

# Abietane diterpenoids synthesized by suspension-cultured cells of *Cephalotaxus fortunei*

Xiao-Hui Xu<sup>a,b</sup>, Wei Zhang<sup>a,c,\*</sup>, Xu-Peng Cao<sup>a</sup>, Song Xue<sup>a</sup>

<sup>a</sup> Marine Bioproducts Engineering Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

<sup>b</sup> Graduate University of the Chinese Academy of Sciences, Beijing 100049, China

<sup>c</sup> Flinders Centre for Marine Bioprocessing and Bioproducts and Department of Medical Biotechnology, School of Medicine, Flinders University, Adelaide, SA 5042, Australia

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## ABSTRACT

Cell suspension cultures of *Cephalotaxus fortunei* were manipulated to produce secondary metabolites of pharmaceutical interest. Seven abietane diterpenoids (six known and one new) were produced by the suspension-cultured *C. fortunei* cells by complementing an elicitation strategy with an in situ product removal strategy. Based on the results of spectrometric analysis, the structure of the new compound was determined to be 20(10→5)-abeo-4,5-seco-5(10),6,8,11,13-abietapentaene-3-one (**1**). The six known compounds were identified as 12-hydroxyabieta-6,8,11,13-tetraene-3-one (**2**), abieta-8,11,13-triene-3 $\beta$ ,12-diol (hinokiol) (**3**), abietatriene-3 $\beta$ -ol (**4**), 11,12-dihydroxy-8,11,13-abietatriene-3,7-dione (**5**), 11-hydroxy-12-methoxyabieta-8,11,13-triene-3,7-dione (**6**), and 3 $\beta$ ,11-dihydroxy-12-methoxyabieta-8,11,13-triene-7-one (**7**). This is the first time that diterpenoids have been isolated and identified from this pharmaceutically important plant. These results suggest that the strategies we applied were effective methods for synthesizing abietane diterpenoids via a suspension culture of *C. fortunei* cells.

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## 1. Introduction

*Cephalotaxus fortunei*, Hook. f. is an indigenous plant to China, from which alkaloids of pharmaceutical importance such as harringtonine and homoharringtonine have been previously isolated (Zhang et al., 1998; Hu et al., 1995; Bocar et al., 2003). Because complex natural products are not economically produced by total chemical synthesis with the exception of some small molecules, most useful metabolites are still obtained from wild or cultivated plant resources (Mizukami and Hayashi, 2010). A plant cell-culture process could be an alternative method for producing these useful secondary metabolites, and commercial production of several secondary metabolites has been achieved (McCoy and O'Connor, 2008; Kolewe et al., 2008; Smetanska, 2008). In this context, a suspension-cultured cell line of *C. fortunei* was used to produce valuable secondary metabolites. By applying a process intensification strategy of elicitation integrated with in situ product removal, seven abietane diterpenoids new to the plant *C. fortunei* were produced by the suspension-cultured *C. fortunei* cells. We report here the isolation and identification of one new and six known abietane diterpenoids from *C. fortunei* suspension cell cultures.

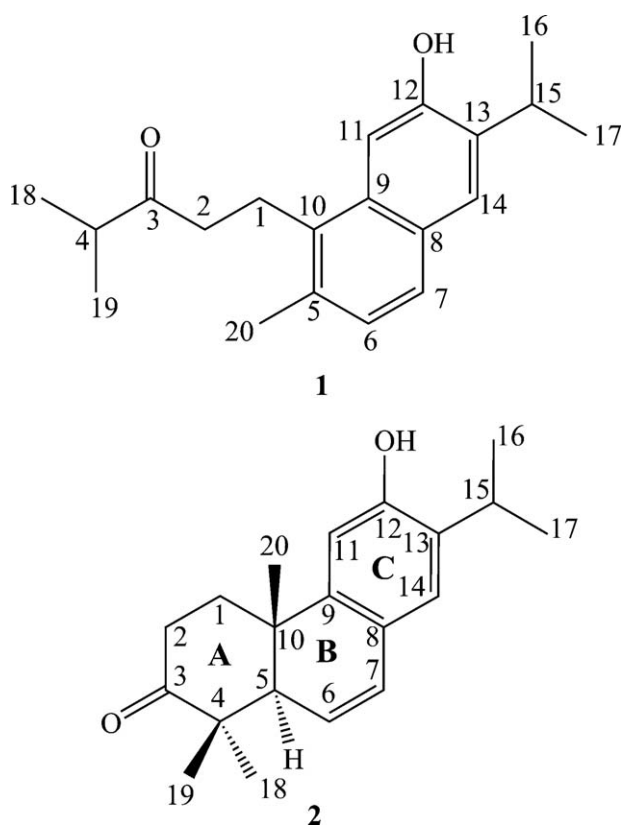
## 2. Results and discussion

By using elicitation and in situ product removal simultaneously, seven abietane diterpenoids were synthesized by the suspension-cultured *C. fortunei* cells. The structural elucidation and identification of these compounds were based on spectral data analysis including MS, 1D and 2D NMR. The seven abietane diterpenoids included one new abietane diterpenoid, 20(10→5)-abeo-4,5-seco-5(10),6,8,11,13-abietapentaene-3-one (**1**), and six known abietane diterpenoids, 12-hydroxyabieta-6,8,11,13-tetraene-3-one (**2**), abieta-8,11,13-triene-3 $\beta$ ,12-diol (hinokiol) (**3**) (Zhao et al., 1998), abietatriene-3 $\beta$ -ol (**4**) (Urones et al., 1988; Chamy et al., 1991), 11,12-dihydroxy-8,11,13-abietatriene-3,7-dione (**5**) (Li et al., 2003), 11-hydroxy-12-methoxyabieta-8,11,13-triene-3,7-dione (**6**) (Yang et al., 1998), and 3 $\beta$ ,11-dihydroxy-12-methoxyabieta-8,11,13-triene-7-one (**7**) (Li et al., 2002; Orihara et al., 2002; Monacelli et al., 2002) (Fig. 1).

Compound **1** was obtained as pale yellow oil. The molecular formula for **1** was determined to be C<sub>20</sub>H<sub>26</sub>O<sub>2</sub> by high resolution EIMS data that gave a molecular ion peak at [M]<sup>+</sup> *m/z* 298.1939 (calcd. 298.1933). The <sup>13</sup>C NMR spectrum of **1** (Table 1) revealed the presence of 20 carbon signals, which were sorted by a DEPT experiment into five methyls, two methylenes, six methines, and seven quaternary carbon signals. A keto carbonyl carbon signal was observed at  $\delta_C$  213.6. The <sup>1</sup>H NMR spectrum of **1** (Table 1) showed the presence of signals for two isopropyl groups. The first isopropyl group was observed as a methine peak at  $\delta_H$  3.41 (1H, sept,

\* Corresponding author at: Marine Bioproducts Engineering Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China. Tel.: +86 411 84379069; fax: +86 411 84379069.

E-mail address: [weizhang@dicp.ac.cn](mailto:weizhang@dicp.ac.cn) (W. Zhang).

Fig. 1. Structures of compounds **1** and **2**.

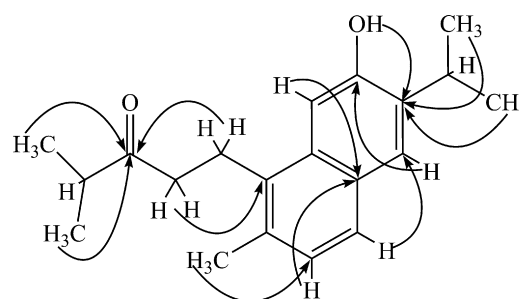
$J = 6.9$  Hz) and two methyl signals at  $\delta_{\text{H}}$  1.32 (6H, d,  $J = 6.9$  Hz). According to the HMBC correlations (Fig. 2), the second isopropyl group attached to the keto carbonyl carbon and was observed as a methine peak at  $\delta_{\text{H}}$  2.67 (1H, sept,  $J = 6.9$  Hz) and two methyl signals at  $\delta_{\text{H}}$  1.08 (6H, d,  $J = 6.9$  Hz). In addition to the two isopropyl groups, the  $^1\text{H}$ – $^1\text{H}$  COSY spectrum also showed the existence of a  $-\text{CH}_2-\text{CH}_2-$  system ( $\delta_{\text{H}}$  2.73 and 3.16), which also correlated to the keto carbonyl carbon in the HMBC spectrum. From these data, a partial structure from C-1  $\rightarrow$  2  $\rightarrow$  3  $\rightarrow$  4  $\rightarrow$  18, 19 of **1** was assembled.

The remaining signals in the  $^1\text{H}$  NMR spectrum were one downfield singlet methyl ( $\delta_{\text{H}}$  2.42), two singlet aromatic protons ( $\delta_{\text{H}}$

Table 1

$^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Compound **1** (acetone- $d_6$ ,  $\delta$  in ppm).

Position	$\delta_{\text{H}}$ (multiplicity, $J$ in Hz)	$\delta_{\text{C}}$	HSQC	$^1\text{H}$ – $^1\text{H}$ COSY
1	2.73 (2H, t, 8.1)	40.4	$\text{CH}_2$	H-2
2	3.16 (2H, t, 8.1)	23.6	$\text{CH}_2$	H-1
3	–	213.6	C	–
4	2.67 (1H, sept, 6.9)	41.3	CH	H-18; H-19
5	–	133.0	C	–
6	7.07 (1H, d, 8.3)	126.9	CH	H-7
7	7.52 (1H, d, 8.3)	126.7	CH	H-6
8	–	129.0	C	–
9	–	133.0	C	–
10	–	132.8	C	–
11	7.29 (1H, s)	105.8	CH	–
12	–	154.9	C	–
13	–	136.9	C	–
14	7.61 (1H, s)	126.5	CH	–
15	3.41 (1H, sept, 6.9)	28.2	CH	H-16; H-17
16	1.32 (3H, d, 6.9)	23.0	$\text{CH}_3$	H-15
17	1.32 (3H, d, 6.9)	23.0	$\text{CH}_3$	H-15
18	1.08 (3H, d, 6.9)	18.5	$\text{CH}_3$	H-4
19	1.08 (3H, d, 6.9)	18.5	$\text{CH}_3$	H-4
20	2.42 (3H, s)	20.0	$\text{CH}_3$	–
12-OH	8.59 (1H, s)	–	–	–

Fig. 2. Key HMBC correlations of compound **1**.

7.29 and 7.61), two mutually coupled doublet aromatic protons ( $\delta_{\text{H}}$  7.07 and 7.52), and one singlet hydroxyl proton ( $\delta_{\text{H}}$  8.59). Together with the information on the remaining 10 aromatic carbons in the  $^{13}\text{C}$  NMR spectrum, these data indicate that a naphthyl group carrying one methyl, one hydroxyl, and one isopropyl group is present in **1**. The substituted positions and assignments were confirmed by an HMBC experiment. Additionally, the former partial structure from C-1 to C-4 was assigned to C-10 on the basis of the HMBC correlations between C-10 and H-2. One structurally related compound, 20(10 $\rightarrow$ 5)-abeo-4,5-seco-5(10),6,8,11,13-podocarpapentaen-3-one, reported from the callus of *Securinega suffruticosa*, exhibited very similar NMR spectral data (Yuan et al., 2005). Compound **1** differs from 20(10 $\rightarrow$ 5)-abeo-4,5-seco-5(10),6,8,11,13-podocarpapentaen-3-one only by the presence of an isopropyl group instead of a methyl at C-13. Moreover, the structure of compound **1** was further confirmed by comparing  $^1\text{H}$  NMR data with that of a synthetic compound named 2-isopropyl-3-methoxy-6-methyl-5-(4-methyl-3-oxopentyl)naphthalene owing to only a substituent difference between these two compounds (Matsumoto et al., 1996). Based on these evidences, the structure of compound **1** was established as 20(10 $\rightarrow$ 5)-abeo-4,5-seco-5(10),6,8,11,13-abietapentaene-3-one (**1**).

Compound **2** was obtained as a yellow powder. The HR-EIMS data of **2** exhibited a molecular ion peak at  $[M]^+ m/z$  298.1943 (calcd. 298.1933), which established the molecular formula as  $\text{C}_{20}\text{H}_{26}\text{O}_2$ , consistent with the 20 carbon signals in the  $^{13}\text{C}$  NMR spectrum and the 26 proton signals in the  $^1\text{H}$  NMR spectrum. The NMR spectra of **2** showed the presence of a tetrasubstituted benzene moiety [ $\delta_{\text{H}}$  6.74 (1H, s) and  $\delta_{\text{C}}$  110.27 (CH),  $\delta_{\text{H}}$  6.94 (1H, s) and  $\delta_{\text{C}}$  125.75 (CH), and  $\delta_{\text{C}}$  125.30 (C), 132.94 (C), 145.86 (C), and 155.25 (C)], an isopropyl residue [ $\delta_{\text{H}}$  3.26 (1H, sept,  $J = 6.95$  Hz), 1.19 (3H, d,  $J = 6.95$  Hz), and 1.22 (3H, d,  $J = 6.95$  Hz) and  $\delta_{\text{C}}$  27.38 (CH), 22.84 ( $\text{CH}_3$ ), and 22.76 ( $\text{CH}_3$ )], and three methyls [ $\delta_{\text{H}}$  1.11 (3H, s), 1.21 (3H, s), and 1.25 (3H, s) and  $\delta_{\text{C}}$  25.39 ( $\text{CH}_3$ ), 20.30 ( $\text{CH}_3$ ), and 23.01 ( $\text{CH}_3$ )], which are indicative that compound **2** was an abietane diterpenoid with an aromatic C-ring. The assignment of all protonated carbons was established by DEPT and HSQC experiments. A keto carbonyl carbon signal was observed in the  $^{13}\text{C}$  NMR and DEPT spectra of **2** at  $\delta_{\text{C}}$  214.31 and was positioned at C-3 on the basis of its HMBC correlations (Fig. 3) to H-1, H-2, H-18 and H-19. The  $^1\text{H}$ – $^1\text{H}$  COSY spectrum showed the existence of  $-\text{CH}_2-\text{CH}_2-$  and  $-\text{CH}=\text{CH}=\text{CH}-$  systems that could be assigned to positions 1, 2 of ring A and positions 5, 6, and 7 of ring B, respectively. The *cis*-olefin between C-6 and C-7 was suggested by chemical shifts ( $\delta_{\text{C}}$  125.30 and 129.76,  $\delta_{\text{H}}$  5.81 and 6.59) and the coupling constant of their connected protons ( $J = 9.6$  Hz). The phenolic hydroxyl group was assigned to C-12 because of the chemical shift ( $\delta_{\text{C}}$  155.25). Based on the HMBC spectrum, the connections to the carbon skeleton were established and the substitution pattern was confirmed. Accordingly, compound **2** was determined to be 12-hydroxyabieta-6,8,11,13-tetraene-3-one.

So far, only one study in the literature (Ulubelen et al., 1988) has described the isolation and identification of **2**. In that study, the  $^1\text{H}$

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