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A novel phenolic compound from *Pinus yunnanensis*

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A rare type of phenolic compound, namely, planchol E (**1**), was isolated from the cones and seeds of *Pinus yunnanensis* together with 16 known abietane diterpenoids (**2**–**17**). The structure of planchol E was established on the basis of extensive spectroscopic analysis, and it was found that the new compound did not show cytotoxic activity against several cancer cell lines.

Keywords: *Pinus yunnanensis*; pinaceae; planchol E; abietane diterpenoid

1. Introduction

Pinus, with more than 100 species, is the largest genus of conifers and the most widespread genus of trees in the Northern Hemisphere. There are 22 species and 10 varieties in China. In the Southwest region of Yunnan Province, *Pinus yunnanensis* is an important economic tree in the forest zone [1]. Previous reports show that a number of compounds, mainly diterpenoids, have been isolated from the bark, twigs, and needles of this plant [2,3]. However, no systematic investigation on the chemical constituents of the cones and seeds is available up to now. As part of BioBioPha to assemble a large-scale natural product library, which is very valuable in the discovery of new drug leads from nature [4–6], our current research on the cones and seeds of *P. yunnanensis* afforded a rare type of phenolic compound, namely, planchol E (**1**), together with 16 known abietanes (**2**–**17**) (Figure 1). This paper reports the isolation, structure elucidation, and cytotoxic activity of planchol E.

2. Results and discussion

Compound **1**, obtained as amorphous powder, had the molecular formula C₁₄H₁₂O₇ based on the HR-ESI-MS (pos.), showing a quasi-molecular ion peak at *m/z* 293.0653 (calcd for C₁₄H₁₃O₇, 293.0661) with nine degrees of unsaturation. The IR spectrum showed the absorption bands of hydroxy (3415 cm⁻¹), γ -lactone (1760 cm⁻¹), conjugated carbonyl (1658, 1633 cm⁻¹), and aromatic ring (1590 cm⁻¹) groups. The ¹H-NMR spectrum (Table 1) exhibited two *m*-coupled aromatic proton doublets at δ_{H} 5.93 (1H, d, *J* = 1.9 Hz) and 5.90 (1H, d, *J* = 1.9 Hz), two oxygen-bearing methine protons at δ_{H} 4.98 (1H, d, *J* = 2.3 Hz) and 4.53 (1H, d, *J* = 2.3 Hz), three spin-coupled protons at δ_{H} 3.04 (1H, dd, *J* = 18.9, 11.5 Hz), 2.73 (1H, dd, *J* = 18.9, 5.3 Hz), and 3.26 (1H, dd, *J* = 11.5, 5.3 Hz), and a methyl singlet at δ_{H} 1.62 (3H, s). The ¹³C-NMR (DEPT) spectrum (Table 1) revealed 14 carbon resonances, including an aromatic ketone at δ_{C} 187.4 (s); an ester carbonyl at δ_{C} 174.0 (s); a set

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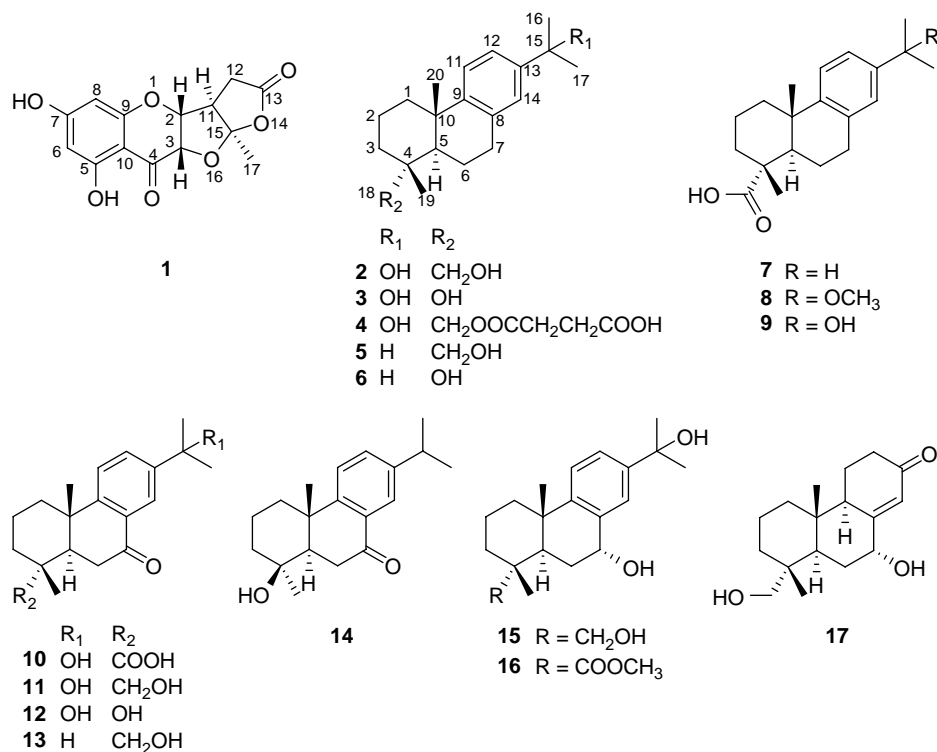


Figure 1. Structures of compounds 1–17.

Table 1. ¹H- and ¹³C-NMR spectral data and HMBC correlations of planchol E (**1**) in DMSO-*d*₆.

No.	δ_{H}	δ_{C}	HMBC (H \rightarrow C)
2	4.98 (d, 2.3)	84.1 (d)	C-3, C-12, C-15
3	4.53 (d, 2.3)	76.3 (d)	C-2, C-4, C-10
4		187.4 (s)	
5		164.0 (s)	
6	5.93 (d, 1.9)	96.5 (d)	C-4 ^a , C-5, C-7, C-8, C-10
7		168.0 (s)	
8	5.90 (d, 1.9)	95.2 (d)	C-4 ^a , C-6, C-7, C-9, C-10
9		161.3 (s)	
10		100.4 (s)	
11	3.26 (dd, 11.5, 5.3)	50.0 (d)	C-2, C-3, C-12, C-13, C-17
12	3.04 (H _{α} , dd, 18.9, 11.5) 2.73 (H _{β} , dd, 18.9, 5.3)	30.9 (t)	C-2, C-11, C-13, C-15
13		174.0 (s)	
15		116.1 (s)	
17	1.62 (s)	24.3 (q)	C-11, C-15
5-OH	11.7 (br s)		
7-OH	11.1 (vbr s)		

Note: ^aWeak but significant four-bond HMBC correlations.

of signals at δ_C 168.0 (s), 164.0 (s), 161.3 (s), 100.4 (s), 96.5 (d), and 95.2 (d) assignable to a 1,2,4,6-tetrasubstituted and 2,4,6-trioxygenated aromatic ring; two oxygen-bearing methine carbons at δ_C 84.1 (d) and 76.3 (d); and the signals at δ_C 116.1 (s), 50.0 (d), 30.9 (t), and 24.3 (q). According to the degrees of unsaturation, this molecule contained three rings except for a benzene ring. The above NMR character was very similar to that of a known compound planchol A [7]. Nevertheless, there was an obvious difference: an up-field methylene resonance was absent and replaced by an aromatic ketone carbonyl carbon at δ_C 187.4 (s) in **1**, suggesting that the C-4 methylene was converted into a ketone group. This deduction can be validated by the observable HMBC correlations (Table 1) from the protons at δ_H 4.53 (1H, d, $J = 2.3$ Hz, H-3), 5.93 (1H, d, $J = 1.9$ Hz, H-6), and 5.90 (1H, d, $J = 1.9$ Hz, H-8) to the carbon at δ_C 187.4 (s, C-4). The relative configuration was deduced to be consistent with that of planchol A, based on the ROESY experiment (Figure 2) and their accordant coupling constants. Concretely, the ROESY correlations of H-2 \leftrightarrow H-3, H-2 \leftrightarrow H-12 β , and H-3 \leftrightarrow H-12 β indicated that these protons were cofacial and β -oriented, whereas the correlations of H-11 \leftrightarrow Me-17 and H-11 \leftrightarrow H-12 α revealed their α -orientation. Therefore, the structure of **1** was established and named as planchol E.

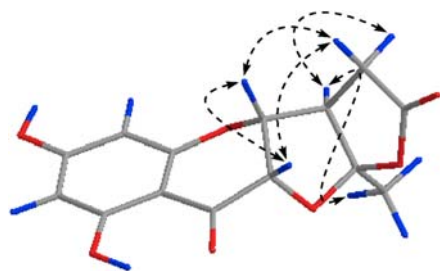


Figure 2. Observed ROESY correlations of **1**.

By comparison with their spectroscopic data with those previously reported, 16 known diterpenoids were identified as daturabietatriene (**2**) [8], 18-norabieta-8,11,13-triene-4,15-diol (**3**) [9], abiesadine I (**4**) [10], dehydroabietinol (**5**) [11], 18-norabieta-8,11,13-trien-4-ol (**6**) [12], dehydroabietic acid (**7**) [13], abiesadine N (**8**) [3,10], 15-hydroxydehydroabietic acid (**9**) [14], 15-hydroxy-7-oxodehydroabietic acid (**10**) [15], 15,18-dihydroxyabieta-8,11,13-trien-7-one (**11**) [16], 18-nor-4,15-dihydroxyabieta-8,11,13-trien-7-one (**12**) [16], 7-oxodehydroabietinol (**13**) [11], 19-nor-4-hydroxyabieta-8,11,13-trien-7-one (**14**) [12], abieta-8,11,13-triene-7 α ,15,18-triol (**15**) [17], methyl 7 α ,15-dihydroxydehydroabietate (**16**) [18], and 7 α ,15-dihydroxy podocarp-8(14)-en-13-one (**17**) [19]. The present study showed that abietane diterpenoids were the fat-soluble representative constituents of the cones and seeds, and moreover, compounds **3**, **4**, **11**, **12**, **14**, **15**, and **17** were obtained for the first time from the genus *Pinus*. We think that planchol E (**1**) possesses a certain biogenetic relationship with dihydroflavonols. Given the interesting cytotoxic activity of natural products with γ -lactone unit, **1** was evaluated for its cytotoxic activity using the MTT method [20]; however, this compound did not show the expected activity ($IC_{50} > 40 \mu M$) against HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines.

3. Experimental

3.1 General experimental procedures

Optical rotation was measured on a Jasco P-1020 (Jasco International Co., Ltd., Tokyo, Japan) automatic digital polarimeter. UV spectroscopic data were obtained from online HPLC analysis. IR spectrum was recorded using a Bruker Tensor 27 FT-IR (Bruker Optics GmbH, Ettlingen, Germany) spectrometer with KBr pellets. NMR spectra were carried

out on a Bruker Avance III 600 MHz (Bruker BioSpin GmbH, Rheinstetten, Germany) spectrometer with deuterated DMSO signals (δ_{H} 2.49 ppm, δ_{C} 39.5 ppm) as internal standard. HR-ESI-MS was recorded on an API QSTAR Pulsar i (MDS Sciex, Concord, Ontario, Canada) mass spectrometer. Silica gel 200-300 mesh (Qingdao Marine Chemical, Inc., Qingdao, China), Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), and MCI gel CHP 20P (75–150 μm , Mitsubishi Chemical Corp., Tokyo, Japan) were used for normal pressure column chromatography. MPLC was performed on a Büchi Sepacore System equipping pump manager C-615, pump modules C-605, and fraction collector C-660 (Büchi Labortechnik AG, Flawil, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm , Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan). Fractions were monitored and analyzed by TLC (Qingdao Marine Chemical, Inc., China), in combination with Agilent 1200 series HPLC system equipped with Eclipse XDB-C18 column (5 μm , 4.6 \times 150 mm).

3.2 Plant material

The cones and seeds of *P. yunnanensis* were collected in Yangbi County of Yunnan Province, China, in April 2009, and identified by one of the authors (Prof. G.M. Liu). A voucher specimen (No. BBP2010012PY) has been deposited at Dali University.

3.3 Extraction and isolation

The air-dried cones and seeds of *P. yunnanensis* (10 kg) were cut into small pieces and immediately extracted with 85% EtOH (3 \times 25 L, each 1 d) at room temperature. After evaporation of the solvent under vacuum, the EtOH extract was partitioned between CHCl_3 and H_2O (1:1) to obtain a CHCl_3 layer (165 g), which was subjected to silica gel column

chromatography eluted with a gradient of increasing acetone in petroleum ether (v/v = 10:0, 9:1, 7:3, 4:6, 1:9, 0:10; each \sim 3 L) to afford six fractions (I–VI) in succession. Fraction II (24 g) was repeatedly isolated by silica gel (petroleum ether:acetone = 100:0 \rightarrow 100:8), MCI (50 \rightarrow 80% MeOH in H_2O), Sephadex LH-20 (CHCl_3 :MeOH = 1:1), and medium pressure liquid chromatography (MPLC) columns (70 \rightarrow 90% MeOH in H_2O) to give compounds **5** (19 mg), **6** (25 mg), **7** (20 mg), **8** (25 mg), **9** (15 mg), **13** (10 mg), and **14** (10 mg). Fraction III (9 g) was subjected to a silica gel column (CHCl_3 :MeOH = 100:0 \rightarrow 100:1) to afford four subfractions (IIIa–III d), and subfraction IIIa (200 mg) was further purified on silica gel (CHCl_3 :MeOH = 100:0 \rightarrow 100:1) to obtain **10** (50 mg). Fraction IV (28 g) was separated by silica gel (CHCl_3 :MeOH = 100:0 \rightarrow 80:1) to afford three subfractions (IVa–IVc), and subfraction IVa (1.2 g) was subjected to Sephadex LH-20 (CHCl_3 :MeOH = 1:1) and MPLC (60 \rightarrow 90% MeOH in H_2O) to give **2** (20 mg), **3** (20 mg), **4** (10 mg), **11** (40 mg), and **16** (30 mg). Fraction V (12 g) was further isolated and purified by silica gel (CHCl_3 :MeOH = 100:0 \rightarrow 60:1) and Sephadex LH-20 (MeOH) to afford **1** (6.0 mg) and **12** (30 mg). Fraction VI (16 g) was repeatedly chromatographed on silica gel (CHCl_3 :MeOH = 100:0 \rightarrow 50:1) to yield **15** (40 mg) and **17** (8.0 mg).

3.3.1 Planchol E (I)

Amorphous powder, $[\alpha]_{\text{D}}^{16} - 26.0$ (*c* 0.28, MeOH). UV λ_{max} (MeOH): 214, 301, 345 (sh) nm. IR (KBr): 3415, 1760, 1658, 1633, 1590, 1380, 1286, 1170, 1092, 921 cm^{-1} . ^1H - and ^{13}C -NMR spectral data (see Table 1). EI-MS: *m/z* 292 (37, $[\text{M}]^+$), 248 (4), 205 (12), 178 (14), 153 (100), 152 (94), 141 (32), 124 (17), 95 (15). HR-ESI-MS (pos.): *m/z* 293.0653 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{14}\text{H}_{13}\text{O}_7$, 293.0661).

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