Ent-Abietane and *ent*-Labdane Diterpenoids from *Isodon parvifolius*

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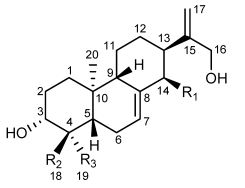
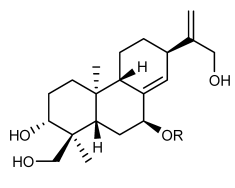
A phytochemical investigation on the leaves of *Isodon parvifolius* yielded three new *ent*-abietanoids parvifolines L—N (1—3), together with five known *ent*-abietanoids (4—8) and one known *ent*-labdane diterpenoid (9). Their structures were determined on the basis of extensive spectroscopic analysis.

Key words *Isodon parvifolius*; Labiatae; *ent*-abietanoid; parvifoline L; parvifoline M; parvifoline N

In previous phytochemical investigations of the genus *Isodon* (family Labiatae), tetracyclic *ent*-kauranoids were the most extensively studied because of their various activities, including antibacterial, anti-inflammatory and especially antitumor activities.[1)] However, some tricyclic diterpenoids were also reported in several *Isodon* species.[1—6)] *Isodon parvifolius* (Batalin) H. Hara, a perennial undershrub, is mainly distributed in the Tibet Autonomous Region and Sichuan Province, People's Republic of China. In early studies, *ent*-isopimarane type tricyclic diterpenoids were isolated from this plant originating from the Tibet Autonomous Region.[7)] In our search for new natural substances, a further study of this species collected from Sichuan Province, led to the isolation of three new *ent*-abietanoids parvifolines L—N (**1**—**3**), along with five known *ent*-abietanoids, rubescensin P (**4**),[5)] rubescensin I (**5**),[5)] enanderianin P (**6**),[8)] rubescensin J (**7**),[5)] and 14β,16-dihydroxy-3α,18-[(1-methylethane-1,1-diyl)dioxy]-*ent*-abieta-7,15(17)-diene (**8**),[5)] and one known *ent*-labdane type diterpenoid, 8β,13β-oxidoeperu-14-en-18-oic-acid (**9**).[9)] This is the first example of *ent*-abietane and *ent*-labdane type diterpenoids from *I. parvifolius*. The isolation and structural elucidation of the new diterpenoids were described in this paper.

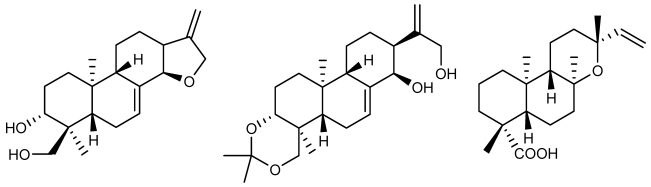


	R_1	R_2	R_3
1	OH	CH_3	CH_2OH
2	OCH_3	CH_2OH	CH_3
5	OH	CH_2OH	CH_3
6	OH	CHO	CH_3



3 R = CH_3

4 R = H



7 **8** **9**

Results and Discussion

Compound **1** was obtained as white amorphous powder. Its molecular formula was determined to be $C_{20}H_{32}O_4$ from the positive high resolution electrospray ionization mass spectroscopy (HR-ESI-MS) quasi-molecular ion peak at *m*/*z* 359.2198 $[M+Na]^+$, indicating five degrees of unsaturation. From the ^{13}C-NMR spectra, two olefinic quaternary carbons (δ_C 152.7, 141.2), an olefinic methine (δ_C 124.1), an olefinic methylene (δ_C 110.8), two oxygenated methines (δ_C 80.6, 74.6), two oxygenated methylenes (δ_C 64.8, 64.5), three methines (δ_C 51.1, 48.6, 47.4), five methylenes (δ_C 38.3, 28.7, 25.8, 23.8, 23.4), two nonoxygenated quaternary carbons (δ_C 42.5, 35.0) and two methyls (δ_C 23.7, 16.4) were observed. Compared with the classical *ent*-kaurane diterpenoids, one characteristic nonoxygenated quaternary carbon was absent in the high field of the ^{13}C-NMR spectrum of **1**. The information mentioned above and the tricyclic diterpenoids isolated from the plants of the same genus, suggested that compound **1** was an *ent*-abietanoid.

The NMR data of **1** were very similar to those of rubescensin I (**5**).[5)] The principal differences between them were the chemical shifts at C-3, C-4, C-5, C-18 and C-19. Therefore, **1** was assumed to be an epimer of **5**, differing from each other only in the configuration of C-4. The hydroxyl group was removed from C-18 in **5** to C-19 in **1**, which was supported by the downfield shift of C-3 (δ_C 80.6) and C-5 (δ_C 51.1) in **1** comparing with that of C-3 (δ_C 74.1) and C-5 (δ_C 43.0) in **5** due to the loss of γ-gauche steric compression effect between the 18-OH and H-3β and H-5β. Moreover, in the rotating frame Overhauser enhancement spectroscopy (ROESY) experiment (Fig. 1), the correlation of Me-18 with H-5, which in turn correlated with H-3 and H-9 unambiguously confirmed the above deduction. All the NMR data of **1** were assigned based on detailed analysis of its heteronuclear multiple bond connectivity (HMBC) and ROESY spectra. Thus, the structure of **1** was determined to

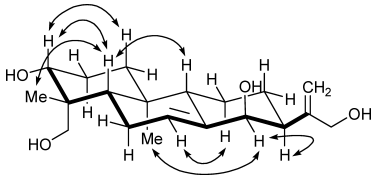


Fig. 1. Selected ROESY Correlations of **1**

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

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.80[a)] 1.14[a)]	38.3 t	1.80 br d, 13.1 1.20[a)]	38.1 t	1.59 br d, 13.2 1.19[a)]	37.0 t
2	1.95[a)] 1.95[a)]	28.7 t	1.90[a)] 1.90[a)]	27.9 t	1.95—1.85[a)] 1.95—1.85[a)]	27.9 t
3	3.58 m	80.6 d	4.18 dd, 5.8, 9.6	73.6 d	4.17 dd, 5.1, 10.5	75.0 d
4		42.5 s		43.0 s		42.7 s
5	1.26 dd, 5.5, 10.9	51.1 d	1.93[a)]	43.0 d	2.20 br d, 10.9	41.6 d
6	2.25[a)] 1.69 br d, 11.1	23.4 t	2.25 m 2.01 m	23.3 t	2.02 br d, 10.9 1.67[a)]	29.1 t
7	5.66 br s	124.1 d	5.61 d, 5.1	126.6 d	3.58 br s	81.9 d
8		141.2 s		135.6 s		136.5 s
9	2.29[a)]	48.6 d	2.03[a)]	48.6 d	2.13 br s	46.7 d
10		35.0 s		35.1 s		38.6 s
11	1.98[a)] 1.98[a)]	23.8 t	1.70 m 1.20 m	24.3 t	1.68[a)] 1.32[a)]	22.5 t
12	1.80[a)] 1.14[a)]	25.8 t	1.93—1.90[a)] 1.93—1.90[a)]	25.4 t	1.89[a)] 1.31[a)]	30.0 t
13	2.47 br d, 12.6	47.4 d	2.44 br d, 12.2	46.0 d	2.90 br s	40.1 d
14	4.56 br s	74.6 d	3.77 br s	83.9 d	5.75 br s	131.9 d
15		152.7 s		152.3 s		155.4 s
16	4.65 d, 13.4 4.55 d, 13.4	64.8 t	4.58 d, 14.1 4.50 d, 14.1	64.9 t	4.47 2H, br s	64.3 t
17	5.57 br s 5.28 br s	110.8 t	5.54 br s 5.18 br s	109.6 t	5.54 br s 5.11 br s	108.2 t
18	1.42 3H, s	23.7 q	4.12 d, 10.4 3.66 d, 10.4	67.6 t	4.12 d, 10.3 3.74 d, 10.3	69.4 t
19	4.60 d, 10.7 3.76 d, 10.7	64.5 t	1.15 3H, s	13.2 q	1.14 3H, s	12.7 q
20	0.81 3H, s	16.4 q	0.89 3H, s	15.9 q	0.87 3H, s	14.7 q
OMe			3.05 3H, s	55.0 q	3.20 3H, s	54.8 q

a) Overlapped signals.

be 3α,14β,16,19-tetrahydroxy-*ent*-abieta-7,15(17)-diene, and named parvifoline L.

The molecular formula of compound **2** was $C_{21}H_{34}O_4$ as deduced from the HR-ESI-MS, *m/z* 373.2355 $[M+Na]^+$. The NMR data of **2** were in good agreement with those of **5**, except for the additional signals for a methoxyl group δ_H 3.05 (3H, s) and δ_C 55.0 (q). The C-14 (δ_C 83.9) signal of **2** appearing more downfield than that of **5** (δ_C 74.8), linked the methoxyl group at C-14 in **2**. This was further confirmed by the HMBC correlation between the methoxyl protons (δ_H 3.05) and C-14 (δ_C 83.9). Furthermore, correlations from H-14 (δ_H 3.77) to OMe, C-7, C-9, C-12 and C-15 provided additional evidence for this linkage. The relative stereochemistry of **2** was the same as that of **5**, which was also ascertained by the ROESY correlations. Therefore, compound **2** was elucidated as 3α,16,18-trihydroxy-14β-methoxy-*ent*-abieta-7,15(17)-diene, and called parvifoline M.

Compound **3** was assigned the molecular formula $C_{21}H_{34}O_4$ by HR-ESI-MS (*m/z* 373.2351 $[M+Na]^+$). The general features of its NMR spectra closely resembled those of rubescensin P (**4**),[5] except for the extra methoxyl group signals δ_H 3.20 (3H, s) and δ_C 54.8 (q). In the HMBC spectrum, the correlation between the methoxyl protons (δ_H 3.20) and C-7 (δ_C 81.9) showed that the methoxyl group at the C-7 position was evident in **3** instead of a hydroxyl group at the same position in **4**. Meanwhile, the orientations of all the substitutes in **3** were identical to those of **4**, which was proved by the results of the ROESY experiment. Consequently, parvifoline N (**3**) was characterized as 3α,16,18-trihydroxy-7β-methoxy-*ent*-abieta-8(14),15(17)-diene.

From the leaves of *I. Parvifolius* collected from Sichuan Province, we did not obtain *ent*-isopimarane type diterpenoids which were isolated from this plant collected from Tibet Autonomous Region previously, but obtained the *ent*-abietane and *ent*-labdane tricyclic diterpenoids. This result should be attributed to the different ecological environment of the two places, since the collection season is identical.

Experimental

General Experiment Procedures Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. MS spectra were performed on a VG Autospec-3000 spectrometer under 70 eV. 1D and 2D NMR spectra were recorded on Brucker AM-400 and DRX-500 spectrameters. Unless specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Semipreparative HPLC was performed on an Agilent 1100 apparatus equipped with a UV detector and Zorbax SB-C-18 (Agilent, 9.4 mm×25 cm) column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatography with a Shimadzu PRC-ODS (K) column. Column chromatography was performed with silica gel (200—300 mesh, Qing-dao Marine Chemical Factory, Qing-dao, P. R. China), silica gel H (60 μm, Qing-dao Marine Chemical Factory) and MCI gel (75—150 μm, Mitsubishi Chemical Corporation, Japan).

Plant Material The leaves of *Isodon parvifolius* were collected in Mao County, north of Sichuan Province, People's Republic of China, in August 2004. The sample was identified by Prof. 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.

Extraction and Isolation The air-dried and powdered leaves of *I. parvifolius* (6.0 kg) were extracted with Me_2CO (3×10 l) each two days at room temperature and filtered. The filtrate was evaporated to give a residue, which was dispersed in H_2O (4 l) and extracted with petroleum ether (60—90 °C, 3×2 l) and EtOAc (4×2.5 l) successively. The EtOAc extract (400 g) was decolorized on MCI gel, eluted with 90% MeOH–H_2O to yield a pale yellow gum (350 g). The gum was subjected to column chromatography over a silica gel (200—300 mesh, 2.0 kg) column, eluted with $CHCl_3$–Me_2CO (1 : 0—0 : 1, gradient system) to obtain fractions 1—5. Fraction 1 (8.0 g) was chromatographed on a normal silica gel column eluted stepwise with a mixture of petroleum ether and acetone (10 : 1—4 : 1). Compound **9** (0.5 g) was crystallized from the petroleum ether–acetone 6 : 1 eluent. Fraction 2 (80.0 g), Fr. 3 (60.0 g) and Fr. 4 (60.0 g) were rechromatographed on normal silica gel, in turn, using identical gradient elution petroleum ether–2-propanol (from 10 : 1 to 2 : 1) to provide subfractions 2a—2d, 3a—3c and 4a—4d, respectively. Compound **8** (10 mg) was isolated from subfraction 2b (10.0 g) eluted with petroleum ether–acetone (5 : 1). Subfraction 2c (1.3 g) was purified by $CHCl_3$–Me_2CO (6 : 1) to give **7** (0.5 g). Successive column chromatography by a silica gel column ($CHCl_3$–MeOH, 20 : 1) and semipreparative HPLC (45% MeOH–H_2O) led to the isolation of **4** (5 mg) and **5** (22 mg). Subfraction 3c (0.6 g) was further separated *via* preparative HPLC (45% MeOH–H_2O) to get **1** (11 mg) and **6** (18 mg). Compound **3** (30 mg) was obtained from subfraction 4b by preparative HPLC (60% MeOH–H_2O). Compound **2** (13 mg) was separated from subfraction 4d eluted with $CHCl_3$–MeOH (10 : 1), followed by preparative HPLC (50% MeOH–H_2O).

Compound **1**: White amorphous powder (MeOH). $[\alpha]_D^{28.4}$+55.52° (c= 0.35, MeOH). UV λ_{max} (MeOH) nm (log ε): 204 (3.64). IR (KBr) ν_{max} cm^{-1}: 3415, 2967, 2932, 2865, 2852, 1647, 1442, 1054, 1033. ^{1}H- and ^{13}C-NMR data: see Table 1. Positive ESI-MS: m/z 359 $[M+Na]^+$; positive HR-ESI-MS $[M+Na]^+$ m/z 359.2198 (Calcd for $C_{20}H_{32}O_4Na$ $[M+Na]^+$, 359.2198).

Compound **2**: White amorphous powder (MeOH). $[\alpha]_D^{28.0}$+23.49° (c= 0.56, MeOH). UV λ_{max} (MeOH) nm (log ε): 204 (3.82). IR (KBr) ν_{max} cm^{-1}: 3407, 2934, 2868, 1642, 1446, 1082, 1059, 1033. ^{1}H- and ^{13}C-NMR data: see Table 1. Positive ESI-MS: m/z 373 $[M+Na]^+$; positive HR-ESI-MS $[M+Na]^+$ m/z 373.2355 (Calcd for $C_{21}H_{34}O_4Na$ $[M+Na]^+$, 373.2354).

Compound **3**: White amorphous powder (MeOH). $[\alpha]_D^{20.8}$+54.84° (c=1.10, MeOH). UV λ_{max} (MeOH) nm (log ε): 204 (4.04). IR (KBr) ν_{max} cm^{-1}: 3417, 2966, 2937, 2867, 2817, 1648, 1446, 1108, 1084, 1064, 1040. ^{1}H- and ^{13}C-NMR data: see Table 1. Positive ESI-MS: m/z 373 $[M+Na]^+$; positive HR-ESI-MS $[M+Na]^+$ m/z 373.2351 (Calcd for $C_{21}H_{34}O_4Na$ $[M+Na]^+$, 373.2354).

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