New *ent*-Abietane and *ent*-Kaurane Diterpenoids from *Isodon rubescens*

Xu Liu,[a,b] Rui Zhan,[a] Wei-Guang Wang,[a] Xue Du,[a] Xiao-Nian Li,[a] Jian-Hong Yang,[a] Peng Zhang,[b] Yan Li,[a] Jian-Xin Pu,[a] Ji-Zhou Wu,*[,b] and Han-Dong Sun*[,a]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences; Kunming 650204, P.R. China: and* [b] *Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, Tongji School of Pharmacy, Huazhong University of Science and Technology; Wuhan 430030, Hubei, P.R. China.*

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Five new *ent*-abietane diterpenoids, *ent*-abierubesins A–E (1–5), and two new *ent*-kauranoids, hubeirubesins A and B (6, 7), together with three known diterpenoids (8–10), were isolated from the aerial parts of *Isodon rubescens*. Their structures were identified by means of extensive spectroscopic analysis, and the absolute stereochemistry of 1 was determined by single-crystal X-ray diffraction experiment.

Key words *Isodon rubescens*; *ent*-abietane; *ent*-abierubesin; *ent*-kaurane; hubeirubesin

As an important class of diterpenoids, *ent*-abietanoids are not so widely spread and structurally diverse as their enantiomers, abietanoids. Among *ent*-abietanes, 12,17-olide-type ones are in a majority and isolated from plants of Euphorbiaceae and Acanthaceae families.[1)] However, another type of *ent*-abietanes without 12,17-olide moieties have been obtained from the genus *Isodon* of Lamiaceae family.[2,3)] Our previous investigations on chemical constituents of *Isodon rubescens* (Hemsl.) Hara yielded mainly *ent*-kauranoids.[4–6)] However, five new *ent*-abietanoids, *ent*-abierubesins A–E (**1**–**5**), and two new *ent*-kauranoids, hubeirubesins A and B (**6**, **7**), together with another three known diterpenoids (**8**–**10**), were isolated from this species in our present research. Although belonging to *ent*-abietanes, compound **5** was quite different from other *ent*-abietanes (**1**–**4**, **8**, **9**) in functional groups of A- and B-rings. In contrast, **5** exhibited the same structural characteristics (even including the configuration of C-16) as the known *ent*-kauranoid (**10**) so that **5** might be assumed as an 8,15-*seco* product of **10**. Current paper describes the isolation, structure elucidation, and cytotoxic evaluation of selected compounds.

Results and Discussion

The aerial parts of *I. rubescens* (10 kg) were extracted with 90% aqueous EtOH at room temperature and then the extract was partitioned between EtOAc and H_2O. Repeated chromatography of the EtOAc soluble portion (550 g) yielded five new (**1**–**5**) and two known (**8**, **9**) *ent*-abietane diterpenoids, together with two new (**6**, **7**) and one known (**10**) *ent*-kaurane diterpenoids (Fig. 1). By comparing the spectroscopic data with literature values, three known compounds (**8**–**10**) were identified as hebeiabinin B (**8**),[7)] *ent*-abienervonin C (**9**),[8)] and rubescensin G (**10**),[9)] respectively.

ent-Abierubesin A (**1**) was obtained as colorless pieces in MeOH. The molecular formula was determined by positive high resolution-electrospray ionization mass spectrometry (HR-ESI-MS) as $C_{20}H_{32}O_5$ (*m*/*z* 375.2157 $[M+Na]^+$), indicating five degrees of unsaturation. In ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1**, signals of a ketone carbonyl, a trisubstituted olefine, nine methylenes (including three oxygenated ones), three methines, one oxygenated and two nonoxygenated quaternary carbons, and two methyl groups were present (Tables 1, 3). Compound **1** retained three degrees of unsaturation except above-mentioned olefinic and carbonyl groups, and lacked one nonoxygenated quaternary carbon relative to the classical *ent*-kaurane skeleton, suggesting **1** to be a tricyclic diterpenoid. Comparison of NMR spectra of **1** and the known tricyclic *ent*-abietanoid, hebeiabinin B (**8**),[7)] revealed great similarities except for one carbonyl group in **1** instead of the oxymethine group at C-3 in **8**. This difference was confirmed by heteronuclear multiple bond connectivity (HMBC) correlations from H-5 (δ_H 2.54), H_2-18 (δ_H 4.17, 3.51), and H_3-19 (δ_H 1.01) to C-3 (δ_C 216.2).

Based on the assumption of the same configurations of **1** and **8**, rotating frame Overhauser effect spectroscopy (ROESY) correlations of H-5/H-9/H_2-18 and H_3-19/H_3-20, together with correlations of both H-13 and H_3-20 with H-11α, suggested that H-5/H-9/H_2-18 were all in β-orientations on the contrary to α-orientations of H_3-19/H_3-20/H-13 (Fig. 2). **1** was crystallized in MeOH to afford a crystal of the orthorhombic space group $P2_12_12_1$ which was analyzed by X-ray crystallography (CCDC 894929, Fig. 3). Bearing on five oxygen atoms in the molecular, the final refinement on Cu$K\alpha$ data resulted in a Flack parameter of 0.01(12),[10)] allowing an unambiguous assignment of the absolute configuration of **1** as an *ent*-abietanoid with charity centers of 4*S*, 5*S*, 9*R*, 10*S*, and 13*R*. Thus, compound **1** was elucidated as 15,16,17,18-tetrahydroxy-*ent*-abiet-7-en-3-one.

The molecular formula of *ent*-abierubesin B (**2**) was $C_{20}H_{34}O_5$ with four degrees of unsaturation, judging from the pseudo molecular ion peak at 377.2297 ($[M+Na]^+$, Calcd for $C_{20}H_{34}O_5Na$: 377.2303) in its HR-ESI-MS spectrum. 1D-NMR data of **1** were quite similar to those of **8**. Based on heteronuclear singular quantum correlation (HSQC) and HMBC experiments, two compounds were concluded to share the same planar structure. Differently, C-3 (δ_C 81.1) and C-5 (δ_C 51.8) in **2**, comparing with the same carbons in **8**, shifted downfield by $\Delta\delta_C$ 7.5 and 8.8, respectively. In stereochemistry, key ROESY correlations of H_2-19/H_3-20 indicated the same α-orientations of CH_2OH-19 and CH_3-20. So compound **2** was confirmed as the C-4 epimer of **8**, namely 3α,15,16,17,19-pentahydroxy-*ent*-abiet-7-ene.

ent-Abierubesins C (**3**) and D (**4**) were both obtained as white amorphous powder, with the same molecular formula

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: ywjz@mails.tjmu.edu.cn; hdsun@mail.kib.ac.cn

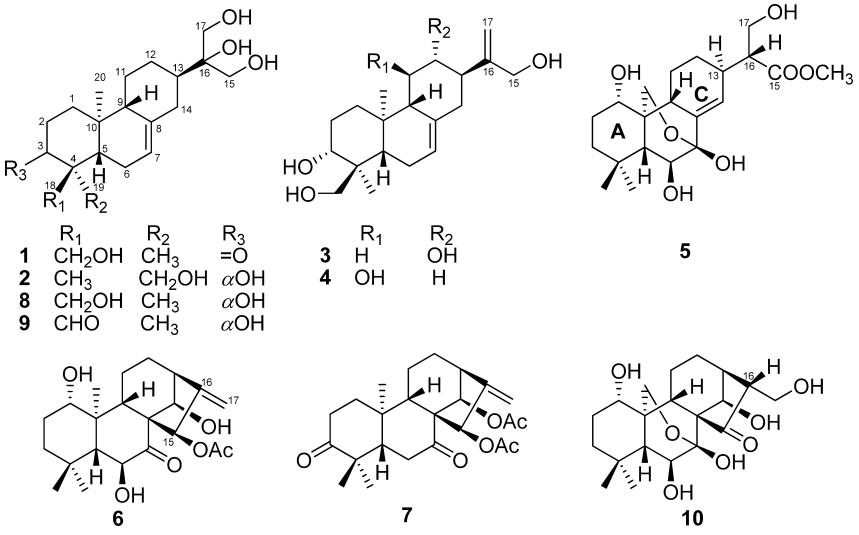

Fig. 1. Structures of Compounds **1**–**10**

of $C_{20}H_{32}O_4$ indicated by positive HR-ESI-MS, *m/z* 359.2193 and 359.2195 ($[M+Na]^+$, Calcd for $C_{20}H_{32}O_4Na$: 359.2198), respectively. Comparing ^{13}C-NMR and DEPT spectra of **3** and **4** with those of laxiflorin O,[11] we found just one more hydroxy group substituting at C-ring in each of **3** and **4** than in laxiflorin O. The position and orientation of additional hydroxy groups of **3** and **4** was confirmed by selected HMBC, ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), and ROESY correlations. Concretely in **3**, HMBC correlations of H-9, H_2-11, H-13, and H_2-14 with C-12 (δ_C 74.0) and the ROESY correlation of H-12/H-9β confirmed an α-oriented hydroxy group at C-12 in **3**, in accordance with a spin system of H-9/H_2-11/H-12/H-13/H_2-14 resulting from ^{1}H–^{1}H COSY correlations. In a similar method, the HO-11β in compound **4** was confirmed on the basis of HMBC correlations from H-9, H_2-12, H-13, and H_3-20 (long-range ^4J correlation) to C-11 (δ_C 72.4) and key ROESY correlations of H-11/H-13α/H_3-20, corresponding to the spin system of H-9/H-11/H_2-12/H-13/H_2-14. Accordingly, compounds **3** and **4** were established as 3α,12α,15,18-tetrahydroxy-*ent*-abiet-7,16-diene and 3α,11β,15,18-tetrahydroxy-*ent*-abiet-7,16-diene, respectively.

ent-Abierubesin E (**5**) gave a pseudo molecular ion peak at *m/z* 419.2052 in its HR-ESI-MS spectrum, corresponding to a molecular formula of $C_{21}H_{32}O_7$. The ^{13}C-NMR and DEPT data exhibited an ester carbonyl, a trisubstituted C=C group, a hemiketal group, two OCH groups, two OCH_2 groups, a OCH_3 group, two nonoxygenated quarternary carbons, and other signals. Found by comparing the ^{13}C-NMR data of **5** and rubescensin G (**10**), two compounds took similar A- and B-rings, just like a common 7,20-epoxy-*ent*-karanoid. Differently, **5** showed the presence of an ester carbonyl and a trisubstituted C=C group instead of a ketone group at C-15, a quarternary carbon at C-8 and an oxymethine at C-14 in **10**. The different substructure of **5** was easily deduced as two joint spin systems, namely H-9/H_2-11/H_2-12/H-13/H-14 and H-13/H-16/H_2-17, due to HSQC and ^{1}H–^{1}H COSY spectra. In addition, HMBC correlations from hydrogens of OCH_3 and CH-16 groups to the ester carbonyl (C-15) and from H-9 to C-14 implied an methyl ester at C-15 and a closed C-ring.

The relative stereochemistry of compound **5** except for C-16 could be easily judged from the same ROESY correlations as **10**. After the energy-minimized conformation of **5** was carefully observed in Chem3D software, C-ring was found to be a half-chair conformation unlike the boat one of **10** (C-8/-9/-12/-13/-14 almost in one plane in Fig. 4). This change arose on account of the coplanar double bond in C-ring. To minimize steric hindrance, the C-13/C-16 bond tended to be staggered conformation so as to make H-13/H-16 in anti- or gauche-orientation. According to the Karplus equation[12] and its application in elucidating the configuration of natural molecules,[13] the large coupling constant ($^3J_{H\text{-}13/H\text{-}16}$=8.8 Hz) and no ROESY correlation of H-13/H-16 indicated two anti-oriented vicinal protons. Furthermore, obvious ROESY correlations of H-13α/H_2-17, H-12α/H-17, H-12β/H-16, H-14/H-16, and H-14/$C\underline{H}_3O$–C-15, as visualized in the Newman projection (Fig. 4), helped to locate C-14/C-15 and C-12/C-17 at two opposite gauche-orientations, respectively. Consequently, the relative configuration of C-16 was deduced as *R**, in agreement with that of the same carbon in **10**. Compound **5** was accordingly established as methyl (16*R**)-1α,6β,7,17-tetrahydroxy-7α,20-epoxy-*ent*-abiet-8(14)-en-15-oate.

Hubeirubesin A (**6**) exhibited pseudo molecular ion peak at *m/z* 415.2089 ($[M+Na]^+$, Calcd for $C_{22}H_{32}O_6Na$: 415.2096) in HR-ESI-MS spectrum, indicating its molecular formula as $C_{22}H_{32}O_6$. Characteristic ^{13}C-NMR and DEPT data (Tables 2, 3) implied that **6** might bear an *ent*-kaurane skeleton with an acetoxy group. On the basis of the maximum absorption wavelength at 207 nm in UV spectrum and the similarities of ^{13}C-NMR spectra of **6** and isodoglutinosin A,[14] it was guessed that the acetoxymethine (δ_H 7.51; δ_C 74.8) in **6** replaced the ketone carbonyl at C-15 in isodoglutinosin A, leaving the only unconjugated ketone group (δ_C 210.7) at C-7 in **6**.[15] HMBC correlations of H-15 with C-7, C-8, C-9, C-16, C-17 and the carbonyl at δ_C 171.0, and of H-5 (δ_H 1.52) and H-6 (δ_H 5.23) with C-7 proved above guess (Fig. 5). In the ROESY spectrum, correlations of H-6/H_3-19/H_3-20, H-14/H_3-20, H-1/H-5β/H-9β and $C\underline{H}_3$COO-15/H-9β/H-11β/H-12β established β-oriented HO-6, HO-14, and AcO-15 groups, and an α-oriented HO-1 group. Compound **6** was therefore confirmed as 1α,6β,14β-trihydroxy-15β-acetoxy-*ent*-kuar-16-en-7-one.

HR-EI-MS spectrum of hubeirubesin B (**7**) revealed $[M]^+$ ion peak at *m/z* 416.2193, corresponding to the

Table 1. ^{1}H-NMR Data (in Pyridine-d_5) of Compounds **1**–**5** (600 MHz, δ in ppm, J in Hz)

No.	1	2	3	4	5
1	1.86[a]	1.77[a]	1.85 dd, 13.0, 3.2	3.26 m	3.86 dd, 11.0, 5.4
	1.54[a]	1.11[a]	1.26 m	1.71 m	
2	2.55[a]	2.00[a]	1.93[a]	2.12[a]	1.96[a]
	2.41[a]	1.94[a]	1.92[a]	1.99 m	1.95[a]
3		3.68 dd, 11.5, 3.8	4.29 brs	4.38 dd, 11.7, 3.9	1.47 m
					1.38 m
5β	2.54[a]	1.31 dd, 12.2, 4.2	1.97 m	2.08[a]	1.66 d, 2.7
6	1.97 m	2.00[a]	2.04[a]	2.09[a]	4.29 d, 2.7
	1.87[a]	1.93[a]	2.04[a]	2.08[a]	
7	5.41 brs	5.42 brs	5.40 brs	5.55 brs	
9β	1.80[a]	1.69 d, 12.2	1.92[a]	2.14[a]	2.65 m
11	1.77[a]	1.77[a]	2.31[a]	3.93 m	2.41 m
	1.10 m	1.11[a]	1.52 m		2.18[a]
12	2.26 d, 13.0	2.29 d, 12.7	3.95 m	2.48[a]	2.14[a]
	1.52[a]	1.60 m		1.85 m	1.58 m
13α	2.12 m	2.12 m	2.33[a]	2.32 m	3.01 brs
14	2.80 brd, 13.8	2.84 brd, 12.7	2.44 brd, 10.6	2.48[a]	6.59 s
	2.40[a]	2.50 t, 12.7	2.32[a]	2.08[a]	
15	4.24[a,b]	4.29[a,b]	4.65[b]	4.48[b]	
16					2.90 td, 8.8, 4.5
17	4.24[a,b]	4.29[a,b]	5.65 s	5.09 s	4.26 dd, 10.6, 8.8
			5.29 s	5.48 s	4.18 dd, 10.6, 4.5
18	4.17 d, 10.6	1.52 3H, s	4.17 dd, 10.6, 4.6	4.22 d, 10.5	1.18 3H, s
	3.51 d, 10.6		3.69 dd, 10.6, 3.2	3.74 d, 10.5	
19	1.01 3H, s	4.63 d, 10.8	1.15 3H, s	1.23 3H, s	1.20 3H, s
		3.79 d, 10.8			
20	0.91 3H, s	0.80 3H, s	0.97 3H, s	1.34 3H, s	4.41 d, 9.8
					4.33 d, 9.8
HO-3			6.02 s	5.96[a]	
HO-11				5.95[a]	
HO-12			6.12 d, 4.2		
HO-15	6.29[a]		6.75 s	6.62 s	
HO-16	5.21 s				
HO-17	6.29[a]				
HO-18	6.39 s		6.53 brs	6.54 s	
OMe					3.59 3H, s

a) Overlapped signals. *b*) AB spin system.

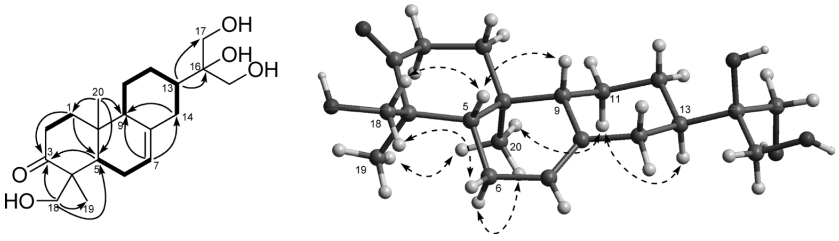

Fig. 2. Key HMBC (H↷C), ^{1}H–^{1}H COSY (▬), and ROESY (↷) Correlations of **1**

molecular formula $C_{24}H_{32}O_6$. The ^{13}C-NMR and DEPT spectra of **7**, together with the maximum UV absorption wavelength at 206 nm just like **6**, exhibited two acetoxy groups, two oxymethines, an unconjugated olefine, and two ketone carbonyls. The NMR data of **7** were very similar to those of rabdosinatol[16] except for one carbonyl at δ_C 207.0 in **7** instead of the oxymethine at C-7 or C-14 in rabdosinatol. Obvious HMBC correlations of H-5 (δ_H 1.94) and H_2-6 (δ_H 3.11, 2.46) with the carbonyl and of H-14 (δ_H 5.80) with C-12 (δ_C 33.9) and C-16 (δ_C 151.2) assigned the carbonyl at C-7 unambiguously. β-Orientations of the acetoxy substituents at C-14 and C-15 were confirmed by key ROESY correlations of H-14/H_3-20 and C$\underline{H}_3$COO–15/H-9β/H-11β. Accordingly, compound **7** was established as 14β,15β-diacetoxy-*ent*-kuar-16-en-3,7-dione.

8,15-*seco*-*ent*-Kauranoids, such as rubescensins T[17] and U,[18] and *ent*-abietanoids have almost the same skeleton but are distinguished by the orientation of the substituent (*e.g.* –H, –OH, or –OOH) at C-8 all the time.[2] On the contrary, cleavage of C-8/C-15 bond of *ent*-kauranes was confirmed to yield *ent*-abietanes rather than 8,15-*seco*-*ent*-kauranes by several chemists at first,[19–22] and was later thought to reverse the configurations of C-8 and C-9.[23] In addition, more and more *ent*-abietane analogues (with H-8α and H-9β) bearing

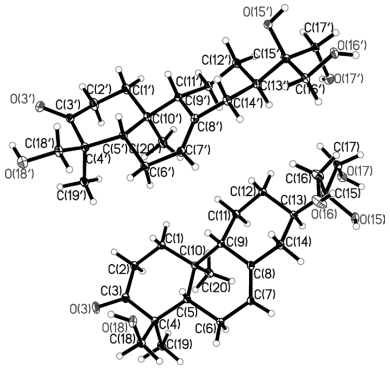

Fig. 3. X-Ray Crystallographic Structure of **1**

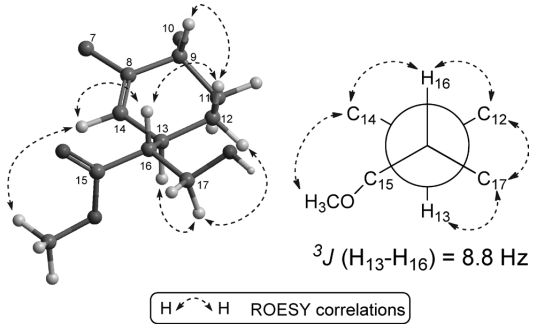

Fig. 4. Determination of Stereochemistry of C-16 of Compound **5** on the Basis of $^3J_{\text{H-13/H-16}}$ Constant and Key ROESY Correlations Illustrated in Chem3D Model and Newman Projection of the Relevant Fragment

Table 2. ^{1}H-NMR Data (in Pyridine-d_5) of Compounds **6** and **7** (600 MHz, δ in ppm, J in Hz)[a)]

No.	**6**	**7**	No.	**6**	**7**
1	3.84 m	1.96[a)]	12	2.17[a)]	1.97[a)]
		1.36 m		1.77 m	1.73 m
2	1.96 m	2.69 m	13α	3.06 brs	2.94 brs
	1.85 m	2.43 m	14α	5.03 s	5.80 s
3	1.61 m		15α	7.51 s	7.16 s
	1.28[a)]		17	5.47 s	5.43 s
5β	1.52 d, 12.3	1.94 dd, 14.3, 2.7		5.18 s	5.21 s
6	5.23 d, 12.3	3.11 t, 14.3	18	1.32 3H, s	0.99 3H, s
		2.46 dd, 14.3, 2.7	19	1.29 3H, s	0.98 3H, s
9β	2.76 d, 8.1	2.37 d, 8.4	20	1.73 3H, s	1.42 3H, s
11	3.49 dd 15.4, 5.4	1.83 dd, 14.9, 7.4	OAc-14		1.90 3H, s
	2.15[a)]	1.36 m	OAc-15	2.10 3H, s	2.11 3H, s

a) Overlapped signals.

Table 3. ^{13}C-NMR Data (in Pyridine-d_5) of Compounds **1**–**7** (δ in ppm)

No.	**1**[a)]	**2**[a)]	**3**[b)]	**4**[b)]	**5**[b)]	**6**[c)]	**7**[c)]
1	36.4 t	38.7 t	38.5 t	41.3 t	73.2 d	80.3 d	38.7 t
2	36.5 t	29.2 t	28.4 t	28.8 t	30.9 t	30.9 t	34.6 t
3	216.2 s	81.1 d	73.6 d	73.6 d	40.2 t	40.0 t	214.5 s
4	53.2 s	42.9 s	43.5 s	44.0 s	34.2 s	35.3 s	47.8 s
5	44.0 d	51.8 d	43.1 d	44.0 d	61.8 d	57.9 d	53.9 d
6	24.5 t	23.9 t	23.8 t	23.7 t	73.7 d	74.8 d	39.0 t
7	120.4 d	120.6 d	120.9 d	124.3 d	97.6 s	210.7 s	207.0 s
8	138.9 s	138.5 s	136.8 s	137.0 s	143.3 s	65.8 s	64.2 s
9	52.0 d	53.5 d	51.5 d	60.3 d	49.8 d	53.5 d	51.0 d
10	35.2 s	35.7 s	35.6 s	37.5 s	43.8 s	46.3 s	39.0 s
11	26.7 t	26.6 t	36.2 t	72.4 d	26.7 t	19.7 t	18.2 t
12	27.2 t	27.2 t	74.0 d	44.2 t	28.9 t	34.3 t	33.9 t
13	42.9 d	42.7 d	50.9 d	39.4 d	37.4 d	50.6 d	47.1 d
14	36.4 t	36.3 t	40.8 t	42.8 t	123.8 d	77.1 d	78.6 d
15	65.3 t	65.4 t	65.8 t	64.9 t	175.7 s	74.8 d	74.1 d
16	76.0 s	76.0 s	154.0 s	155.3 s	55.8 d	153.7 s	151.2 s
17	65.4 t	65.4 t	109.9 t	107.5 t	62.1 t	109.5 t	110.3 t
18	67.2 t	24.2 q	67.5 t	67.8 t	32.7 q	35.7 q	26.1 q
19	19.4 q	65.0 t	13.6 q	14.1 q	22.3 q	23.3 q	21.3 q
20	16.0 q	16.8 q	16.4 q	16.2 q	65.0 t	16.4 q	17.3 q
OMe					51.8 q		
OAc							171.1, 21.1
OAc						171.0, 21.6	170.8, 21.3

a) Recorded in 100 MHz. *b*) Recorded in 150 MHz. *c*) Recorded in 125 MHz.

similar characteristic substituents as *ent*-kauranes have been not only identified from *Isodon* species, but also presumed to be biogenerated from 8,15-*seco*-*ent*-kauranes.[3,11,18,22,24,25)] Similarly, **5** resembled **10** so much in structure (including configurations) that **5** can be assumed to derive from **10** by means of usual cleave, dehydroxylation, and oxidation. In summary,

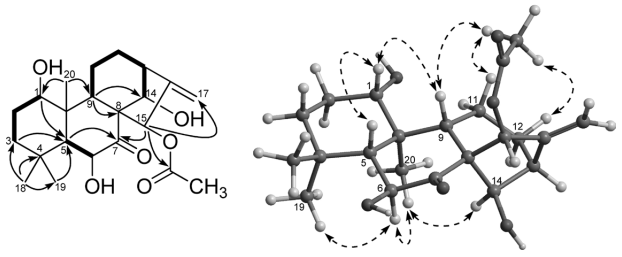

Fig. 5. Key HMBC (H↷C), ^{1}H–^{1}H COSY (▬), and ROESY (↶) Correlations of **6**

distinguishing *ent*-abietane and 8,15-*seco*-*ent*-kaurane by the configuration of C-8 seems to be useful and authentic only in categorizing structures, but not in predicting biogenesis.

For the quantity limit of **5**, the cytotoxicity of only compounds **1**–**4** and **6**–**10** against HL-60, SMMC-7721, A-549, MCF-7 and SW480 cell lines was evaluated by reported MTT method.[26] But all tested compounds were not cytotoxic (IC_{50} values $>40\,\mu$M) against any selected cell line.

Experimental

General Procedures Melting points were measured on an XR4A apparatus that were uncorrected. X-Ray data was determined using a Bruker APEX DUO instrument. Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A BioRad FtS-135 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. NMR spectra were recorded on Bruker AM-400, AM-500, and DRX-600 spectrometers. HR-ESI-MS and HR-electron impact (EI)-MS were performed on a VG Autospec-3000 spectrometer and a Waters AutoSpec Premier spectrometer P776, respectively. Column chromatography (CC) was performed with silica gel (100–200 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany) and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Preparative HPLC and semi-preparative HPLC were performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} (9.4 mm×25 cm) column and on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column, respectively. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH. Petrol ether (PE, 60–90°C), EtOAc, $CHCl_3$, acetone, MeOH, EtOH were analytical grade and produced by Sinopharm Chemical Reagent Co., Ltd., China. All solvents were distilled prior to use.

Plant Material The aerial parts of *Isodon rubescens* (Hemsl.) Hara were collected in Jianshi County, Hubei Province, P. R. of China, in September 2010, and identified by Prof. Ying-Ming Wang. A voucher specimen (No. IP20081006) has been deposited in Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, Tongji School of Pharmacy, Huazhong University of Science and Technology.

Extraction and Isolation The air-dried and powdered aerial parts of *I. rubescens* (10 kg) were extracted 4 times with 95% aqueous EtOH at room temperature to yield an extract, which was suspended in H_2O and extracted with EtOAc. The EtOAc partition (550 g) was applied to silica gel CC, eluting with $CHCl_3$–acetone (1 : 0–0 : 1 gradient system) to give fractions A–F. Fraction E (52 g) was decolored with MCI gel column (90% MeOH–H_2O), and then was subjected to silica gel CC, eluted with $CHCl_3$–acetone 20 : 1 to 1 : 1), give nine subfractions (Fr-E1–Fr-E9). Fr-E9/1–Fr-E9/4 was obtained by separating the Fr-E9 (5 g) with RP-18 CC (30–90% MeOH–H_2O). Then preparative HPLC (30–60% MeOH–H_2O, 35°C, 3 mL/min) on Fr-E9/2 (500 mg) led to several subfractions and pure compounds **1** (150 mg), **2** (34 mg), **8** (23 mg), **9** (15 mg), and **10** (21 mg). Furthermore, **3** (6 mg) and **4** (10 mg) resulted from semi-preparative HPLC (28% MeOH–H_2O, 35°C, 3 mL/min) on subfractions of Fr-E9/2. Compounds **6** (28 mg) and **10** (140 mg) were easily achieved from Fr-E9/3 by silica gel CC ($CHCl_3$–MeOH 20 : 1), and **5** (2 mg) was from the residue of above Fr-E9/3 by semi-preparative HPLC. Fraction B (120 g) was decolored samely as fraction E, and then was subjected to silica gel CC (petrol ether–acetone 20 : 1 to 1 : 1), to give Fr-B1–Fr-B5. The subfraction Fr-B4 (12 g) was further applied to silica gel CC, and eluted with $CHCl_3$–acetone (40 : 1, 20 : 1, 10 : 1) to give Fr-B4/1–Fr-B4/8. Finally, **7** (3 mg) was yielded by semi-preparative HPLC on Fr-B4/4 (120 mg).

ent-Abierubesin A (**1**): Colorless pieces in MeOH; mp: 202–204°C; $[\alpha]_D^{23}$ +32.5 (c=0.08, MeOH); UV (MeOH) λ_{max} (logε) nm: 208 (2.83), 250 (1.43), 293 (1.2); IR (KBr) ν_{max} cm^{-1}: 3431, 2939, 1695, 1633, 1455, 1433, 1071, 1049, 830, 587; positive HR-ESI-MS *m/z*: 375.2157 ([M+Na]$^+$, Calcd for $C_{20}H_{32}O_5Na$: 375.2147); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2, respectively.

X-Ray crystal structure analysis of **1**: $C_{40}H_{64}O_{10}$, molecular weight (M_W)=704.9, orthorhombic, space group $P2_12_12_1$, Z=4, a=6.44160(10) Å, b=22.4994(2) Å, c=24.9788(2) Å, $\alpha=\beta=\gamma$=90°, V=3620.23(7) Å^3, μ(Cu$K\alpha$)=0.738 mm^{-1}, ρ_{calc}=1.293 g/cm^3; S=1.060, final R indices: R_1=0.0354, wR_2=0.0972 for 6474 observed from 6502 independent and 57507 measured reflections (θ_{max}=69.69, $I>2\sigma(I)$ criterion and 464 parameters); maximum and minimum residues are 0.989 and −0.350 eÅ^{-3}, respectively. The Flack parameter value was x=0.01(12), indicating that the absolute structure has been determined correctly.

ent-Abierubesin B (**2**): White amorphous powder; $[\alpha]_D^{24}$ +4.6 (c=0.05, MeOH); UV (MeOH) λ_{max} (logε) nm: 207 (2.77); IR (KBr) ν_{max} cm^{-1}: 3440, 2934, 1630, 1053, 1024; positive HR-ESI-MS *m/z*: 377.2297 ([M+Na]$^+$, Calcd for $C_{20}H_{34}O_5Na$: 377.2303); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 3.

ent-Abierubesin C (**3**): White amorphous powder; $[\alpha]_D^{25}$ −9.8 (c=0.09, MeOH); UV (MeOH) λ_{max} (logε) nm: 205 (2.94); IR (KBr) ν_{max} cm^{-1}: 3440, 2928, 1629, 1448, 1272, 1250, 1053, 916; positive HR-ESI-MS *m/z*: 359.2193 ([M+Na]$^+$, Calcd for $C_{20}H_{32}O_4Na$: 359.2198); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 3.

ent-Abierubesin D (**4**): White amorphous powder; $[\alpha]_D^{25}$ −17.9 (c=0.04, MeOH); UV (MeOH) λ_{max} (logε) nm: 204 (3.01), 253 (2.25); IR (KBr) ν_{max} cm^{-1}: 3428, 2928, 2871, 1713, 1641, 1550, 1449, 1384, 1306, 1060; positive HR-ESI-MS *m/z*: 359.2195 ([M+Na]$^+$, Calcd for $C_{20}H_{32}O_4Na$: 359.2198); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2.

ent-Abierubesin E (**5**): White amorphous powder; $[\alpha]_D^{25}$ +0.6 (c=0.08, MeOH); UV (MeOH) λ_{max} (logε) nm: 203 (3.13); IR (KBr) ν_{max} cm^{-1}: 3440, 2928, 1629, 1448, 1272, 1250, 1053, 916; positive HR-ESI-MS *m/z*: 419.2052 ([M+Na]$^+$, Calcd for $C_{21}H_{32}O_7Na$: 419.2045); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 3.

Hubeirubesin A (**6**): White amorphous powder; $[\alpha]_D^{23}$ −59.5

(c=0.10, MeOH); UV (MeOH) λ_{max} (logε) nm: 207 (2.95); IR (KBr) ν_{max} cm^{-1}: 3442, 2933, 2871, 1743, 1640, 1372, 1241, 1059; positive HR-ESI-MS m/z: 415.2089 ([M+Na]$^+$, Calcd for $C_{22}H_{32}O_6Na$: 415.2096); ^{1}H- and ^{13}C-NMR data, see Tables 2 and 3.

Hubeirubesin B (**7**): White amorphous powder; $[\alpha]_D^{22}$ −37.0 (c=0.20, MeOH); UV (MeOH) λ_{max} (logε) nm: 206 (2.76); IR (KBr) ν_{max} cm^{-1}: 3438, 2959, 2938, 1742, 1709, 1630, 1459, 1373, 1228, 1073; positive HR-EI-MS m/z: 416.2193 ([M]$^+$, Calcd for $C_{24}H_{32}O_6$: 416.2199); ^{1}H- and ^{13}C-NMR data, see Tables 2 and 3.

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