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Rubesanolides A and B: Diterpenoids from *Isodon rubescens*

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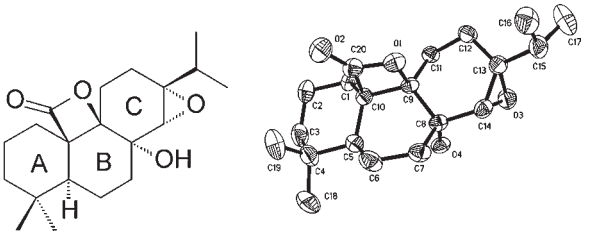
ABSTRACT



Rubesanolide A (**1**)

From the medicinal plant *Isodon rubescens*, we isolated two novel diterpenes, rubesanolides A (1) and B (2). The compounds contain a unique *β*-lactone subgroup. This is the first discovery for a natural diterpene having rings A, B, and C in chair, boat, and twist-chair conformations, respectively. The structures were elucidated by analysis of spectroscopic data, and the absolute configuration of 1 was determined by X-ray diffraction.

Isodon (formerly *Rabdosia*), a genus of Labiatae (= Lamiaceae) family, is well-known for producing bioactive diterpenoids with diverse skeletons, especially *ent*-kaurane diterpenoids. More than 600 new compounds have been identified from this genus previously.[1,2]

Isodon rubescens (Hemsl.) Hara is a well-known folk medicine in China for treatment of respiratory and gastrointestinal bacterial infections, inflammation, and cancer.[2] An herbal product, made from the extract of this plant, has been developed as a Chinese medicine to treat sore throat and inflammation in 1977.[3] Six samples of this species collected from different regions of China had been previously investigated phytochemically,[2] which led to the isolation of more than 70 new diterpenoids including several with novel skeletons such as dimeric *ent*-kauranoids. Our further investigation on this plant led to the isolation of two novel diterpenoids, rubesanolides A (**1**) and B (**2**), with an unprecedented *β*-lactone group formed between C-9 and C-20. The compounds have a very different conformation from that of abietane diterpenes (Figure 1), in which, all three rings (A, B, and C) are in chair conformations. However, in rubesanolides A (**1**) and B (**2**), the three six-member rings form chair, boat, and twisted-chair conformations,

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(1) Sun, H. D.; Xu, Y. L.; Jiang, B. *Diterpenoids from Isodon Species*; Science Press: Beijing, 2001.

(2) Sun, H. D.; Huang, S. X.; Han, Q. B. *Nat. Prod. Rep* **2006**, *23*, 673–698.

(3) In the Pharmacopoecia of People's Repulic of China; People's Health Press: Beijing, 1977; p 186.

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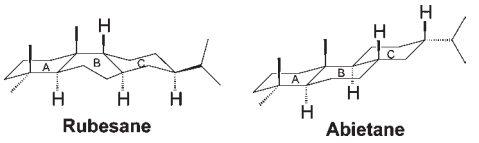


Figure 1. Comparison of the configurations between rubesane and abietane diterpenes.

respectively. The rings between A and B and between B and C are *trans*-fused, which is the first time that a diterpene having such a conformation in the skeleton has been discovered.

The leaves of *I. rubescens* were collected in Longli, Guizhou, China, in October, 2006. The plant was identified by Professor Deyuan Chen of the Guiyang College of Traditional Chinese Medicine, and the voucher specimen is deposited at the Guiyang College of Traditional Chinese Medicine. The dried and milled plant material (8.5 kg) was extracted with 100% MeOH and then concentrated in vacuo to give a crude extract (1450 g), which was treated with activated charcoal in MeOH to remove most of the chlorophylls. The filtered solution was concentrated and absorbed in silica gel, which was subjected to a silica gel column and eluted with a gradient solvent system of petroleum ether/EtOAc and EtOAc/MeOH to yield five fractions. The petroleum ether/EtOAc 9:1 fraction was further separated by repeated silica gel column chromatography to afford rubesanolides A (**1**) (15 mg) and B (**2**) (5 mg).

Rubesanolide A (**1**),[4] [α]12 D +145.8° (*c* 0.165, MeOH), was isolated as colorless laminate crystals (MeOH). Its positive EIMS showed an $[M]^+$ at *m*/*z* 334, corresponding to a molecular formula of $C_{20}H_{30}O_4$, requiring six units of unsaturation. The molecular formula was further confirmed by HR-ESIMS ($[M+Na]^+$, found 357.2035, calcd 357.2041), as well as ^{13}C and DEPT NMR spectra. Its IR spectrum showed the absorption bands at 3451 and 1804 cm^{-1}, indicating the presence of hydroxyl and carbonyl groups, respectively. The ^1H, ^{13}C, and DEPT NMR spectra (Table 1) showed characteristic signals of a methine group ([δ_H 2.18 (br dd, J = 13.1, 3.7 Hz, H-5); δ_C42.2 (d, C-5)], two tertiary methyl groups [δ_H 0.94 (s, Me-18) and 0.99 (s, Me-19)], an isopropyl group [δ_H 1.57 (m, H-15), 0.98 (d, J = 7 Hz, Me-16), and 0.93 (d, J = 7 Hz, Me-17); δ_C 34.3 (d, C-15), 17.6 (q, C-16), and 18.13 (q, C-17)], a carbonyl group [δ_C 174.1 (s, C-20)], an oxymethine carbon [δ_H 2.73 (s, H-14); δ_C 63.3 (d, C-14)], three oxyquaternary carbons [δ_C 67.8 (s, C-8), 80.4 (s, C-9), and 68.1 (s, C-13)], and two quaternary carbons [δ_C 33.4 (s, C-4) and 61.2 (s, C-10)]. Eight methylene groups were also clearly observed in the DEPT spectrum. On the basis of these data

(4) Rubesanolide A (**1**): colorless laminate crystals (MeOH); mp 138−140 °C; $[\alpha]_D^{12}$ +145.8° (*c* 0.165, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.38) nm, CD (MeOH) (225 nm, $\Delta\varepsilon$ +3.10); IR (KBr) λ_{max} 3451, 2961, 1804, 1641, 1430, 1367, 1208, 1139, 1096 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; positive EIMS showed an $[M]^+$ at *m*/*z* 334; HR-ESIMS ($[M+Na]^+$ *m*/*z* 357.2035, calcd 357.2041 for $C_{20}H_{30}O_4Na$).

Table 1. NMR Spectroscopic Data (δ in ppm, J in Hz) of Rubesanolides A (**1**)[a] and B (**2**)[b]

1			**2**		
position	δ_H, mult	δ_C, mult	position	δ_H, mult	δ_C, mult
1α	1.26 (td, 13.3, 4.2)	28.2 t	1α	1.43 (td, 13.6, 4.0)	30.7 t
1β	2.15 (br d, 13.3)		1β	2.15 (brd, 13.4)	
2α	1.56 m	19.1 t	2α	1.59 (br dqu, [c]13.7, 3.3)	20.2 t
2β	1.78 (br qt, 13.8, 3.3)		2β	1.76 (br qt, 13.6, 3.3)	
3α	1.19 (br td, 13.9, 3.4)	41.2 t	3α	1.23 (br td, 13.4, 3.4)	42.5 t
3β	1.45 (br d, 13.3)		3β	1.48 (br d, 13.1)	
4	–	33.4 s	4	–	34.5 s
5	2.18 (br dd, 13.1, 3.7)	42.2 d	5	1.98 (br dd, 10.8, 6.6)	42.8 d
6α	1.97 m	18.09 t	6α	1.94 (br ddt, 13.7, 10.7, 6.7)	19.1 t
6β	1.52 m		6β	1.51 m	
7α	1.92 m	29.2 t	7α	2.12 (br ddd, 14.4, 10.0, 3.7)	27.8 t
7β	2.04 m		7β	1.80 (br ddd, 14.8, 10.2, 6.5)	
8	–	67.8 s	8	–	73.2 s
9	–	80.4 s	9	–	82.7 s
10	–	61.2 s	10	–	61.9 s
11α	1.72 (br ddd, 14.2, 8.2, 5.3)	19.5 t	11α	2.67 (dq, 18.3, 2.5)	28.9 t
11β	1.67 (br ddd, 14.4, 6.3, 2.9)		11β	2.17 (br dd, 18.5, 5.1)	
12α	2.02 m	19.5 t	12	5.38 m	116.4 d
12β	1.99 m				
13	–	68.1 s	13	–	145.2 s
14	2.73 s	63.3 d	14	3.99 br s	72.9 d
15	1.57 m	34.3 d	15	2.60 (br sep,[d] 7.0)	29.6 d
16	0.98 (d, 7.0)	17.6 q	16	1.05 (d, 6.9)	20.9 q
17	0.93 (d, 7.0)	18.13 q	17	0.99 (d, 6.9)	22.8 q
18	0.94 s	31.4 q	18	0.91 s	31.5 q
19	0.99 s	20.2 q	19	1.05 s	21.1 q
20	–	174.1 s	20	–	176.3 s
8-OH	2.87 br s				

[a] Data were recorded in $CDCl_3$ on a Bruker AM 400MHz spectrometer (^1H, ^{13}C, DEPT, HMBC, HMQC, COSY, and ROESY); chemical shift [δ values are given in ppm with reference to the signal of $CDCl_3$ (δ 7.24 ppm)] for ^1H and to the center peak of the signal of $CDCl_3$ (δ 77.1 ppm) for ^{13}C. [b] Data were recorded in CD_3OD on an INOVA 400MHz spectrometer (^1H, ^{13}C, DEPT, HMBC, HMQC, COSY, and ROESY); chemical shift [δ values are given in ppm with reference to the signal of CD_3OD (δ 3.34 ppm)] for ^1H and to the center peak of the signal of CD_3OD (δ 49.1 ppm) for ^{13}C. [c] qu represents quintet. [d] sep represents septet.

and chemotaxonomic considerations, compound **1** was determined to be a diterpenoid.

The presence of the HMBC correlations from the proton at δ_H 2.73 (H-14) to C-8, -9, -12, -13, and -15 and from the methine proton at δ_H 1.57 (H-15) to C-12, -13, and -14 indicated that the two oxygenated carbons of δ_C 63.3 (d) and 68.1 (s) are C-14 and C-13, respectively (Figure 2). The fact that the H-14 signal [δ_H 2.73 (s)] and the ^{13}C NMR signals of C-13 [δ_C 68.1 (s)] and C-14 [δ_C 63.3 (d)] are significantly shifted upfield in comparison with those of normal oxymethine and oxyquaternary carbon groups indicated an epoxide ring group formed between C-13 and C-14. The presence of the HMBC correlations of H_2-1 and

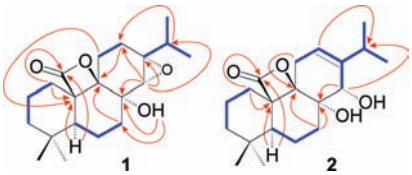


Figure 2. Key COSY (− in blue) and HMBC (→ in red) correlations for **1** and **2**.

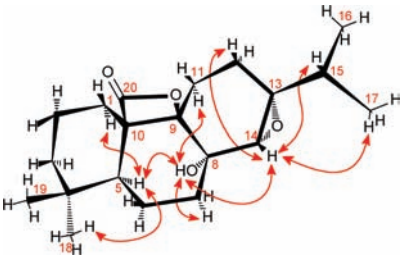


Figure 3. Key ROESY correlations of **1**.

H-5 to the ^{13}C NMR signal at δ_C 174.1 determined the carbonyl carbon at C-20. The presence of the HMBC correlations from the proton at δ_H 2.87 (OH) to the ^{13}C NMR signal at δ_C 67.8 (C-8), 29.2 (C-7), and 63.3 (C-14) indicated a hydroxy group at C-8, and the presence of the HMBC correlations from H_2-7, H_2-11, H_2-12, and H-14 to the ^{13}C NMR signal at δ_C 80.4 determined C-9 as an oxyquaternary carbon. When the three rings, the epoxy group, and one double bond (the carbonyl group) were considered, one undefined double-bond equivalent remained in the diterpenoid. This undefined double-bond equivalent must belong to an additional ring since no other double bond was observed in the NMR spectra. The additional ring was thus determined as a β-lactone formed between C-9 and C-20. A ROESY experiment was used to establish the relative stereochemistry of **1** (Figure 3). In the ROESY spectrum, the presence of the correlations between H-5 and H-1α/H-3α/H-7α/H_3-18, between 8-OH and H-5α/H-7α/H-11α, and between H-14 and H_2-7/H-12β/H-15 determined both H-5 and 8-OH as α-oriented and H-14 as β-oriented. The CD spectrum of **1** showed a positive Cotton effect (225 nm, $\Delta\varepsilon$ +3.10) indicative of the n→π exiton of the β-lactone group.[5] The CD data, together with the significantly large optical rotation datum, determined **1** as an enantiomerically pure compound.

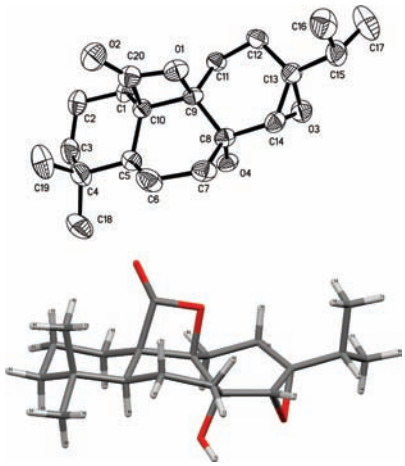


Figure 4. X-ray crystallographic structure of **1**.

To confirm the structure and to determine its absolute configuration, **1** was crystallized in MeOH to afford a crystal of the monoclinic space group $P2_1$, which was analyzed by X-ray crystallography.[6] The absolute configuration was determined by the measurement of the Flack parameter, which is calculated during the structural refinement.[7,8] It measures the proportion of a pair of enantiomers. If the value is 0, the structure provided by the X-ray is the correct absolute structure, but if the value is 1, the inverted structure should be the correct absolute structure for the molecule. If the value is near 0, the crystal is racemic, in which case, the absolute structure of the molecule cannot be determined by the X-ray data. In our study, the final refinement on the Cu Kα data of the crystal of **1** resulted in a Flack parameter of 0.00 (19), allowing an unambiguous assignment of the absolute structure as shown in Figure 4. The six chiral centers, C-5, C-8, C-9, C-10, C-13, C-14, were thus determined as *S*, *S*, *R*, *R*, *R*, *S*, respectively. Accordingly, the structure of **1** was established and given the trivial name rubesanolide A.

Rubesanolide B (**2**),[9] $[\alpha]_D^{31}$ +15.1° (*c* 0.531, MeOH), a white amorphous powder, was determined to have a molecular formula of $C_{20}H_{30}O_4$ from analysis of the NMR spectral data and an HR-ESIMS ion at *m*/*z* 357.2040 ($[M+Na]^+$, calcd 357.2041). The NMR spectra of **2** were very similar to those of **1** (Table 1), indicating that **2** was

(5) Wang, X. N.; Shao, P. L.; Lv, H.; Ye, S. *Org. Lett.* **2009**, *11*, 4029–4031.

(6) Colorless laminate crystals of rubesanolide A (from MeOH) belong to the monoclinic space group $P2_1$. The crystal data: $C_{20}H_{30}O_4$, M = 334, a = 6.301(3) Å, b = 11.371(8) Å, c = 13.236(8) Å, β = 95.602 (16)°, V = 943.8(10) Å^3, Z = 2, d = 1.177g/cm^3. A crystal of dimensions 0.02 × 0.27 × 0.46 mm^3 was used for measurements on a Rigaku MicroMax 002+ diffractometer with a graphite monochromator (ω-κ scans, 2θmax = 145.72°), Cu Kα radiation. The total number of independent reflections measured was 3259, of which 2900 were observed ($|F|^2 \geq 2\sigma|F|^2$). The crystal structure was solved and refined by the direct method SHELXS-97 (Sheldrick, G. M. University of Gottingen: Gottingen, Germany, 1997), expanded using difference Fourier techniques and full-matrix least-squares calculations. Final indices: R_1 = 0.0403, wR_2 = 0.1039 ($w = 1/\sigma|F|^2$), s = 1.016. Crystallographic data for the structure of 1 have been deposited in the Cambridge Crystallographic Data Centre (Deposition No. CCDC798778). Copies of these data can be obtained, free of charge, on application to the CCDC via the Internet at www.ccdc.com.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).

(7) Flack, H. D.; Bernardinelli, G. *Chirality* **2008**, *20*, 681–690.

(8) Flack, H. D. *Act Crystallogr.* **1983**, *A39*, 876–881.

(9) Rubesanolide B (**2**): a white amorphous powder; $[\alpha]_D^{31}$ +15.1° (*c* 0.531, MeOH); UV (MeOH) λ_{max} (log ε) 204 (1.01) nm, IR (KBr) λ_{max} 3452, 2961, 1787, 1638, 1457, 1391, 1208, 1146, 1042 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; positive EIMS showed a $[M]^+$ at *m*/*z* 334; HR-ESIMS ($[M+Na]^+$ *m*/*z* 357.2040, calcd 357.2041 for $C_{20}H_{30}O_4Na$).

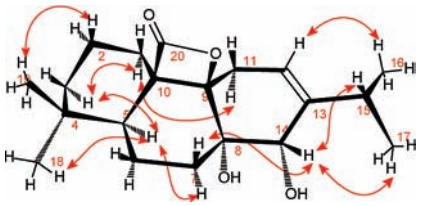
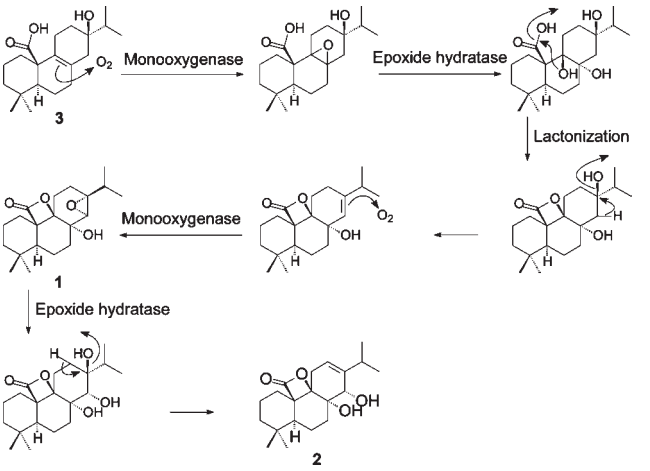


Figure 5. Key NOESY correlations of **2**.

also a diterpene with the same skeleton as that of **1**. Similar to **1**, **2** also contains a β-lactone group between C-9 and C-20, evidenced by the presence of the ^{13}C NMR signals at δ_C 61.9 (s, C-10), 82.7 (s, C-9), and 176.3 (s, C-20). Further analysis of the 1D NMR data (^1H, ^{13}C, and DEPT) showed that **2** contained no epoxide group that was found in **1**. Instead, the signals of a carbon−carbon double bond [δ_H 5.38 (m); δ_C 116.4 (d) and 145.2 (s)] were observed in the ^1H and ^{13}C NMR spectra of **2**. This double bond was assigned to between C-12 and C-13 on the basis of the presence of the HMBC correlations of H-12 to C-9, C-14, and C-15. An oxymethine group was also observed in **2** (Figure 2). It was determined as a hydroxyl group due to its chemical shifts [δ_H 3.99 (br s); δ_C 72.9 (d)] and calculation of the double-bond equivalents. The hydroxyl group was determined at C-14 by the presence of the HMBC correlations of H-14 to C-8, C-13, and C-15. The presence of the NOEs between H-1α and H-3α and between H-5 and H-3α/H_3-18 determined that H-5 is α-oriented. The presence of the NOEs between H-14 and H-7β/H_3-17 and between H-12 and H_3-16 determined that H-14 is β-oriented (Figure 5). The relative configuration of **2** is thus determined to be 5*S**, 8*S**, 9*R**, 10*R**, 14*S**, respectively, and given the trivial name rubesanolide B.

Rubesanolides A (**1**) and B (**2**) are unique diterpenoids wih a β-lactone group between C-9 and C-20. They may be derived from abietane diterpenes. A plausible biosynthetic origin of **1** and **2** from lophanic acid **3** is proposed (Scheme 1).[10] Lophanic acid was found to be a major component (0.46%) in an *Isodon* species (*Isodon lophanthoides*)[11,12] and is therefore considered to be a plausible biosynthetic precursor of **1** and **2**. The carbon−carbon double bond of lophanic acid (**3**) is transformed by monoxygenase to an epoxide, which is converted to dihydroxyl groups by epoxide hydratase. Lactonization of one of the hydroxyl groups (9-OH) with the C-20 carboxylic acid group then results in a β-lactone group. The β-lactone intermediate undergoes a series of reactions including another epoxidation and expoxide hydrolysis to produce rubesanolides A (**1**) and B (**2**).

Scheme 1. Proposed Biogenetic Pathway of **1** and **2**



Compounds **1** and **2** were evaluated for their cytotoxicity against several human tumor cell lines, including A549, K562, Hela, and MCF7 cell lines by the MTT[13] and SRB[14,15] methods as previously reported. However, no cytotoxicity against these cell lines were observed for the two compounds at a concentration of 10 μg/mL. Compound **2** was further evaluated for its cancer chemopreventive potential based on their ability to inhibit tumor necrosis factor alpha (TNF-α)-induced NF-κB activity, nitric oxide (NO) production, quinone reductase 1 (QR1), and aromatase activities.[16,17] At a concentration of 10 μg/mL, it showed only 14.9% inhibtion activity against aromatase and nearly 0% inhibitory effects in the other assays.

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Supporting Information Available. 1D and 2D NMR, MS, UV, IR spectra of rubesanolides A (**1**) and B (**2**), CD spectrum of **1**, and detailed experimental procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

(10) Dewick, P. M. In *Medicinal Natural Products: A Biosynthetic Approach*, 2nd ed.; John Wiley & Sons, Ltd., The Atrium, Southern Gate, Chichester, West Sussex, U.K., 2002.

(11) Wang, Z. Q.; Xu, F. M.; Dong, H. Z.; Node, M.; Fuji, K. *Tianran Chanwu Yanjiu Yu Kaifa* **1995**, *7*, 24–28.

(12) Jiang, B.; Lu, Z. Q.; Zhang, H. J.; Zhao, Q. S.; Sun, H. D. *Fitoterapia* **2000**, *71*, 360–364.

(13) Niu, X. M.; Li, M. L.; Zhao, Q. S.; Mei, S. X.; Na, Z.; Wang, S. J.; Lin, Z. W.; Sun, H. D. *Planta Med.* **2002**, *68*, 528–533.

(14) Monks, A.; Scuduero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.

(15) Jutiviboonsuk, A.; Zhang, H. J.; Tan, G. T.; Ma, C. Y.; Hung, N. V.; Cuong, N. M.; Bunyapraphatsara, N.; Soejarto, D. D.; Fong, H. H. S. *Phytochemistry* **2005**, *66*, 2745–2751.

(16) Homhual, S.; Zhang, H. J.; Bunyapraphatsara, N.; Kondratyuk, T.; Santarsiero, B. D.; Mesecar, A. D.; Herunsalee, A.; Chaukul, W.; Pezzuto, J. M.; Fong, H. H. S. *Planta Med.* **2006**, *72*, 255–260.

(17) Yang, J. H.; Kondratyuk, T. P.; Marler, L. E.; Qiu, X.; Choi, Y. S.; Cao, H. M.; Sturdy, M.; Pegan, S.; Liu, Y; Wang, L. Q.; Mesecar, A. D.; van Breemen, R. B.; Pezzuto, J, M.; Fong, H. H. S.; Chen, Y. G.; Zhang, H. J. *Phytochemistry* **2010**, *71*, 641–647.