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A new *ent*-kaurane diterpenoid from *Isodon phyllostachys*

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Abstract

A new *ent*-kaurane diterpenoid, phyllostachysin C (**1**), together with five known compounds, sculponeatins B and C, nodosin, ursolic acid and 2α-hydroxyursolic acid, were isolated from the leaves of *Isodon phyllostachys*. The structure of **1** was elucidated on the basis of its spectral properties.

Keywords: *Isodon phyllostachys*; *ent*-Kauranoids; Phyllostachysin C

1. Introduction

Isodon phyllostachys (Diels) Hara (Lamiaceae), distributed widely in the north-west of Yunnan and south-west of Sichuan of China, has been used as antiphlogistic or an antibiotic agent in folk medicine. We reported previously two new *ent*-kauranoids, phyllostachysin A [1] and B [2], and further examination of the diterpenoids in this plant collected in Sichuan province led to the isolation of a new compound, phyllostachysin C (**1**), and five known isolates, sculponeatins B and C [3], nodosin [4], ursolic acid and 2α-hydroxyursolic acid. We now present the isolation and characterization of **1**.

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2. Experimental

2.1. Plant material

The leaves of *I. phyllostachys* were collected in Muli of Sichuan province in August 1987, and air-dried. The identity of plant material was verified by Prof. Xi-Wen Li, and a voucher specimen (870831-KIB) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica.

2.2. Extraction and isolation

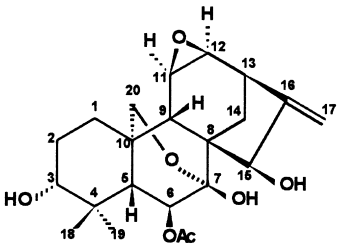
The dried and powdered leaves (2.2 kg) were extracted with Et_2O and concentrated. The residue was dissolved in MeOH and decolored by activated charcoal. After the removal of MeOH, the extract (92 g) was subjected to Si-gel CC eluting with petrol–chloroform and chloroform–acetone. Each fraction was further purified by recrystallization to yield **1** (550 mg), sculponeatins B (1.3 g) and C (3.3 g), nodosin (2.5 g), ursolic acid (115 mg) and 2α-hydroxyursolic acid (50 mg).

Phyllostachysin C (**1**). $C_{22}H_{30}O_7$, colorless needles, m.p. 203–205°C, $[\alpha]_D^{22}$: −102.0° (*c* 0.55, C_5H_5N); IRmax (KBr): 3550, 3520, 3330, 1740, 1655, 1225, 1100, 1055, 1038, 1020 cm^{-1}; ^{1}H-NMR (400 MHz, Py-d_5): 1.63 (1H, *m*, H-1α), 1.82 (1H, *overlap*, H-1β), 1.86 (2H, *m*, H_2-2α and β), 3.50 (1H, *br d*, *J* 9.2 Hz, H-3β), 1.55 (1H, *d*, *J* 4.2 Hz, H-5β), 5.76 (1H, *d*, *J* 4.2 Hz, H-6α), 2.57 (1H, *br s*, H-9β), 3.19 (1H, *t*, *J* 4.3 Hz, H-11α), 3.22 (1H, *t*, *J* 4.3 Hz, H-12α), 2.96 (1H, *br s*, H-13α), 2.56 (1H, *d*, *J* 12.1 Hz, H-14α), 2.07 (1H, *dd*, *J* 4.6, 12.1 Hz, H-14β), 5.01 (1H, *br s*, H-15α), 5.54 (1H, *br s*, H-17a), 5.25 (1H, *br s*, H-17b), 1.20 (3H, *s*, Me-18), 1.30 (3H, *s*, Me-19), 4.20 (1H, *ABd*, *J* 9.2 Hz, H-20a), 4.44 (1H, *ABd*, *J* 9.2 Hz, H-20b), 2.19 (3H, *s*, Me-OAc); ^{13}C-NMR (100 MHz, Py-d_5): 28.4 (C-1), 27.9 (C-2), 77.2 (C-3), 40.1 (C-4), 58.4 (C-5), 74.4 (C-6), 96.4 (C-7), 51.4 (C-8), 41.1 (C-9), 37.5 (C-10), 50.8 (C-11), 53.6 (C-12), 38.3 (C-13), 26.6 (C-14), 74.7 (C-15), 153.4 (C-16), 109.5 (C-17), 27.8 (C-18), 15.9 (C-19), 67.9 (C-20), 169.2 (C- OAc), 21.5 (C- OAc); EI-MS (70 eV) *m / z*: 406 $[M]^+$ (1), 346 $[M\text{-}AcOH]^+$ (35), 328 $[M\text{-}AcOH\text{-}H_2O]^+$ (8), 318 (3), 300 (4), 282 (10), 167 (100), 149 (67); HREI-MS *m / z*: 406.1986 (calcd for $C_{22}H_{30}O_7$, 406.1992).

3. Results and discussion

The ethereal extract from the leaves of *I. phyllostachys* was subjected to Si-gel column chromatography eluting with petrol–chloroform and chloroform–acetone. Each fraction was further purified repeatedly to yield phyllostachysin C (**1**) and five known compounds.

Phyllostachysin C (**1**) was obtained as colorless needles and had a molecular formula of $C_{22}H_{30}O_7$ deduced from HREI-MS (obsd 406.1986, calcd 406.1992) and the analysis of its ^{13}C-NMR spectrum. It contained an acetoxyl group [^{1}H-NMR: δ 2.19 (3H, *s*); ^{13}C-NMR: δ 169.2 (*s*) and 21.5 (*q*)], *exo*-methylene group [IRmax (KBr): 1655 cm^{-1}; ^{1}H-NMR: δ 5.25 and 5.54 (each 1H, *br s*); ^{13}C-NMR: δ 109.5 (*t*)



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and 153.4 (*s*)], an oxygen-bearing methylene [^{1}H-NMR: δ 4.20 and 4.44 (each 1H, *ABd*, J = 9.2 Hz); ^{13}C-NMR: δ 67.9 (*t*)], and a ketalic group [^{13}C-NMR: δ 96.4 (*s*)]. Moreover, signals due to two tertiary methyl groups, three methylenes, eight methines including five oxygenated ones and three quaternary carbons were also observed in the ^{13}C-NMR spectrum of **1**. These facts, with consideration of the structures of diterpenoids isolated so far from the *Isodon* genus [5,6], established the basic skeleton of **1** as 7β-hydroxy-7α, 20-epoxy-*ent*-kaur-16-ene.

According to the ^{1}H–^{1}H COSY and HMQC experiments, all protons and related carbons were assigned, which provided information on the five oxygenated methines. The signals at δ 3.50 (1H, *br d*, J = 9.2 Hz, H-3) and δ 77.2 (*d*, C-3), and the downfield shifts of C-2 and C-4 disclosed that a hydroxyl group was located at C-3. The acetoxyl group was at C-6 through the signals of H-6 (δ 5.76, 1H, *d*, J = 4.2 Hz) and C-6 (δ 74.4, *d*). The signals at δ 5.01 (1H, *s*, H-15) and δ 74.7 (*d*, C-15) were indicative of a 15-hydroxyl group. The signals at δ 3.19 (1H, *t*, J = 4.3), 3.22 (1H, *t*, J = 4.3 Hz), δ 50.8 (*d*) and 53.6 (*d*) were ascribable to H-11, H-12, C-11 and C-12, respectively, which showed the presence of a 11, 12-epoxide ring.

The following NOESY cross peaks: H-3β with H-1β and H-5β; H-6α with Me-18, 19; H-15α with H-14β, indicated that 3-OH, 6-OAc and 15-OH possessed α-, β- and β-orientation, respectively. The upfield shifts of C-19, 18 and C-9 owing to the γ-steric compression effects of 3α- and 15β-OH also supported partial results above. Additionally, the β-configuration of the 11, 12-epoxide ring was decided by the NOE effects of H-11α with H-14α and H-1α, and the peak form of H-12 (*t*, J = 4.3 Hz). Thus, the structure of phyllostachysin C (**1**) was identified as shown.

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