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Diterpenoids from the needles and twigs of the cultivated endangered pine *Pinus kwangtungensis* and their PTP1B inhibitory effects

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ABSTRACT

Pinus kwangtungensis is an endangered pine species native to China. In the present study, 15 diterpenoids including three new labdane-type analogs were isolated and characterized during a pioneer phytochemical investigation on a mass-limited sample of the needles and twigs of this plant, which is growing in a Cantonese garden. The new structures, (4*S*,5*R*,9*S*,10*R*)-6-oxo-labd-7,13-dien-16,15- olid-19-oic acid (**1**), 15(*S*)-*n*-butoxypinusolidic acid (**2**), and *β*-D-glucopyranosyl- (4*S*,5*R*,9*S*,10*R*)-labda-8(17),13-dien-15,16-olid-19-oate (**3**), were established by extensive spectroscopic methods and some chemical transformations. Among the isolates, lambertianic acid (**10**) and cassipourol (**15**) showed inhibitory activities against human protein tyrosine phosphatase 1 B (PTP1B), a target for the treatment of type-II diabetes and obesity, with IC_{50} values of 25.5 and 11.2 μM, respectively.

1. Introduction

Naturally occurring compounds from the rare and endangered plants have been documented to show greater potential for drug discovery (Ibrahim et al., 2013; Zhu et al., 2011). It is an urgent need to prioritize protection and utilization of these fragile plants at risk and facing extinction. Since 2013, we have launched a new program to systematically identify novel bioactive natural products from the rare and endangered plants endemic to China (Hu et al., 2016a,b; Ma et al., 2016; Wang et al., 2015, 2016; Wu et al., 2016; Xiong et al., 2015, 2016). In particular, great attentions have been paid to the rare and endangered species in the Pinaceae family, which ranks among the top-20 privileged drug-prolific families that produced high numbers of approved drugs (Fig. 1, Zhu et al., 2011). In our previous studies, a number of novel terpenoids with interesting bioactivities were obtained, e.g., dabeshanensins A–K from the endangered plant *Pinus dabeshanensis* (Hu et al., 2016a), and beshanzuenones A–D from the critically endangered plant *Abies beshanzuensis* (Hu et al., 2016b).

Among the 39 Pinaceae plants recorded in the China Plant Red Data Book (CPRDB) (Fu and Jin, 1992), *Pinus kwangtungensis* Chun ex Tsiang is characterized as a five-needled pine that generally inhabits the summits, cliffs or slopes of some remote mountains in southern China (Tian et al., 2008). Global warming and long-term deforestation threaten its existence and genetic integrity, and this species is listed as “threatened” in the CPRDB (Fu and Jin, 1992). To our knowledge, only a composition of pimaric-type resin acids from this plant has been previously analyzed (Chen et al., 2008). As part of our ongoing research towards the discovery of novel bioactive agents from the wild and/or cultivated endangered Pinaceae plants (Hu et al., 2016a,b), three new (**1–3**) and 12 known (**4–15**) diterpenoids were isolated from the mass-limited (290 g, dried) needles and twigs of the title plant, which is growing in the Foshan Botanical Garden at Canton (kwangtung) of China. Herein, we report their isolation and structure elucidation, as well as their inhibitory activities against the enzyme of human protein tyrosine phosphatase 1 B (PTP1B).

2. Results and discussion

From the 90% MeOH extract of the needles and twigs of *P. kwangtungensis*, ten labdane-type (**1–10**), two abietane-type (**11**, **12**), two podocarpane-type (**13**, **14**), and one retinane-type (**15**) diterpenoids were obtained and characterized (Fig. 2). By comparison of their spectroscopic data and physicochemical properties with those reported in the literature, the known ones were identified as 15(*R*)-*n*-butoxypinusolidic acid (**4**, Kim et al., 2012), adenanthoside C (**5**, Wu et al., 2015), pinosolide acid (= pinusolidic acid, **6**, Calderón et al., 1987; Fang et al., 1989; Yang and Han, 1998), pinusolide (**7**, Hu et al., 2016a; Raldugin et al., 1970), 15*ξ*-hydroxypinusolidic acid [= 15-oxo-

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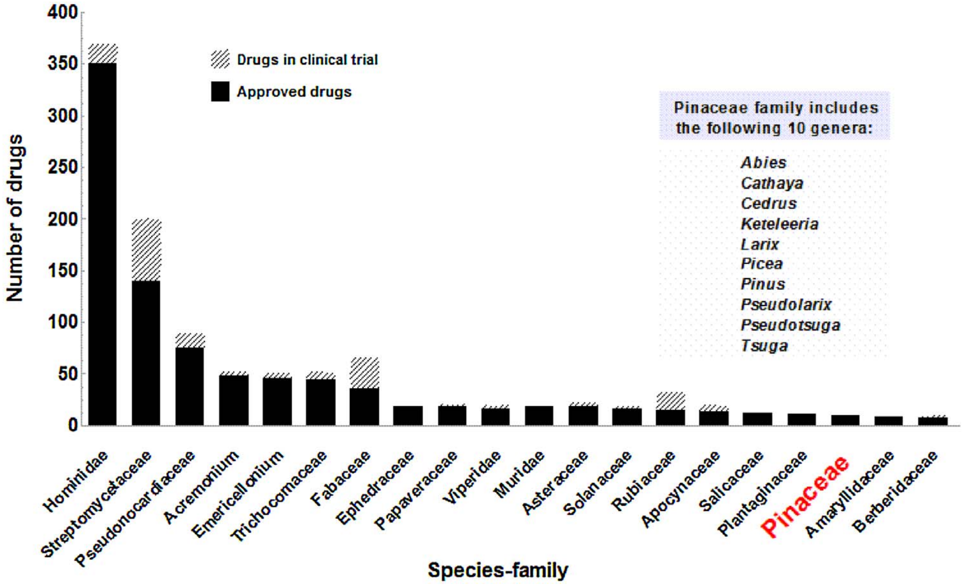


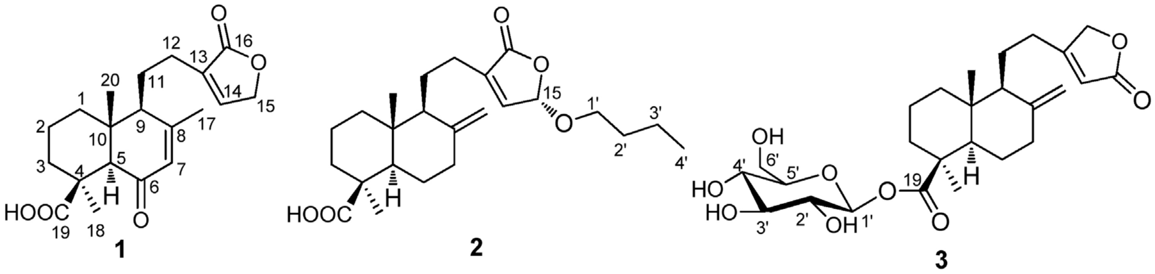
Fig. 1. Pinaceae ranks among the top-20 privileged drug-prolific families that produced high numbers of approved drugs (Zhu et al., 2011).

8(17),13-labdadiene-16,19-dioic acid, lactol form, **8**, Asili et al., 2004], 16-hydroxy-8(17),13-labdadien-15,16-olid-19-oic acid [= 16-oxo-8(17),13- labdadiene-15,19-dioic acid, lactol form, **9**, Asili et al., 2004], lambertianic acid (**10**, Asili et al., 2004; Dauben and German, 1966; Fang et al., 1991), abieta-7,13-diene-18-oic acid (**11**, Wang et al., 2008; Yang et al., 2010), 7*a*,15-dihydroxy-8,11,13-abietatrien-18-oic acid (= 7*a*,15-dihydroxy-dehydroabietic acid, **12**, Prinz et al., 2002; Yang et al., 2010), 8(14)-podocarpen-13-on-18-oic acid (**13**, Yang et al., 2008), abiesanordine E (**14**, Yang et al., 2008), and cassipourol (**15**, Chaturvedula et al., 2006), respectively.

Compound **1** was obtained as a colorless oil and its molecular formula was determined to be $C_{20}H_{26}O_5$ from a *pseudo*-molecular ion at *m/z* 347.1853 ($[M+H]^+$, calcd 347.1853) in its positive HRESIMS and from its ^{13}C NMR data, requiring eight degrees of unsaturation. The IR spectrum of **1** showed the existence of α,*β*-unsaturated γ-lactone (1756 cm^{-1}), carboxyl (1721 cm^{-1}) and conjugated enone (1678 cm^{-1}) functionalities. Its ^1H NMR data (Table 1) contained signals typical for two tertiary methyls (δ 1.45, s, Me-18; 0.81, s, Me-20), one vinylic methyl (δ 2.10, s, Me-17), one oxymethylene (δ 4.83, 2H, br s, H_2-15), and two olefinic protons (δ 6.00, s, H-7; 7.21, br s, H-14). The ^{13}C NMR spectrum of **1** (Table 1) exhibited twenty carbon resonances, which were classified by DEPT and HSQC NMR experiments as three methyls, six methylenes (one oxygenated at δ 70.3), four methines (two olefinic at δ 127.0 and 145.0), four quaternary carbons (two olefinic at δ 133.5 and 164.0), and three carbonyl groups (δ 174.0, 176.4, and 205.3). The above spectroscopic data resembled those of pinosolide acid (**6**, Calderón et al., 1987; Fang et al., 1989), suggested that compound **1** is also a bicyclic labdane-type diterpenoid featuring an α,*β*-unsaturated γ-lactone group. Differing from the exocyclic double bond $\Delta^{8,17}$ presented in **6**, an α,*β*-unsaturated ketone group was found for **1**, which was concluded to be $\Delta^{7,8}$-6-one from the key HMBC correlations from H-5 to C-6, from H-7 to C-5/C-17, and from Me-17 to C-7/C-8 (Fig. 3).

The relative configuration of **1** was assigned as shown in Fig. 3 by the observed NOE correlations of Me-18/H-5, H-5/H-9, and Me-20/H_b-11. In consistency with this deduction, the ^{13}C chemical shift of Me-18 (δ_C 28.3) is typical of an equatorial disposition. In general, the axial-oriented methyl is resonating at a higher field (Δ = *ca.* −10 ppm) than such a group at the equatorial position due to the *δ-syn* axial effect with Me-20 (Barrero and Altarejos, 1993; San Feliciano et al., 1993). Its absolute configuration was then deduced by analysis of the electronic circular dichroism (ECD) spectrum, from which a diagnostic negative Cotton effect at 243 nm ($\Delta\varepsilon$ −13.4) due to the $\pi-\pi^*$ transition of the conjugated enone group was observed. As summarized by Burgstahler et al., the Cotton effect in the region of the ultraviolet maximum (230–260 nm) in a cyclic conjugated enone is usually dominated by allylic axial perturbation(s) of the double bond (Burgstahler and Barkhurst, 1970). Thus, the absolute configuration at C-9 in **1** could be assigned as *S* due to the left-handed allylic axial chirality contribution of H-9 adjacent to the unsaturated ketone chromophore (Burgstahler and Barkhurst, 1970). Consequently, the structure of compound **1** was characterized as (4*S*,5*R*,9*S*,10*R*)-6-oxo-labd-7,13-dien-16,15-olid-19-oic acid.

Compound **2** was assigned the molecular formula $C_{24}H_{36}O_5$ by the positive mode HRESIMS (*m/z* 405.2636 $[M + H]^+$) and its ^{13}C NMR data. The IR absorption bands at 1766 and 1722 cm^{-1} gave hints of the presence of α,*β*-unsaturated γ-lactone and carboxyl functionalities. Similar to pinosolide acid (**6**), the presence of two singlet methyls [δ_H 1.25 (s, Me-18), δ_C 29.0; 0.61 (s, Me-20), δ_C 12.8], an exocyclic double bond [δ_H 4.58 and 4.90 (each br s, H_2-17), δ_C 106.9], an α,*β*-



1 **2** **3**

Fig. 2. Chemical structures of diterpenoids **1–3**.