Rubescensins S and T: Seco-*ent*-Kaurane Diterpenoids from *Isodon rubescens* var. *taihangensis*

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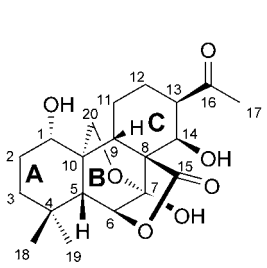
Two new diterpenoids, rubescensin S (=(1α,6β,14β)-7α,20-epoxy-1,7,14-trihydroxy-16-oxo-15,16-seco-*ent*-kauran-6,15-olide; **1**) and rubescensin T (=(1α,6β,11β,20*S*)-7α,20-epoxy-1,6,7-trihydroxy-20-methoxy-8,15-seco-*ent*-kaur-16-en-11,15-olide; **2**) were isolated from the Chinese medicinal herb *Isodon rubescens* var. *taihangensis*. Compound **1** possesses a unique, unprecedented 15,16-seco-*ent*-kaurane skeleton. Both compounds exhibited cytotoxic activities against K562 human leukemia cells.

Introduction. – The genus *Isodon* (family Labiatae) is well-known as a rich source of *ent*-kaurane diterpenoids. Following the first reported antitumor constituent enmein, a 6,7-seco-*ent*-kauranoid from *I. japonica* [1], *ca.* 400 *ent*-kauranoids have been isolated from the plants of this genus [2]. Many of these diterpenoids display various biological activities, such as antibacterial [3], anti-inflammatory [4], stomachic [2], and especially antitumor actions [5–8]. Some *ent*-kaurane derivatives even displayed significant anti-HIV activities [9]. During our continuous phytochemical investigation of *I. rubescens* (HEMSL.) HERA and its varieties, we have reported more than 50 diterpenoids, and many of them showed inhibitory effects against K562 human leukemia cells [10–15]. Our search for bioactive substances from *I. rubescens* var. *taihangensis* GAO et LI [16] has now led to the isolation of two novel seco-*ent*-kauranoids named rubescensins S (**1**) and T (**2**). Details of the isolation, structural determination, and bioactivities of these two new compounds are presented here.



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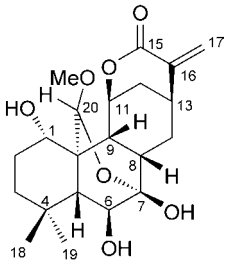

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Results and Discussion. – Compound **1** was determined to have the molecular formula $C_{20}H_{28}O_7$ (seven degrees of unsaturation) by its strong $[M+H]^+$ ion at *m*/*z*

381.1928 in the positive HR-FAB mass spectrum. The NMR spectra (*Table*) exhibited signals for 25 H-atoms attached to C-atoms, along with those for 20 C-atoms. On the basis of careful analysis of ^{1}H-, ^{13}C-, and 2D-NMR data, compound **1** was identified as (1α,6β,14β)-7α,20-epoxy-1,7,14-trihydroxy-16-oxo-15,16-seco-*ent*-kauran-6,15-olide, and named rubescensin S. This is the first 15,16-seco-*ent*-kaurane diterpenoid isolated from a natural resource.

Table. *^{1}H- and ^{13}C-NMR Data, Including HMBC Correlations, of Compounds* **1** *and* **2**. Parameters: 500 (^{1}H) and 125 MHz (^{13}C); solvent: C_5D_5N; δ in ppm, J in Hz. Asterisks (*) mark overlapping signals.

	1			**2**		
	δ_C	δ_H	HMBC (position)	δ_C	δ_H	HMBC (position)
H_β–C(1)	73.2 (*d*)	3.70 (*dd*, *J* = 10.0, 5.5)	3, 5, 20	78.5 (*d*)	3.65 (*dd*, *J* = 9.0, 4.8)	5, 9, 10, 20
H_α–C(2)	29.9 (*t*)	1.87 (*m*)	1, 4, 10	29.8 (*t*)	1.79 (*m*)	1, 3, 4
H_β–C(2)		1.89 (*m*)	1, 3, 4		1.88 (*m*)	1, 10
H_α–C(3)	38.9 (*t*)	1.36 (*dt*, *J* = 13.0, 3.0)	1, 4, 5, 18, 19	39.0 (*t*)	1.39 (br. *d*, *J* = 13.0)	1, 4, 5, 18, 19
H_β–C(3)		1.27 (*dt*, *J* = 13.0, 4.7)	1, 5, 18, 19		1.26 (*dt*, *J* = 13.0, 2.5)	5, 18, 19
C(4)	33.0 (*s*)	–		33.3 (*s*)	–	
H_β–C(5)	57.3 (*d*)	1.59 (*s*)	1, 6, 7, 18, 19, 20	63.1 (*d*)	1.57 (*s*)	1, 6, 7, 18, 19, 20
H_α–C(6)	80.6 (*d*)	4.61 (*s*)	4, 5, 7, 8, 10, 15	73.2 (*d*)	4.08 (*s*)	4, 5, 7, 8, 10
C(7)	101.1 (*s*)	–		100.7 (*s*)	–	
C(8) or H_β–C(8)	50.1 (*s*)	–		28.6 (*d*)	3.27 (*m*)	
H_β–C(9)	52.4 (*d*)	2.14*	1, 5, 7, 8, 10, 14, 15, 20	47.5 (*d*)	2.80 (*d*, *J* = 10.5)	1, 5, 7, 8, 10, 14, 20
C(10)	41.3 (*s*)	–		45.1 (*s*)	–	
H_α–C(11)	21.6 (*t*)	2.17*	8, 9, 12, 13	73.2 (*d*)	5.82 (*s*)	8, 9, 10, 12, 13, 15
H_β–C(11)		2.31 (*m*)	9, 10, 13		–	
H_α–C(12)	24.8 (*t*)	2.03 (*m*)	9, 11, 13, 14, 16	27.0 (*t*)	2.70 (br. *d*, *J* = 13.5)	9, 11, 13, 16
H_β–C(12)		1.92 (*m*)	9, 13, 16		1.91 (*m*)	9, 13, 14, 16
H_α–C(13)	48.6 (*d*)	4.12 (*dt*, *J* = 10.5, 2.5)	8, 11, 12, 14, 16, 17	32.3 (*d*)	2.94 (*m*)	11, 15, 16, 17
H_α–C(14)	66.7 (*t*)	5.26 (*d*, *J* = 10.5)	7, 8, 9, 13, 15, 16	29.6 (*t*)	2.55 (*dt*, 13.5, 3.5)	7, 8, 9, 13, 16
H_β–C(14)		–			2.19 (*m*)	7, 9, 13, 16
C(15)	177.0 (*s*)	–		165.3 (*s*)	–	
C(16)	211.2 (*s*)	–		140.9 (*s*)	–	
H–C(17)	30.2 (*q*)	2.23 (*s*)	13, 16	127.0 (*t*)	6.52, 5.45 (2*s*)	13, 15, 16
Me(18)	31.6 (*q*)	0.94 (*s*)	3, 4, 5, 19	32.4 (*q*)	1.09 (*s*)	3, 4, 5, 19
Me(19)	21.3 (*q*)	1.01 (*s*)	3, 4, 5, 18	21.6 (*q*)	0.99 (*s*)	3, 4, 5, 18
CH_2(20) or H–C(20)	64.3 (*t*)	4.68 (*d*, *J* = 10.5), 4.48 (*d*, *J* = 10.5)	1, 5, 7, 9, 10	100.9 (*d*)	5.42 (*s*)	1, 2, 5, 7, 9, 10
MeO	–	–		53.4 (*q*)	3.48 (*s*)	20

In the ^{13}C-NMR spectrum of **1** (see the *Table*), the characteristic signals of the skeleton of a 7β-hydroxy-7α,20-epoxykaurane were present: two non-oxy Me groups (δ_C 31.6 and 21.3), three non-oxy CH (δ_C 57.3, 52.4, and 48.6), three non-oxy quaternary C-atoms (δ_C 33.0, 50.1, and 41.3), one OCH_2 (δ_C 64.3), and one hemiketal C-atom (δ_C 101.1). By comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of a known 7α,20-epoxy-*ent*-kauranoid, oridonin (**3**), the major diterpene constituent of this plant [10], it was confirmed that compound **1** was also a 7α,20-epoxy-*ent*-kauranoid, and was very similar with **3** regarding rings *A*–*C*. This assignment was supported by detailed analyses of the COSY, HMQC, and HMBC data of **1** (*Table*). Three OCH moieties of **1**, as those of **3**, were assigned to C(1), C(6), and C(14) by the HMBC correlations of H–C(1) with C(5), C(9), and C(20), of H–C(6) with C(4), C(5), C(7), and C(8), and of H–C(14) with C(7), C(8), C(9), and C(13), respectively, as well as by the COSY interactions of H–C(6) with H–C(5) and of H–C(14) with H–C(13).

By contrast, the data for the remaining portion of the structure were quite distinctive from those of known kauranes previously reported from this plant. These data included signals of a lactone C=O C-atom, and an isolated Me group whose H-atoms displayed HMBC coupling with a ketone C-atom, together composing an acetyl (Ac) group. The key to the structural elucidation of **1** was the location of the lactone and the Ac groups. The lactone C=O C-atom was assigned to C(15) linked to C(6) through an oxy-bridge on the basis of the HMBC correlations of H–C(9), H–C(14), and H–C(6) with this C-atom. This linkage was supported by the significant downfield shift of C(6) (δ_C 80.6). Accordingly, the Ac group was linked to C(13) due to HMBC correlations between the Ac H-atoms with C(13), and H–C(14) with the Ac C=O C-atom. These assignments led to a structurally new compound with a unique 15,16-seco-kaurane skeleton.

The ROESY spectrum was used to establish the relative configuration of **1**. Although we have not yet determined the absolute configuration, we assumed the *ent*-kaurane configuration found in the kauranes from the genus *Isodon* plants. The key NOEs of H–C(1) with H_β–C(5) and H_β–C(9), of H–C(6) with Me(19), and of H–C(14) with H_α–C(11) indicated the presence of H_β–C(1), H_α–C(6), and H_α-C(14), and suggested the chair and twisted boat conformations for rings *A*–*C*, as displayed in *Fig. 1*.

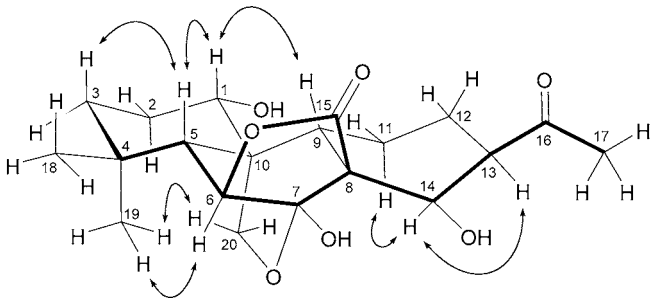
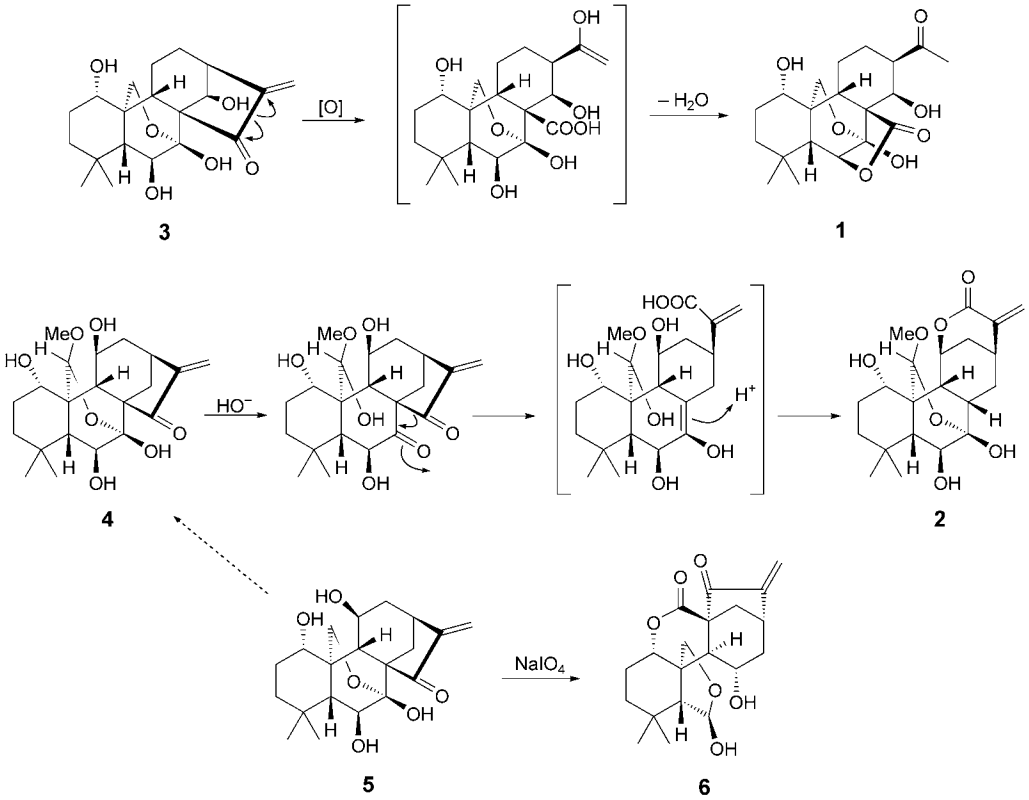


Fig. 1. *Key ROESY correlations of rubescensin S* (**1**)

The molecular formula of rubescensin T (**2**) was determined to be $C_{21}H_{30}O_7$ (seven degrees of unsaturation) by analysis of its NMR, EI-MS, and positive HR-FAB-MS spectra. The ^{1}H- and ^{13}C-NMR specta closely matched with those of rabdoternin G (**4**), a known *ent*-kauranoid obtained from the genus *Isodon* [17] (see the *Scheme* below). Significant differences included the presence of a new non-oxy CH signal at δ_C 28.6, an α,β-unsaturated lactone C=O signal at δ_C 165.3, and the absence of a non-oxy quaternary C-atom and an α,β-unsaturated C=O C-atom. Moreover, the new methine resonance (δ_C 3.27) displayed an apparent ^{1}H,^{1}H-COSY correlation with H–C(9) (δ_H 2.80), leading to the placement of this methine at C(8). Another resonance (δ_H 5.82) also exhibited ^{1}H,^{1}H-COSY coupling with H–C(9), being assignable to H–C(11), and showed a clear HMBC interaction with the above-mentioned lactone C=O C-atom. These observations strongly indicated that the C(8)–C(15) bond was cleaved, allowing the α,β-unsaturated C=O group to form a lactone ring with the 11-OH group. Thus, the establishment of a rare 8,15-seco-kaurane moiety was achieved. The (20*S*)-config-

Scheme. *Supposed Biogenesis of* **1** *and* **2**, *and Chemical Transformation of* **5** *to* **6**



uration was assigned on the basis of an NOE interaction between H–C(20) and the H-atoms of the Me(19) group in the ROESY spectrum. Accordingly, compound **2** was elucidated to be (1α,6β,11β,20*S*)-7α,20-epoxy-1,6,7-trihydroxy-20-methoxy-8,15-seco-*ent*-kaur-16-en-11,15-olide as confirmed by a detailed analysis of its HMBC (*Table*) and ROESY (*Fig. 2*) spectra.

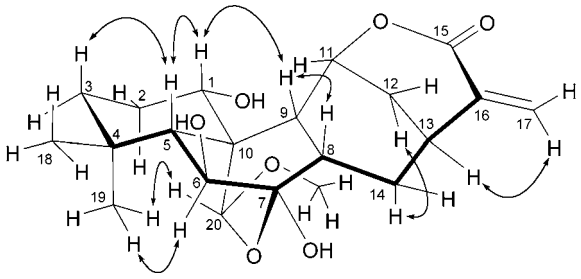


Fig. 2. *Key ROESY correlations of rubescensin T* (**2**)

To date, over 100 bioactive 6,7-seco-*ent*-kauranoids with various oxygenations have been reported from the genus *Isodon*, following the first example enmein. This kind of seco-skeleton could be chemically transformed from a 'normal' *ent*-kaurane, *e.g.*, lasiodonin (**5**), to a 6,7-seco-*ent*-kauranoid, epinodosin (**6**) [18]. The isolation of these two novel seco-*ent*-kauranoids might lead to more new natural (or semi-natural) bioactive substances with rich oxygenation patterns, as enmein did. A possible biogenesis pathway of compounds **1** and **2** from the major diterpene components, oridonin **3** and lasiodonin **5**, of this plant is proposed in the *Scheme* [19]. The possibility that **1** and **2** were artifacts produced during extraction and purification can be excluded because the isolation conditions were very mild and did not involve temperatures above 60° or of the use of acids or bases.

Rubescensins S (**1**) and T (**2**) both exhibited cytotoxic acitivities against K562 human leukemia cells (IC_{50} = 7.03 and 6.18 μg/ml, resp.), with cisplatin (IC_{50} = 1.14 μg/ml) [14] being the positive control.

Experimental Part

General. Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Bio-Rad FtS-135* spectrometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker DRX-500* spectrometer; δ in ppm, J in Hz; Me_4Si as internal standard, measured in C_5D_5N. Mass spectra: *VG Autospec-3000* spectrometer; 70 eV (EI); in m/z (rel. %).

Plant Material. The plants were collected in the Hebi Prefecture, Henan Province, China, in August 2000, and identified by Prof. *Zhong-Wen Lin*. A voucher specimen (KIB-2000-10 Lin) was deposited at the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The air-dried leaves (10 kg) were extracted at r.t. overnight with 70% aq. acetone (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) was subjected to column chromatography (CC) (SiO_2, 100–200 mesh, 3.0 kg; $CHCl_3$/acetone 1:0 → 0:1): *Fr. I–IX*. After repeated CC (SiO_2, gradient mixtures of $CHCl_3$/acetone), *Fr. V* afforded a subfraction (15 mg), which exhibited an interesting blue spot on a TLC plate (SiO_2), when heated at 200° after dipping in 10% ethanolic sulphuric acid. This subfraction was subjected to prep. RP-HPLC (*HP-1100*, *ZORBAX SB-C18* column, 9.4 × 250 mm; H_2O/MeOH 2:3, 3 ml/min, 204-nm detection, t_R 26.4 min) to give pure **2** (8.0 mg). A search for the similar blue spot disclosed **1** in another subfraction (12.3 mg) from *Fr. VI*. Pure **1** (6.9 mg) was isolated by the same RP-HPLC separation (t_R 21.6 min).

(1α,6β,14β)-7α,20-Epoxy-1,7,14-trihydroxy-16-oxo-15,16-seco-ent-*kauran-6,15-olide* (= *Rubescensin S*; **1**). White amorphous powder. t_R 21.6 min (*ZORBAX SV-C18* column (9.4 × 250 mm); H_2O/MeOH 3:2, flow rate 3 ml/min, detection at 204 nm). $[\alpha]_D^{22.3} = +8.0$ (c = 0.31, acetone). IR (KBr): 3476, 2953, 2927, 2864, 1778, 1694, 1147, 1023. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 380 (1, M^+), 362 (60), 320 (47), 276 (25), 196 (70), 178 (52), 150 (77), 107 (100). FAB-MS: 381 ($[M+H]^+$). HR-FAB-MS: 381.1928 ($[M+H]^+$, $C_{20}H_{29}O_7^+$; calc. 381.1913).

(1α,6β,11β,20S)-7α,20-Epoxy-1,6,7-trihydroxy-20-methoxy-8,15-seco-ent-*kaur-16-en-11,15-olide* (= *Rubescensin T*; **2**). White amorphous powder. t_R 26.4 min (*ZORBAX SV-C18* column (9.4 × 250 mm); H_2O/MeOH 3:2, flow rate 3 ml/min, detection at 204 nm). $[\alpha]_D^{22.1} = +60.9°$ (c = 0.12, acetone). IR (KBr): 3443, 2932, 1705, 1700, 1623, 1295, 1160, 1067. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 394 (1, M^+), 376 (4), 362 (63), 344 (41), 334 (10), 316 (91), 298 (23), 288 (26), 275 (44), 263 (46), 245 (22), 231 (18), 81 (100). FAB-MS: 395 ($[M+H]^+$). HR-FAB-MS: 395.2066 ($[M+H]^+$, $C_{21}H_{31}O_7^+$; calc. l395.2070).

Cytotoxicity Assay. Compounds **1** and **2** were evaluated for their cytotoxic activities against the K562 human leukemia cell line, using the improved MTT method previously described [14], with cisplatin as the positive control (IC_{50} = 1.14 μg/ml). The IC_{50} values were determined to be 7.03 μg/ml (**1**) and 6.18 μg/ml (**2**). The OD data were recorded in X ± S, and the IC_{50} values were calculated with the GWBASIC software.

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