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SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 5187–5190

A new rearranged and a new *seco-ent*-kaurane diterpenoids from *Isodon parvifolius*

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Received 20 March 2006; revised 3 May 2006; accepted 8 May 2006

Abstract—Parvifoline X (**1**), a new rearranged *ent*-kaurane diterpenoid, and parvifoline Y (**2**), a new 8,15-*seco*-*ent*-kaurane diterpenoid, were isolated from the leaves of *Isodon parvifolius*. Their structures were elucidated by spectroscopic methods including 2D NMR analysis, and supported by a biogenetic pathway. Parvifoline X (**1**), possessing a new 15(8→11)-*abeo*-7α,20-epoxy-*ent*-kaurane skeleton, was found from the genus *Isodon* for the first time. Compounds **1** and **2** were evaluated for their inhibitory activity against A549, HT-29, and K562 cell lines. Parvifoline Y (**2**) was the most cytotoxic against A549 cells with an IC_{50} value of 4.97 μM.
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The genus *Isodon* belonging to the Labiatae contain much amount of *ent*-kaurane diterpenoids. Thus far, more than 500 new *ent*-kaurane diterpenoids have been isolated from the genus *Isodon* by our group,[1] including many interesting novel *ent*-kaurane diterpenoids such as 1:1 complexes of natural *ent*-kauranoids,[2] a natural equimolecular mixture of two epimeric *ent*-kauranoids,[3] 6,7:8,15-*seco*-*ent*-kauranoids,[4] 8,15-*seco*-*ent*-kauranoids,[5] 15,16-*seco*-*ent*-kauranoid,[5] 20-*nor*-*ent*-kauranoid,[6] symmetric and asymmetric dimeric *ent*-kauranoids,[7] and novel C_{19} skeleton *ent*-kauranoid.[8] Many of these diterpenoids display various biological activities, such as antibacterial,[9] anti-inflammatory,[10] and especially antitumor actions.[11] Previous chemical studies of the leaves of *Isodon parvifolius* (Batalin) H. Hara evidenced the presence of bioactive diterpenoids.[12] As part of our continuation on search for novel and biologically active structures from genus *Isodon*, two minor new *ent*-kauranoids, namely parvifolines X (**1**) and Y (**2**), were isolated from the leaves of *I. parvifolius*.[13] Their structures were identified by extensive NMR spectroscopic means including ^1H–^1H COSY, HMQC, HMBC, and ROESY techniques. In addition, a possible biogenetic pathway from one of the major known diterpenoids, lasiodonin (**3**)[12g] was also proposed for them. Their cytotoxic activity against A549, HT-29, and K562 cell lines was also evaluated. In this letter, we wish to report the isolation and structure elucidation of the new diterpenoids, and the cytotoxic activity.

An acetone extract of the leaves of *I. parvifolius* was partitionated in turn with petroleum ether (60–90 °C), ethyl acetate, and *n*-butanol against water. Parvifolines X (**1**)[14] and Y (**2**)[15] were isolated from the ethyl acetate partition and *n*-butanol partition, respectively, by repeated normal and reverse silica gel column chromatography and semipreparative reversed-phase HPLC.

Compound **1** was obtained as colorless needles, mp 185–187 °C, $[\alpha]_D^{24.3}$ −135.3 (*c* 0.25, MeOH). Its HRESIMS exhibited a sodiated molecular ion peak at *m*/*z*

Keywords: *Isodon parvifolius*; Labiatae; Parvifolines X and Y; NMR data; Cytotoxicity.
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doi:10.1016/j.tetlet.2006.05.025

371.1825, consistent with a molecular formula of $C_{20}H_{28}O_5$. Its IR spectrum displayed the presence of hydroxyl (3495, 3453 cm^{-1}) and carbonyl (1738 cm^{-1}) groups. The ^1H NMR spectrum of **1** (Table 1) showed the signals for two tertiary methyls at δ_H 1.17 (3 H, s) and 1.09 (3H, s), one secondary methyl at δ_H 1.44 (3H, d, $J = 6.8$ Hz), two oxygenated methylene protons at δ_H 4.51 (1H, br d, $J = 8.0$ Hz) and 4.05 (1H, br d, $J = 8.0$ Hz), and two methine protons at δ_H 4.83 (1H, br s) and 4.23 (1H, br s), each bearing a hydroxyl group. The ^{13}C NMR data (Table 1) indicated the presence of a free ketone carbon at δ_C 212.5, two quaternary sp^2 carbons at δ_C 139.3 and 135.7, one hemiketal quaternary carbon at δ_C 99.1, two oxygenated methine carbons at δ_C 75.5 and 70.0, and one oxygenated methylene at δ_C 65.5, as well as three methyls, four methylenes, four methines, and two quaternary carbons. Compared with the classical *ent*-kaurane diterpenoids isolated from the genus *Isodon*, such as lasiodonin (**3**), one quaternary sp^3 carbon was absent, while two quaternary sp^2 carbons were present. These information suggested that compound **1** must be a rearranged *ent*-kaurane diterpenoid that shared partly structural features with lasiodonin (**3**). Further comparison of the NMR data of **1** with those of **3** revealed that they primarily differed from each other in rings C, D, and E. A detailed analysis of ^1H–^1H COSY data of compound **1** revealed three major spin systems. Two of these were H-1/H_2-2/H_2-3 and H-5/H-6, which were extended to form rings A, B, and C through HMBC correlations from H-1 to C-9, C-10, and C-20, from H-3 to C-1, C-5, C-18, and C-19, from H-5 to C-6, C-9, C-10, C-18, C-19, and C-20, and from H-6 to C-4, C-7, and C-8. Another spin system deduced from the ^1H–^1H COSY spectrum corresponded to H-11/H_2-12/H-13/H_2-14/H-16/H_3-17 unit, which were extended in turn via HMBC correlations to form rings D and E. Thus the scaffold of compound **1** was figured out (Fig. 1).

The relative stereochemistry of **1** was fixed by using ROESY spectrum (Fig. 2). The ROESY correlations of H-1/H-11, H-1/H-5β, H-5β/H_3-18, H-6/H_3-19, H-16/H-12α, H-16/H-13α, and H_3-17/H-14β, indicated the stereochemistry of H-1β, H-6α, H-11α, and H-16α. A computer modeled 3D structure of **1** (Fig. 2, on which the key ROESY correlations and corresponding

Table 1. ^1H and ^{13}C NMR assignments of compounds **1**[a] and **2**[b] in C_5D_5N[c]

Position	1		2	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	70.0	4.83 br s	78.8	5.79 br s
2	29.2	1.99 (2H) m	30.1	β 1.91 m
				α 1.75 m
3	39.7	α 1.45 m	39.3	α 1.42 br d (13.4)
		β 1.35 m		β 1.31–1.25 (overlap)
4	33.3		33.6	
5	57.1	β 1.39 br s	63.6	β 1.59 (overlap)
6	75.5	α 4.23 br s	73.6	α 4.11–4.08 (overlap)
7	99.1		101.1	
8	135.7		29.0	β 3.28 m
9	139.3		47.7	β 2.83 (overlap)
10	46.5		45.1	
11	46.1	α 4.13 br s	73.6	α 3.68 br s
12	33.0	1.90 (2H) br s	27.3	α 2.83 (overlap)
				β 1.99 br d (13.4)
13	35.8	α 2.53 br s	32.7	α 3.00 br s
14	27.1	α 2.84 dd (18.2, 4.0)	29.9	α 2.67 dt (12.8, 3.1)
		β 2.73 br d (18.2)		β 2.21 m
15	212.5		165.6	
16	48.1	α 2.38 m	141.3	
17	12.1	1.44 (3H) d (6.8)	127.2	a 6.53 br s
				b 5.49 br s
18	33.5	1.09 (3H) s	32.9	1.10 (3H) s
19	22.3	1.17 (3H) s	22.0	1.03 (3H) s
20	65.5	a 4.51 br d (8.0)	100.2	5.54 s
		b 4.05 br d (8.0)		
OH-1		6.34 br s		Not observed
OH-6		6.22 br s		Not observed
OH-7		Not observed		Not observed
OBu			67.6	a 4.11–4.08 (overlap)
				b 3.51 dd (16.5, 7.1)
			31.8	1.59–1.40 (2H) (overlap)
			19.8	1.31–1.25 (2H) (overlap)
			13.9	0.82 (3H) t (7.3)

[a] ^1H and ^{13}C NMR spectra were acquired at 500 and 125 MHz, respectively.
[b] ^1H and ^{13}C NMR spectra were acquired at 400 and 100 MHz, respectively.
[c] TMS was used as internal standard; assignments were based on ^1H–^1H COSY, HMQC, HMBC, and ROESY spectra.

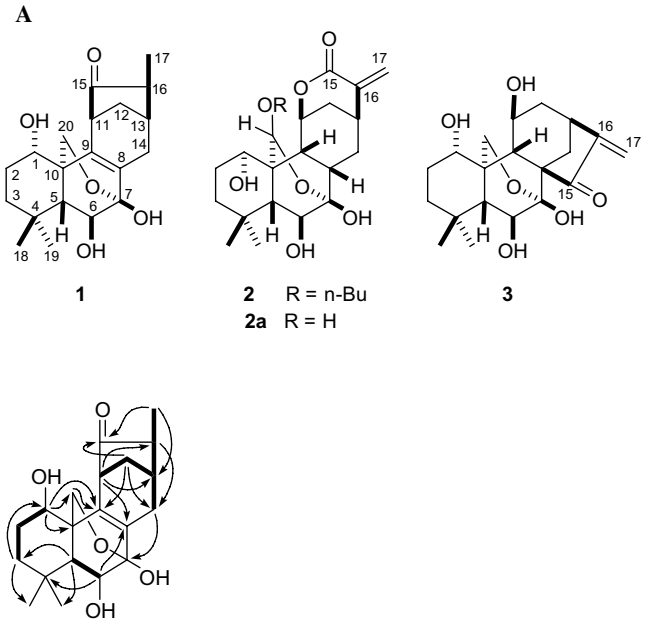


Figure 1. ^{1}H–^{1}H COSY (▬) and selected HMBC (H→C) correlations of compound **1**.

interatomic distances were depicted) was generated by using the molecular modeling program CS Chem 3D Pro Version 6.0, using MM2 force field calculations for energy minimization. The relative stereochemistry and favorable conformation of **1** offered by computer modeling were consistent with those of **1** assigned by ROESY spectrum. Consequently, compound **1** was determined as 16(*R*)-methyl-1α,6β,7β-trihydroxy-15(8→11)-*abeo*-7α,20-epoxy-*ent*-kaur-8(9)-en-15-one and given the trivial name parvifoline X.

Although the absolute stereochemistry of **1** was not established by CD spectrum, this compound is presumed to be an *ent*-kaurane-type diterpenoid by consideration of the presence of *ent*-kaurane-type diterpenoid as main components in the genus *Isodon*.

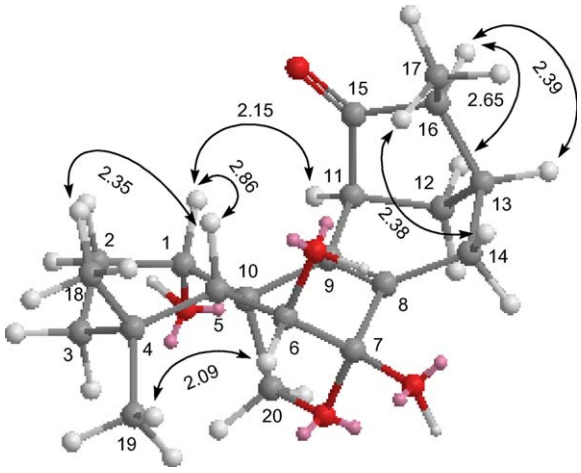
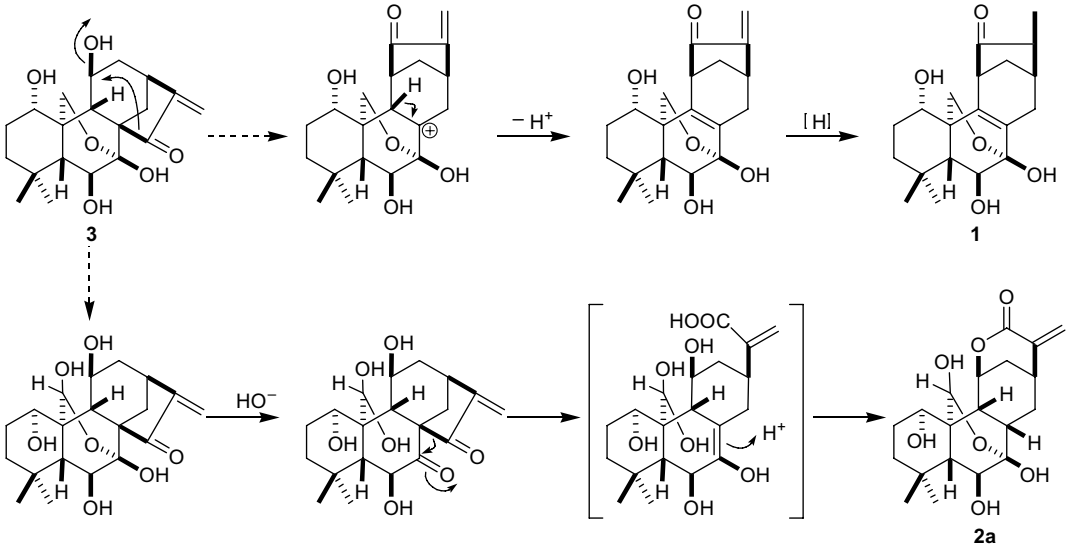


Figure 2. Key ROESY correlations and corresponding interatomic distances (Å) of compound **1**.

From a biogenetic point of view, compound **1** might be biosynthetically formed from lasiodonin (**3**) by eliminating of the hydroxyl group at C-11 and 1,3-rearrangement (Scheme 1).

Compound **1** representing a new 15(8→11)-*abeo*-7α,20-epoxy-*ent*-kaurane skeleton diterpenoid, was isolated from the genus *Isodon* for the first time. This new compound, however, further expands the diversities of *ent*-kaurane diterpenoids in genus *Isodon*.

Compound **2**, $[\alpha]_D^{25.3}$ +91.2 (*c* 0.47, MeOH), was isolated as colorless needles. A molecular formula of $C_{24}H_{36}O_7$ was determined for **2** by HRESIMS. Both the ^{1}H and ^{13}C NMR spectroscopic data of compound **2** (Table 1) were closely comparable to those of rubescensin T,[5] except for the signals due to a butoxyl group at C-20 in **2** rather than a methoxyl group at the same position in rubescensin T, which was proved by HMBC correlations from H-20 (δ_H 5.54) to one oxygenated methylene (δ_C 67.6). The (20*S*)-configuration was assigned on the basis of a ROESY cross-peak between H-20 and H_3-19. All



Scheme 1. Biogenetic pathway proposed for compounds **1** and **2a**.

the above-mentioned spectroscopic observations constructed the structure of parvifoline Y (**2**) as 20(*S*)-1α,6β,7β-trihydroxy-20-butoxy-8,15-*seco*-7α,20-epoxy-*ent*-kaur-16-en-11,15-olide. Since *n*-butanol was used during isolation processes, compound **2** was likely to be an artifact of isolation produced from **2a**, which was a new natural product. A possible biogenetic pathway of compound **2a** from **3** was also proposed in Scheme 1.[5]

The cytotoxic activity of compounds **1** and **2** was evaluated in A549, HT-29, and K562 cell lines. Compound **2** exhibited modest activity against the above cell lines, with IC_{50} value of 4.97, 12.44, and 5.81 μM, respectively. While compound **1** was completely inactive in these cell lines with $IC_{50} > 100$ μM.[16] This result further confirmed that the cyclopentanone conjugated with an exomethylene group is the active center.[9]

Acknowledgments

Financial support of the Natural Science Foundation of Yunnan Province (No. 2004C0008Z), and the National Natural Science Foundation of China (No. 20502026 to Q.-B. Han.) are gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.05.025.

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13. The leaves of *Isodon parvifolius* (6 kg) were collected in Mao County, north of Sichuan Province, People's Republic of China, in August 2004. The sample was identified by Professor Xi-Wen Li, and a voucher specimen (KIB 04081802) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.
14. *Parvifoline X* (**1**): colorless needles, mp 185–187 °C; $[\alpha]_D^{24.3}$ −135.3 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.68) nm; IR (KBr) ν_{max} 3495, 3453, 2944, 2919, 1738, 1627, 1065, 913, 539 cm^{-1}; positive ESIMS: *m*/*z* 371 $[M+Na]^+$, 719 $[2M+Na]^+$; positive HRESIMS $[M+Na]^+$ *m*/*z* 371.1825 (calcd for $C_{20}H_{28}O_5Na$, 371.1834); ^1H and ^{13}C NMR, see Table 1.
15. *Parvifoline Y* (**2**): colorless needles, mp 216–218 °C; $[\alpha]_D^{25.3}$ +91.2 (*c* 0.47, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.01) nm; IR (KBr) ν_{max} 3443, 2960, 2932, 2864, 1710, 1623, 1340, 1141, 984 cm^{-1}; positive ESIMS: *m*/*z* 459 $[M+Na]^+$, 895 $[2M+Na]^+$; positive HRESIMS $[M+Na]^+$ *m*/*z* 459.2353 (calcd for $C_{24}H_{36}O_7Na$, 459.2358); ^1H and ^{13}C NMR, see Table 1.
16. Performed according to the literature: Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766. A549 = human lung cancer cells; HT-29 = human colon cancer cells; K562 = human leukemia cells.