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Abietane diterpenoids from *Lycopodium complanatum*

YanMei Weng[a], Xia Yu[a], Jing Li[a], QiuZhu Dong[a], FuShuang Li[c], Fei Cheng[a,b], Yan Zhang[a], CaiPing Yao[a], ZhenXing Zou[a,b], WenHao Zhou[a], GuiShan Tan[a,b], KangPing Xu[a,*]

[a] Xiangya School of Pharmaceutical Sciences, Central South University, Changsha 410013, PR China
[b] Xiangya Hospital of Central South University, Changsha 410008, PR China
[c] Whitehead Institute for Biomedical Research, 455 Main Street, Cambridge, MA 02142, USA

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ABSTRACT

Five new abietane diterpenoids (complanatins A-E, **1–5**) have been isolated from the club moss *Lycopodium complanatum*, along with two known abietane diterpenoids (xanthoperol and sugiol). Their structures were determined by comprehensive analysis of 1D, 2D NMR, CD and HRESIMS data. The cytotoxic effects of five compounds (**1–4**, **7**) were evaluated in three human lung cancer cell lines (MSTO-211H, NCI-H2052 and NCI-H226). Compounds **3** and **4** exhibited cytotoxic activities against the three cell lines. In addition, a plausible biogenetic pathway of compounds **1–7** was proposed.

1. Introduction

Lycopodium complanatum belongs to the family of the Club moss (Lycopodiaceae), and mainly distributes in the temperate and subtropical area [1]. It's used as a Traditional Chinese Medicine for the treatment of arthritic pain, quadriplegia and contusion [2]. To date, many *lycopodium* alkaloids [3–6] especially the lycopodane type alkaloids [7], and serratane triterpenoids [8,9] have been discovered in *Lycopodium complanatum*. The reported bioactivities of these compounds include cytotoxicity, cholinesterase inhibition, antioxidant and neurological effects [7,9–13]. Recently a new hydroquinone diterpenoid (lycoxanthol) [14], two new abietane type diterpenoids (lycopod-abietanes A and B) [15] and a known abietane diterpenoid (sugiol) [16] were obtained from *Lycopodium lucidulum* Michx., *Lycopodium deuterodensum* and *Lycopodium obscurum* L., respectively. Our previous study has reported several neolignans and serratane triterpenoids with inhibitory activities of xanthine oxidase from *Palhinhaea cernua* (Lycopodiaceae) [17]. Herein, we report the isolation and structure elucidation of five new abietane diterpenoids (complanatins A-E, **1–5**) together with two known abietane diterpenoids (xanthoperol [18,19] and sugiol [16,20]), and cytotoxic activities of compounds **1–4**, **7** (Fig. 1).

2. Experimental

2.1. General experiment procedures

Column chromatographic (CC) separations were carried out on silica gel (300–400 or 80–100 mesh, Qingdao Permanent Sea Silica Ltd., Qingdao, China), Sephadex LH-20 gel (GE Healthcare, Uppsala, Sweden) and polyamide (80–100 or 30–60 mesh, Taizhou Luqiao Sijia Biochemical Plastics Factory, Taizhou, China). Thin-layer chromatography (TLC) and preparative TLC were performed using silica gel $HSGF_{254}$ plates (Yantai Jiangyou Silica Development Ltd., Yantai, China). The spots were visualized under UV light at 254/365 nm and daylight with 10% H_2SO_4 in EtOH or amine followed by heating or absorbing, respectively. The semi-preparative HPLC separations were conducted using Agilent 1260 system with a C_{18} column (5 μm, 250 × 10 mm i.d.; SilGreen, Beijing, China). The fractions were monitored by TLC analysis. All the solvents were of analytical grade and purchased from Cologne Chemical Co., Ltd., Chengdu, China.

Optical rotations were obtained on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). CD spectra were measured on an Applied Photophysics spectrometer (Chirascan, New Haven, USA). NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on a Bruker AVANCEIII instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) with TMS as internal standard. Chemical shifts (δ) were reported in ppm and the coupling constants (J) in Hz. The high resolution electrospray ionization mass spectroscopy (HRESIMS) was performed using an Agilent Technologies liquid chromatograph system connected to a Q-TOF mass spectrometer in positive ionization mode.

2.2. Plant material

The club moss *Lycopodium complanatum* was collected in July 2015 from ShaoYang, Hunan Province, People's Republic of China. The plant

* Corresponding author.
E-mail address: xukp395@csu.edu.cn (K. Xu).

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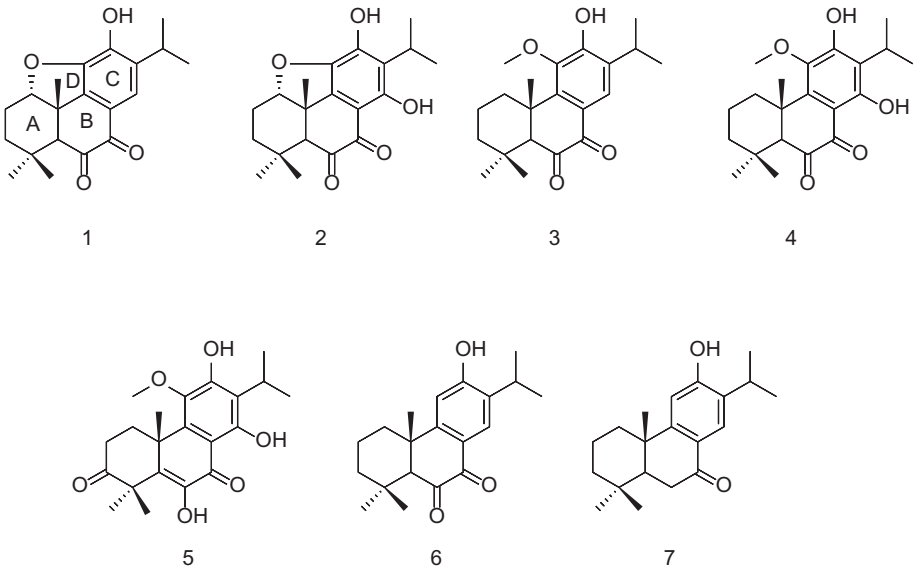

Fig. 1. Structures of compounds **1–7** from *Lycopodium complanatum*.

was identified by Prof. KangPing Xu (Xiangya School of Pharmaceutical Sciences, Central South University). The voucher specimen (No. 20150923) was deposited at the Herbarium library of Xiangya School of Pharmaceutical Sciences, Central South University.

2.3. Extraction and isolation

The dried whole herbs *Lycopodium complanatum* (15.0 kg) were extracted three times by reflux with 70% EtOH. The combined solvent was evaporated under reduced pressure to obtain crude ethanol extract, which was then suspended in water and partitioned with petroleum ether (PE), EtOAc and n-BuOH, 3 × 15 L, successively.

The EtOAc extract (245.0 g) was subjected to column chromatography on silica gel eluting with a gradient of CH_2Cl_2/MeOH (100:0 to 0:100) to obtain nine fractions (A-I) according to TLC patterns. Fraction B (14.3 g) was separated by silica gel using a gradient solvent (100:2:0 to 2:2:1, PE/EtOAc/MeOH) to afford three fractions (B1-B3). Fraction B2 (9.8 g) was observed to develop crystals upon solvent removal, which crystalline fraction was repeatedly rinsed with petroleum ether to afford compound **7** (17.0 mg). The latter was repeatedly applied to silica gel column chromatography and elution of a gradient of PE/EtOAc (5,0.3 to 2:1) for fraction B2, to provide six subfractions including B2.2 (0.94 g), B2.3 (3.49 g) and B2.4 (0.32 g).

Subfraction B2.2 was eluted with a gradient system (100:1 to 100:8, PE/EtOAc) through a silica gel column, in which compound **4** (8.2 mg) was obtained by using preparative TLC on silica gel $HSGF_{254}$ plates with PE-acetone (3:1, R_f = 0.6) as solvent system, followed by further purification on a Sephadex LH-20 column (MeOH). Subfraction B2.3 was repeatedly chromatographed over silica gel with isocratic elution of PE/EtOAc (10:1) to afford four subfractions (B2.3.1–4). Fraction B2.3.1 (0.33 g) was applied to a polyamide column with gradient system of EtOH/H_2O (1:9 to 0:1). Compound **3** (10.6 mg) and compound **5** (0.4 mg) were isolated by using semi-preparative HPLC with solvent system of ACN/H_2O (3:2) from subfraction B2.3.1.2, respectively. Fraction B2.3.2 (0.83 g) was separated through polyamide column chromatography and gradient elution was a mixture of EtOH/H_2O (1:9 to 0:1) to produce five subfractions. The second subfraction was isolated and purified through a Sephadex LH-20 column (20% MeOH/H_2O) to get compound **6** (0.5 mg). The third subfraction was purified by a silica gel column eluting with PE/EtOAc (100:1 to 3:1) to yield compound **2** (2.3 mg). Fraction B2.4 was subjected to a polyamide column eluting with EtOH/H_2O (1:9 to 0:1) and further purification by Sephadex LH-20 (MeOH) to yield compound **1** (5.3 mg).

2.3.1. Complanatin A (1)

Complanatin A (**1**): a dark red amorphous powder; [α]25 D + 79.50 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 215 (2.48), 271 (1.86), 351 (1.76) nm; CD (*c* = 0.24 mg/mL, MeOH) 218 (+11.60), 270 (−2.61), 296 (−3.40), 355 (+2.60) nm; ^{1}H NMR (500 MHz in $CDCl_3$) and ^{13}C NMR (125 MHz in $CDCl_3$) data see Table 1. HRESIMS *m/z* 329.1757 $[M + H]^+$ (calcd for $C_{20}H_{25}O_4$, 329.1753).

2.3.2. Complanatin B (2)

Complanatin B (**2**): a dark red amorphous powder; [α]25 D − 75.29 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (2.34), 284 (1.83), 353 (1.85) nm; CD (*c* = 0.26 mg/mL, MeOH) 223 (+10.49), 286 (−5.05), 354 (+5.42) nm; ^{1}H NMR (500 MHz in $CDCl_3$) and ^{13}C NMR (125 MHz in $CDCl_3$) data see Table 1. HRESIMS *m/z* 345.1714 $[M + H]^+$ (calcd for $C_{20}H_{25}O_5$, 345.1702).

2.3.3. Complanatin C (3)

Complanatin C (3): an orange amorphous powder; [α]25 D + 89.53 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.32), 255 (1.37), 351 (1.61) nm; CD (*c* = 0.11 mg/mL, MeOH) 201 (+16.67), 261 (−6.86), 315 (−5.13), 380(+4.72) nm; ^{1}H NMR (500 MHz in $CDCl_3$) and ^{13}C NMR (125 MHz in $CDCl_3$) data see Table 1. HRESIMS *m/z* 345.2074 $[M + H]^+$ (calcd for $C_{21}H_{29}O_4$, 345.2066).

2.3.4. Complanatin D (4)

Complanatin D (4): a dark red amorphous powder; [α]25 D − 294.67 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (2.39), 268 (1.52), 400 (1.80) nm; CD (*c* = 0.16 mg/mL, MeOH) 206 (+6.92), 227 (+1.54), 255 (−1.48), 279 (−0.70), 352 (+1.32) nm; ^{1}H NMR (500 MHz in $CDCl_3$) and ^{13}C NMR (125 MHz in $CDCl_3$) data see Table 1. HRESIMS *m/z* 361.2013 $[M + H]^+$ (calcd for $C_{21}H_{29}O_5$, 361.2015).

2.3.5. Complanatin E (5)

Complanatin E (5): a yellow amorphous powder; HPLC-UV (ACN-H_2O) λ_{max}: 204, 260, 284, 325, 370 nm; ^{1}H NMR (500 MHz in $CDCl_3$)