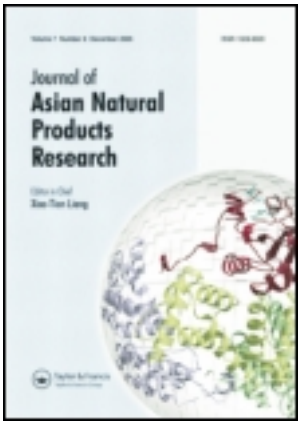

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

A novel phenolic compound from Pinus yunnanensis

Ting Lei [a b], Yan Li [c], Dong-Mei Li [b], Guang-Ming Liu [b], Ji-Kai Liu [c] & Fei Wang [a c]

[a] BioBioPha Co., Ltd., Kunming, 650204, China

[b] School of Pharmaceutical Sciences, Dali University, Dali, 671000, China

[c] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650204, China

Available online: 29 Apr 2011

To cite this article: Ting Lei, Yan Li, Dong-Mei Li, Guang-Ming Liu, Ji-Kai Liu & Fei Wang (2011): A novel phenolic compound from Pinus yunnanensis , Journal of Asian Natural Products Research, 13:05, 425-429

To link to this article: http://dx.doi.org/10.1080/10286020.2011.565747

Journal of Asian Natural Products Research
Vol. 13, No. 5, May 2011, 425–429

A novel phenolic compound from *Pinus yunnanensis*

Ting Lei[ab], Yan Li[c], Dong-Mei Li[b], Guang-Ming Liu[b]*, Ji-Kai Liu[c] and Fei Wang[ac]*

[a]*BioBioPha Co., Ltd., Kunming 650204, China;* [b]*School of Pharmaceutical Sciences, Dali University, Dali 671000, China;* [c]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China*

(*Received 21 December 2010; final version received 21 February 2011*)

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 $C_{14}H_{12}O_7$ based on the HR-ESI-MS (pos.), showing a quasi-molecular ion peak at *m*/*z* 293.0653 (calcd for $C_{14}H_{13}O_7$, 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 ^{1}H-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 ^{13}C-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

*Corresponding authors. Email: wangfei@mail.kib.ac.cn; lgm888999@yahoo.com.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.565747
http://www.informaworld.com

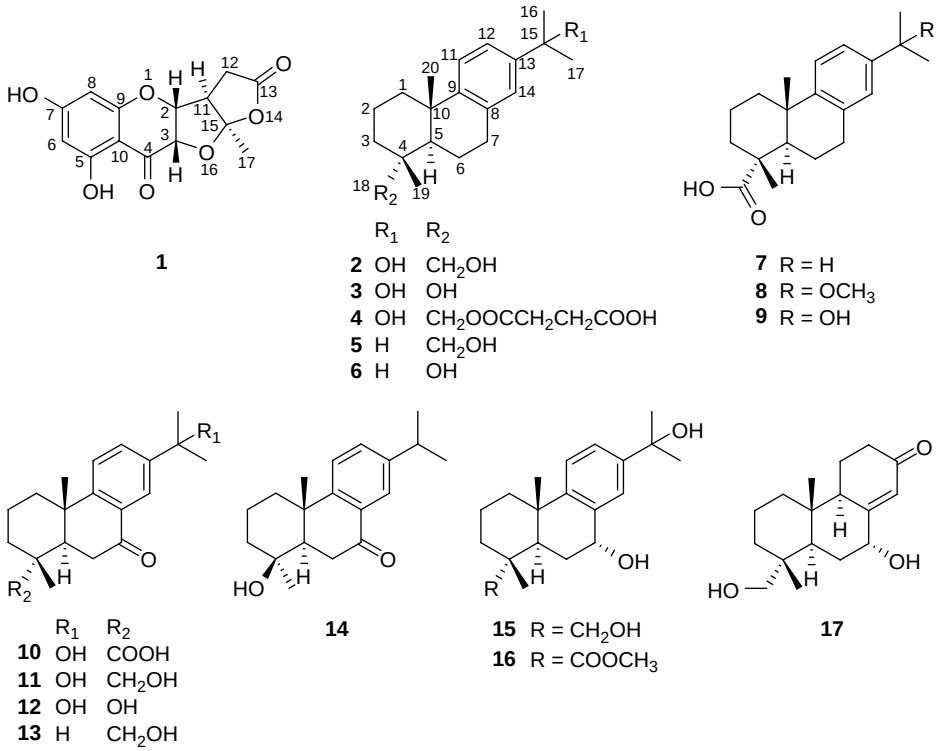

Figure 1. Structures of compounds **1**–**17**.

Table 1. ^{1}H- and ^{13}C-NMR spectral data and HMBC correlations of planchol E (**1**) in DMSO-d_6.

No.	δ_H	δ_C	HMBC (H → 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: [a] Weak 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 ↔ H-3, H-2 ↔ H-12β, and H-3 ↔ H-12β indicated that these protons were cofacial and β-oriented, whereas the correlations of H-11 ↔ Me-17 and H-11 ↔ H-12α revealed their α-orientation. Therefore, the structure of **1** was established and named as planchol E.

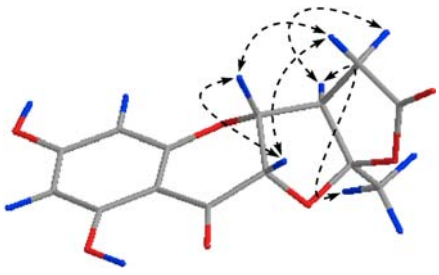

Figure 2. Observed ROESY correlations of **1**.

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-7α,15,18-triol (**15**) [17], methyl 7α,15-dihydroxydehydroabietate (**16**) [18], and 7α,15-dihydroxypodocarp-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 ($IC_{50} > 40$ μM) 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 × 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 × 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 ~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 → 100:8), MCI (50 → 80% MeOH in H_2O), Sephadex LH-20 ($CHCl_3$:MeOH = 1:1), and medium pressure liquid chromatography (MPLC) columns (70 → 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 → 100:1) to afford four subfractions (IIIa–IIId), and subfraction IIIa (200 mg) was further purified on silica gel ($CHCl_3$:MeOH = 100:0 → 100:1) to obtain **10** (50 mg). Fraction IV (28 g) was separated by silica gel ($CHCl_3$:MeOH = 100:0 → 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 → 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 → 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 → 50:1) to yield **15** (40 mg) and **17** (8.0 mg).

3.3.1 Planchol E (*1*)

Amorphous powder, $[\alpha]_D^{16}$ −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}. ^{1}H- and ^{13}C-NMR spectral data (see Table 1). EI-MS: *m/z* 292 (37, $[M]^+$), 248 (4), 205 (12), 178 (14), 153 (100), 152 (94), 141 (32), 124 (17), 95 (15). HR-ESI-MS (pos.): *m/z* 293.0653 $[M + H]^+$ (calcd for $C_{14}H_{13}O_7$, 293.0661).

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (No. 30860365), the 'Western Light' Program of Chinese Academy of Sciences, and Natural Product Library Program of BioBioPha.

References

[1] Y. Tsiang and P.Y. Li, *Flora of China* (Science Press, Beijing, 1977), Vol. 63, p. 204.
[2] T. Feng, X.H. Cai, Q.G. Tan, and X.D. Luo, *Z. Naturforsch.* **65b**, 765 (2010).
[3] B. Wang, J. Ju, X.F. He, T. Yuan, and J.M. Yue, *Helv. Chim. Acta* **93**, 490 (2010).
[4] F. Wang, Y. Gao, L. Zhang, B. Bai, Y.N. Hu, Z.J. Dong, Q.W. Zhai, H.J. Zhu, and J.K. Liu, *Org. Lett.* **12**, 3196 (2010).
[5] F. Wang, F.C. Ren, Y.J. Li, and J.K. Liu, *Chem. Pharm. Bull.* **58**, 1267 (2010).
[6] Y.M. Wang, H. Xiao, J.K. Liu, and F. Wang, *J. Asian Nat. Prod. Res.* **12**, 220 (2010).
[7] J. Chang and R. Case, *Planta Med.* **71**, 955 (2005).
[8] J.M.M. del Corral, M. Gordaliza, M.A. Salinero, and A.S. Feliciano, *Magn. Reson. Chem.* **32**, 774 (1994).
[9] H. Ohtsu, R. Tanaka, and S. Matsunaga, *J. Nat. Prod.* **61**, 406 (1998).
[10] X.W. Yang, L. Feng, S.M. Li, X.H. Liu, Y.L. Li, L. Wu, Y.H. Shen, J.M. Tian, X. Zhang, X.R. Liu, N. Wang, Y.H. Liu, and W.D. Zhang, *Bioorg. Med. Chem.* **18**, 744 (2010).
[11] M.A. González, D. Pérez-Guaita, J. Correa-Royero, B. Zapata, L. Agudelo, A. Mesa-Arango, and L. Betancur-Galvis, *Eur. J. Med. Chem.* **45**, 811 (2010).
[12] C.K. Lee, J.M. Fang, and Y.S. Cheng, *Phytochemistry* **39**, 391 (1995).
[13] T.A. van Beek, F.W. Claassen, J. Dorado, M. Godejohann, R. Sierra-Alvarez, and J.B.P.A. Wijnberg, *J. Nat. Prod.* **70**, 154 (2007).
[14] E.J. Alvarez-Manzaneda, R. Chahboun, J.J. Guardia, M. Lachkar, A. Dahdouh, A. Lara, and I. Messouri, *Tetrahedron Lett.* **47**, 2577 (2006).
[15] T. Matsumoto, S. Imai, Y. Sunaoka, and T. Yoshinari, *Bull. Chem. Soc. Jpn.* **61**, 723 (1988).
[16] H. Ohtsu, R. Tanaka, and S. Matsunaga, *J. Nat. Prod.* **61**, 1307 (1998).
[17] H. Ohtsu, R. Tanaka, Y. In, S. Matsunaga, H. Tokuda, and H. Nishino, *Planta Med.* **67**, 55 (2001).
[18] S. Prinz, U. Müllner, J. Heilmann, K. Winkelmann, O. Sticher, E. Haslinger, and A. Hüfner, *J. Nat. Prod.* **65**, 1530 (2002).
[19] H. Ohtsu, R. Tanaka, Y. In, S. Matsunaga, H. Tokuda, and H. Nishino, *Can. J. Chem.* **78**, 31 (2000).
[20] T. Mosmann, *J. Immunol. Methods* **65**, 55 (1983).