

New *ent*-Abietanoids from *Isodon rubescens*

by Quan-Bin Han^a), Rong-Tao Li^a), Ji-Xia Zhang^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, Yunnan, P.R. China

(Phone: +86-871-522 3251; fax: +86-871-521 6343; e-mail: hdsun@mail.kib.ac.cn or han_dongsun@hotmail.com)

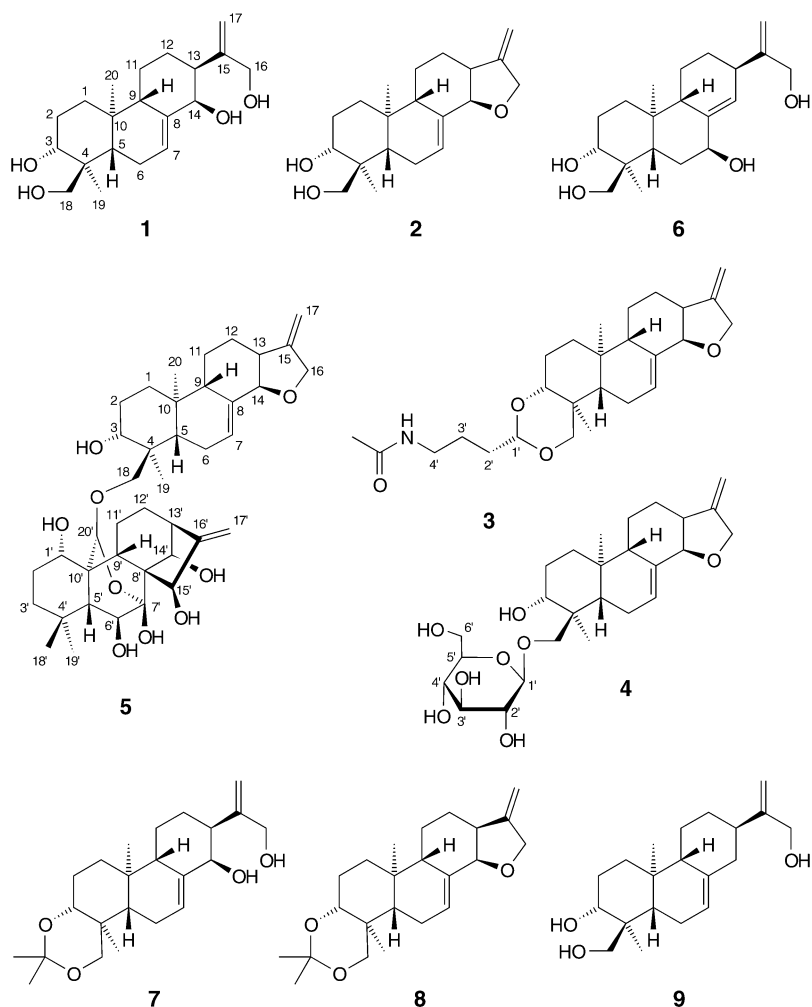
^b) Department of Chemistry, Xinxiang Medical College, Xinxiang 453000, Henan, P.R. China

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 1H - and ^{13}C -NMR data (Table I) indicated **1** to be an *ent*-abietanoid similar to laxiflorin O (**9**) reported from *I. eriocalyx* var. *laxiflora* [3]. Compound **1** was elucidated as (3 α ,14 β)-*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 tricyclic diterpenoid. Comparison of the 1H - and ^{13}C -NMR spectra of **1** and the known tricyclic *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**.

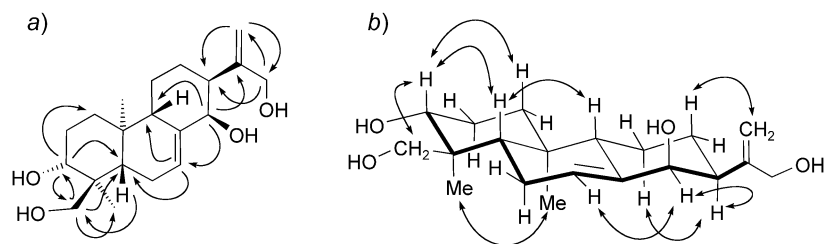


The above assignment was also corroborated by HMBC experiments (*Fig. 1,a*). The $\text{CH}_2(17)=\text{C}(15)$ moiety was confirmed by correlations of $\text{CH}_2(17)$ (δ 5.57 and 5.28) with $\text{C}(13)$ (δ 47.6) and $\text{C}(16)$ (δ 64.6). The other $\text{C}=\text{C}$ bond was assigned to $\text{C}(7)$ and $\text{C}(8)$ on the ground of the observed HMBC correlation of $\text{H}-\text{C}(7)$ at δ_{H} 5.66 with $\text{C}(5)$ at δ_{C} 43.0 and $\text{C}(9)$ at δ_{C} 48.5. Due to the presence of the HMBC correlations of $\text{H}-\text{C}(3)$ with $\text{C}(1)$, $\text{C}(5)$, and $\text{C}(18)$, of $\text{H}-\text{C}(14)$ with $\text{C}(7)$, $\text{C}(9)$, and $\text{C}(15)$, of $\text{CH}_2(16)$ with $\text{C}(13)$ and $\text{C}(17)$, and of $\text{CH}_2(18)$ with $\text{C}(3)$, $\text{C}(5)$, and $\text{C}(19)$, the four OH groups were placed at $\text{C}(3)$, $\text{C}(14)$, $\text{C}(16)$, and $\text{C}(18)$, 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. ^1H - and/or ^{13}C -NMR Data of Compounds **1**, **2**, **7**, and **8**. 400 and 100 MHz, resp.; $\text{C}_5\text{D}_5\text{N}$; δ in ppm, J in Hz. Asterisks (*) mark overlapping signals.

	1		7	2		8
	^{13}C	^1H	^{13}C	^{13}C	^1H	^{13}C
$\text{CH}_2(1)$	38.1 (t)	1.82, 1.18 (2m)	38.2 (t)	37.5 (t)	1.79, 1.18 (2m)	37.9 (t)
$\text{CH}_2(2)$	27.9 (t)	1.92, 1.85 (2m)	24.3 (t)	28.8 (t)	1.89, 1.62 (2m)	24.3 (t)
$\text{H}_\beta\text{-C}(3)$	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)
$\text{C}(4)$	43.3 (s)	–	36.7 (s)	42.7 (s)	–	36.6 (s)
$\text{H}_\beta\text{-C}(5)$	43.0 (d)	1.90*	45.6 (d)	42.3 (d)	1.94*	45.0 (d)
$\text{CH}_2(6)$	23.4 (t)	2.05–2.00*	22.4 (t)	23.5 (t)	2.09–2.03*	22.8 (t)
$\text{H-C}(7)$	124.3 (d)	5.66 (d, $J = 2.1$)	123.2 (d)	129.5 (d)	5.66 (d, $J = 2.1$)	128.8 (d)
$\text{C}(8)$	141.2 (s)	–	141.5 (s)	134.6 (s)	–	135.4 (s)
$\text{H}_\beta\text{-C}(9)$	48.5 (d)	2.40*	48.5 (d)	49.2 (d)	2.05*	49.5 (d)
$\text{C}(10)$	35.1 (s)	–	35.1 (s)	34.8 (s)	–	35.1 (s)
$\text{CH}_2(11)$	23.9 (t)	1.75, 1.20 (2m)	23.6 (t)	23.7 (t)	1.93, 1.04 (2m)	23.6 (t)
$\text{CH}_2(12)$	25.7 (t)	2.20, 1.65 (2m)	25.3 (t)	27.5 (t)	1.65, 1.42 (2m)	28.9 (t)
$\text{H}_\alpha\text{-C}(13)$	47.6 (d)	2.50 (br. d, $J = 12.4$)	47.3 (d)	45.8 (d)	2.41 (m)	45.9 (d)
$\text{H}_\alpha\text{-C}(14)$	74.8 (d)	4.58 (br. s)	74.5 (d)	83.4 (d)	4.18 (br. s)	83.5 (d)
$\text{C}(15)$	152.8 (s)	–	152.5 (s)	154.5 (s)	–	154.6 (s)
$\text{CH}_2(16)$	64.6 (t)	4.65, 4.56 (2d, $J = 14.0$)	64.7 (t)	69.4 (t)	4.55, 4.26 (2d, $J = 14.0$)	69.6 (t)
$\text{CH}_2(17)$	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)
$\text{CH}_2(18)$	66.1 (t)	4.10, 3.59 (2d, $J = 10.8$)	72.2 (t)	67.1 (t)	4.06, 3.60 (2d, $J = 10.8$)	72.1 (t)
$\text{Me-C}(19)$	13.0 (q)	1.15 (s)	12.9 (q)	12.6 (q)	1.12 (s)	12.7 (q)
$\text{Me-C}(20)$	15.0 (q)	0.92 (s)	15.9 (q)	15.0 (q)	0.86 (s)	15.3 (q)
$\text{Me}_2\text{C-O}$	–	–	99.0 (s)	–	–	99.0 (s)
			30.2 (q)			30.2 (q)
			19.4 (q)			19.4 (q)

Fig. 1. Key HMBC ($\text{H} \rightarrow \text{C}$) and ROESY correlations observed in **1**

from the ROEs of $\text{H}_\beta\text{-C}(3)$ with both $\text{H}_\beta\text{-C}(5)$ and $\text{CH}_2(18)$, and of $\text{H}_\alpha\text{-C}(14)$ with both $\text{H}_\alpha\text{-C}(13)$ and $\text{H-C}(7)$.

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 $\text{C}_{20}\text{H}_{30}\text{O}_3$, as determined by HR-EI-MS (m/z 318.2193; calc. 318.2195). Comparison of the ^1H -, ^{13}C -, and DEPT-NMR data of **2** and **1** revealed that **2** was also an *ent*-abietanoid, differing from **1** only at $\text{C}(14)$ and $\text{C}(16)$. The clear HMBCs correlations between $\text{H-C}(14)$ (δ_{H} 4.18) and $\text{C}(16)$ (δ_{C} 69.4), and between $\text{CH}_2(16)$ (δ_{H} 4.55 and 4.26) and $\text{C}(14)$ indicated the presence of an O-bridge between $\text{C}(14)$ and $\text{C}(16)$, 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 H₂O). Thus, **2** was elucidated as (3 α)-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 C₂₆H₃₉NO₄. On the basis of careful analysis of the ¹H-, ¹³C-, and 2D-NMR data (Table 2), compound **3** was identified as 3 α ,(3 α ,14 β)-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. ¹H- and ¹³C-NMR Data of Compounds **3**, **4**, and **6**. 400 and 100 MHz, resp.; C₅D₅N; 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 (<i>t</i>)	1.73, 1.05 (2 <i>m</i>)	37.6 (<i>t</i>)	1.68, 1.00 (2 <i>m</i>)	37.1 (<i>t</i>)	1.63, 1.25 (2 <i>m</i>)
CH ₂ (2)	24.2 (<i>t</i>)	1.70–1.18*	29.0 (<i>t</i>)	1.81, 1.52 (2 <i>m</i>)	27.9 (<i>t</i>)	1.90*
H β –C(3)	85.0 (<i>d</i>)	3.13 (<i>m</i>)	73.0 (<i>d</i>)	4.11*	74.9 (<i>d</i>)	4.23 (<i>m</i>)
C(4)	36.5 (<i>s</i>)	–	43.1 (<i>s</i>)	–	42.6 (<i>s</i>)	–
H β –C(5)	45.1 (<i>d</i>)	1.00*	42.8 (<i>d</i>)	1.83*	40.9 (<i>d</i>)	2.47 (<i>dd</i> , J = 13.0, 2.0)
CH ₂ (6)	22.9 (<i>t</i>)	1.70–1.18*	23.8 (<i>t</i>)	2.07, 1.95 (2 <i>m</i>)	30.5 (<i>t</i>)	2.09 (<i>d</i> , J = 13.0) 1.77 (<i>m</i>)
H–C(7)	128.8 (<i>d</i>)	5.78 (<i>d</i> , J = 2.0)	129.8 (<i>d</i>)	5.79 (<i>d</i> , J = 2.2)	72.3 (<i>d</i>)	4.49 (br. <i>s</i>)
C(8)	135.5 (<i>s</i>)	–	134.5 (<i>s</i>)	–	141.8 (<i>s</i>)	–
H β –C(9)	49.4 (<i>d</i>)	1.93*	49.1 (<i>d</i>)	1.90*	46.5 (<i>d</i>)	2.58 (br. <i>s</i>)
C(10)	35.1 (<i>s</i>)	–	35.1 (<i>s</i>)	–	38.7 (<i>s</i>)	–
CH ₂ (11)	23.6 (<i>t</i>)	1.70–1.18*	23.9 (<i>t</i>)	1.87, 0.97 (2 <i>m</i>)	22.6 (<i>t</i>)	1.70, 1.36 (2 <i>m</i>)
CH ₂ (12)	24.2 (<i>t</i>)	1.70–1.18*	27.5 (<i>t</i>)	1.57, 1.36 (2 <i>m</i>)	29.7 (<i>t</i>)	1.92, 1.32 (2 <i>m</i>)
H α –C(13)	45.9 (<i>d</i>)	2.37 (<i>m</i>)	46.1 (<i>t</i>)	2.38 (<i>m</i>)	39.4 (<i>d</i>)	2.89 (<i>m</i>)
H–C(14)	83.5 (<i>d</i>)	4.14 (<i>d</i> , J = 4.0, H α)	83.6 (<i>d</i>)	4.15 (<i>d</i> , J = 4.0, H α)	128.7 (<i>d</i>)	5.89 (br. <i>s</i>)
C(15)	154.6 (<i>s</i>)	–	154.9 (<i>s</i>)	–	155.1 (<i>s</i>)	–
CH ₂ (16)	69.6 (<i>t</i>)	4.53, 4.23 (2 <i>d</i> , J = 13.2)	69.6 (<i>t</i>)	4.53, 4.25 (2 <i>d</i> , J = 14.0)	64.2 (<i>t</i>)	4.41*
CH ₂ (17)	103.2 (<i>t</i>)	5.03, 4.84 (2 br. <i>s</i>)	103.2 (<i>t</i>)	5.00, 4.85 (2 br. <i>s</i>)	107.8 (<i>t</i>)	5.43, 4.99 (2 br. <i>s</i>)
CH ₂ (18)	77.9 (<i>t</i>)	3.69, 3.08 (2 <i>d</i> , J = 10.8)	76.0 (<i>t</i>)	4.21, 3.53 (2 <i>d</i> , J = 10.4)	69.4 (<i>t</i>)	4.17, 3.82 (2 <i>d</i> , J = 10.4)
Me–C(19)	13.6 (<i>q</i>)	1.17 (<i>s</i>)	12.8 (<i>q</i>)	1.05 (<i>s</i>)	12.8 (<i>q</i>)	1.18 (<i>s</i>)
Me–C(20)	15.3 (<i>q</i>)	0.73 (<i>s</i>)	15.2 (<i>q</i>)	0.78 (<i>s</i>)	14.6 (<i>q</i>)	0.90 (<i>s</i>)
H–C(1')	102.6 (<i>d</i>)	4.67 (br. <i>s</i>)	105.8 (<i>d</i>)	4.84 (<i>d</i> , J = 7.6)	–	–
H–C(2')	32.8 (<i>t</i>)	1.90–1.78 (2 H)*	74.9 (<i>d</i>)	4.04 (<i>dd</i> , J = 6.4, 2.0)	–	–
H–C(3')	24.7 (<i>t</i>)	1.90–1.78 (2 H)*	78.7 (<i>d</i>)	4.24*	–	–
H–C(4')	39.6 (<i>t</i>)	3.48 (2 H)*	72.2 (<i>d</i>)	4.13*	–	–
H–C(5')	–	–	78.6 (<i>d</i>)	4.02 (<i>m</i>)	–	–
CH ₂ (6')	–	–	63.2 (<i>t</i>)	4.62 (<i>d</i> , J = 11.0), 4.32 (<i>dd</i> , J = 11.0, 8.0)	–	–
NHAc	169.8 (<i>s</i>) 23.1 (<i>q</i>)	8.44 (br. <i>s</i> , NH) 1.99 (<i>s</i>)	–	–	–	–

Examination of the ^1H - 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 $\text{CH}_2(4')$ 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 $^1\text{H}, ^1\text{H}$ -COSY experiment, as well as from the HMBC correlations (Fig. 2) of both the $\text{CH}_2(4')$ (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 $^1\text{H}, ^1\text{H}$ -COSY spectrum, the overlapping $\text{CH}_2(2')$ and $\text{CH}_2(3')$ resonances (δ_{H} 1.90–1.78) exhibited correlations with the H-atoms of CH(1') and $\text{CH}_2(4')$ (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 $_{\beta}$ –C(3) and $\text{CH}_2(18)$, confirming β -configuration at C(1').

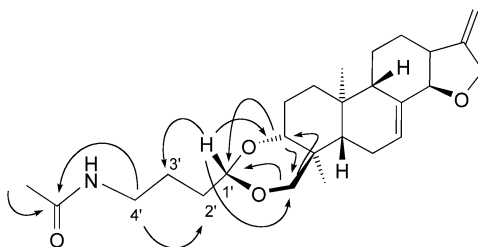


Fig. 2. Key HMBC (H \rightarrow C) correlations observed in **3**

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 $\text{C}_{26}\text{H}_{40}\text{O}_8$, as determined by HR-EI-MS (480.2708 (M^+ ; calc. 480.2723)). Analysis of the ^1H - 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 ^1H - 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 $\text{CH}_2(18)$ (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 $\text{C}_{40}\text{H}_{58}\text{O}_9$, as determined by both HR-FAB-MS and ^1H - 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 ^1H - 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 7 α ,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 CH₂(18) and C(20') (Fig. 3).

Table 3. ^1H - and ^{13}C -NMR Data of Compound **5**. Note that **5a** and **5b** are ether-brided fragment structures (see chemical formula). 400 and 100 MHz, resp.; C₅D₅N; δ in ppm, J in Hz. Asterisks (*) mark overlapping signals.

5a			5b		
	^{13}C	^1H		^{13}C	^1H
CH ₂ (1)	37.9 (<i>t</i>)	1.78, 1.11 (<i>2m</i>)	H _{β} –C(1')	75.9 (<i>d</i>)	3.66 (<i>dd</i> , J = 9.6, 4.0)
CH ₂ (2)	29.1 (<i>t</i>)	1.91*, 1.65*	CH ₂ (2')	31.0 (<i>t</i>)	1.91*, 1.75*
H _{β} –C(3)	73.4 (<i>d</i>)	3.95*	CH ₂ (3')	39.3 (<i>t</i>)	1.36*, 1.29*
C(4)	42.3 (<i>s</i>)	–	C(4')	33.6 (<i>s</i>)	–
H _{β} –C(5)	42.5 (<i>d</i>)	2.09 (<i>dd</i> , J = 9.6, 2.4)	H _{β} –C(5')	56.7 (<i>d</i>)	1.62 (<i>d</i> , J = 5.0)
CH ₂ (6)	23.7 (<i>t</i>)	2.52, 2.00 (<i>2m</i>)	H _{α} –C(6')	71.6 (<i>d</i>)	4.11 (<i>d</i> , J = 5.0)
H–C(7)	130.8 (<i>d</i>)	5.59 (<i>m</i>)	C(7')	101.5 (<i>s</i>)	–
C(8)	133.7 (<i>s</i>)	–	C(8')	52.4 (<i>s</i>)	–
H _{β} –C(9)	50.5 (<i>d</i>)	1.97*	H _{β} –C(9')	45.0 (<i>d</i>)	2.89 (<i>m</i>)
C(10)	34.9 (<i>s</i>)	–	C(10')	43.5 (<i>s</i>)	–
CH ₂ (11)	23.3 (<i>t</i>)	1.86*, 1.00*	CH ₂ (11')	21.6 (<i>t</i>)	2.67, 2.24 (<i>2m</i>)
CH ₂ (12)	27.8 (<i>t</i>)	1.60, 1.26 (<i>2m</i>)	CH ₂ (12')	33.9 (<i>t</i>)	2.55 (<i>m</i>), 1.74*
H _{α} –C(13)	45.3 (<i>d</i>)	2.39 (<i>m</i>)	H _{α} –C(13')	46.4 (<i>d</i>)	2.93 (<i>d</i> , J = 7.2)
H _{α} –C(14)	83.5 (<i>d</i>)	4.12*	H _{α} –C(14')	75.9 (<i>d</i>)	5.16 (<i>s</i>)
C(15)	153.7 (<i>s</i>)	–	C(15')	73.1 (<i>d</i>)	5.65 (<i>s</i>)
CH ₂ (16)	69.2 (<i>t</i>)	4.45, 4.13 (<i>2d</i> , J = 10.4)	CH ₂ (16')	161.2 (<i>s</i>)	–
CH ₂ (17)	103.8 (<i>t</i>)	5.02, 4.85 (2 br. <i>s</i>)	CH ₂ (17')	109.0 (<i>t</i>)	5.65, 5.37 (2 br. <i>s</i>)
CH ₂ (18)	71.7 (<i>t</i>)	3.96, 3.87 (<i>2d</i> , J = 7.0)	Me–C(18')	33.5 (<i>q</i>)	1.16 (<i>s</i>)
Me–C(19)	12.5 (<i>q</i>)	1.02 (<i>s</i>)	Me–C(19')	22.3 (<i>q</i>)	1.05 (<i>s</i>)
Me–C(20)	15.0 (<i>q</i>)	0.81 (<i>s</i>)	H–C(20')	101.8 (<i>d</i>)	5.76 (<i>s</i>)

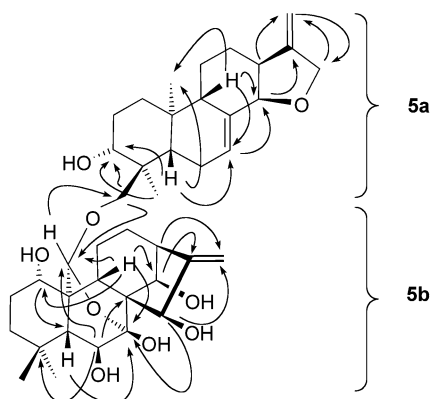


Fig. 3. Key HMBC (H \rightarrow C) correlations observed in **5**

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 CH₂(18), confirming the linkage between the two moieties of **5**.

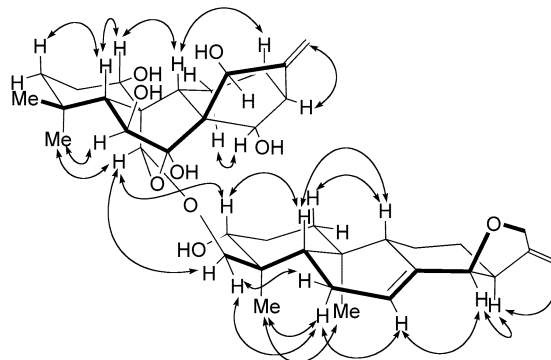


Fig. 4. Key ROESY correlations observed in **5**

Rubescensin P (**6**), an amorphous powder, had the same molecular formula (C₂₀H₃₂O₄) as **1**, as revealed by HR-EI-MS. Comparison of the ¹³C-NMR data of **6** (Table 2) and **1** (Table 1) indicated that they both had two OCH₂, two OCH, two olefinic quaternary C-atoms, an olefinic CH, and an olefinic CH₂, 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 (3 α ,7 β)-*ent*-abieta-8(14),15(17)-diene-3,7,16,18-tetraol.

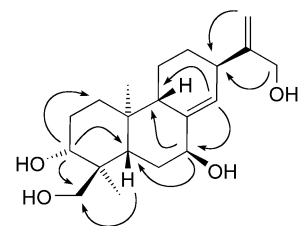


Fig. 5. Key HMBC (H \rightarrow C) correlations observed in **6**

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 $\text{CH}_2(18)$ (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*-abiet-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 cytotoxicity 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 $\mu\text{g/ml}$, which is in the range of cisplatin (1.44 $\mu\text{g/ml}$).

Experimental Part

General. Optical rotations: JASCO DIP-370 digital polarimeter. UV Spectra: Shimadzu UV-210A spectrometer; λ_{max} in nm, ($\log \epsilon$). 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 $\text{C}_5\text{D}_5\text{N}$; 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 ($3\times$), 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); $\text{CHCl}_3/\text{acetone}$ 1:0 \rightarrow 0:1), affording *Fractions I–IX*. After repeated CC (SiO_2 ; gradient mixtures of $\text{CHCl}_3/\text{MeOH}$), *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 ($3\times$), 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 ; $\text{CHCl}_3/\text{acetone}$ 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 $\text{CHCl}_3/\text{MeOH}$), followed by repeated prep. TLC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ 10:1).

(3 α ,14 β)-*ent*-Abieta-7,15(17)-diene-3,14,16,18-tetraol (*Rubescensin I*; **1**). White amorphous powder. $[\alpha]_{\text{D}}^{25} = +38.9$ ($c = 0.32$, MeOH). UV (MeOH): 203 (4.74). IR (KBr): 3417, 2933, 2870, 1385, 1087, 1056, 917. ^1H - 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^+ , $\text{C}_{20}\text{H}_{32}\text{O}_4^+$; calc.: 336.2301).

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

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

1071, 1031, 950. ^1H - 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^+ , $\text{C}_{20}\text{H}_{30}\text{O}_3^+$; calc.: 318.2195).

(3 α ,14 β)-3,18-[(1*S*)-4-(Acetylamino)butane-1,1-diyl]dioxyl-14,16-epoxy-ent-abieta-7,15(17)-diene (*Rubescensin K*; **3**). White amorphous powder. $[\alpha]_{\text{D}}^{20} = +7.2$ ($c = 1.25$, AcOEt). UV (MeOH): 204 (4.29). IR (KBr): 3416, 2933, 2856, 1652, 1645, 1558, 1444, 1382, 1114, 1026. ^1H - 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^+ , $\text{C}_{29}\text{H}_{39}\text{NO}_4^+$; calc.: 429.2879).

(3 α ,14 β)-14,16-Epoxy-18-[(β -D-glucopyranosyl)oxy]-ent-abieta-7,15(17)-dien-3-ol (*Rubescensin L*; **4**). White amorphous powder. $[\alpha]_{\text{D}}^{20} = -35.7$ ($c = 0.224$, pyridine). UV (MeOH): 205 (3.91). IR (KBr): 3441, 3410, 2933, 2861, 1662, 1635, 1079, 1023. ^1H - 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^+ , $\text{C}_{26}\text{H}_{40}\text{O}_8^+$; calc.: 480.2723).

(1 α ,6 β ,7 β ,14 β ,15 β ,20*R*)-7 α ,20-Epoxy-20-[(3 α ,14 β)-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. $[\alpha]_{\text{D}}^{20} = -22.5$ ($c = 0.355$, MeOH). UV (MeOH): 205 (4.34). IR (KBr): 3417, 2932, 2863, 1357, 1253, 1171, 1087, 1017, 981, 896. ^1H - and ^{13}C -NMR: see Table 3. FAB-MS: 683 ($[M+1]^+$), 365 ($[M_{\text{SB}} - \text{H}_2\text{O} + 1]^+$). HR-FAB-MS: 683.4137 ($[M+H]^+$, $\text{C}_{40}\text{H}_{59}\text{O}_9^+$; calc.: 683.4159).

(3 α ,7 β)-ent-Abieta-8(14),15(17)-diene-3,7,16,18-tetraol (*Rubescensin P*; **6**). White amorphous powder. $[\alpha]_{\text{D}}^{27} = +72.9$ ($c = 0.29$, MeOH). UV (MeOH): 204 (3.87). IR (KBr): 3291, 2936, 2863, 1387, 1307, 1052. ^1H - 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^+ , $\text{C}_{20}\text{H}_{32}\text{O}_4^+$; calc.: 336.2301).

(3 α ,14 β)-3,18-[(1-Methylethane-1,1-diyl)dioxyl]-ent-abieta-7,15(17)-diene-14,16-diol (**7**). White amorphous powder. $[\alpha]_{\text{D}}^{27} = +20.8$ ($c = 0.35$, MeOH). UV (MeOH): 204 (3.39). IR (KBr): 3442, 2933, 2861, 1559, 1540, 1507, 1457, 1382, 1207, 1096. ^1H -NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz): 5.62 (br. s, H-C(7)); 5.57 (br. s, H $_{\text{a}}$ -C(17)); 5.28 (br. s, H $_{\text{b}}$ -C(17)); 4.65 (d , $J = 14.0$, H $_{\text{a}}$ -C(16)); 4.57 (br. s, H $_{\text{a}}$ -C(14)); 4.56 (d , $J = 14.0$, H $_{\text{b}}$ -C(16)); 3.50 (overlapped, H $_{\text{b}}$ -C(3)); 3.48 (d , $J = 10.6$, H $_{\text{a}}$ -C(18)); 3.26 (d , $J = 10.6$, H $_{\text{b}}$ -C(18)); 2.47 (br. d , $J = 12.4$, H $_{\text{a}}$ -C(13)); 2.31 (overlapped, H $_{\text{b}}$ -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 $_{\text{b}}$ -C(5)); 0.81 (s, Me(20)). ^{13}C -NMR ($\text{C}_5\text{D}_5\text{N}$, 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^+ , $\text{C}_{23}\text{H}_{36}\text{O}_4^+$; calc.: 376.2614).

(3 α ,14 β)-14,16-Epoxy-3,18-[(1-methylethane-1,1-diyl)dioxyl]-ent-abieta-7,15(17)-diene-14,16-diol (**8**). White amorphous powder. $[\alpha]_{\text{D}}^{26} = +15.4$ ($c = 0.29$, $\text{C}_5\text{H}_5\text{N}$). UV (MeOH): 204 (4.09). IR (KBr): 3441, 2986, 2929, 2856, 1383, 1209, 1098, 1027. ^1H -NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz): 5.80 (br. s, H-C(7)); 5.02 (br. s, H $_{\text{a}}$ -C(17)); 4.84 (br. s, H $_{\text{b}}$ -C(17)); 4.54 (d , $J = 14.0$, H $_{\text{a}}$ -C(16)); 4.24 (d , $J = 14.0$, H $_{\text{b}}$ -C(16)); 4.16 (br. s, H $_{\text{a}}$ -C(14)); 3.50 (dd , $J = 10.6$, 4.8, H $_{\text{b}}$ -C(3)); 3.44 (d , $J = 10.6$, H $_{\text{a}}$ -C(18)); 3.32 (d , $J = 10.6$, H $_{\text{b}}$ -C(18)); 2.39 (m , H $_{\text{a}}$ -C(13)); 2.04–1.90 (overlapped, CH $_2$ (6)); 1.98 (overlapped, H $_{\text{b}}$ -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 $_{\text{b}}$ -C(5)); 0.78 (s, Me(20)). ^{13}C -NMR ($\text{C}_5\text{D}_5\text{N}$, 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^+ , $\text{C}_{23}\text{H}_{34}\text{O}_5^+$; calc.: 358.2508).

REFERENCES

- [1] Q. B. Han, S. X. Mei, B. Jiang, A. H. Zhao, H. D. Sun, *Chin. J. Org. Chem.* **2003**, 23, 270.
- [2] Q. B. Han, B. Jiang, J. X. Zhang, Xue-Mei Niu, H. D. Sun, *Helv. Chim. Acta* **2003**, 86, 773.
- [3] X. M. Niu, S. H. Li, Q. S. Zhao, S. X. Mei, Z. W. Lin, H. D. Sun, Y. Lu, C. Wang, Q. T. Zheng, *Helv. Chim. Acta* **2003**, 86, 299.
- [4] Y. H. Gong, in 'The ^{13}C -NMR Data of Natural Organic Compounds', Yunnan Sciences and Technology Press, Kunming, 1986, p. 398.
- [5] Y. Takeda, K. Takeda, T. Fujita, H. D. Sun, Y. Minami, *Chem. Pharm. Bull.* **1990**, 38, 439.
- [6] Y. Takeda, K. Takeda, T. Fujita, H. D. Sun, Y. Minami, *Phytochemistry* **1994**, 35, 1513.
- [7] Q. B. Han, M. L. Li, S. H. Li, Y. K. Mou, Z. W. Lin, H. D. Sun, *Chem. Pharm. Bull.* **2003**, 51, 790.
- [8] Y. Takeda, H. Otsuka, *Stud. Nat. Prod. Chem.* **1995**, 15, 111.

Received November 19, 2003