

ent-Abietane Diterpenoids from *Isodon xerophilus*

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Three new *ent*-abietanoids, named xerophilusins XIV–XVI, and four known analogues, as well as four known chemical constituents were isolated from the leaves of *Isodon xerophilus*. Their structures were elucidated by extensive spectroscopic studies, and comparison with literature data. In addition, the cytotoxic activity of the *ent*-abietanoids against chronic myelogenous leukemia (K562), stomach adenocarcinoma (MKN45), and hepatocellular carcinoma (HepG2) human cell lines was investigated and no activities were observed.

Key words: *Isodon xerophilus*, Lamiaceae, *ent*-Abietanoid, Xerophilusins XIV–XVI

INTRODUCTION

Isodon xerophilus (C. Y. Wu et H. W. Li) H. Hara is a perennial shrub native to Yunnan Province. It has long been used as an antitumor, anti-inflammatory, and antibacterial agent by local people. Previous investigations discovered a series of bioactive *ent*-kaurane diterpenoids from this plant (Li et al., 2007). Further study of this plant led to the isolation of eleven other compounds including three new *ent*-abietanoids named as xerophilusins XIV–XVI (**1–3**). On the basis of the spectroscopic analysis and comparison with reported data, they were elucidated as 3 α ,16,17,18-tetrahydroxy-*ent*-abieta-7-ene (**1**), 15,16,17-trihydroxy-3 α ,18-[(1-methylethane-1,1-diyl)dioxy]-*ent*-abieta-7-ene (**2**), 3 α ,15,18-trihydroxy-16,17-[(1-methylethane-1,1-diyl)dioxy]-*ent*-abieta-7-ene (**3**), hebeiabinin B (**4**) (Huang et al., 2007), maoyecrystals G and H (**5** and **6**) (Han et al., 2003), rubescensin I (**7**) (Han et al., 2004), pinoresinol (**8**) (Vermes et al., 1991), 5,8-dihydroxy-4',6,7-trimethoxy-flavone-8-O- β -D-glucopyranoside (**9**) (Na et al., 2001), patriscabratine (**10**) (Gu et al., 2002), and 9,16-dioxooctadec-10,12,14-trienoic acid (**11**) (Herz and Kulanthaivel, 1984),

respectively. Among them, compounds **10** and **11** were first found in Lamiaceae. Besides, the cytotoxicity of compounds **1–7** against K562, MKN45, and HepG2 human cell lines was also evaluated. This paper describes the isolation and structural elucidation of these compounds and the cytotoxicity assay result.

MATERIALS AND METHODS

General experimental procedure

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were carried out on a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) and high-resolution electrospray ionization (HRESI) mass spectra were acquired on an API QSTAR time-of-flight mass spectrometer. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatographic apparatus with a Shimadzu PRC-ODS (K) column (34 mm × 15 cm, 25 mL/min). Thin layer chromatography (TLC) was run on HSGF₂₅₄ silica gel plates (10–40 μ m, Qingdao Marine Chemical Inc.). Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc.), Lichroprep RP-18 gel (40–63 μ m, Merck), and MCI gel (75–150 μ m, Mitsubishi Chemical Corporation). All solvents including petroleum ether (60–90°C) were

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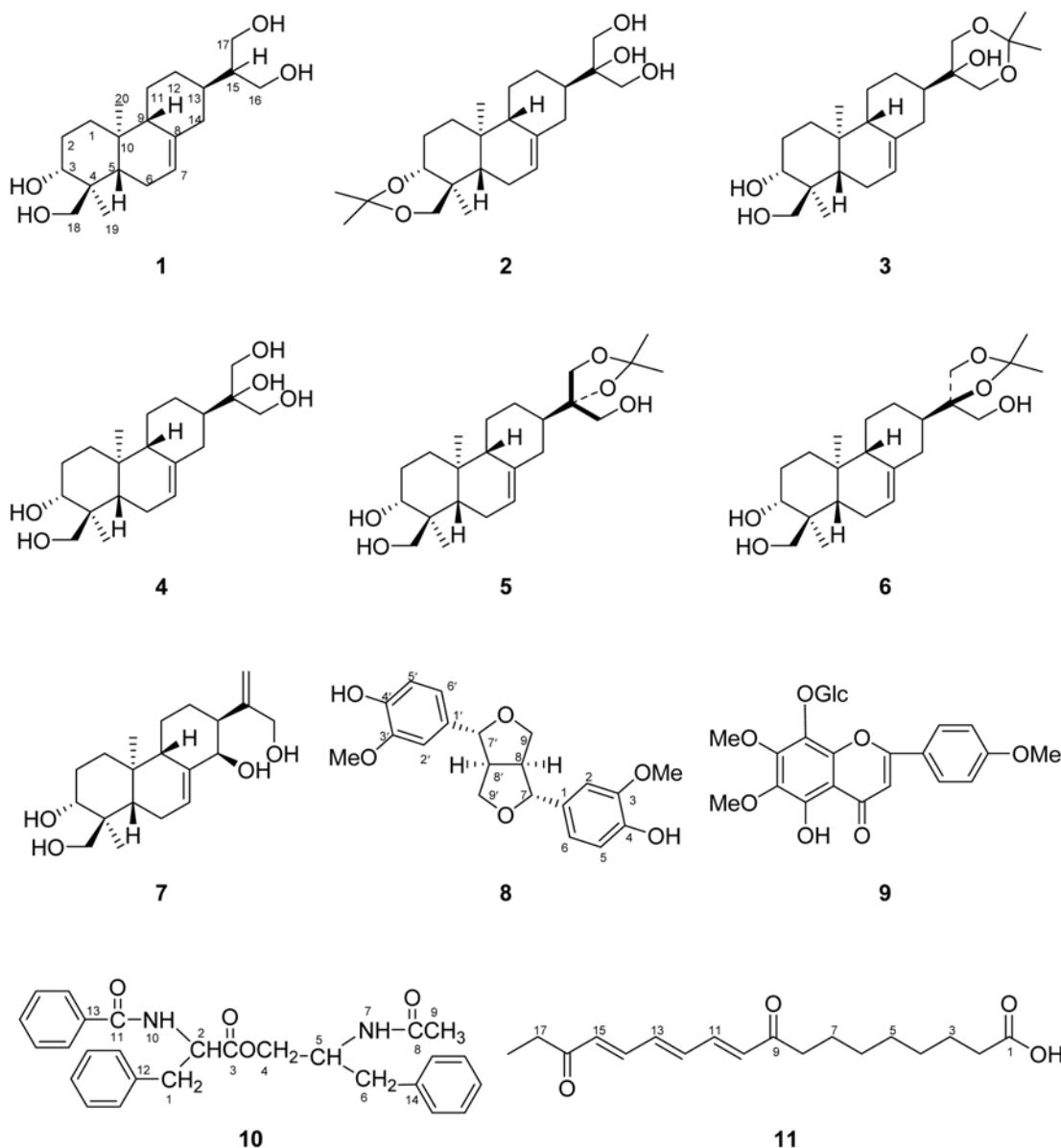


Fig. 1. Structures of compounds 1-11

distilled prior to use.

Plant materials

The leaves of *Isodon xerophilus* were collected in Yuanyang County of Yunnan Province, People's Republic of China, in August 2001. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 01082815) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Cytotoxicity assay

The cytotoxicity of compounds 1-7 was tested against chronic myelogenous leukemia (K562), stomach adenocarcinoma (MKN45), and hepatocellular carcinoma (HepG2) human cell lines. The detailed experimental procedures have been described in previously published paper (Li et al., 2007).

Extraction and isolation

The air-dried, milled plant material (7.5 kg) was soaked with acetone (3×12 L, each 3 days) at room temperature and filtered. The filtrate was evaporated *in vacuo* to afford a residue, which was dissolved in H_2O (4 L) and then extracted with petroleum ether (3

$\times 3$ L) and ethyl acetate (3×4 L), sequentially. The EtOAc extract (504 g) was decolorized using MCI gel, eluted with 90% MeOH-H₂O, to yield a yellowish gum (423 g). The gum was separated on a silica gel column, eluted with CHCl₃-Me₂CO (1:0 to 0:1, gradient system), to obtain seven fractions, A-G. Separation of fraction B (33.4 g) by silica gel column chromatography, eluted with petroleum ether-acetone (6:1 to 2:1, gradient system), yielded four subfractions, B1-B4. Compounds **10** (0.65 g) and **11** (8 mg) were obtained from subfraction B1 by silica gel column chromatography eluted with petroleum ether-2-propanol (12:1). Fraction C (112.5 g) was applied to a silica gel column, eluted with a gradient system (petroleum ether-acetone, 4:1 to 1:1), to afford four main subfractions,

C1-C4. Compound **8** (22 mg) was isolated from sub-fraction C3 by RP-18 column chromatography eluted with 40% MeOH-H₂O. Fraction F (28.7 g) was subjected to a RP-18 column (30% to 60% MeOH-H₂O, gradient system) to provide F1 (0.62 g), F2 (1.30 g), and F3 (0.21 g), respectively. Compounds **5** and **6** (40 mg) were purified from F1 by silica gel column chromatography, eluted with petroleum ether-acetone (3:1). Then, F2 was applied to a silica gel column and separated by gradient elution (CHCl₃-MeOH, 30:1 to 10:1) to afford F2a (0.15 g), F2b (0.12 g), and F2c (0.50 g), respectively. Preparative HPLC (30% MeOH-H₂O) was used to purify compounds **2** (70 mg), **3** (7 mg), and **4** (40 mg) from F2b. Fraction F3 was separated using a silica gel column (CHCl₃-MeOH, 50:1 to 20:1, gradient

Table I. ¹H- and ¹³C-NMR data of compounds **1-3**

No.	1		2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	1.76 m 1.18	38.1 t	1.75 ^a 1.12 ^a	38.3 t	1.85 m 1.18 m	38.1 t
2	1.78 2H, m	27.9 t	1.74 ^a 1.53 ^a	24.5 t	1.90 ^a	27.9 t
3	4.26 ^a	73.7 d	3.60 dd, 11.6, 3.4	77.6 d	4.23 m	73.8 d
4		43.1 s		36.9 s		43.0 s
5	1.71 ^a	43.0 d	1.05 ^a	46.0 d	1.51 ^a	43.0 d
6	2.52 br d, 12.5	39.7 t	1.85 m 1.87 ^a	22.6 t	2.02 m 1.50 ^a	23.5 t
7	5.33 br s	120.3 d	5.35 br s	119.5 d	5.41 br s	120.7 d
8		137.8 s		138.5 s		137.3 s
9	1.68 ^a	52.9 d	1.71 ^a	53.0 d	1.73 ^a	52.8 d
10		35.3 s		35.5 s		35.3 s
11	1.88 m 1.88 m	23.4 t	1.75 ^a 1.09 ^a	25.8 t	1.90 ^a 1.08 m	25.7 t
12	1.86 ^a 1.12 ^a	30.2 t	2.25 m 1.65 m	26.7 t	1.76 ^a 1.40 ^a	25.6 t
13	1.79 m	37.1 d	2.07 m	42.2 d	1.90 ^a	43.2 d
14	1.68 ^a 1.09 m	26.0 t	2.81 br d, 14.1 2.48 m	35.9 t	2.45 m 2.28 m	34.6 t
15	1.70 ^a	49.2 d		75.5 s		68.2 s
16		62.2 t		65.0 t	4.04 2H, dd, 11.6, 4.1	68.0 t
17	4.24-4.15 4H, m	62.1 t	4.24 4H, br s	65.0 t	3.94 2H, t, 11.8	68.0 t
18	4.12 d, 9.5 3.65 d, 9.5	67.7 t	3.54 d, 10.6 3.43 d, 10.6	72.5 t	4.11 d, 10.2 3.65 d, 10.2	67.9 t
19	1.13 3H, s	13.1 q	1.22 3H, s	13.0 q	1.16 3H, s	13.1 q
20	0.88 3H, s	15.9 q	0.79 3H, s	15.9 q	0.93 3H, s	15.9 q
S				99.1		98.3
			1.52 3H, s	30.3	1.47 3H, s	26.9
			1.50 3H, s	19.5	1.48 3H, s	20.9

Measured in C₅D₅N; δ in ppm; J in Hz; **1** at 400 MHz; **2** and **3** at 500 MHz; S = isopropylidene; ^aOverlapped signals

system) to give subfractions F3a-F3c, and compound **9** (33 mg). Subfraction F3b was further purified by preparative HPLC (40% MeOH-H₂O) to provide compounds **1** (10 mg) and **7** (15 mg), respectively.

Xerophilusin XIV (1)

A white amorphous powder; $[\alpha]_D^{28} + 36.6^\circ$ (MeOH, *c* 0.83); UV (MeOH) λ_{max} : 206 nm; IR (KBr): 3386, 2930, 1630, 1444, 1383, 1051, 1033 cm⁻¹; HRESIMS (positive) *m/z* 361.2363 [M + Na]⁺ (calcd for C₂₀H₃₄O₄Na, 361.2354); ESIMS (positive) *m/z* 361 [M + Na]⁺, 677 [2M + 1]⁺, 699 [2M + Na]⁺; ¹H-NMR (400 MHz, C₅D₅N) and ¹³C-NMR (100 MHz, C₅D₅N): see Table I.

Xerophilusin XV (2)

A white amorphous powder; $[\alpha]_D^{28} + 43.7^\circ$ (MeOH, *c* 0.79); UV (MeOH) λ_{max} : 205 nm; IR (KBr): 3419, 2990, 2938, 2891, 2857, 1640, 1461, 1446, 1380, 1365, 1259, 1209, 1106, 1038 cm⁻¹; HRESIMS (positive) *m/z* 417.2605 [M + Na]⁺ (calcd for C₂₃H₃₈O₅Na, 417.2616); ESIMS (positive) *m/z* 417 [M + Na]⁺, 811 [2M + Na]⁺; ¹H-NMR (500 MHz, C₅D₅N) and ¹³C-NMR (125 MHz, C₅D₅N): see Table I.

Xerophilusin XVI (3)

A white amorphous powder; $[\alpha]_D^{28} + 12.6^\circ$ (MeOH, *c* 0.57); UV (MeOH) λ_{max} : 205 nm; IR (KBr): 3431, 2988, 2934, 2868, 1631, 1447, 1382, 1251, 1199, 1155, 1059, 1033 cm⁻¹; HRESIMS (positive) *m/z* 417.2610 [M + Na]⁺ (calcd for C₂₃H₃₈O₅Na, 417.2616); ESIMS (positive) *m/z* 417 [M + Na]⁺, 811 [2M + Na]⁺; ¹H-NMR (500 MHz, C₅D₅N) and ¹³C-NMR (125 MHz, C₅D₅N): see Table I.

RESULTS AND DISCUSSION

The molecular formula of compound **1** was determined to be C₂₀H₃₄O₄ by the quasi-molecular ion peak at *m/z* 361.2363 [M + Na]⁺ in the HRESIMS, corresponding to four degrees of unsaturation. The ¹³C- and DEPT NMR spectra of **1** displayed twenty carbon signals including one trisubstituted double bond, nine methylenes, five methines, two quaternary carbons, and two methyls (Table I). Considering its unsaturation, compound **1** should possess three rings, which suggested that it is a tricyclic diterpene. These information and the tricyclic diterpenoids isolated from the plants of the same genus, suggested that compound **1** is an *ent*-abietanoid (Sun et al., 2006). The ¹H- and ¹³C-NMR spectra of **1** were very similar to those of hebeiabinin B (**4**) except for the absence of one hydroxyl group. The HMBC correlations of both H-13 and H-15 with C-16 and C-17 (Fig. 2) indicated that one of the hydroxyl

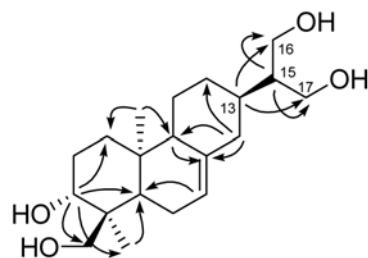


Fig. 2. Key HMBC correlations of **1**

groups of C-15 in **4** does not exist in **1**. Moreover, because of the ROESY correlations between H-3 and H-18, the hydroxyl group at C-3 was determined to be α -orientated. Thus, compound **1** was identified as 3 α ,16,17,18-tetrahydroxy-*ent*-abieta-7-ene.

Compounds **2** and **3** were determined to have the same molecular formula C₂₃H₃₈O₅ by HRESIMS. In their ¹H- and ¹³C-NMR spectra, besides the signals of an isopropylidene group, there were signals of an *ent*-abietanoid, also identical to those of **4** (Table I). In the HMBC spectrum of **2**, H-3 and H-18 correlating to the quaternary carbon at δ 99.1 proved that the isopropylidene group is linked at C-3 and C-18. Analogously, the HMBC correlations of H-16 and H-17 with the quaternary carbon at δ 98.3 proved that the isopropylidene group is linked at C-16 and C-17 in **3**. Therefore, compounds **2** and **3** were elucidated as 15,16,17-trihydroxy-3 α ,18-[(1-methylethane-1,1-diyl)dioxy]-*ent*-abieta-7-ene and 3 α ,15,18-trihydroxy-16,17-[(1-methylethane-1,1-diyl)dioxy]-*ent*-abieta-7-ene, respectively.

Compounds **1-7** showed noncytotoxic activity against K562, MKN45, and HepG2 cell lines (IC₅₀ > 10 μ M). These *ent*-abietanoids may be the noncytotoxic constituents in *I. xerophilus*.

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