 g) was applied to a silica gel column eluted in a step gradient manner with $CHCl_3$–Me_2CO (from 60:1 to 20:1), to give compounds **7** (25 mg) and **3** (7 mg). Fraction D (15.6 g) was subjected to RP-18 chromatograph eluted with a CH_3OH–H_2O (30%–100%) gradient system to afford four main fractions D1–D4. Subfraction D1 (0.4 g) was applied to silica gel CC, eluted with $CHCl_3$–Me_2CO (25:1), to give compound **1** (11 mg). Compound **12** (4.3 g) was obtained from D2 (9.2 g) by recrystallization from CH_3OH. The remainder of D2 (4.8 g) was applied to a silica gel column using $CHCl_3$–Me_2CO (15:1) as solvent, and finally purified by semipreparative HPLC (CH_3OH–CH_3CN–H_2O, 60:5:35) to yield compounds **9** (4 mg) and **11** (55 mg). Separation of fraction D3 (1.1 g) by silica gel CC eluted with $CHCl_3$–Me_2CO (10:1) yielded compound **10** (25 mg). Compounds **5** (8 mg) and **6** (5 mg) were obtained from subfraction D4 (1.2 g) by repeated silica gel CC eluted with $CHCl_3$–Me_2CO (8:1). Fraction E (5.5 g) was divided into subfractions E1–E4 by passage over a RP-18 column, which was eluted with CH_3OH–H_2O (from 30% to 100%). Compound **2** (56 mg) was obtained from E1 (1.2 g) by silica gel CC, which was eluted with $CHCl_3$–Me_2CO (4:1). Subfraction E3 (0.6 g) was subjected to silica gel CC using $CHCl_3$–isopropyl alcohol (15:1) as eluant to

give **8** (15 mg). E4 (35 mg) was finally purified by semipreparative HPLC (CH_3OH–H_2O, 80:20) to afford compound **4** (6 mg).

4.3.1. Hebeiabinin A (*1*)

Amorphous powder; $[\alpha]_D^{19}$ − 8.41 (*c* 0.15, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3400, 2931, 1709, 1688, 1622, 1457, 1370, 1072, 982; For ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) spectra, see Table 1; HRESIMS (positive ion) *m/z* 369.1685 (calc. for $C_{20}H_{26}O_5Na$ $[M+Na]^+$, 369.1677).

4.3.2. Hebeiabinin B (*2*)

Amorphous solid; m.p. 181–183 °C; $[\alpha]_D^{19}$ − 34.8 (*c* 0.23, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3425, 2933, 1632, 1384, 1053, 1036, 1044, 989; For ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) spectra, see Table 1; HRESIMS (positive ion) *m/z* 377.2299 (calc. for $C_{20}H_{34}O_5Na$ $[M+Na]^+$, 377.2303).

4.3.3. Hebeiabinin C (*3*)

Colorless needles; m.p. 190–192 °C; $[\alpha]_D^{19}$ − 46.3 (*c* 0.30, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3409, 2943, 1729, 1639, 1436, 1015, 898; For ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) spectra, see Table 1; HRESIMS (positive ion) *m/z* 339.1937 (calc. for $C_{20}H_{28}O_3Na$ $[M+Na]^+$, 339.1936).

4.3.4. Hebeiabinin D (*4*)

Amorphous powder; $[\alpha]_D^{19}$ − 37.4 (*c* 0.14, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3418, 2934, 1709, 1642, 1451, 1092, 988; For ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz), see Tables 2 and 3; HRESIMS (positive ion) *m/z* 739.4025 (calc. for $C_{40}H_{60}O_{11}Na$ $[M+Na]^+$, 739.4033).

4.3.5. Hebeiabinin E (*5*)

Amorphous powder; $[\alpha]_D^{19}$ − 5.9 (*c* 0.21, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3420, 2933, 1705, 1641, 1456, 1089, 1020, 978; For ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz), see Tables 2 and 3; HRESIMS (positive ion) *m/z* 681.3980 (calc. for $C_{40}H_{56}O_9Na$ $[M+Na]^+$, 681.4002).

4.3.6. Reduction of hebeiabinin C (*3*)

Hebeiabinin C (**3**, 2.0 mg) was added to MeOH (2 mL) and $NaBH_4$ (2.0 mg), with the whole stirred and kept at room temperature for 30 min. Me_2CO (2 mL) was then added for 10 min, with the solvent then evaporated and the residue subjected to RP-18 column eluted with CH_3OH–H_2O (0:1 and 1:0) as eluant to give rubescensin J (**7**) (1.8 mg).

4.4. Cytotoxicity assay

Cytotoxicity of compounds against suspended tumor cells was determined by trypan blue exclusion method and against adherent cells was determined by sulforhodamine B (SRB) assay. Cells were plated in 96-well plate 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. After compound treatment, cells were counted (suspended cells) or fixed and stained with SRB (adherent cells) as described in literature (Monks et al., 1991).

Table 2
^1H NMR spectroscopic data (ppm) for compounds **4** and **5** (*J* in Hz)

No.	4	5	No.	4	5
1α	1.76 *br d* (13.5)	1.76 *br d* (13.5)	1′	–	–
1β	1.13 overlapped	1.08–1.12 *m*	–	3.54–3.57 *m*	3.54–3.57 *m*
2α	1.80–1.87 *m*	1.82–1.89 *m*	2′	1.86 overlapped	1.82–1.89 *m*
2β	1.80–1.87 *m*	1.82–1.89 *m*	–	1.48–1.52 *m*	1.82–1.89 *m*
3α	–	–	3′	1.29–1.37 *m*	1.30–1.37 *m*
3β	3.90 *dd* (11.0, 3.7)	3.90 overlapped	–	1.29–1.37 *m*	1.30–1.37 *m*
5	1.87 overlapped	1.98 overlapped	5′	1.41 *d* (6.8)	1.42 *d* (6.8)
6α	1.87–1.92 *m*	2.01 overlapped	6′	4.13 *dd* (10.4, 6.8)	4.14 overlapped
6β	1.87–1.92 *m*	2.34–2.40 *m*	–	–	–
7	5.65 *br d* (3.6)	5.93 *br d* (4.0)	7′	–	–
9	1.85 overlapped	2.00 overlapped	9′	1.97 *dd* (13.1, 6.5)	2.01 overlapped
11α	1.80 overlapped	1.61 overlapped	11′	2.81–2.85 *m*	2.83–2.87 *m*
11β	1.14 overlapped	1.04 overlapped	–	2.22 overlapped	2.23 overlapped
12α	2.28 *br d* (13.5)	1.62 overlapped	12′	2.44–2.50 *m*	2.71–2.77 *m*
12	2.27 *t* like (13.0)	1.36 overlapped	–	1.58–1.66 *m*	1.50–1.56 *m*
13	2.10–2.14 *m*	2.34–2.42 *m*	13′	3.27 *br d* (9.8)	3.43 *br d* (9.6)
14α	2.90 *br d* (13.5)	4.08 *br d* (4.0)	14′	5.44 *br s*	5.41 *br s*
14β	2.57 *t* like (13.0)	–	–	–	–
16	4.23 overlapped	4.45, 4.14 2 *d* (13.3)	16′	–	–
17	4.23 overlapped	5.01, 4.82 2 *s*	17′	6.20, 5.35 *2br s*	6.25, 5.52 *2br s*
18	3.86,4.03 2 *d* (9.2)	3.87–3.94 2 *H*	18′	1.23 *s*	1.25 *s*
19	0.92 *s*	1.01 *s*	19′	1.00 *s*	1.02 *s*
20	0.82 *s*	0.81 *s*	20′	5.77 *br s*	5.74 *br s*

Table 3
^{13}C NMR spectroscopic data (ppm) for compounds **4** and **5**

No.	4	5	No.	4	5
1	38.1	37.9	1′	75.4	75.5
2	28.0	27.9	2′	31.0	31.1
3	72.1	71.8	3′	39.4	39.5
4	42.3	42.4	4′	34.1	34.1
5	43.2	42.8	5′	59.8	59.8
6	23.2	23.5	6′	74.6	74.6
7	119.7	130.1	7′	99.7	99.7
8	138.6	134.4	8′	62.1	62.0
9	53.7	50.4	9′	53.3	53.6
10	35.3	35.0	10′	43.9	43.8
11	26.1	23.8	11′	23.2	23.5
12	26.7	29.1	12′	31.3	31.4
13	42.2	45.6	13′	44.3	44.2
14	36.1	83.6	14′	73.6	73.8
15	75.3	154.1	15′	210.2	210.2
16	64.9	69.4	16′	153.2	153.4
17	64.9	103.7	17′	119.0	119.0
18	71.9	71.9	18′	33.5	33.6
19	13.2	12.8	19′	22.3	22.3
20	16.0	15.2	20′	102.3	102.0

Table 4
Cytotoxicity data of compounds **1**, **4**, **5**, **11**, and **12** with IC_{50} values (μM)[a]

	A549	HT-29	K562
1	53.21	15.88	42.71
4	5.05	5.03	7.66
5	0.91	1.81	4.07
11	2.16	5.81	6.84
12	9.54	12.31	7.86

[a] Amrubicin hydrochloride (positive control): $IC_{50} = 0.82$ (A549), 4.36 (HT-29), and 1.26 (K562), respectively.

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