Two Novel *ent*-Kaurene Diterpenoids from *Isodon rubescens*

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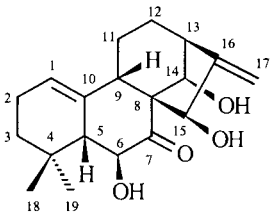
A novel 20-nor-*ent*-kaurene diterpenoid, rubescensin N (**1**), and a new 7,20-epoxy-*ent*-kaurene diterpenoid, rubescensin O (**2**), along with the seven known diterpenoids rabdoternins A–F and xerophilusin N, were isolated from *Isodon rubescens* collected in Jiyuan prefecture, Henan Province, China. Their structures were established by extensive spectroscopic analysis. Compound **1** is the first example of a naturally occurring 20-nor-*ent*-kaurene diterpenoid from the *Isodon* genus plants.

Introduction. – *Isodon rubescens* (HEMSL.) HARA, recorded in the Pharmacopoeia of People's Republic of China (edition of 1977), is a famous folk medicine for its antibacterial, anti-inflammatory, and antitumor activities. More than twenty *ent*-kaurene diterpenoids have been reported from the leaves of *I. rubescens* collected in different regions [1–17]. Our careful reinvestigation of this plant collected in Jiyuan prefecture of Henan Province led to the isolation of a novel 20-nor-*ent*-kaurene diterpenoid, rubescensin N (**1**), and a new 7,20-epoxy-*ent*-kaurene diterpenoid, rubescensin O (**2**), along with the seven known compounds rabdoternins A–F and xerophilusin N. In this paper, we wish to report the isolation and structural elucidation of the two new compounds.

Results and Discussion. – Rubescensin N (**1**), obtained as microcrystals from MeOH, gave a molecular ion peak at *m/z* 318 in the EI-MS, consistent with the molecular formula $C_{19}H_{26}O_4$ determined by the HR-EI-MS, which suggested that compound **1** would be a rare nor-diterpenoid. This deduction was confirmed by the ^{13}C- and DEPT-NMR spectrum exhibiting signals for 19 C-atoms. On the basis of careful analysis of the ^1H- and ^{13}C-NMR data (*Table*) and 2D-NMR spectra, compound **1** was identified as 6*β*,14*β*,15*β*-trihydroxy-20-nor-*ent*-kaur-1(10),16(17)-dien-7-one and named rubescensin N. This is the first nor-diterpenoid isolated from *Isodon* genus plants.

In the ^{13}C-NMR spectrum of **1**, the signals of a carbonyl group (*δ* 211.3), two olefinic quaternary C-atoms (*δ* 157.9 and 136.1), an olefinic CH_2 moiety (*δ* 107.6), an olefinic CH (*δ* 123.1 d), three OCH (*δ* 77.6, 76.9, and 72.6), three non-oxy CH (*δ* 55.7, 51.3, and 44.0), two non-oxy quaternary C-atoms (*δ* 63.8 and 33.1), four non-oxy CH_2 (*δ* 38.4, 31.9, 22.9, and 19.5), and two non-oxy Me groups (*δ* 32.3 and 22.1) were present. Thus, compound **1** revealed only two 'fatty' quaternary C-atoms instead of the characteristic three ones of the normal *ent*-kaurene skeleton, the characteristic signal for C(10) being absent, as indicated by comparison of the ^{13}C-NMR data (see *Table*) with those of rabdoternin B (**3**) [18] and phyllostachysin B (**4**) [19]. Moreover, besides the exocyclic CH_2 group normally located at C(16) in most *ent*-kaurenoids from the *Isodon* genus plants, there was an extra C=C bond in **1**, assigned to be between C(1) and C(10) on the ground of the HMBCs (*Fig. 1*) of the olefinic proton



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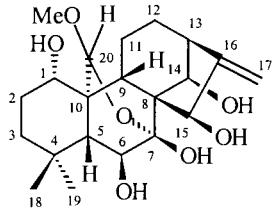

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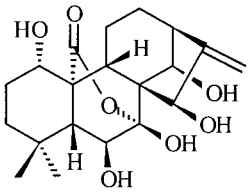

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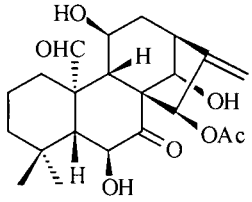

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Table. *^1H-* (400 MHz) *and/or ^{13}C-* (100 MHz) *NMR Data of* **1**–**3**. C_5D_5N; δ in ppm, J in Hz.

	1		**2**		**3**
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
H_β–C(1)	123.1 (*d*)	5.77 (*dd*, *J* = 2.0, 4.0)	74.3 (*d*)	3.70 (*dd*, *J* = 4.8, 11.6)	73.6 (*d*)
H_α–C(2)	22.9 (*t*)	2.13 (*m*)	31.3 (*t*)	2.34 (*m*)	32.9 (*t*)
H_β–C(2)	–	1.98 (*m*)	–	1.82 (*m*)	–
H_α–C(3)	38.4 (*t*)	1.28–1.31 (*overlapped*)	40.7 (*t*)	1.40–1.56 (*overlapped*)	39.3 (*t*)
H_β–C(3)	–	1.28–1.31(*overlapped*)	–	1.40–1.56 (*overlapped*)	–
C(4)	33.1 (*s*)	–	33.2 (*s*)	–	34.3 (*s*)
H_β–C(5)	55.7 (*d*)	2.23 (*d*, *J* = 12.0)	56.6 (*d*)	2.08 (*d*, *J* = 8.0)	55.2 (*d*)
H_α–C(6)	76.9 (*d*)	4.68 (*d*, *J* = 12.0)	73.6 (*d*)	4.45 (*d*, *J* = 8.0)	72.2 (*d*)
C(7)	211.3 (*s*)	–	101.0 (*s*)	–	108.6 (*s*)
C(8)	63.8 (*s*)	–	53.7 (*s*)	–	53.6 (*s*)
H_β–C(9)	44.0 (*d*)	3.18 (*m*)	48.3 (*d*)	2.72 (*m*)	44.6 (*d*)
C(10)	136.1 (*s*)	–	44.4 (*s*)	–	48.6 (*s*)
H_α–C(11)	19.5 (*t*)	1.83 (*m*)	19.6 (*t*)	2.00 – 2.20 (*overlapped*)	21.0 (*t*)
H_β–C(11)	–	2.45 (*m*)	–	2.00 – 2.20 (*overlapped*)	–
H_α–C(12)	31.9 (*t*)	2.04 (*m*)	33.8 (*t*)	2.42 (*m*)	31.1 (*t*)
H_β–C(12)	–	1.70 (*m*)	–	1.68 (*m*)	–
H_α–C(13)	51.3 (*d*)	3.04 (*m*)	45.7 (*d*)	2.88 (*d*, *J* = 8.8)	45.6 (*d*)
H_α–C(14)	77.6 (*d*)	4.50 (*s*)	75.7 (*d*)	5.13 (*s*)	74.7 (*d*)
H_α–C(15)	72.6 (*d*)	6.17 (*s*)	72.7 (*d*)	5.67 (*s*)	72.6 (*d*)
C(16)	157.9 (*s*)	–	161.0 (*s*)	–	159.5 (*s*)
CH_2(17)	107.6 (*t*)	5.65, 5.29 (2*s*)	109.4 (*t*)	5.73, 5.37 (2*s*)	110.2 (*t*)
Me–C(18)	32.3 (*q*)	1.32 (*s*)	35.8 (*q*)	1.40 (*s*)	31.2 (*q*)
Me–C(19)	22.1 (*q*)	1.15 (*s*)	23.2 (*q*)	1.36 (*s*)	21.0 (*q*)
H–C(20)	–	–	101.0 (*d*)	5.72 (*s*)	176.2 (s)
MeO	–	–	55.0 (*q*)	3.53 (*s*)	–

H–C(1) at δ 5.77 (*dd*, $J = 2.0$, 4.0 Hz) with C(3) (δ 38.4, *t*), C(5) (δ 55.7, *d*), and C(9) (δ 44.0, *d*), and of the olefinic quarternary C(10) (δ 136.1) with CH_2(11) (δ 1.83 and 2.45, each 1 H, *m*), H–C(5) (δ 2.23, 1 H, *d*, J = 12.0 Hz), and H–C(9) (δ 3.18, *m*). These data, combined with the absence of ^{1}H- and ^{13}C-NMR signals for Me(20), confirmed the novel 20-nor-*ent*-kaurene skeleton for **1**.

The remaining oxygenation functionalities of **1** were established accordingly. The CH_2(17)=C(16) moiety was confirmed by the long-range correlations between CH_2(17) and C(13) and C(15) (HMBC). Due to the presence of the HMBC (*Fig. 1*) H–C(15)/C(17) and C(9), H–C(14)/C(15), C(16), and C(9), and H–C(6)/C(4), C(5), and C(7), the three OH groups were placed at C(15), C(14), and C(6), respectively. In addition, the HMBCs between a carbonyl C-atom (δ 211.3, *s*) and H–C(15) and H–C(6) established that this carbonyl group was located at C(7).

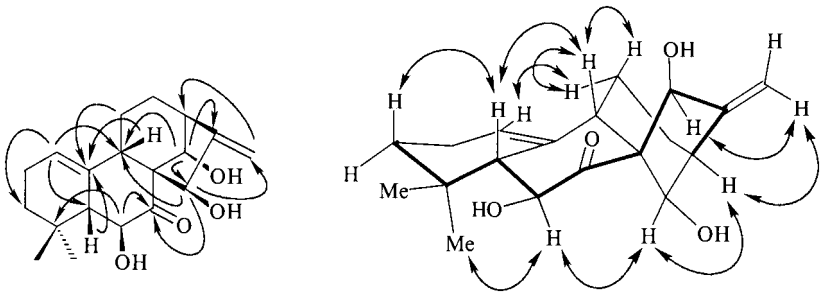
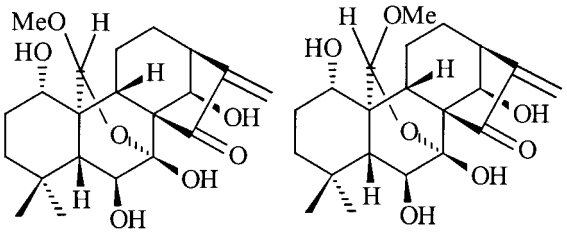


Fig. 1. *Selected HMBC* (from H to C) *and key ROESY correlations of* **1**

The relative configuration of **1** was defined by the ROESY experiment (*Fig. 1*). Significant ROEs between Me(19) (δ 1.15, *s*) and H–C(6) (δ 4.68, *d*, $J = 12.0$ Hz) and between H–C(14) and H_α–C(13) and H_α–C(6) disclosed that both OH–C(6) and OH–C(14) were β-oriented. The β-orientation of OH–C(15) was suggested by the absence of any ROE of H–C(15) and the abnormal upfield shift of C(9) (δ 44.0, *d*) due to the γ-steric compression effect between OH–C(15) and C(9) [20].

Rubescensin O (**2**), obtained as needles from MeOH, had the molecular formula $C_{21}H_{32}O_7$ as established by HR-EI-MS. Comparison of the ^{13}C- and DEPT-NMR data of **2** and **3** led to the deductions that they only differed at C(20) and that the ester carbonyl group at C(20) in **3** was replaced by a ketal moiety in **2**. These deductions were confirmed by the observation of a MeO signal in the NMR spectra of **2** ($\delta_{(H)}$ 3.53 and $\delta_{(C)}$ 55.0), which was correlated to that of the ketal atom C(20) (δ 101.0) in the HMBC plot. Moreover, a pair of known 20-epimers, the rabdoternins E and F [21], were also isolated besides **1** and **2**. Comparison of the ^{13}C-NMR data for C(20) of these two compounds with those of **2** established the same orientation of MeO in **2** as that in rabdoternin E. This assignment was corroborated by the clearly exhibited ROESY correlations between H–C(20) and H_α–C(11) as shown in *Fig. 2*. Therefore, **2** was elucidated as (20*R*)-7α,20-epoxy-20-methoxy-*ent*-kaur-16-ene-1α,6β,7β,14β,15β-pentol.

The possibility that compound **1** is an artifact produced in the separation can be excluded because the isolation conditions did not involve the use of temperatures above 60° or of acid and alkali. Based on the structures of the compounds so far isolated from *I. rubescens* and considering the isolation of the analog phyllostachysin B (**4**) from *Isodon* plants [19] and the relatively large amounts of **3**, a plausible biosynthetic origin for the skeleton of **1** is proposed, in which **1** is biosynthesized from **3**, as shown in the *Scheme*. The mechanism for the reaction from **3** to **5** would be a hydrolysis of a ketal



rabdoternin E rabdoternin F

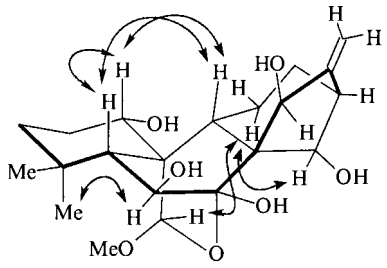
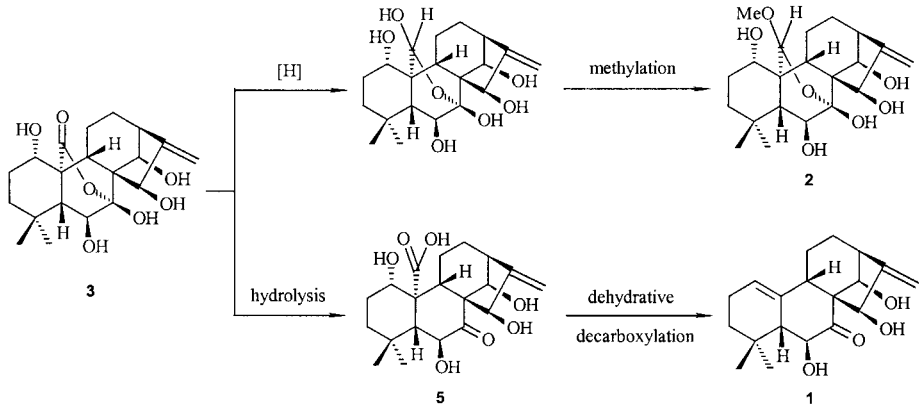


Fig. 2. *Key ROESY correlations of* **2**

[22], while the second step would be a dehydrative decarboxylation of a β-hydroxy-carboxylic acid [23][24]. Also **2** could be produced from **3** by a reduction followed by methylation (*Scheme*).

Scheme. *Supposed Biogenesis of* **1** *and* **2**



Experimental Part

General. Optical rotations: *Jasco DIP-370* digital polarimeter. UV Spectra: *Shimadzu UV-210A* spectrometer; λ_{max}(log ε) in nm. IR Spectra: *Bio-Rad-FtS-135* spectrometer; KBr pellets; in cm^{-1}. Melting points: *XRC-1* micro melting-point apparatus; uncorrected. 1D and 2D NMR Spectra: *Bruker AM-400* and

DRX-500 spectrometers; δ in ppm, J in Hz; Me_4Si as internal standard, measured in C_5D_5N. MS: *VG-Autospec-3000* spectrometer; 70 eV for EI; *m/z* (rel. %).

Plant Material. The plant material was collected in Jiyuan Prefecture, Henan Province (Aug. 1999) and identified by Prof. *Zhong-Wen Lin*; a voucher specimen (KIB-99-10-13 Lin) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Science.

Extraction and Isolation. The air-dried leaves (13 kg) were extracted three times with 70% Me_2CO at r.t. overnight, and the extract was filtered. The filtrate was evaporated and the resulting residue partitioned between H_2O and AcOEt. The AcOEt fraction (424 g) was subjected to column chromatography (silica gel (100–200 mesh, 3.0 kg), $CHCl_3/Me_2CO$ 1:0 → 0:1): *Fractions I–IX*. After repeated chromatography (silica gel, gradient mixtures $CHCl_3/Me_2CO$), *Fr. VI* afforded **2** (80 mg). In the same way, **1** (4 mg) was isolated from *Fr. VII*.

Rubescensin N (= *(6β,14β,15β)-6,14,15-Trihydroxy-20-nor*-ent-*kaur-1(10),16(17)-dien-7-one*; **1**): Microcrystals from MeOH. M.p. 110–112°. $[\alpha]_D^{27} = +18.05$ ($c = 0.28$, MeOH). UV (MeOH): 204 (3.90). IR (KBr): 3442, 2928, 1700, 1634, 1558, 1540, 1521, 1249, 1125, 1072, 765. ^{1}H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz): *Table*. EI-MS: 318 (70, M^+), 300 (100), 282 (22), 271 (25), 254 (12), 244 (16), 226 (16), 105 (18), 91 (44), 77 (34). HR-EI-MS: 318.1829 ($C_{19}H_{26}O_4^+$; calc. 318.1831).

Rubescensin O (= *(1α,6β,7β,14β,15β,20R)-7,20-Epoxy-20-methoxy*-ent-*kaur-16-ene-1,6,7,14,15-pentol*; **2**): Microcrystals from MeOH. M.p. 144–146°. $[\alpha]_D^{19} = -23.96$ ($c = 0.79$, MeOH). UV (MeOH): 204 (3.54). IR (KBr): 3390, 2932, 2869, 1250, 1199, 1144, 1074, 993. 956, 660. ^{1}H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz): *Table*. EI-MS: 396 (8, M^+), 378 (20), 360 (10), 342 (14), 328 (16), 310 (22), 300 (33), 282 (26), 271 (27), 254 (20), 226 (24). HR-EI-MS: 396.2166 ($C_{21}H_{32}O_7^+$; calc. 396.2148).

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