New *ent*-Abietanoids from *Isodon rubescens*

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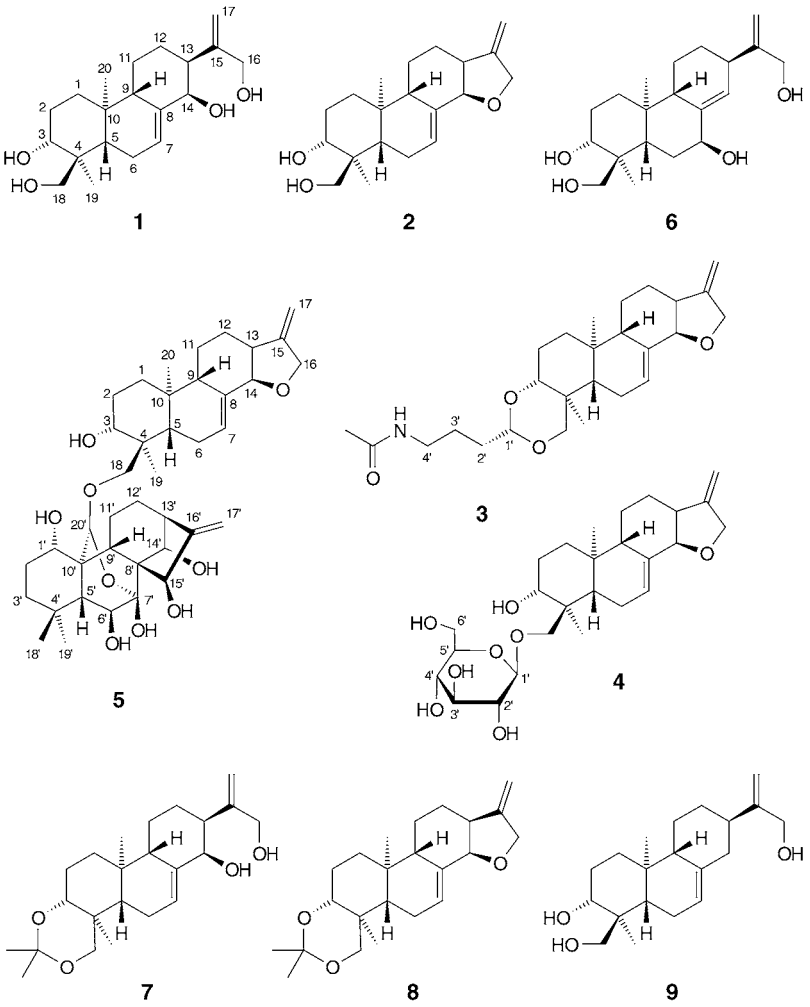
Six new *ent*-abietane diterpenoids, rubescensins I–M (**1**–**5**) and P (**6**), along with two related acetonide derivatives (**7** and **8**), were isolated from *Isodon rubescens*. Their structures were elucidated by detailed spectroscopic analysis. Compound **5** is the first N-containing diterpenoid from the genus *Isodon*, exhibiting notable cytotoxicity against human tumor K562 cells.

Introduction. – In our previous papers [1][2], 20 tetracyclic *ent*-kaurane diterpenoids were reported from *Isodon rubescens* (HEMSL.) HARA. Our continuing search for biologically active principles from this plant, collected in two different prefectures of the Henan Province of China, has now led to the isolation of six new *ent*-abietane diterpenoids named rubescensins I–M (**1**–**5**) and P (**6**), together with two related acetonide derivatives (**7** and **8**). In this paper, we report the isolation and structural elucidation of these new compounds.

Results and Discussion. – *Structure Elucidation*. Rubescensin I (**1**), obtained as an amorphous powder, gave rise to a molecular-ion peak at *m*/*z* 336.2315 in the HR-EI mass spectrum, consistent with the molecular formula $C_{20}H_{32}O_4$. Careful analysis of the ^{1}H- and ^{13}C-NMR data (*Table 1*) indicated **1** to be an *ent*-abietanoid similar to laxiflorin O (**9**) reported from *I. eriocalyx* var. *laxiflora* [3]. Compound **1** was elucidated as (3α,14β)-*ent*-abieta-7,15(17)-diene-3,4,16,18-tetraol by extensive analysis of its 2D-NMR spectra, and by comparison of the 1D-NMR spectra of **1** and **9**.

In the ^{13}C-NMR spectrum of **1**, signals of two olefinic quaternary C-atoms (δ 141.2 and 152.8), an olefinic CH_2 (110.8), an olefinic CH (124.3), two O–CH_2 (66.1, 64.6), five CH_2 (38.1, 27.9, 25.7, 23.9, 23.4), two O–CH (74.8, 74.1), three CH (48.5, 47.6, 43.0), two nonoxygenated quaternary C-atoms (43.3, 35.1), and two Me groups (13.0, 15.9) were present. Thus, compound **1** was lacking one nonoxygenated quaternary C-atom relative to the classical *ent*-kaurane skeleton, suggesting **1** to be a tricylic diterpenoid. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** and the known tricylic *ent*-abietanoid laxiflorin O (**9**) [3] revealed great similarities, except for one more OH group at C(14) of **1**. Thus, **1** can be regarded as an *ent*-abietane diterpenoid corresponding to 14-hydroxylaxiflorin O. This was further supported by positive $[\alpha]_D$ values for **1** and **9**.



The above assignment was also corroborated by HMBC experiments (*Fig. 1,a*). The $CH_2(17)$=C(15) moiety was confirmed by correlations of $CH_2(17)$ (δ 5.57 and 5.28) with C(13) (δ 47.6) and C(16) (δ 64.6). The other C=C bond was assigned to C(7) and C(8) on the ground of the observed HMBC correlation of H–C(7) at δ_H 5.66 with C(5) at δ_C 43.0 and C(9) at δ_C 48.5. Due to the presence of the HMBC correlations of H–C(3) with C(1), C(5), and C(18), of H–C(14) with C(7), C(9), and C(15), of $CH_2(16)$ with C(13) and C(17), and of $CH_2(18)$ with C(3), C(5), and C(19), the four OH groups were placed at C(3), C(14), C(16), and C(18), respectively (*Table 1* and *Fig. 1,a*).

The relative configuration of **1** was derived by a ROESY experiment (*Fig. 1,b*). The α-orientation of the 3-OH and the β-orientation of the 14-OH groups were apparent

Table 1. *^{1}H- and/or ^{13}C-NMR Data of Compounds* **1**, **2**, **7**, *and* **8**. 400 and 100 MHz, resp.; C_5D_5N; δ in ppm, J in Hz. Asterisks (*) mark overlapping signals.

	1		**7**	**2**		**8**
	^{13}C	^{1}H	^{13}C	^{13}C	^{1}H	^{13}C
CH_2(1)	38.1 (*t*)	1.82, 1.18 (2*m*)	38.2 (*t*)	37.5 (*t*)	1.79, 1.18 (2*m*)	37.9 (*t*)
CH_2(2)	27.9 (*t*)	1.92, 1.85 (2*m*)	24.3 (*t*)	28.8 (*t*)	1.89, 1.62 (2*m*)	24.3 (*t*)
H_β–C(3)	74.1 (*d*)	4.14 (*dd*, J = 10.8, 4.9)	77.4 (*d*)	73.1 (*d*)	4.17 (*dd*, J = 10.8, 4.9)	77.3 (*d*)
C(4)	43.3 (*s*)	–	36.7 (*s*)	42.7 (*s*)	–	36.6 (*s*)
H_β–C(5)	43.0 (*d*)	1.90*	45.6 (*d*)	42.3 (*d*)	1.94*	45.0 (*d*)
CH_2(6)	23.4 (*t*)	2.05–2.00*	22.4 (*t*)	23.5 (*t*)	2.09–2.03*	22.8 (*t*)
H–C(7)	124.3 (*d*)	5.66 (*d*, J = 2.1)	123.2 (*d*)	129.5 (*d*)	5.66 (*d*, J = 2.1)	128.8 (*d*)
C(8)	141.2 (*s*)	–	141.5 (*s*)	134.6 (*s*)	–	135.4 (*s*)
H_β–(9)	48.5 (*d*)	2.40*	48.5 (*d*)	49.2 (*d*)	2.05*	49.5 (*d*)
C(10)	35.1 (*s*)	–	35.1 (*s*)	34.8 (*s*)	–	35.1 (*s*)
CH_2(11)	23.9 (*t*)	1.75, 1.20 (2*m*)	23.6 (*t*)	23.7 (*t*)	1.93, 1.04 (2*m*)	23.6 (*t*)
CH_2(12)	25.7 (*t*)	2.20, 1.65 (2*m*)	25.3 (*t*)	27.5 (*t*)	1.65, 1.42 (2*m*)	28.9 (*t*)
H_α–C(13)	47.6 (*d*)	2.50 (br. *d*, J = 12.4)	47.3 (*d*)	45.8 (*d*)	2.41 (*m*)	45.9 (*d*)
H_α–C(14)	74.8 (*d*)	4.58 (br. *s*)	74.5 (*d*)	83.4 (*d*)	4.18 (br. *s*)	83.5 (*d*)
C(15)	152.8 (*s*)	–	152.5 (*s*)	154.5 (*s*)	–	154.6 (*s*)
CH_2(16)	64.6 (*t*)	4.65, 4.56 (2*d*, J = 14.0)	64.7 (*t*)	69.4 (*t*)	4.55, 4.26 (2*d*, J = 14.0)	69.6 (*t*)
CH_2(17)	110.8 (*t*)	5.57, 5.28 (2 br. *s*)	110.7 (*t*)	102.9 (*t*)	5.01, 4.84 (2 br. *s*)	103.2 (*t*)
CH_2(18)	66.1 (*t*)	4.10, 3.59 (2*d*, J = 10.8)	72.2 (*t*)	67.1 (*t*)	4.06, 3.60 (2*d*, J = 10.8)	72.1 (*t*)
Me–C(19)	13.0 (*q*)	1.15 (*s*)	12.9 (*q*)	12.6 (*q*)	1.12 (*s*)	12.7 (*q*)
Me–C(20)	15.0 (*q*)	0.92 (*s*)	15.9 (*q*)	15.0 (*q*)	0.86 (*s*)	15.3 (*q*)
Me_2C–O	–	–	99.0 (*s*)	–	–	99.0 (*s*)
			30.2 (*q*)			30.2 (*q*)
			19.4 (*q*)			19.4 (*q*)

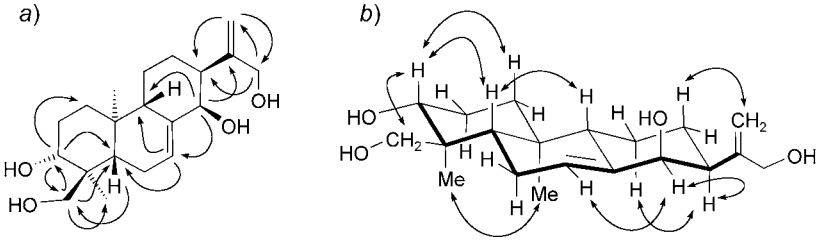


Fig. 1. *Key HMBC* (H → C) *and ROESY correlations observed in* **1**

from the ROEs of H_β–C(3) with both H_β–C(5) and CH_2(18), and of H_α–C(14) with both H_α–C(13) and H–C(7).

Compound **1** showed an unprecedented color change on TLC plates (SiO_2) from green to red to purple to blue when being baked at 200° after dipping in 10% ethanolic H_2SO_4. So did rubescensin *J* (**2**), having the molecular formula $C_{20}H_{30}O_3$, as determined by HR-EI-MS (*m*/*z* 318.2193; calc. 318.2195). Comparison of the ^{1}H-, ^{13}C-, and DEPT-NMR data of **2** and **1** revealed that **2** was also an *ent*-abietanoid, differing from **1** only at C(14) and C(16). The clear HMBCs correlations between H–C(14) (δ_H 4.18) and C(16) (δ_C 69.4), and between CH_2(16) (δ_H 4.55 and 4.26) and C(14) indicated the presence of an O-bridge between C(14) and C(16), causing significant downfield

chemical shifts of C(14) (δ_C 83.4) and C(16) (δ_C 69.4). This was consistent with a molecular weight being 18 amu lower than that of **1** (condensation of C(14)–OH and C(16)–OH under loss of H_2O). Thus, **2** was elucidated as (3α)-14,16-epoxy-*ent*-abieta-7,15(17)-diene-3,18-diol.

Rubescensin K (**3**) was also an *ent*-abietanoid, as indicated by the same characteristic color changes in the TLC test. It exhibited an odd molecular-ion peak at *m*/*z* 429 in the EI mass spectrum, and an $[M+1]^+$ signal at *m*/*z* 430 in the FAB mass spectrum, suggesting that it might contain a N-atom. The HR-EI-MS data (M^+ signal at *m*/*z* 429.2871) verified this assumption, giving rise to the molecular formula $C_{26}H_{39}NO_4$. On the basis of careful analysis of the ^{1}H-, ^{13}C-, and 2D-NMR data (*Table 2*), compound **3** was identified as 3α,(3α,14β)-3,18-{[(1*S*)-4-(acetylamino)butane-1,1-diyl]dioxy}-14,16-epoxy-*ent*-abieta-7,15(17)-diene, and was named rubescensin K, which is the first N-containing diterpenoid isolated from *Isodon* plants.

Table 2. *^{1}H- and ^{13}C-NMR Data of Compounds* **3**, **4**, *and* **6**. 400 and 100 MHz, resp.; C_5D_5N; δ in ppm, *J* in Hz. Asterisks (*) mark overlapping signals.

	3		**4**		**6**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
CH_2(1)	37.9 (*t*)	1.73, 1.05 (2*m*)	37.6 (*t*)	1.68, 1.00 (2*m*)	37.1 (*t*)	1.63, 1.25 (2*m*)
CH_2(2)	24.2 (*t*)	1.70–1.18*	29.0 (*t*)	1.81, 1.52 (2*m*)	27.9 (*t*)	1.90*
H_β–C(3)	85.0 (*d*)	3.13 (*m*)	73.0 (*d*)	4.11*	74.9 (*d*)	4.23 (*m*)
C(4)	36.5 (*s*)	–	43.1 (*s*)	–	42.6 (*s*)	–
H_β–C(5)	45.1 (*d*)	1.00*	42.8 (*d*)	1.83*	40.9 (*d*)	2.47 (*dd*, *J* = 13.0, 2.0)
CH_2(6)	22.9 (*t*)	1.70–1.18*	23.8 (*t*)	2.07, 1.95 (2*m*)	30.5 (*t*)	2.09 (*d*, *J* = 13.0) 1.77 (*m*)
H–C(7)	128.8 (*d*)	5.78 (*d*, *J* = 2.0)	129.8 (*d*)	5.79 (*d*, *J* = 2.2)	72.3 (*d*)	4.49 (br. *s*)
C(8)	135.5 (*s*)	–	134.5 (*s*)	–	141.8 (*s*)	–
H_β–C(9)	49.4 (*d*)	1.93*	49.1 (*d*)	1.90*	46.5 (*d*)	2.58 (br. *s*)
C(10)	35.1 (*s*)	–	35.1 (*s*)	–	38.7 (*s*)	–
CH_2(11)	23.6 (*t*)	1.70–1.18*	23.9 (*t*)	1.87, 0.97 (2*m*)	22.6 (*t*)	1.70, 1.36 (2*m*)
CH_2(12)	24.2 (*t*)	1.70–1.18*	27.5 (*t*)	1.57, 1.36 (2*m*)	29.7 (*t*)	1.92, 1.32 (2*m*)
H_α–C(13)	45.9 (*d*)	2.37 (*m*)	46.1 (*t*)	2.38 (*m*)	39.4 (*d*)	2.89 (*m*)
H–C(14)	83.5 (*d*)	4.14 (*d*, *J* = 4.0, H_α)	83.6 (*d*)	4.15 (*d*, *J* = 4.0, H_α)	128.7 (*d*)	5.89 (br. *s*)
C(15)	154.6 (*s*)	–	154.9 (*s*)	–	155.1 (*s*)	–
CH_2(16)	69.6 (*t*)	4.53, 4.23 (2*d*, *J* = 13.2)	69.6 (*t*)	4.53, 4.25 (2*d*, *J* = 14.0)	64.2 (*t*)	4.41*
CH_2(17)	103.2 (*t*)	5.03, 4.84 (2 br. *s*)	103.2 (*t*)	5.00, 4.85 (2 br. *s*)	107.8 (*t*)	5.43, 4.99 (2 br. *s*)
CH_2(18)	77.9 (*t*)	3.69, 3.08 (2*d*, *J* = 10.8)	76.0 (*t*)	4.21, 3.53 (2*d*, *J* = 10.4)	69.4 (*t*)	4.17, 3.82 (2*d*, *J* = 10.4)
Me–C(19)	13.6 (*q*)	1.17 (*s*)	12.8 (*q*)	1.05 (*s*)	12.8 (*q*)	1.18 (*s*)
Me–C(20)	15.3 (*q*)	0.73 (*s*)	15.2 (*q*)	0.78 (*s*)	14.6 (*q*)	0.90 (*s*)
H–C(1′)	102.6 (*d*)	4.67 (br. *s*)	105.8 (*d*)	4.84 (*d*, *J* = 7.6)	–	–
H–C(2′)	32.8 (*t*)	1.90–1.78 (2 H)*	74.9 (*d*)	4.04 (*dd*, *J* = 6.4, 2.0)	–	–
H–C(3′)	24.7 (*t*)	1.90–1.78 (2 H)*	78.7 (*d*)	4.24*	–	–
H–C(4′)	39.6 (*t*)	3.48 (2 H)*	72.2 (*d*)	4.13*	–	–
H–C(5′)	–	–	78.6 (*d*)	4.02 (*m*)	–	–
CH_2(6′)	–	–	63.2 (*t*)	4.62 (*d*, *J* = 11.0), 4.32 (*dd*, *J* = 11.0, 8.0)	–	–
NHAc	169.8 (*s*)	8.44 (br. *s*, NH)	–	–	–	–
	23.1 (*q*)	1.99 (*s*)				

Examination of the ^{1}H- and ^{13}C-NMR data of **3** revealed that the molecule consisted of two portions, one of which closely resembled **2**, except for the downfield-shifted C(3) and C(18) ^{13}C-NMR signals at δ_C 85.0 and 77.9, respectively. The other portion contained one N- and six C-atoms, including one Me (δ_C 23.1), three CH_2 (24.7, 32.8, 39.6), a C=O group (169.8), and one highly oxygenated CH (102.6). The $CH_2(4')$ group resonating at δ_C 39.6 and δ_H 3.48 (*m*) was linked with an acetamide NH (δ_H 8.44), as deduced from a ^{1}H,^{1}H-COSY experiment, as well as from the HMBC correlations (*Fig. 2*) of both the $CH_2(4')$ (δ_H 3.48) and a Me group (δ_H 1.99) with the C=O C-atom (δ_C 169.8). H–C(1′) (δ_C 102.6; δ_H 4.67) was linked with C(3) and C(18) according to a HMBC experiment. In the ^{1}H,^{1}H-COSY spectrum, the overlapping $CH_2(2')$ and $CH_2(3')$ resonances (δ_H 1.90–1.78) exhibited correlations with the H-atoms of CH(1′) and $CH_2(4')$ (δ_H 3.48), respectively, suggesting that C(1′) to C(4′) were anchored in a line, as confirmed by the HMBC experiment (*Fig. 2*). The H–C(1′) H-atom was involved in NOEs with H_β–C(3) and $CH_2(18)$, confirming β-configuration at C(1′).

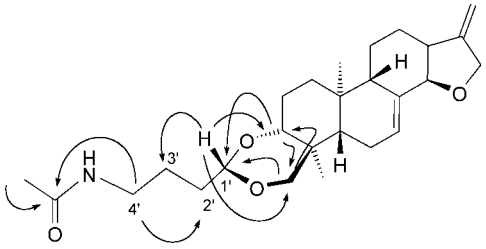


Fig. 2. *Key HMBC* (H → C) *correlations observed in* **3**

Rubescensin L (**4**), a white amorphous powder, gave rise to a molecular-ion peak at *m*/*z* 480 in the EI mass spectrum, in accord with the molecular formula $C_{26}H_{40}O_8$, as determined by HR-EI-MS (480.2708 (M^+; calc. 480.2723)). Analysis of the ^{1}H- and ^{13}C-NMR data indicated that **4** was a glucoside of **2**. The significant downfield shift of C(18) (δ_C 76.0) of **4**, in combination with the ^{1}H- and ^{13}C-NMR data and coupling patterns of the glucose moiety with reference data [4], suggested that a β-D-glucose unit was linked at C(18), which was confirmed by HMBCs correlations between $CH_2(18)$ (δ_H 4.21 and 3.53) and C(1′) (δ_C 105.8), and between H–C(1′) (δ_H 4.84) and C(18). Therefore, **4** was established as the 18-*O*-β-D-glycopyranoside of **2**.

Rubescensin M (**5**) was obtained as a white amorphous powder. FAB-MS exhibited an $[M+1]^+$ peak at *m*/*z* 683, consistent with the molecular formula $C_{40}H_{58}O_9$, as determined by both HR-FAB-MS and ^{1}H- and ^{13}C-NMR (*Table 3*). Analysis of the spectral data, including 2D-NMR, indicated that **5** was composed of two diterpene moieties (partial structures **5a** and **5b**), as shown in *Fig. 3*. Thereby, based on NMR, **5a** was identical with **2**, and **5b** was suggested to be a 7,20-epoxy-*ent*-kaurane due to the characteristic signals of three nonoxygenated quaternary C-atoms [C(4′), C(8′), C(10′) at δ_C 33.6, 52.4, 43.5, resp.], two Me groups at quaternary C-atoms [C(18′) and C(19′) at δ_C 33.5 and 22.3, resp.], a hemiketal C-atom [C(7′) at δ_C 101.5], and three nonoxygenated CH groups [C(5′), C(9′), C(13′) at δ_C 56.7, 45.0, 46.4, resp.]. Further comparison of the ^{1}H- and ^{13}C-NMR data of **5b** and rabdoternins B and F [5][6], two known 7,20-epoxy-*ent*-kauranoids that had been isolated as well, suggested that **5b** was

a 7α,20-epoxy-*ent*-kaur-16-ene-1,6,7,14,15-pentaol, strongly resembling rabdoternin B, except for the oxygenation pattern of C(20), and differing from rabdoternin F only at C(15). Furthermore, it was deduced that **5a** and **5b** were linked together by an oxy bridge between C(18) (δ_C 71.7) and C(20′) (δ_C 101.8) on the basis of HMBC correlations between H–C(20) and C(18), as well as between CH_2(18) and C(20′) (*Fig. 3*).

Table 3. *^{1}H- and ^{13}C-NMR Data of Compound* **5**. Note that **5a** and **5b** are ether-bridged fragment structures (see chemical formula). 400 and 100 MHz, resp.; C_5D_5N; δ in ppm, J in Hz. Asterisks (*) mark overlapping signals.

	5a			**5b**	
	^{13}C	^{1}H		^{13}C	^{1}H
CH_2(1)	37.9 (*t*)	1.78, 1.11 (2*m*)	H_β–C(1′)	75.9 (*d*)	3.66 (*dd*, *J* = 9.6, 4.0)
CH_2(2)	29.1 (*t*)	1.91*, 1.65*	CH_2(2′)	31.0 (*t*)	1.91*, 1.75*
H_β–C(3)	73.4 (*d*)	3.95*	CH_2(3′)	39.3 (*t*)	1.36*, 1.29*
C(4)	42.3 (*s*)	–	C(4′)	33.6 (*s*)	–
H_β–C(5)	42.5 (*d*)	2.09 (*dd*, *J* = 9.6, 2.4)	H_β–C(5′)	56.7 (*d*)	1.62 (*d*, *J* = 5.0)
CH_2(6)	23.7 (*t*)	2.52, 2.00 (2*m*)	H_α–C(6′)	71.6 (*d*)	4.11 (*d*, *J* = 5.0)
H–C(7)	130.8 (*d*)	5.59 (*m*)	C(7′)	101.5 (*s*)	–
C(8)	133.7 (*s*)	–	C(8′)	52.4 (*s*)	–
H_β–C(9)	50.5 (*d*)	1.97*	H_β–C(9′)	45.0 (*d*)	2.89 (*m*)
C(10)	34.9 (*s*)	–	C(10′)	43.5 (*s*)	–
CH_2(11)	23.3 (*t*)	1.86*, 1.00*	CH_2(11′)	21.6 (*t*)	2.67, 2.24 (2*m*)
CH_2(12)	27.8 (*t*)	1.60, 1.26 (2*m*)	CH_2(12′)	33.9 (*t*)	2.55 (*m*), 1.74*
H_α–C(13)	45.3 (*d*)	2.39 (*m*)	H_α–C(13′)	46.4 (*d*)	2.93 (*d*, *J* = 7.2)
H_α–C(14)	83.5 (*d*)	4.12*	H_α–C(14′)	75.9 (*d*)	5.16 (*s*)
C(15)	153.7 (*s*)	–	C(15′)	73.1 (*d*)	5.65 (*s*)
CH_2(16)	69.2 (*t*)	4.45, 4.13 (2*d*, *J* = 10.4)	CH_2(16′)	161.2 (*s*)	–
CH_2(17)	103.8 (*t*)	5.02, 4.85 (2 br. *s*)	CH_2(17′)	109.0 (*t*)	5.65, 5.37 (2 br. *s*)
CH_2(18)	71.7 (*t*)	3.96, 3.87 (2*d*, *J* = 7.0)	Me–C(18′)	33.5 (*q*)	1.16 (*s*)
Me–C(19)	12.5 (*q*)	1.02 (*s*)	Me–C(19′)	22.3 (*q*)	1.05 (*s*)
Me–C(20)	15.0 (*q*)	0.81 (*s*)	H–C(20′)	101.8 (*d*)	5.76 (*s*)

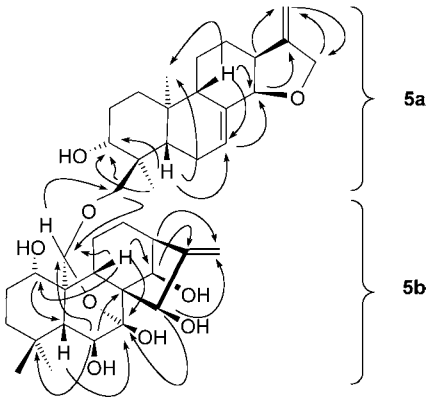


Fig. 3. *Key HMBC* (H → C) *correlations observed in* **5**

The relative configuration of **5** was confirmed by a ROESY experiments (*Fig. 4*). The orientations of the OH groups at C(1′), C(6′), and C(14′) were shown to be α, β, and β, respectively, as deduced from the NOEs between H_β–C(1′) (δ_H 3.66) and H_β–C(5′) (δ_H 1.62), and between H_β–C(9′) (δ_H 2.89) and H_α–C(6′) (δ_H 4.11) and Me(19′) (δ_H 1.05), as well as between H_α–C(14′) (δ_H 5.16) and H_α–C(11′) (δ_H 2.67), respectively. However, there was no NOE for H–C(15′), suggesting an α-orientation, which was confirmed by the steric effect between H_β–C(9′) and HO–C(15′) indicated by an upfield shift of C(9′) (δ_C 45.0). Finally, the relative (*S*)-configuration at C(20′) was determined by the key NOE between H–C(20′) and Me(19′). There were also NOEs between H–C(20′) and H_β–C(3), and between H–C(20′) and CH_2(18), confirming the linkage between the two moieties of **5**.

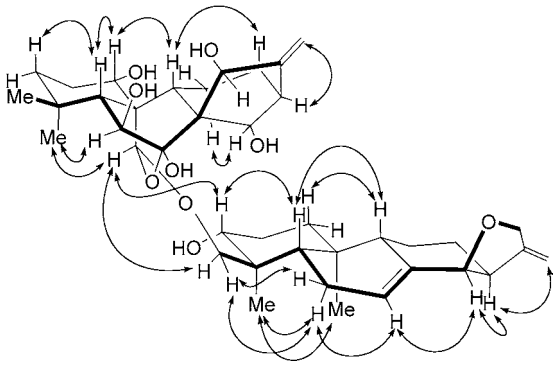


Fig. 4. *Key ROESY correlations observed in* **5**

Rubescensin P (**6**), an amorphous powder, had the same molecular formula ($C_{20}H_{32}O_4$) as **1**, as revealed by HR-EI-MS. Comparison of the ^{13}C-NMR data of **6** (*Table 2*) and **1** (*Table 1*) indicated that they both had two OCH_2, two OCH, two olefinic quaternary C-atoms, an olefinic CH, and an olefinic CH_2, with differences in rings B and C, which were detailed by HMBC correlations between H–C(14) (δ_H 5.89) and both C(15) (δ_C 155.1) and C(9) (δ_C 46.5) (*Fig. 5*), suggesting the presence of a C=C bond between C(8) (δ_C 141.8) and C(14) (δ_C 128.7). The β-orientation of HO–C(7) was indicated by the HMBC correlations between H_α–C(7) and both C(5) and C(9), and by the NOE of H_α–C(7) and H–C(14). Thus, **6** was determined to be (3α,7β)-*ent*-abieta-8(14),15(17)-diene-3,7,16,18-tetraol.

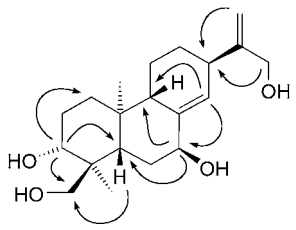


Fig. 5. *Key HMBC* (H → C) *correlations observed in* **6**

The ^{13}C-NMR spectrum of **7** closely resembled that of **1**, except for three extra C-atom signals at δ_C 99.0, 30.2, and 19.4, suggesting that **7** was an acetonide derivative of **1**. The acetonide group was located between C(3) and C(18), as indicated by HMBC correlations between both H–C(3) (δ_H 3.50) and CH_2(18) (δ_H 3.48 and 3.26) with the quaternary C-atom (δ_C 99.0) of the Me_2C group. The relative configurations at C(3) and C(14) were assigned by the NOEs in the ROESY spectrum of **7**. Therefore, **7** was identified as (3*α*,14*β*)-3,18-[(1-methylethane-1,1-diyl)dioxy]-*ent*-abieta-7,15(17)-diene-14,16-diol; in the same way, compound **8**[1]) was elucidated as the acetonide of **2**.

The isolation of *ent*-abietanoids from an *Isodon* plant, a genus notable as a rich source of tetracyclic *ent*-kauranoids [8], may suggest a potential biogenesis path from *ent*-kaurane to *ent*-abietane, because these *ent*-abietanoids, without the key H_α–C(8) of the *ent*-abietane skeleton, could also be regarded as 8,15-*seco*-*ent*-kauranoids.

Cytotoxicity. The new compounds **1**–**8** were tested for their cytotoxity against human-tumor K562 cells by a method previously described [7]. Only compound **3** exhibited a significant inhibitory effect, with an IC_{50} value of 0.49 μg/ml, which is in the range of cisplatin (1.44 μg/ml).

Experimental Part

General. Optical rotations: *JASCO DIP-370* digital polarimeter. UV Spectra: *Shimadzu UV-210A* spectrometer; λ_{max} in nm, (log ε). IR Spectra: *Bio-Rad FtS-135* spectrometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; in C_5D_5N; δ in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz. Mass spectra: *VG Autospec-3000* spectrometer (70 eV for EI); in *m*/*z* (rel. %).

Plant Material. Plants of *Isodon rubescens* were collected in the Jiyuan and Hebi Prefectures in August 1999 and August 2000, resp., Henan Province of China. They were identified by Prof. *Zhong-Wen Lin*. Voucher specimens (KIB-99-10-13 Lin and KIB-2000-8 Lin) were deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Science.

Extraction and Isolation. a) *Plant Material from the Jiyuan Prefecture.* The air-dried leaves (13 kg) of *I. rubescens* from the Jiyuan Prefecture were extracted with 70% aq. acetone at r.t. overnight (3×), and the extract was filtered. The filtrate was evaporated, and the resulting residue was partitioned between H_2O and AcOEt. The AcOEt fraction (424 g of dry extract) was purified by column chromatography (CC) (3 kg of SiO_2 (100–200 mesh); $CHCl_3$/acetone 1:0 → 0:1), affording *Fractions I–IX*. After repeated CC (SiO_2; gradient mixtures of $CHCl_3$/MeOH), *Fraction VII* afforded **1** (150 mg), **7** (14 mg), and **2** (110 mg). In the same way, **3** (8 mg), **4** (5 mg), and **5** (6 mg) were isolated from *Fraction VIII*.

b) *Plant Material from the Hebi Prefecture.* The air-dried leaves (10 kg) of *I. rubescens* from the Hebi Prefecture were extracted with 70% aq. acetone at r.t. overnight (3×), and the extract was filtered. The filtrate was evaporated, and the resulting residue was partitioned between H_2O and AcOEt. The AcOEt fraction (400 g of dry extract) was purified by CC (SiO_2; $CHCl_3$; $CHCl_3$/acetone 9:1, 8:2, 7:3, 6:4; acetone) to afford *Fractions I–VI*. Compounds **1** (50 mg), **7** (6 mg), **2** (80 mg), and **8** (20 mg) were isolated from *Fraction V*, and compounds **4** (20 mg) and **6** (23 mg) were obtained from *Fraction VI* by repeated CC (SiO_2; gradient mixtures of $CHCl_3$/MeOH), followed by repeated prep. TLC (SiO_2; $CHCl_3$/MeOH 10:1).

(3α,14β)-ent-*Abieta-7,15(17)-diene-3,14,16,18-tetraol* (*Rubescensin I*; **1**). White amorphous powder. $[\alpha]_D^{21} = +38.9$ ($c = 0.32$, MeOH). UV (MeOH): 203 (4.74). IR (KBr): 3417, 2933, 2870, 1385, 1087, 1056, 917. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 336 (65, M^+), 318 (80), 300 (85), 282 (42), 269 (54), 167 (90), 149 (100). HR-EI-MS: 336.2315 (M^+, $C_{20}H_{32}O_4^+$; calc.: 336.2301).

(3α,14β)-14,16-Epoxy-ent-*abieta-7,15(17)-diene-3,18-diol* (*Rubescensin J*; **2**). White amorphous powder. $[\alpha]_D^{26} = -24.8$ ($c = 0.30$, MeOH). UV (MeOH): 204 (3.77). IR (KBr): 3418, 2935, 2863, 1733, 1712, 1559, 1226,

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[1]) Compounds **7** and **8** may well be artifacts of **1** and **2**, respectively, formed during acetone extraction and purification.

1071, 1031, 950. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 318 (10, M^+), 300 (6), 269 (8), 241 (5), 162 (31), 149 (100). HR-EI-MS: 318.2193 (M^+, $C_{20}H_{30}O_3^+$; calc.: 318.2195).

*(3α,14β)-3,18-{[(1*S*)-4-(Acetylamino)butane-1,1-diyl]dioxy}-14,16-epoxy*-ent-*abieta-7,15(17)-diene* (*Rubescensin K*; **3**). White amorphous powder. $[\alpha]_D^{20} = +7.2$ ($c = 1.25$, AcOEt). UV (MeOH): 204 (4.29). IR (KBr): 3416, 2933, 2856, 1652, 1645, 1558, 1444, 1382, 1114, 1026. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 429 (10, M^+), 386 (3), 355 (2), 329 (8), 300 (8), 284 (9), 149 (100). FAB-MS: 430 ($[M+1]^+$). HR-EI-MS: 429.2871 (M^+, $C_{29}H_{39}NO_4^+$; calc. 429.2879),

*(3α,14β)-14,16-Epoxy-18-[(β-*D*-glucopyranosyl)oxy]*-ent-*abieta-7,15(17)-dien-3-ol* (*Rubescensin L*; **4**). White amorphous powder. $[\alpha]_D^{20} = -35.7$ ($c = 0.224$, pyridine). UV (MeOH): 205 (3.91). IR (KBr): 3441, 3410, 2933, 2861, 1662, 1635, 1079, 1023. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 480 (4, M^+), 462 (8), 444 (3), 329 (60), 318 (62), 300 (80), 282 (82), 269 (54), 149 (100). HR-EI-MS: 480.2708 (M^+, $C_{26}H_{40}O_8^+$; calc.: 480.2723).

*(1α,6β,7β,14β,15β,20*R*)-7α,20-Epoxy-20-[((3α,14β)-14,16-epoxy-3-hydroxy*-ent-*abieta-7,15(17)-dien-18-yl)oxy]*-ent-*kaur-16-ene-1,6,7,14,15-pentaol* (*Rubescensin M*; **5**). White amorphous powder. $[\alpha]_D^{20} = -22.5$ ($c = 0.355$, MeOH). UV (MeOH): 205 (4.34). IR (KBr): 3417, 2932, 2863, 1357, 1253, 1171, 1087, 1017, 981, 896. ^{1}H- and ^{13}C-NMR: see *Table 3*. FAB-MS: 683 ($[M+1]^+$), 365 ($[M_{\mathbf{5b}} - H_2O + 1]^+$). HR-FAB-MS: 683.4137 ($[M+H]^+$, $C_{40}H_{59}O_9^+$; calc.: 683.4159).

(3α,7β)-ent-*Abieta-8(14),15(17)-diene-3,7,16,18-tetraol* (*Rubescensin P*; **6**). White amorphous powder. $[\alpha]_D^{27} = +72.9$ ($c = 0.29$, MeOH). UV (MeOH): 204 (3.87). IR (KBr): 3291, 2936, 2863, 1387, 1307, 1052. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 336 (1, M^+), 318 (18), 300 (8), 282 (3), 269 (6), 251 (4), 241 (20), 162 (20), 149 (100). HR-EI-MS: 336.2312 (M^+, $C_{20}H_{32}O_4^+$; calc.: 336.2301).

(3α,14β)-3,18-[(1-Methylethane-1,1-diyl)dioxy]-ent-*abieta-7,15(17)-diene-14,16-diol* (**7**). White amorphous powder. $[\alpha]_D^{27} = +20.8$ ($c = 0.35$, MeOH). UV (MeOH): 204 (3.39). IR (KBr): 3442, 2933, 2861, 1559, 1540, 1507, 1457, 1382, 1207, 1096. ^{1}H-NMR (C_5D_5N, 400 MHz): 5.62 (br. *s*, H−C(7)); 5.57 (br. *s*, H_a−C(17)); 5.28 (br. *s*, H_b−C(17)); 4.65 (*d*, $J = 14.0$, H_a−C(16)); 4.57 (br. *s*, H_α−C(14)); 4.56 (*d*, $J = 14.0$, H_b−C(16)); 3.50 (overlapped, H_β−C(3)); 3.48 (*d*, $J = 10.6$, H_a−C(18)); 3.26 (*d*, $J = 10.6$, H_b−C(18)); 2.47 (br. *d*, $J = 12.4$, H_α−C(13)); 2.31 (overlapped, H_β−C(9)); 2.27, 1.65 (2*m*, CH_2(12)); 1.88–1.83 (overlapped, CH_2(6)); 1.82, 1.62 (2*m*, CH_2(2)); 1.78, 1.10 (2*m*, CH_2(1)); 1.70, 1.18 (2*m*, CH_2(11)); 1.21 (*s*, Me(19)); 0.98 (*dd*, $J = 12.0$, 4.4, H_β−C(5)); 0.81 (*s*, Me(20)). ^{13}C-NMR (C_5D_5N, 100 MHz): see *Table 1*. EI-MS: 376 (70, M^+), 361 (62), 358 (35), 343 (20), 340 (45), 300 (42), 283 (30), 282 (30), 265 (46), 232 (30), 167 (66), 149 (88), 55 (100). HR-EI-MS: 376.2621 (M^+, $C_{23}H_{36}O_4^+$; calc.: 376.2614).

(3α,14β)-14,16-Epoxy-3,18-[(1-methylethane-1,1-diyl)dioxy]-ent-*abieta-7,15(17)-diene-14,16-diol* (**8**). White amorphous powder. $[\alpha]_D^{26} = +15.4$ ($c = 0.29$, C_5H_5N). UV (MeOH): 204 (4.09). IR (KBr): 3441, 2986, 2929, 2856, 1383, 1209, 1098, 1027. ^{1}H-NMR (C_5D_5N, 400 MHz): 5.80 (br. *s*, H−C(7)); 5.02 (br. *s*, H_a−C(17)); 4.84 (br. *s*, H_b−C(17)); 4.54 (*d*, $J = 14.0$, H_a−C(16)); 4.24 (*d*, $J = 14.0$, H_b−C(16)); 4.16 (br. *s*, H_α−C(14)); 3.50 (*dd*, $J = 10.6$, 4.8, H_β−C(3)); 3.44 (*d*, $J = 10.6$, H_a−C(18)); 3.32 (*d*, $J = 10.6$, H_b−C(18)); 2.39 (*m*, H_α−C(13)); 2.04–1.90 (overlapped, CH_2(6)); 1.98 (overlapped, H_β−C(9)); 1.84, 1.58 (2*m*, CH_2(2)); 1.72, 1.06 (2*m*, CH_2(1)); 1.93, 1.04 (2*m*, CH_2(11)); 1.60, 1.41 (2*m*, CH_2(12)); 1.22 (*s*, Me(19)); 1.06 (overlapped, H_β−C(5)); 0.78 (*s*, Me(20)). ^{13}C-NMR (C_5D_5N, 100 MHz): see *Table 1*. EI-MS: 358 (68, M^+), 343 (72), 330 (8), 300 (30), 283 (50), 265 (30), 149 (100). FAB-MS: 359 ($[M+1]^+$). HR-EI-MS: 358.2521 (M^+, $C_{23}H_{34}O_3^+$; calc.: 358.2508).

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Received November 19, 2003