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A new abietane diterpene from *Glyptostrobus pensilis*

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ABSTRACT

A new abietane diterpene, glypensin A (**1**) and four known compounds, 12-acetoxy-*ent*-labda-8(17), 13*E*-dien-15-oic acid (**2**), quercetin 3-*O*-α-*L*-arabinofuranoside (**3**), quercetin 3-*O*-β-*D*-galactopyranoside (**4**), β-sitosterol (**5**) were isolated from the branches and leaves of *Glyptostrobus pensilis* (Staut.) Koch. Their structures were determined by MS, 1D- and 2D-NMR means. Compound **1** showed cytotoxicity on human chronic myeloid leukemia cell line K562 (IC_{50} = 21.2 μM).

1. Introduction

Glyptostrobus pensilis is the unique species in the genus *Glyptostrobus* (Taxodiaceae), which only lives in several south districts in China. It has been protected as a living fossil in China and also listed as a threatened species by the IUCN [1]. The leaves and barks of *G. pensilis* are used as one herb to treat rheumatoid arthritis, hypertension, dermatitis and scald. Up to now, only five flavonoids have been reported from this plant [2]. As part of our systematic investigations on chemical and bioactive constituents from Taxodiaceae plants, we carried out extensive chemical studies on the branches and leaves of *G. pensilis*, and obtained one new abietane diterpene and four known compounds (Fig. 1). Meanwhile, compounds **1–4** were tested on human chronic myeloid leukemia cell line K562. The result indicated that compound **1** showed cytotoxicity with an IC_{50} value of 21.2 μM. In this paper, we reported the isolation and identification of compounds **1–5**.

2. Experimental

2.1. Generals

Optical rotations: Horiba SEAP-300. IR: Bio-Red FTS-135. UV: 2401PC. NMR: Bruker AM-400 and DRX-500. MS: VG Autospec-3000.

2.2. Plant

Branches and leaves of *G. pensilis* were collected from Kunming Botany Garden, Yunnan Province, People's Republic of China, in August 2006. It was identified by Prof. Wei-Bang Sun from Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried and powdered branches and leaves (3.5 kg) of *G. pensilis* were extracted with MeOH for three times under room temperature and then concentrated under reduced pressure. The concentrated MeOH extract (372 g) was dissolved in hot water and extracted with petroleum ether, AcOEt and *n*-BuOH, respectively, to afford 75 g petroleum

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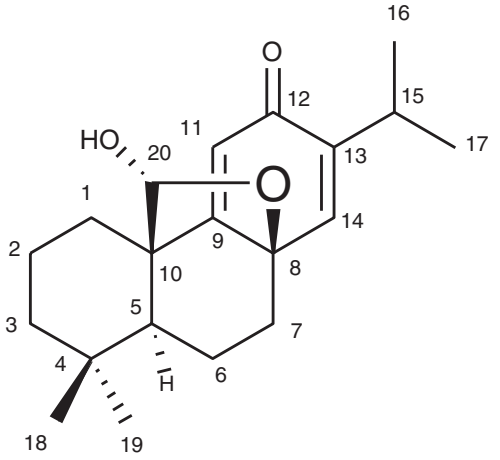

Fig. 1. The structure of compound **1**.

ether extract, 83 g AcOEt extract, 90 g *n*-BuOH extract and 124 g water extract. The petroleum ether extract was purified by CC (900 g silica gel; petroleum ether/acetone mixtures of increasing polarity), to give fractions (Fr.) 1–8. Fr. 4 was eluted with petroleum ether /AcOEt 3:1 to afford **1** (18 mg), **2** (15 mg) and **5** (62 mg). The AcOEt extract was purified by CC (1100 g silica gel; $CHCl_3$/MeOH mixtures of increasing polarity), to give fractions (Fr.) A–F. Fr. C was further purified by CC ($CHCl_3$/MeOH 6:1) and Sephadex LH-20 column ($CHCl_3$/MeOH 1:1) to afford **3** (28 mg) and **4** (36 mg).

*2.4. Reduction of **1** to pisiferal*

Compound **1** (5.5 mg) was resolved in glacial acetic acid (4 ml) and freshly activated zinc powder (100 mg) was added, then the mixture was stirred at room temperature for 4 h. Saturated brine solution (4 ml) was added to the reacted solution and the mixture was extracted with $CHC1_3$ (4×6 ml). The $CHC1_3$ extracts were evaporated in vacuo. The residue was purified on prepare silica gel TLC to give 2.5 mg pure pisiferal (petroleum/acetone 3:1).

Compound **1** (Fig. 1), (5*S*, 8*R*, 10*R*, 20*R*)-8, 20-epoxy-12-oxoabieta-9(11), 13-diene, white plate-like crystal. M. p. 180–182 °C. $[\alpha]_D^{28}=-264.5$ ($c=0.14$, MeOH). UV (MeOH): 202 (4.15), 231 (4.09), 236 (4.09). IR (KBr): 3341, 2946, 2868, 1681, 1644, 1427, 1367, 1125, 995, 909. ^{1}H and ^{13}C NMR spectral data see Table 1. HR-TOF-MS *m/z*: 339.1929 $[M+Na]^+$. Calcd. for $C_{20}H_{28}O_3Na$ 339.1936. EI MS *m/z*: 316 $[M]^+$.

3. Results and discussion

Compound **1** was found to possess the molecular formula of $C_{20}H_{28}O_3$ by positive HR-TOF-MS ($[M+Na]^+$ at *m/z* 339.1929, calcd. 339.1936), which was also confirmed by ^{13}C and DEPT NMR spectra. The appearance of 20 carbons in the ^{13}C NMR spectra, two trisubstituted double bonds (δ_C 168.9 (s) and 116.0 (d), 145.1(s) and 138.7 (d)), a conjugated ketone (δ_C 185.9), an isopropyl group (δ_H 1.07 (d, 3H, 6.9 Hz), 1.05 (d, 3H, 6.9 Hz), 2.96 (1H, m)), two methyls (δ_C 32.8 (q), 21.3 (q)), an hemiacetal group (δ_C 100.0 (d), δ_H 5.66 (d, 1H, 7.3 Hz)), and the seven units of unsaturation suggested that this compound might have an abietane diterpenoid skeleton [3].

Table 1
^{1}H and ^{13}C NMR data of **1** (100 MHz and 400 MHz, CD_3OD, *J* in Hz, δ in ppm).

C	δ_H	δ_C	H–H COSY	HMBC (H→C)	ROESY
1	1.96 (m), 1.46 (m)	26.5	H-2	2,3,5,9,10,20	
2	1.56 (m)	18.7	H-1, 3	1,3,4,10	
3	1.52(m), 1.31 (m)	41.6	H-2	1,5,18,19	
4		34.2			
5	1.39 (dd, 12.1, 5.9)	52.5	H-6	7,9,10,18,19,20	H-19
6	1.75 (m)	19.3	H-5, 7	4,5,7,8,10	
7	2.18 (dd, 13.0, 4.8), 1.27 (dd, 13.0, 4.8)	40.5	H-6	5,6,8,9,14	
8		78.5			
9		168.9			
10		51.7			
11	5.93 (s)	116.0		8,9,10,12,13	
12		185.9			
13		145.1			
14	6.69 (s)	138.7		7,9,12,13,15	
15	2.96 (m)	26.3	H-16, 17	12,13,14,16,17	
16	1.07 (d, 6.9)	22.0	H-15	13,15	
17	1.05 (d, 6.9)	21.8	H-15	13,15	
18	1.03 (s)	21.3		3,4,5,19	H-20
19	0.95 (s)	32.8		3,4,5,18	H-5
20	5.66 (d, 7.3)	100.0	H-OH	5,8,9,10,12	H-18
OH-20	2.40 (d, 7.3)		H-20	5,20	

Comparison with the literature, the 1D NMR data of compound **1** was nearly the same as 8-hydroxy-12-oxoabieta-9(11),13-dien-20-oic acid 8, 20-lactone, except for the appearance of an hemiacetal (δ_H 5.66 (d, 1H, H-20, $J=7.3$ Hz), δ_C 100.0 (d, C-20)) and the disappearance of a lactone carbonyl (δ_C 175.5 (s, C-20)) [4]. In the HMBC spectrum, cross-peaks between δ_H 5.66 (d, 1H, H-20, $J=7.3$ Hz) and δ_C 52.5 (d, C-5), 78.5 (s, C-8), 168.9 (s, C-9), 51.7 (s, C-10) indicated that the hemiacetal group was existed between C-10 and C-8 (Fig. 2).

In the ROESY spectrum, no cross-peaks between δ_H 5.66 (d, 1H, H-20, $J=7.3$ Hz), 2.40 (d, H-OH, $J=7.3$ Hz) and 1.39 (dd, 1H, H-5, $J=12.1$, 5.9 Hz) was observed, which suggested the orientation of C-20 was different from that of H-5. Furthermore, a reduction reaction was carried out on **1** and pisiferal ($C_{20}H_{28}O_2$, EI MS *m/z*: 300 $[M]^+$, $[\alpha]_D^{24}=303.8$ ($c=0.20$, MeOH), δ_H 9.89 (s, 1H, H-20), 6.92 (s, 1H, H-14), 6.58 (s, 1H, H-11), 5.08 (s, 1H, H-OH)) was obtained successfully [4,5], which confirmed that **1** was an abietane diterpene and the absolute configuration of C-5 was *S* and C-10 was *R*.

In the ROESY spectrum, a cross-peak between δ_H 1.39 (dd, 1H, H-5, $J=12.1$, 5.9 Hz,) and δ_H 0.95 (s, 3H, H-19) was observed, which indicated that C-19 was the α-orientation and C-18 was the β-orientation. Meanwhile, a correlation between δ_H 1.03 (s, 3H, H-18) and δ_H 5.66 (d, 1H, H-20, $J=7.3$ Hz) suggested that H-20 was the β-orientation (Fig. 2). Thus, the structure of compound **1** was determined as (5*S*, 8*R*, 10*R*, 20*R*)-8, 20-epoxy-12-oxoabieta-9(11), 13-diene, named glypenin A.

Four known compounds **2, 3, 4** and **5** were respectively determined as: 12-acetoxy-*ent*-labda-8(17), 13*E*-dien-15-oic

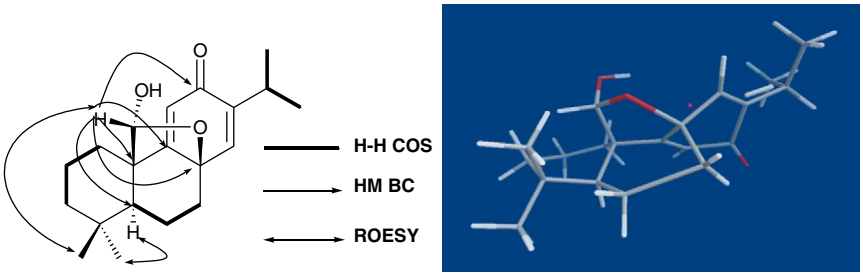

Fig. 2. Key 2D- NMR correlations (left) and 3D molecular modeling (right) of **1**.

acid **(2)** [6], quercetin 3-*O*-α-*L*-arabinofuranoside **(3)** [2], quercetin 3-*O*-β-*D*-galactopyranoside **(4)** [2], and β-sitosterol **(5)** [7].

Compounds **1–4** were tested for *in vitro* activity on K562 human chronic myeloid leukemia cell line, and the result indicated that compound **1** showed cytotoxicity towards K562 with an IC_{50} value of 21.2 μM.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.fitote.2010.08.001.

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