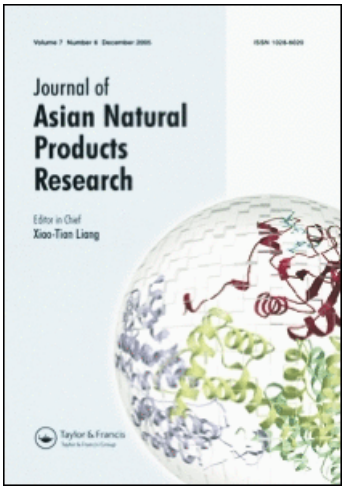


**Journal of Asian Natural Products Research**
Publication details, including instructions for authors and subscription information:
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# New sesquiterpene and triterpene from the fruits of *Cryptomeria fortunei*

Jian Wu[a]; Wei-Min Zhao[a]
[a] Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Online publication date: 20 May 2010

**To cite this Article** Wu, Jian and Zhao, Wei-Min(2010) 'New sesquiterpene and triterpene from the fruits of *Cryptomeria fortunei*', Journal of Asian Natural Products Research, 12: 5, 382 — 387
**To link to this Article: DOI:** 10.1080/10286021003785565
**URL:** http://dx.doi.org/10.1080/10286021003785565

*Journal of Asian Natural Products Research*
Vol. 12, No. 5, May 2010, 382–387



ORIGINAL ARTICLE

# New sesquiterpene and triterpene from the fruits of *Cryptomeria fortunei*

Jian Wu and Wei-Min Zhao*

*Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China*

(*Received 31 December 2009; final version received 16 March 2010*)

A new sesquiterpene (**1**) and a new triterpene (**2**) along with 25 known compounds were isolated from the fruits of *Cryptomeria fortunei* (Taxodiaceae). Their structures were elucidated on the basis of spectroscopic analysis.

**Keywords:** *Cryptomeria fortunei*; Taxodiaceae; triterpene; sesquiterpene



## 1. Introduction

*Cryptomeria fortunei* (Taxodiaceae) is a timber tree distributed widely in China and Japan. Its essential oil showed significant anti-bacterial activity [1]. Previous chemical investigation of the plant resulted in the isolation of biflavones [2], sesquiterpenes [3], abietane-type diterpenes [4–6], and terpene dimers [7], which can be used as chemosystematic markers of conifers [8]. As part of our ongoing program to find new bioactive products, the fruits of *C. fortunei* were investigated systematically. This paper describes the isolation and structural elucidation of a new sesquiterpene (**1**) and a new triterpene (**2**) along with 25 known compounds (Figure 1).

## 2. Results and discussion

Compound **1** was obtained as a colorless oil and showed $[M]^+$ ion at *m*/*z* 238.1930 in its HR-EI-MS, corresponding to the formula $C_{15}H_{26}O_2$. The $^1H$ NMR spectrum of **1** exhibited signals due to three tertiary methyl groups at $\delta_H$ 1.22 (3H, s; 1.06, 3H, s; 0.88, 3H, s), a doublet methyl group at $\delta_H$ 0.93 (3H, d, $J = 7.2$ Hz), and an oxymethine proton signal at $\delta_H$ 3.44 (1H, ddd, $J = 12.0, 8.0, 4.0$ Hz). The $^{13}C$ NMR spectrum of **1** indicated 15 carbon signals separated by the DEPT experiment into four methyl carbons, five methylene carbons, three methine carbons with one linked to an oxygen atom, and three quaternary carbons with two linked to an oxygen atom. After assignment of all protons to their attached carbon atoms according to the HSQC spectrum of **1**, two structural fragments, —$CH_2$—CH—$CH_2$—$CH_2$— and —O—CH—$CH_2$—$CH_2$—CH—$CH_3$, could be deduced from its $^1H$–$^1H$ COSY spectrum. Further analysis of its HMBC spectrum enabled the deduction of a dihydroagarofuran sesquiterpene skeleton to **1** [9] (Table 1). The relative configuration of **1** was established on the basis of the NOESY experiment, in which NOE signals were found between H-1 and H-3β/H-9β; H-12 and H-8β/H-9β; H-6β

*Corresponding author. Email: wmzhao@mail.shcnc.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286021003785565
http://www.informaworld.com

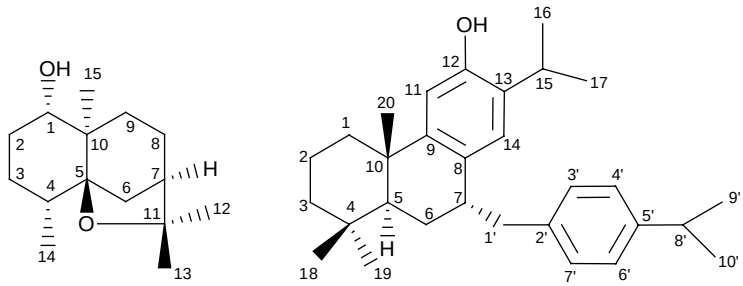


Figure 1. Structures of compounds **1** and **2**.

and H-13; H-2α and H-14/H-15; and H-15 and H-6α, H-8α (Figure 2). Thus, the structure of **1** was characterized as 1α-hydroxyl-β-dihydroagarofuran.

Compound **2** was obtained as a colorless oil with the molecular formula of $C_{30}H_{42}O$ based on the HR-EI-MS and NMR analysis, indicating 10 equivalents of unsaturation. The $^{13}C$ NMR spectrum of **2** revealed 30 carbon resonances separated into seven methyl, five methylene, 10 methine, and eight quaternary carbons by the DEPT experiment. Its $^{1}H$ NMR spectrum displayed six aromatic protons at $\delta_H$ 6.62, 6.86 (each 1H, s; 7.18, 4H, s) and three singlet methyl groups at $\delta_H$ 0.84, 0.94, 1.12. The existence of two isopropyl groups and structural fragments —$CH_2$—$CH_2$—$CH_2$— and —CH—$CH_2$—CH—$CH_2$— could also be deduced from its $^{1}H$–$^{1}H$ COSY and HSQC spectra. Comparison of its $^{13}C$ NMR spectral data with those in the literature [10] suggested **2** to be a ferruginol derivative. Further



Table 1. $^{1}H$ NMR (400 MHz), $^{13}C$ NMR (100 MHz) spectral data and key HMBC correlations of compound **1** (DMSO-$d_6$, $\delta$ in ppm, $J$ in Hz).

| No. | $^{1}H$ NMR | $^{13}C$ NMR | HMBC ($^{1}H \rightarrow {}^{13}C$) |
|---|---|---|---|
| 1 | 3.44 ddd, $J = 12.0, 8.0, 4.0$ | 73.6 d | C-9, C-15 |
| 2α | 1.42 m | 25.9 t | C-3 |
| 2β | 1.32 m | | |
| 3α | 1.22 m | 27.4 t | C-5 |
| 3β | 1.82 m | | C-5, C-14 |
| 4 | 1.55 m | 39.3 d | C-2, C-6 |
| 5 | | 88.5 s | |
| 6α | 1.85 m | 36.8 t | C-8, C-11 |
| 6β | 1.74 m | | C-4, C-8, C-10 |
| 7 | 1.82 m | 43.6 d | C-5, C-9 |
| 8α | 1.60 m | 24.5 t | C-6, C-10 |
| 8β | 1.50 m | | C-6, C-10, C-11 |
| 9α | 1.26 m | 35.0 t | C-5, C-7 |
| 9β | 1.58 m | | C-1, C-15 |
| 10 | | 43.5 s | |
| 11 | | 80.4 s | |
| 12 | 1.22 s | 22.7 q | C-7, C-13 |
| 13 | 1.06 s | 30.4 q | C-7, C-12 |
| 14 | 0.93 d, $J = 7.2$ | 17.5 q | C-3, C-5 |
| 15 | 0.88 s | 15.8 q | C-1, C-5, C-9 |

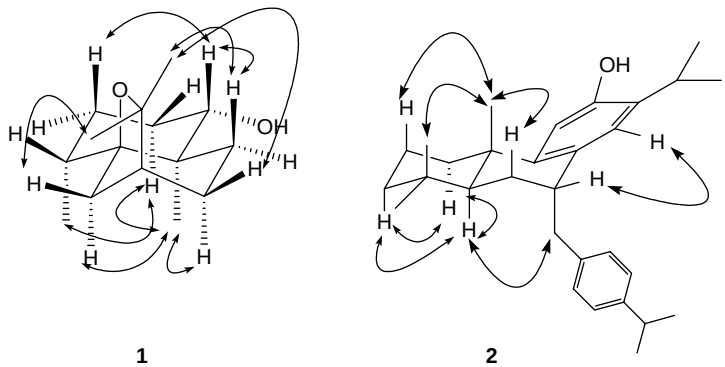


Figure 2. Main NOE correlations in the NOESY spectra of compounds **1** and **2**.



Table 2. $^{1}$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectral data, key HMBC correlations of compound **2**, and $^{13}$C NMR spectral data of ferruginol ($CDCl_3$, $\delta$ in ppm, $J$ in Hz).

| No. | $^{1}$H NMR | $^{13}$C NMR | HMBC ($^{1}$H → $^{13}$C) | $^{13}$C NMR of ferruginol [10] |
|---|---|---|---|---|
| 1α | 2.18 m | 38.8 t | C-3, C-5 | 38.7 t |
| 1β | 1.38 m | | | |
| 2α | 1.58 m | 19.4 t | C-4, C-10 | 19.2 t |
| 2β | 1.72 m | | | |
| 3α | 1.48 m | 41.8 t | C-1, C-5, C-19 | 41.6 t |
| 3β | 1.21 m | | | |
| 4 | | 33.4 s | | 33.2 s |
| 5 | 1.52 m | 45.3 d | C-3, C-7, C-19, C-20 | 50.2 d |
| 6α | 1.24 m | 22.5 t | C-1′, C-10 | 19.1 t |
| 6β | 1.66 m | | | |
| 7 | 3.10 m | 40.1 d | C-2′, C-14 | 29.6 t |
| 8 | | 138.8 s | | 127.3 s |
| 9 | | 148.7 s | | 148.2 s |
| 10 | | 38.0 s | | 37.3 s |
| 11 | 6.62 s | 110.8 d | C-8, C-10, C-13 | 110.9 d |
| 12 | | 151.0 s | | 150.9 s |
| 13 | | 131.0 s | | 131.7 s |
| 14 | 6.86 s | 127.5 d | C-7, C-9, C-12, C-15 | 126.5 d |
| 15 | 3.10 m | 26.9 d | C-12, C-14 | 26.5 d |
| 16 | 1.20 d, $J = 7.5$ | 22.8 q | C-13, C-17 | 22.5 q |
| 17 | 1.22 d, $J = 7.5$ | 22.4 q | C-13, C-16 | 22.7 q |
| 18 | 0.84 s | 21.9 q | C-3, C-5 | 21.5 q |
| 19 | 0.94 s | 33.7 q | C-3, C-5 | 33.2 q |
| 20 | 1.12 s | 25.0 q | | 24.7 q |
| 1′a | 2.90 m | 44.5 t | C-6, C-3′ (7′) | |
| 1′b | 2.72 m | | | |
| 2′ | | 140.4 s | | |
| 3′ (7′) | 7.18 s | 129.3 d | C-1′, C-5′ | |
| 4′ (6′) | 7.18 s | 126.3 d | C-2′, C-8′ | |
| 5′ | | 131.4 s | | |
| 8′ | 2.90 m | 33.7 d | C-4′ (6′) | |
| 9′ (10′) | 1.24 s | 24.1 q | C-5′, C-10′ (9′) | |

analysis of the $^{13}C-^{1}H$ long-range correlation signals in its HMBC spectrum confirmed the existence of the ferruginol fragment and also revealed the attachment of a 4-isopropyl-benzyl group at C-7 (Table 2). Compound **2** was thus a triterpene polymerized by an abietane-type diterpene and a methane-type monoterpene, and its HR-EI-MS exhibited abundant fragment ion signals at *m/z* 285.2217 (285.2216; calcd for $C_{20}H_{29}O$) and 133.1012 (133.1007; calcd for $C_{10}H_{13}$). The relative configuration of **2** was determined according to the NOESY spectrum, in which NOE signals were observed between H-1α and H-5/H-3α; H-2β and Me-20/Me-18; H-3α and H-1α/H-5; H-5 and H-1α/H-3α/H-1′; H-6β and Me-18/Me-20; Me-20 and H-3β/H-6β/H-18; and H-7 and H-14. Finally, compound **2** was elucidated as 7α-*p*-isopropyl-benzyl-ferruginol.

The 25 known compounds isolated from the fruits of *C. fortunei* were identified as isocupressic acid [11], labda-8(17),13-diene-15,19-dioic acid [12], agatholal [13], 15-oxolabda-8(17),13*E*-dien-19-oic acid [6], 19-acetyl-agathadiol [6], imbricataloic acid [12], 15-hydroxylabd-8(17)-en-19-oic acid [14], isochamaecydin [15], chamaecydin [14], 6α-hydrochamaecydin [7], 6β-hydrochamaecydin [7], cryptomeridiol [16], 11-acetoxyeudesman-4α-ol [3], 10β-selinan-4β,11-diol [16], 4-*O*-ethyl-cryptomeridiol [17], 3-eudesmol-1β,11-diol [3], isopimaric acid [18], phyllocladan-16α-ol [19], taxodione [10], 11-hydroxy-12-oxo-7,9,13-abietatriene [20], 6α-hydroxyldemethylcryptojaponol [21], sugiol [22], 12-methoxy-7α,11-dihydroxy-dehydroabietane [23], ferruginol [10], 19-hydroxyferruginol [24], by comparing their spectroscopic data with reported values.

## 3. Experimental

### 3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. EI-MS data were recorded on a MAT-95 mass spectrometer. HR-EI-MS were obtained on a Kratos 1H mass spectrometer. The NMR experiments were run on a Bruker AM 400 spectrometer with TMS as the internal standard. Preparative HPLC was carried out using a Varian SD-1 instrument equipped with a Merck NW25 $C_{18}$ column (20 mm × 250 mm, 10 μm) and a ProStar 320 UV–vis detector. Column chromatographic separations were carried out using Si gel H60 (300–400 mesh), ZCX-II (100–200 mesh) (Qingdao Marine Chemical Group Corporation, Qingdao, China), and MCI gel CHP-20P (Mitsubishi Chemical, Tokyo, Japan). HSGF254 Si gel TLC plates (Yantai Chemical Industrial Institute, Yantai, China) and RP-18 $WF_{254}$ TLC plates (Merck) were used for analytical TLC.

### 3.2 Plant material

The fruits of *C. fortunei* were collected in Wencheng County, Zhejiang Province, in April 2007, and identified by Prof. Jingui Shen of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (No. 20070303SIMM) has been deposited in the herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

### 3.3 Extraction and isolation

Air-dried and powdered fruits (1.0 kg) of *C. fortunei* were extracted with 95% EtOH three times at reflux. The extract was concentrated to dryness *in vacuo* and the aqueous residue was partitioned with ethyl acetate. The ethyl acetate extract (90 g) was subjected to chromatography over MCI with MeOH–$H_2O$ (v/v 7:3; 8:2; 17:3; 9:1; 19:1; 10:0) as the eluant to afford Fr. 1 (7:3–8:2), Fr. 2 (8:2–17:3), Fr. 3 (17:3–9:1), Fr. 4 (9:1–19:1), and Fr. 5 (10:0). Fr. 1 (4 g) was subjected to Si gel ZCX-II with petroleum ether–acetone

(10:1) as the eluant to afford Fr. 1A and Fr. 1B. Compound **1** was separated from Fr. 1A by column chromatography over Si gel H60 with chloroform–acetone (30:1) as the eluant. Repeated chromatography over HPLC and Si gel H60 of Fr. 1B yielded cryptomeridiol (100.9 mg), 10β-selinan-4β,11-diol (7.8 mg), and 3-eudesmol-1β,11-diol (20.2 mg). Fr. 2 (6.0 g) was subjected to Si gel ZCX-II with petroleum ether–acetone (v/v 15:1; 10:1; 8:1; 6:1; 4:1; 2:1) as the eluant to afford Fr. 2A–D. Further chromatography of Fr. 2A over Si gel H60 with petroleum ether as the eluant yielded 11-hydroxy-12-oxo-7,9,13-abietatriene (37.8 mg). Fr. 2B was purified by HPLC (50–80%, 60 min; 80–100%, 30 min; 100% methanol, 10 min) to afford 4-*O*-ethyl-cryptomeridiol (40.5 mg) and 12-methoxy-7α,11-dihydroxy-dehydroabietane (20.6 mg). Fr. 2C was separated into Fr. 2C1 and Fr. 2C2 by chromatography over Si gel H60. Agatholal (23.7 mg), 15-oxolabda-8(17),13*E*-dien-19-oic acid (37.5 mg), 11-acetoxyeudesman-4α-ol (10.5 mg), and sugiol (50.9 mg) were purified from Fr. 2C1 by HPLC (40–80%, 80 min; 80–100%, 10 min; 100% methanol, 30 min). Fr. 2C2 was subjected to HPLC and Si gel to give imbricataloic acid (47.5 mg) and 6α-hydroxyldemethylcryptojaponol (12.3 mg). Repeated chromatography over HPLC and Si gel of Fr. 2D afforded isocupressic acid (35.6 mg), 19-acetylagathadiol (9.6 mg), 15-hydroxylabd-8(17)-en-19-oic acid (70.8 mg), 19-hydroxyferruginol (12.1 mg), and labda-8(17),13-diene-15,19-dioic acid (10.1 mg). Repeated chromatography over Si gel H60 of Fr. 3 (23 g) yielded isopimaric acid (400 mg), phyllocladan-16α-ol (48.4 mg), and taxodione (42.6 mg). Fr. 4 (1.0 g) was subjected to a series of column chromatography over Si gel H60, Sephadex LH-20, and preparative HPLC to afford 6β-hydrochamaecydin (15.1 mg), ferruginol (57.3 mg), and **2** (10.0 mg). Fr. 5 (15 g) was purified by repeated chromatography over Si gel to afford isochamaecydin (5.3 mg), chamaecydin (880 mg), and 6α-hydrochamaecydin (6.5 mg).

#### *3.3.1 1α-Hydroxyl-β-dihydroagarofuran (**1**)*

Colorless oil; $[\alpha]_D^{22}$ −28 ($c = 0.075$, $CHCl_3$); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3336, 2952, 2858, 1741, 1454, 1302, 1149, 1099, 1031, 964, 883; $^1H$ and $^{13}C$ NMR spectral data: see Table 1; EI-MS *m/z*: 238 $[M]^+$; HR-EI-MS *m/z*: 238.1930 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

#### *3.3.2 7α-p-Isopropyl-benzyl-ferruginol (**2**)*

Colorless oil; $[\alpha]_D^{22}$ +65 ($c = 0.17$, $CHCl_3$); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3417, 2960, 2925, 2868, 1707, 1649, 1618, 1508, 1461, 1414, 1165, 1097, 810; $^1H$ and $^{13}C$ NMR spectral data (in $CDCl_3$): see Table 2; $^1H$ NMR spectral data (in $C_5D_5N$): 7.38 (2H, d, $J = 8.0$ Hz), 7.30 (2H, d, $J = 8.0$ Hz), 7.25 (1H, s), 7.17 (1H, s), 3.68 (1H, m), 3.33 (1H, m), 3.18 (1H, dd, $J = 3.9$, 12.2 Hz), 2.86 (2H, m), 2.20 (1H, d, $J = 12.0$ Hz), 1.41 (3H, d, $J = 6.9$ Hz), 1.39 (3H, d, $J = 6.9$ Hz), 1.23 (3H, s), 1.20 (3H, s), 1.14 (3H, s), 0.97 (3H, s), 0.76 (3H, s); EI-MS *m/z*: 418 $[M]^+$; HR-EI-MS *m/z*: 418.3245 $[M]^+$ (calcd for $C_{30}H_{42}O$, 418.3236).

## Acknowledgements

This work was financially supported by the National Science and Technology Major Project 'Key New Drug Creation and Manufacturing Program', China (No. 2009ZX09301-001) and the Key Project of Chinese Academy of Sciences (No. KSCX2-YW-R-184).

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