

## Notes

Two New Lactones from *Cephalotaxus fortunei* var. *alpina*

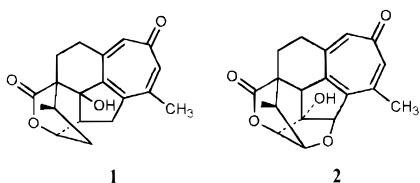
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Two new lactones, fortunulides A (**1**) and B (**2**), together with three known compounds, hainanolidol,<sup>32</sup> acetylcephalotaxine, and wilsonine, were isolated from the needles and stem of *Cephalotaxus fortunei*. Their structures were elucidated on the basis of MS and NMR data.

The genus *Cephalotaxus* is composed of 10 species, eight of which are distributed in China.<sup>1</sup> Investigations of the chemical constituents of extracts of *Cephalotaxus* sp. resulted in the isolation of a number of alkaloids. Several of these, such as harringtonine, isoharringtonine, and homoharringtonine,<sup>2</sup> are of interest due to their antitumor activity. Sun and co-workers<sup>3</sup> reported two nonalkaloid compounds, named hainanolide and hainanolidol, with a new ring skeleton from *Cephalotaxus hainanensis*. Hainanolide has cytotoxic activities against L-615, S180, W256, P-388, and L-1210 cells. In this paper, we report the chemical constituents of *Cephalotaxus fortunei* Hook. f. var. *alpina* Li. Five compounds, fortunulide A (**1**), fortunulide B (**2**), hainanolidol (**3**)<sup>3</sup> acetylcephalotaxine, and wilsonine,<sup>4,5</sup> were isolated from stems and needles of this plant with the aid of aluminum oxide column chromatography. Fortunulides A (**1**) and B (**2**) and hainanolidol belong to the hainanolide<sup>3</sup> skeletal type. All structures were identified by NMR and MS methods, and **1** and **2** were two new compounds. Only four compounds—hainanolide, hainanolidol, **1**, and **2**—are known from higher plants that possess the hainanolide skeleton.



The IR data of **1** (C<sub>19</sub>H<sub>20</sub>O<sub>4</sub>) indicated tropone and lactone moieties, whose presence was supported by UV absorptions at 317.5 and 252 nm, respectively. Comparing the <sup>1</sup>H and <sup>13</sup>C NMR spectrum of **1** (Table 1) and hainanolide,<sup>3</sup> we found that these two compounds have the same carbon skeleton. The <sup>1</sup>H NMR spectra showed the presence of two methyls, connecting with tertiary and troponic carbons, respectively. The two protons of the tropone appeared at δ 6.67, 6.68 (*J* = 2.0 Hz) and showed long-range couplings, indicating that they are in the same plane with a *W*-conformation. The <sup>13</sup>C NMR data also supported the existence of tropone and lactone. Fragment CH (CH<sub>3</sub>)–CH<sub>2</sub>–CH–CH–CH<sub>2</sub> was elucidated on the basis of the <sup>1</sup>H–<sup>1</sup>H and <sup>1</sup>H–<sup>13</sup>C COSY spectra. The IR spectrum showed a

**Table 1.** <sup>13</sup>C and <sup>1</sup>H NMR Data (δ) for Compounds **1** and **2**

no.	<b>1</b>		<b>2</b>	
	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H
1	186.0		186.3	
2	140.8	6.68 (d, <i>J</i> = 2.0 Hz)	141.5	7.10 (d, <i>J</i> = 2.0 Hz)
3	147.0		146.0	
4	146.2		145.6	
5	145.1		145.4	
6	143.8		143.8	
7	138.8	6.67 (d, <i>J</i> = 2.0 Hz)	139.5	6.99 (d, <i>J</i> = 2.0 Hz)
8	28.4	2.52, 3.08 (m, α, βH)	32.3	2.75, 2.83 (m, α, βH)
9	17.9	1.76, 2.65 (m, α, βH)	22.8	1.20, 2.40 (m, α, βH)
10	49.7		47.7	
11	86.8		58.4	3.59 (s)
12	45.6	2.83 (m)	82.0	
13α	35.7	2.43 (dd, <i>J</i> = 19.0, 3.0 Hz)	89.7	5.39 (s)
13β		3.28 (dd, <i>J</i> = 19.0, 9.5 Hz)		
14α	28.8	1.46 (m)	84.8	4.12 (d, <i>J</i> = 6.0 Hz)
14β		1.84 (m)		
15	77.2	4.71 (t, <i>J</i> = 4.4 Hz)	81.6	5.48 (d, <i>J</i> = 6.0 Hz)
16	173.5		173.5	
17	30.0	1.41 (q, <i>J</i> = 6.8 Hz)	41.7	1.77 (q, <i>J</i> = 7.6 Hz)
18	19.3	0.90 (d, <i>J</i> = 6.9 Hz)	14.7	0.79 (d, <i>J</i> = 7.6 Hz)
19	24.4	2.15 (s)	23.6	2.23 (s)

<sup>a</sup> Compound **1** in C<sub>5</sub> D<sub>5</sub>N; **2** in CDCl<sub>3</sub>.

quaternary hydroxyl group (3500–3300, 1150 cm<sup>-1</sup>). Based on the proposed skeleton of the compound, it was concluded that this hydroxyl group could only be at C-11 or C-12. The <sup>1</sup>H–<sup>13</sup>C COSY spectrum showed that H-12 was split to a deformed triplet. So one hydroxyl group, linked with C-11 rather than C-12, was confirmed.

The <sup>1</sup>H–<sup>1</sup>H COSY spectrum of **1** showed that H-12 coupled with H-13β and that H-15 significantly correlated with H-12, H-17, and H-14β, suggesting that H-12, H-15, and H-17 were in β-positions. The α- and β-protons linked with C-13 were doublet doublets with different coupling constants (*J* = 19.0, 3.0 Hz and *J* = 19.0, 9.5 Hz). This phenomenon can be explained as follows: the α- and β-protons at C-13 couple with each other and split into two doublets (*J* = 19.0 Hz), which further couple with H-12, respectively. The coupling constant of 9.5 Hz indicated an axial coupling between the proton at C-12 and the β-proton at C-13, and *J* = 3.0 Hz indicated an axial–equatorial coupling between the H-12 and H-13α. The conformation of **1** was proposed based on the COLOC spectrum. Both C-11 and C-16 coupled with the H-15, from which we can propose that two six-membered rings, composed of C-10, C-11, C-12, C-14, C-15, and C-17 and C-10, C-14, C-15, C-16, and C-17, are both in the boatlike conformation. On

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the basis of the above analyses, the structure of fortunolide A is determined as **1**. All of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals were unambiguously assigned on the basis of 2D NMR experiments.

The spectral data of **2** ( $\text{C}_{19}\text{H}_{18}\text{O}_5$ ) also indicated the presence of lactone and tropone moieties.  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals suggested that **2** has a structure similar to hainanolide. The only difference between them was that **2** had one additional oxygen and an extra degree of unsaturation. After comparing the NMR spectra of the two compounds (**2** and hainanolide), it was concluded that the extra hydroxyl group in **2** was at either C-11 or C-12. In the  $^1\text{H}$  NMR spectrum of **2**, the H-13 signal is a singlet significantly downfield, so the hydroxyl group must be linked with C-12. Otherwise, the H-13 signal would split into a doublet by H-12. H-14 coupled with the H-15, and they both were doublets ( $J = 6.0$  Hz). However, the coupling constant between H-17 and H-14 was too small to be seen in the  $^1\text{H}$  NMR spectrum measured at 400 MHz. These observations indicated that both H-14 and H-15 are axial ( $J = 6-14$  Hz) and that H-17 is equatorial. Thus, the structure of fortunolide B was determined to be **2**.

Hainanolidol was isolated in 1979.<sup>3</sup> By means of  $^1\text{H}-^1\text{H}$  and  $^{13}\text{C}-^1\text{H}$  COSY spectra, we have confirmed the structure of hainanolidol and have assigned all of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals.

## Experimental Section

**General Experimental Procedures.** Melting points were measured with a WC-1 melting point apparatus and were uncorrected. IR spectra were recorded with a Perkin-Elmer 577 spectrometer. UV spectra were obtained with a Shimadzu UV-210A spectrophotometer. MS were measured with a VG-AUTOSPEC mass spectrometer. Optical rotations were obtained on a JASCO DEP-370 polarimeter.  $^1\text{H}$ ,  $^{13}\text{C}$ , and 2D NMR spectra were recorded using a Bruker AM-400 instrument.

**Plant Material.** Stems and needles of *C. fortunei* Hook.f. var. *alpina* Li were collected in Weixi County of Yunnan Province, China. The plant was identified by Assistant Professor Lianfang Li, Forestry Institute of Sciences of Yunnan, China.

**Extraction and Isolation** The air-dried stems and needles (4.5 kg) were extracted with cold 95% EtOH. The solution was

concentrated, and an equal volume of 2% aqueous HCl was added. The insoluble part was separated from the acidic solution by filtration and further extracted twice with 2% aqueous HCl. The combined acidic solutions were neutralized using solid  $\text{Na}_2\text{CO}_3$ , then extracted with  $\text{CHCl}_3$  to afford 14 g of crude extract. The crude extract was subjected to repeated aluminum oxide column chromatography to yield compounds **1** (36 mg), **2** (21 mg), hainanolidol (16 mg), acetylcephalotaxine (57 mg), and wilsonine (30 mg), with yields of  $8.0 \times 10^{-4}$ ,  $4.7 \times 10^{-4}$ ,  $3.6 \times 10^{-4}$ ,  $1.3 \times 10^{-3}$ ,  $6.7 \times 10^{-4}$  % from the air-dried plant material (4.5 kg), respectively.

**Fortunolide A (1):** obtained as pale yellow powder [ $(\text{CH}_3)_2\text{CO}$ ]; mp 263–265 °C;  $[\alpha]_{\text{D}}^{22.5} +136.11^\circ$  (c 0.709,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 252 (4.23), 317.5 (3.90) nm; IR (dry film)  $\nu_{\text{max}}$  3600–3300 (w), 3100–3200, 2940, 2900, 1740, 1615, 1580, 1525, 1440, 1070, 1010, and 910  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, EIMS (70 eV)  $m/z$  312 [ $\text{M}^+$ ], (74), 298 (17), 284 (99), 266 (83), 251 (45), 238 (47), 223 (72), 185 (60), 167 (71), 160 (100), 115 (65), 108 (54), and 55 (88); HREIMS  $m/z$  312. 1362 (calcd for  $\text{C}_{19}\text{H}_{20}\text{O}_4$ , 312.1376).

**Fortunolide B (2):** obtained as yellow needles [ $(\text{CH}_3)_2\text{CO}$ ]; mp 248–250 °C;  $[\alpha]_{\text{D}}^{18.6} +181.82^\circ$  (c 0.506,  $\text{C}_5\text{H}_5\text{N}$ ), UV ( $\text{CH}_3\text{-OH}$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 248.5 (4.26), 320 (3.82) nm; IR (dry film)  $\nu_{\text{max}}$  3300–3000 (w), 2960, 1750, 1615, 1590, 1530, 1365, 1170, 1145, 1070, 1025, 990, and 875  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, EIMS (70 eV)  $m/z$  326 [ $\text{M}^+$ ], (37), 298 [ $\text{M}^+ - \text{H}_2\text{O}$ ], (67), 218 (32), 130 (100), 103 (21), 77 (27); HREIMS  $m/z$  326.1154 (calcd for  $\text{C}_{19}\text{H}_{18}\text{O}_5$ , 326.1123).

**Hainanolidol (3):** obtained as yellow powder [ $(\text{CH}_3)_2\text{CO}$ ]; mp 226.5–227.5 °C; UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 251.5 (4.26), 322 (4.02) nm; IR (dry film)  $\nu_{\text{max}}$  3500–3100 (w), 2943, 2918, 1745, 1610, 1515, 1426, 1375, 1293, 1050, and 890  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR; EIMS (70 eV)  $m/z$  312 [ $\text{M}^+$ ], (75), 284 [ $\text{M}^+ - \text{H}_2\text{O}$ ], (77), 221 (62), 207 (97), 196 (90), 181 (54), 165 (64), 153 (67), 143 (100), 128 (62), 115 (53), and 95 (75).

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