

## Short communication

Three new abietane-type diterpenes from the bark of *Cryptomeria japonica*

Chi-I Chang<sup>a</sup>, Chien-Chih Chen<sup>b,c</sup>, Sheng-Yang Wang<sup>d,e,f</sup>, Hsun-Shuo Chang<sup>g,h</sup>,  
Louis Kuoping Chao<sup>i</sup>, Jih-Jung Chen<sup>j,1</sup>, Cheng-Chi Chen<sup>k,1</sup>, Yueh-Hsiung Kuo<sup>l,m,\*</sup>

<sup>a</sup> Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan

<sup>b</sup> Department of Biotechnology, Hungkuang University, Taichung 443, Taiwan

<sup>c</sup> Department of Nursing, Hungkuang University, Taichung 443, Taiwan

<sup>d</sup> Department of Forestry, National Chung-Hsing University, Taichung 402, Taiwan

<sup>e</sup> Agricultural Biotechnology Center, National Chung-Hsing University, Taichung 402, Taiwan

<sup>f</sup> Agricultural Biotechnology Research Center, Academia Sinica, Taipei 115, Taiwan

<sup>g</sup> School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

<sup>h</sup> Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

<sup>i</sup> Department of Cosmeceutics, China Medical University, Taichung 404, Taiwan

<sup>j</sup> Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung 907, Taiwan

<sup>k</sup> Department of Chemistry, National Taiwan University, Taipei 106, Taiwan

<sup>l</sup> Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung 404, Taiwan

<sup>m</sup> Department of Biotechnology, Asia University, Taichung 413, Taiwan

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## ABSTRACT

Three new abietane-type diterpenoids, 7 $\alpha$ -butoxyabieta-8,12-diene-11,14-dione (**1**), 6 $\alpha$ ,7 $\alpha$ -dihydroxyabieta-8,12-dien-11,14-dione (**2**), and 6 $\alpha$ ,7 $\beta$ -dihydroxyabieta-8,12-diene-11,14-dione (**3**) were isolated from the bark of *Cryptomeria japonica* D. Don. Their structures were established by means of 1D and 2D NMR, IR, UV, and HR-ESI-MS spectra and comparison with the data of known analogues.

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## 1. Introduction

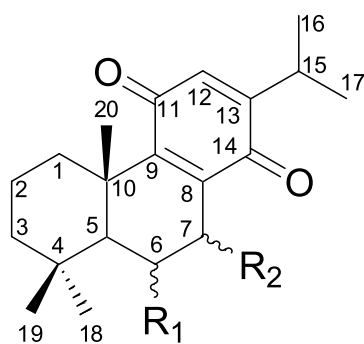
*Cryptomeria japonica* D. Don is the only species existing in the genus *Cryptomeria* (Cupressaceae) and is endemic to Japan known as sugi (Japanese cedar) in Japanese (Gan, 1958). It is widely distributed in Japan and also an important plantation coniferous tree species in Taiwan. Its wood exhibited aromatic, soft, lightweight but strong, waterproof, and reddish-pink in color properties and thus has been used as building materials for Japanese-style houses and other wood products. Previous phytochemical investigations of the leaves, heartwood, and barks of *C. japonica* (Arihara et al., 2004; Chen et al., 2001; Kofujita et al., 2001,

2002; Morita et al., 1995; Nagahama and Tazaki, 1993; Nagahama et al., 1993, 1996a, 1996b, 1998; Narita et al., 2006; Shibuya, 1992; Shieh et al., 1981; Shimizu et al., 1988; Su et al., 1993, 1994a, 1994b, 1995a, 1995b, 1996; Morisawa et al., 2002; Yoshikawa et al., 2006a, 2006b) have indicated that it contains diverse terpenoids, including monoterpenes, sesquiterpenes, and diterpenes. The crude extracts and several isolates from different tissues of this plant have been proven to possess antibacterial (Li et al., 2008), antifungal (Kofujita et al., 2001), cytotoxic (Kofujita et al., 2002), anti-inflammatory (Shyur et al., 2008), antiandrogenic (Tu et al., 2007), and insect antifeedant (Wu et al., 2008) and repellent (Morisawa et al., 2002) properties. In a continued effort to search for new chemical ingredients from the bark of *C. japonica*, a cytotoxic sesquiterpene (C<sub>35</sub>), cryptotrone, with an unprecedented skeleton possessing an abietane diterpene with a unique bicyclic sesquiterpene was published by us (Chen et al., 2010). Here, we further report the isolation and structure elucidation of three new abietane-type diterpenoids (Fig. 1).

\* Corresponding authors at: Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, No. 91, Hsueh-Shih Rd., Taichung City, 404, Taiwan.

E-mail address: [kuoyh@mail.cmu.edu.tw](mailto:kuoyh@mail.cmu.edu.tw) (Y.-H. Kuo).

<sup>1</sup> These authors contributed equally to this article.



- 1  $R_1=H$ ,  $R_2=\alpha\text{-OBU}$   
 2  $R_1=R_2=\alpha\text{-OH}$   
 3  $R_1=\alpha\text{-OH}$ ,  $R_2=\beta\text{-OH}$

Fig. 1. Structures of compounds 1–3.

## 2. Results and discussion

The MeOH extract of the bark of *C. japonica* was partitioned between  $H_2O$  and EtOAc. The EtOAc fraction was purified by repeated silica gel column chromatography and semipreparative NP-HPLC to afford compounds 1–3.

Compound 1 was obtained as a yellow gum. The UV absorption bands at  $\lambda_{\max}$  255 nm and the IR absorption bands at 1653 and  $1600\text{ cm}^{-1}$  indicated the presence of the benzoquinone moiety (Tan et al., 2002). The  $^1H$  NMR spectrum of 1 (Table 1) displayed resonances for three tertiary-linked methyls [ $\delta_H$  0.89, 0.92, and 1.22 (each 3H, s, Me-19, Me-18, and Me-20)], and one oxymethine [ $\delta_H$  4.37 (1H, dd,  $J=3.6, 2.0$  Hz)], an olefinic proton [ $\delta_H$  6.31 (1H, s, H-12)], an isopropyl group attached on the benzoquinone ring [ $\delta_H$  1.07 (3H, d,  $J=6.8$  Hz), 1.10 (3H, d,  $J=6.8$  Hz), and 2.99 (1H, sept,  $J=6.8$  Hz)] (Takaishi et al., 1992). In addition, the proton signals of a

butoxyl group were also observed [ $\delta_H$  0.90 (3H, t,  $J=7.5$  Hz), 1.35 (2H, m), 1.50 (2H, m), and 3.61 (1H, t,  $J=6.8$  Hz)]. The  $^{13}C$  NMR and DEPT experiments reveal 24 carbon signals, consisting of six methyl, six aliphatic methylene, two aliphatic methine, two aliphatic quaternary, one oxygenated methylene, one oxygenated methine, one olefinic methine, three quaternary olefinic, and two carbonyl carbons. Its HR-EI-MS gave a molecular ion at  $m/z$  372.5469, establishing the molecular formula of 1 as  $C_{24}H_{36}O_3$ , with seven degrees of unsaturation. A typical downshifted  $H_{\beta}$ -1 signal at  $\delta_H$  2.61 (1H, br d,  $J=12.8$  Hz) (Chang et al., 2005), 24 carbon signals in the  $^{13}C$  NMR spectrum including 6 carbon signals ( $\delta_C$  131.2, 139.4, 151.1, 153.1, 186.3, and 187.4) attributing from the benzoquinone moiety, along with the HMBC correlations between H-12 ( $\delta_H$  6.31)/C-9 ( $\delta_C$  151.1), C-11 ( $\delta_C$  187.4), and C-15 ( $\delta_C$  26.7) (Fig. 2) hinted that 1 would be a abieta-8,12-diene-11,14-dione diterpene with a butoxy group (Chang et al., 2005; Tan et al., 2002). The butoxyl and isopropyl groups were assigned to located on C-7 and C-13, basing on the HMBC correlations (Fig. 2) between H-7 ( $\delta_H$  4.37)/C-5 ( $\delta_C$  45.6), C-8 ( $\delta_C$  139.4), C-9 ( $\delta_C$  151.1), and C-1' ( $\delta_C$  70.1) and H-15 ( $\delta_H$  2.99)/C-12 ( $\delta_C$  131.2) and C-14 ( $\delta_C$  186.3). The butoxyl group was in  $\alpha$ -axial orientation, which was assured by the equatorial-axial and equatorial-equatorial coupling constants between H-7 and H-6, 3.6 and 2.0 Hz, respectively (Yoshikawa et al., 2006b; Meier et al., 1981; Tezuka et al., 1998; Naman et al., 2016) and the NOE correlations (Fig. 2) between H-6 ( $\delta_H$  2.00)/H-7 and H-6/H-18 ( $\delta_H$  0.92). Based on these observations, compound 1 was elucidated as 7 $\alpha$ -butoxyabieta-8, 12-diene-11,14-dione.

The UV spectrum of 2 showed absorption bands at  $\lambda_{\max}$  256 nm, which were attributed to the benzoquinone moiety. Its IR spectrum exhibited absorption bands for hydroxyl and carbonyl of benzoquinone at 3383 and  $1646\text{ cm}^{-1}$ , respectively. The  $^1H$  NMR spectrum of 2 (Table 1) showed resonances for three tertiary-linked methyls [ $\delta_H$  1.14, 1.19, and 1.34 (each 3H, s, Me-19, Me-18, and Me-20)], two oxymethines [ $\delta_H$  4.00 (1H, dd,  $J=11.6, 4.0$  Hz, H-6) and 4.65 (1H, d,  $J=4.0$  Hz, H-7)], an olefinic proton [ $\delta_H$  6.37 (1H, s, H-12)], and an isopropyl group attached on the benzoquinone ring [ $\delta_H$  1.09 (3H, d,  $J=6.8$  Hz), 1.11 (3H, d,  $J=6.8$  Hz), and 2.98

Table 1

$^1H$  NMR data for compounds 1, 2, and 3. ( $CDCl_3$ ,  $\delta$  in ppm,  $J$  in Hz, 400 MHz for  $^1H$  NMR, 100 MHz for  $^{13}C$  NMR).

| No. | 1          |  | 2          |                             | 3          |                             |
|-----|------------|--|------------|-----------------------------|------------|-----------------------------|
|     | $\delta_C$ | $\delta_H$   | $\delta_C$ | $\delta_H$                  | $\delta_C$ | $\delta_H$                  |
| 1   | 36.0       | 1.17 td (12.8, 3.6) <sup>a</sup> ,<br>2.61 br d (12.8) | 36.6       | 1.23 m, 2.60 dt (12.4, 3.6) | 36.4       | 1.19 m, 2.59 dt (12.4, 4.0) |
| 2   | 19.3       | 1.46 m, 1.70 qt (12.8, 3.6)                            | 19.2       | 1.49 m, 1.68 m              | 18.9       | 1.47 m, 1.65 m              |
| 3   | 41.3       | 1.26 m, 1.42 m   | 42.8       | 1.21 m, 1.41 br d (11.6)    | 42.7       | 1.17 m, 1.41 m              |
| 4   | 33.5       |  | 33.7       |                             | 33.7       |                             |
| 5   | 45.6       | 1.58 m   | 49.4       | 1.63 d (11.6)               | 52.0       | 1.32 d (11.6)               |
| 6   | 23.4       | 1.39 m, 2.00 br d (1.40)                               | 68.6       | 4.00 dd (11.6, 4.0)         | 71.8       | 4.03 dd (11.6, 7.6)         |
| 7   | 69.1       | 4.37 dd (3.6, 2.0)                                     | 65.4       | 4.65 d (4.0)                | 75.0       | 4.54 d (7.6)                |
| 8   | 139.4      |  | 138.4      |                             | 138.4      |                             |
| 9   | 151.1      |  | 151.3      |                             | 151.1      |                             |
| 10  | 39.3       |  | 41.7       |                             | 41.9       |                             |
| 11  | 187.4      |  | 187.5      |                             | 188.6      |                             |
| 12  | 131.2      | 6.31 s   | 132.2      | 6.37 s                      | 132.0      | 6.35 s                      |
| 13  | 153.1      |  | 152.3      |                             | 152.4      |                             |
| 14  | 186.3      |  | 187.0      |                             | 186.9      |                             |
| 15  | 26.7       | 2.99 sept (6.8)  | 26.6       | 2.98 sept (6.8)             | 26.5       | 2.96 sept (6.8)             |
| 16  | 21.6       | 1.10 d (6.8)   | 21.6       | 1.09 d (6.8)                | 21.7       | 1.09 d (6.8)                |
| 17  | 21.7       | 1.07 d (6.8)   | 21.7       | 1.11 d (6.8)                | 21.8       | 1.11 d (6.8)                |
| 18  | 33.4       | 0.92 s   | 36.2       | 1.19 s                      | 36.1       | 1.19 s                      |
| 19  | 22.3       | 0.89 s   | 22.5       | 1.14 s                      | 22.8       | 1.13 s                      |
| 20  | 19.0       | 1.22 s   | 20.8       | 1.34 s                      | 21.6       | 1.42 s                      |
| 1'  | 70.1       | 3.61 t (6.8)   |            |                             |            |                             |
| 2'  | 32.5       | 1.50 m   |            |                             |            |                             |
| 3'  | 19.9       | 1.35 m   |            |                             |            |                             |
| 4'  | 14.3       | 0.90 t (7.5)   |            |                             |            |                             |

<sup>a</sup> Coupling constants are presented in Hz.

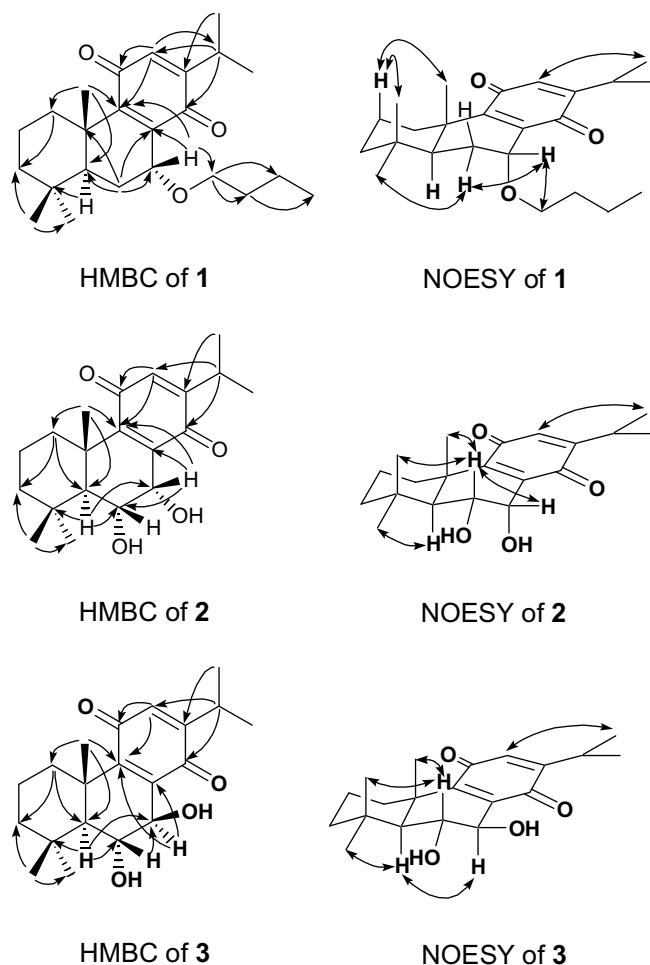


Fig. 2. Key HMBC and NOESY correlations of compounds 1–3.

(1H, sept,  $J=6.8$  Hz)] (Takaishi et al., 1992). A typical downshifted  $H_{\beta-1}$  signal of abieta-8,12-diene-11,14-dione at  $\delta_H$  2.60 (1H, dt,  $J=12.4$ , 3.6 Hz) was also found (Chang et al., 2005). 20 carbon signals were observed in the  $^{13}C$  NMR spectrum of **2** and were differentiated by DEPT experiments as five methyl, three aliphatic methylene, two aliphatic methine, two aliphatic quaternary, two oxygenated methine, one olefinic methine, three quaternary olefinic, and two carbonyl carbons. The molecular formula of **2** was assigned as  $C_{20}H_{28}O_4$  on the basis of a HR-EI-MS molecular ion at  $m/z$  332.4381, representing seven degrees of unsaturation. Similarities in the  $^1H$  and  $^{13}C$  NMR spectroscopic data hinted that **2** should be an analogue of **1**. By comparing the  $^{13}C$  NMR data of **2** with that of **1**, the major differences were the  $^{13}C$  NMR chemical shifts of C-5, C-6, C-7, C-10, Me-19, Me-18, and Me-20. An additional oxymethine [ $\delta_H$  4.00 (1H, dd,  $J=11.6$ , 4.0 Hz, H-6)] showed  $^1H$ - $^1H$  correlations with H-7 [ $\delta_H$  4.65 (1H, d,  $J=4.0$  Hz, H-7)] and H-5 [ $\delta_H$  1.63 (1H, d,  $J=11.6$  Hz, H-5)] suggested that the additional hydroxyl group was attached on C-6 ( $\delta_C$  68.6). The smaller axial-equatorial coupling constant, 4.0 Hz, derived from the coupling of H-6 and H-7 and the larger coupling constant, 11.6 Hz, between the *trans*-diaxial protons H-5 and H-6 and the NOE correlations between H-6/H-7, Me-19 ( $\delta_H$  1.14), and Me-20 ( $\delta_H$  1.34) and H-5/Me-20 (Fig. 2) hinted two hydroxyl groups were located at C-6 and C-7 and were in  $\alpha$ -equatorial and  $\alpha$ -axial orientation, respectively (Yoshikawa et al., 2006b; Meier et al., 1981; Hirasawa et al., 2007). From the above evidences, compound **2** was thus formulated as 6 $\alpha$ ,7 $\alpha$ -dihydroxyabieta-8,12-dien-11,14-dione.

The HR-EI-MS of **3** revealed a molecular ion at  $m/z$  332.4378, and the molecular formula was determined to be  $C_{20}H_{28}O_4$ , indicating seven degrees of unsaturation. The IR spectrum displayed absorption bands for hydroxyl ( $3462\text{ cm}^{-1}$ ) group and benzoquinone moiety ( $1646$  and  $1600\text{ cm}^{-1}$ ). The  $^1H$  NMR spectrum of **3** (Table 1) indicated the presence of three tertiary-linked methyls [ $\delta_H$  1.13, 1.19, and 1.42 (each 3H, s, Me-19, Me-18, and Me-20)], two oxymethines [ $\delta_H$  4.03 (1H, dd,  $J=11.6$ , 7.6 Hz, H-6) and 4.54 (1H, d,  $J=7.6$  Hz, H-7)], an olefinic proton [ $\delta_H$  6.35 (1H, s, H-12)], an isopropyl group on the benzoquinone [ $\delta_H$  1.09 (3H, d,  $J=6.8$  Hz), 1.11 (3H, d,  $J=6.8$  Hz), and 2.96 (1H, sept,  $J=6.8$  Hz)] (Takaishi et al., 1992), and a typical  $H_{\beta-1}$  of abieta-8,12-diene-11,14-dione [ $\delta_H$  2.59 (1H, dt,  $J=12.4$ , 4.0 Hz)] (Chang et al., 2005). The  $^1H$  and  $^{13}C$  NMR data were similar to those of **2**, except for the signals of C-5, C-6, C-7, and Me-20. An ABX coupling system [ $\delta_H$  1.32 (1H, d,  $J=11.6$  Hz, H-5),  $\delta_H$  4.03 (1H, dd,  $J=11.6$ , 7.6 Hz, H-6), and 4.54 (1H, d,  $J=7.6$  Hz, H-7)] was observed. The two hydroxyl groups were located at C-6 and C-7, respectively, which were assured by the HMBC correlations between H-6 ( $\delta_H$  4.03)/C-5 ( $\delta_C$  52.0), C-7 ( $\delta_C$  75.0), and C-10 ( $\delta_C$  41.9) and H-7 ( $\delta_H$  4.54)/C-6 ( $\delta_C$  71.8), C-8 ( $\delta_C$  138.4), and C-9 ( $\delta_C$  151.1) (Fig. 2). The axial-axial coupling between H-6 and H-7 with a  $J$  value, 7.6 Hz, and the NOE correlations (Fig. 2) between H-5/Me-18 ( $\delta_H$  1.19), H-5/H-7, H-6/Me-19 ( $\delta_H$  1.13), and H-6/Me-20 ( $\delta_H$  1.42), suggested that the hydroxyl group at C-7 ( $\delta_C$  75.0) was in  $\beta$ -equatorial orientation in **3**, instead of in  $\alpha$ -axial orientation in **2** (Yoshikawa et al., 2006b; Meier et al., 1981; Hirasawa et al., 2007). Thus, compound **3** was identified as 6 $\alpha$ ,7 $\beta$ -dihydroxyabieta-8,12-diene-11,14-dione.

### 3. Experimental

#### 3.1. General experimental procedures

Optical rotations were measured on a Jasco-DIP-180 polarimeter. UV spectra were recorded on a Shimadzu UV-1601PC spectrophotometer. Infrared (IR) spectra were measured on a Perkin-Elmer-983G FT-IR spectrophotometer.  $^1H$  and  $^{13}C$  NMR and 2D NMR spectra were obtained on a Varian-Unity-Plus-400 spectrometer with tetramethylsilane (TMS) as the internal standard. EI-MS and HR-EI-MS were measured with a Jeol-JMS-HX300 mass spectrometer. Silica gel (230–400 mesh; Merck & Co., Inc.) was used for column chromatography (CC), and pre-coated silica gel (60 F-254; Merck & Co., Inc.) plates were used for TLC. The spots on TLC were detected by spraying with 5%  $H_2SO_4$  and then heating at  $100^\circ C$ . Semi-preparative HPLC was performed using a normal phase column (Purospher STAR Si,  $5\ \mu m$ ,  $250 \times 10\ mm$ ; Merck & Co., Inc.) on a LDC Analytical-III system.

#### 3.2. Plant material

The bark of *C. japonica* D. Don was collected in Sitou, Taiwan in June 2000. The plant material was identified by Dr. Yen-Hsueh Tseng, Department of Forestry, National Chung-Hsing University. A voucher specimen (TCF13443) has been deposited at the Herbarium of the same university.

#### 3.3. Extraction and isolation

The air-dried bark of *C. japonica* (16.0 kg) was extracted with MeOH (100 L) three times (7 days each time) at room temperature. The combined MeOH extract was evaporated under reduced pressure to afford a crude extract (480 g), which was suspended in  $H_2O$  (1 L), and then partitioned between  $H_2O$  and EtOAc (1 L) for three times. The EtOAc fraction (430 g) was subjected to a silica gel (4.0 kg) column using *n*-hexane–EtOAc and EtOAc–MeOH mixtures as solvent systems to obtain 11 fractions. Fr. 2 from *n*-hexane–

EtOAc (19:1) elution (29.4 g) was further purified through a silica gel column (5 × 45 cm), eluted with *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> (1:0–0:1) to obtain eight fractions, 2A–2G. Further purification of subfraction 2B (2.3 g) by HPLC gave **1** (3.6 mg, *t<sub>R</sub>* = 32.2 min) using *n*-hexane–EtOAc (19:1). Fr. 5 from *n*-hexane–EtOAc (7:3) elution (21.6 g) was further purified through a silica gel column (5 × 45 cm), eluted with *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub>–EtOAc (8:8:1–0:1:1) to obtain fifteen fractions, 5A–5O. Further purification of subfraction 5B (1.6 g) by HPLC gave **3** (3.7 mg, *t<sub>R</sub>* = 38.1 min) using *n*-hexane–EtOAc (19:1). Further purification of subfraction 5C by HPLC gave **2** (3.1 mg, *t<sub>R</sub>* = 45.2 min) using *n*-hexane–EtOAc (19:1).

### 3.3.1. 7 $\alpha$ -butoxyabieta-8, 12-diene-11,14-dione (**1**)

Yellow gum; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = –20.5° (c 0.27, CHCl<sub>3</sub>); IR (dry film)  $\nu_{\max}$  1653, 1600, 1467, 1367, 1228, 1089, 983, 897 cm<sup>–1</sup>; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 255 (4.21) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; EI-MS (%) *m/z* 372 (4) [M]<sup>+</sup>, 316 (3), 298 (100), 283 (94), 269 (11), 255 (13), 242 (19), 229 (27), 215 (20), 204 (13). HR-EI-MS [M]<sup>+</sup> *m/z* 372.5469 (calcd for C<sub>24</sub>H<sub>36</sub>O<sub>3</sub> 372.5466).

### 3.3.2. 6 $\alpha$ ,7 $\alpha$ -dihydroxyabieta-8,12- dien-11,14-dione (**2**)

Yellow gum; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = –10.5° (c 0.80, CHCl<sub>3</sub>); IR (dry film)  $\nu_{\max}$  3383, 1646, 1600, 1460, 1381, 1228, 1076, 963, 738 cm<sup>–1</sup>; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 256 (4.18) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; EI-MS (%) *m/z* 332 (15) [M]<sup>+</sup>, 316 (39), 314 (M<sup>+</sup>–H<sub>2</sub>O, 43), 299 (54), 286 (100), 271 (43), 257 (16), 243 (35), 229 (42), 215 (37), 203 (20), 187 (18). HR-EI-MS [M]<sup>+</sup> *m/z* 332.4381 (calcd for C<sub>20</sub>H<sub>28</sub>O<sub>4</sub> 332.4388).

### 3.3.3. 6 $\alpha$ ,7 $\beta$ -dihydroxyabieta- 8,12-diene-11,14-dione (**3**)

Yellow gum; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = –24.5° (c 1.10, CHCl<sub>3</sub>); IR (dry film)  $\nu_{\max}$  3462, 1646, 1600, 1467, 1381, 1241, 1096, 897, 738 cm<sup>–1</sup>; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 258 (4.09) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; EI-MS (%) *m/z* 332 (100) [M]<sup>+</sup>, 316 (65), 314 (M<sup>+</sup>–H<sub>2</sub>O, 77), 299 (86), 286 (34), 271 (54), 257 (17), 243 (40), 229 (42), 219 (48), 215 (30), 203 (23), 187 (15). HR-EI-MS [M]<sup>+</sup> *m/z* 332.4378 (calcd for C<sub>20</sub>H<sub>28</sub>O<sub>4</sub> 332.4388).

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.phytol.2016.11.008>.

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