

A NEW TRICYCLIC 6-7-5-RING DITERPENE FROM

PLIOCENE FOSSIL *TSUGA DUMOSA*

You-Xing Zhao, Cheng-Sen Li,[†] Xiao-Dong Luo, and Jun Zhou *

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China

E-mail: jzhou@mail.kib.ac.cn

[†]Institute of Botany, Chinese Academy of Sciences, Beijing 100093, People's Republic of China

Abstract - A new diterpene named (+)-parryin-15,16-*O*-isopropylidene (**1**) and three known isopimara analogues (**2-4**) were isolated from the Pliocene lignified wood of *Tsuga dumosa* collected in Changning coalmine, Yunnan, China. Their structures were elucidated by spectroscopic analysis.

Tsuga dumosa is an economically important conifer indigenous to the Yunnan Province of China.¹ An interesting fact that Pliocene lignified wood of *Tsuga dumosa* was preserved morphologically in coalmine has raised questions about its phytochemical constituents. Previous investigation of fossil plant showed the presence of natural constituents.²⁻⁵ To probe the organic constituents of this fossil *T. dumosa*, we undertook the chemical investigation of Pliocene lignified wood of *T. dumosa* collected from an open coalmine in Changning of Yunnan Province, China. This paper describes the isolation and elucidation of a new tricyclic 6-7-5-ring diterpene, (+)-parryin-15,16-*O*-isopropylidene (**1**) and three known isopimara analogues,⁶ (-)-isopimara-8 (14)-en-15,16-*O*-isopropylidene (**2**), isopimara-8-en-15,16-*O*-isopropylidene (**3**) and isopimara- 8-en-15,16 -diol (**4**).

Compound (**1**) was obtained as viscous oil. HRESIMS gave the formula as C₂₃H₃₈O₄ (at *m/z* 379.2866 [M+1]⁺, calcd 379.2848). IR spectrum showed a carbonyl (1689 cm⁻¹) and a hydroxyl (3455 cm⁻¹) groups, which were confirmed by signals at δ_{C} 213.6 (C-8) and δ_{H} 4.45 (s) in NMR spectrum, respectively. Its ¹H NMR spectrum exhibited six methyl signals at δ_{H} 1.44, 1.26 (s, H-1', 3'), 1.14 (s, H-17), 0.93 (s, H-18), 0.91 (s, H-20) and 0.80 (s, H-19). The ¹³C NMR

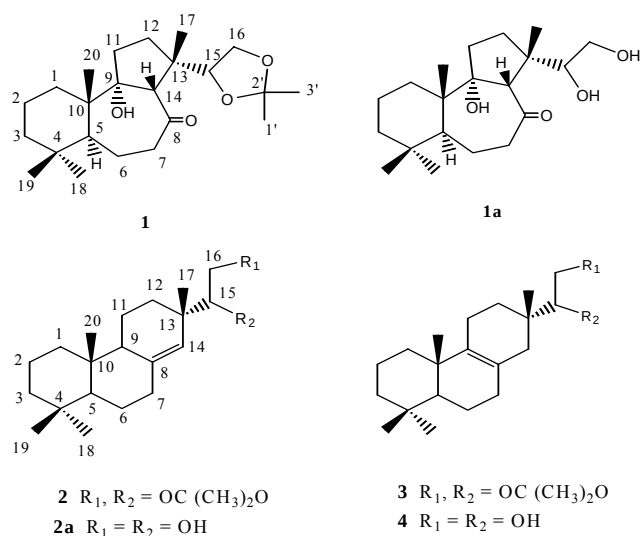
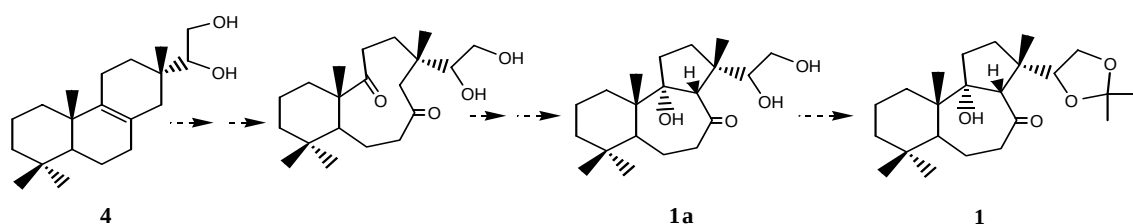


Figure 1. Structures of compounds (**1-4**)

spectrum of **1** revealing 23 carbon atoms were similar to those of parryin⁷ except for C-15 ($\delta_{\text{C}} 77.7$) and C-16 ($\delta_{\text{C}} 66.2$) attached to oxygen atoms and three additional carbon signals attributable to C-1', C-2' and C-3', suggesting that **1** has the skeleton of parryin. The tricyclic 6-7-5-ring structure of **1** could be determined by its HMBC spectrum, showing key correlations of signals at $\delta_{\text{H}} 2.56$ (s, H-14) with $\delta_{\text{C}} 213.6$ (C-8), 42.6 (C-10) and 77.7 (C-15), $\delta_{\text{H}} 4.51$ (H-15) with $\delta_{\text{C}} 30.0$ (C-12) and 64.7 (C-14), $\delta_{\text{H}} 2.09$ (H-5) with $\delta_{\text{C}} 85.3$ (C-9) and $\delta_{\text{H}} 1.14$ (H-17) with $\delta_{\text{C}} 64.7$ (C-14). The stereochemistry of tricyclic 6-7-5-ring was further supported by its important correlations between H-14 / H-17, H-14 / H-20, H-5 / H-18 and H-5 / -OH in ROESY spectrum and similar optical rotation to parryin. Thus, compound (**1**) was elucidated to be (+)-parryin-15,16 -O-isopropylidene.

Compound (**1-3**) may be the products resulted from the condensation of compounds (**1a**, **2a** and **4**) in acetone solution during isolation process. This assumption was further confirmed by the fact that acidolysis of **1** with acetic acid gave **1a**. The tricyclic 6-7-5-ring diterpene isolated from Pliocene fossil wood may be produced by reasonable biological or chemical path during the period of burial in sediment. The probable origin of **1** may derive from **2a** as described in literature⁷ and/or **4** as shown in **Scheme 1**.



Scheme 1. Hypothetical synthetic pathway to compound (**1**)

EXPERIMENTAL

General Experimental Procedures -- Optical rotations were taken on a JASCO DIP-370 digital polarimeter. IR spectra were measured on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. MS spectra were recorded on a VG Auto Spec-3000 spectrometer. 1D and 2D NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as internal standard.

Plant Material -- The Pliocene lignified wood of *Tsuga dumosa* was collected from an open coalmine in Yunnan Province, People's Republic of China, and identified by professor Cheng-Sen Li. A voucher specimen (LL-1-3) has been deposited in Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation -- The powder lignified wood of *Tsuga dumosa* (2.8 kg) was extracted with MeOH (8 L $\times$ 3) under reflux for 12 h. The extract (27.0 g) was chromatographed over silica gel and eluted with CHCl₃-MeOH (from CHCl₃ to CHCl₃-MeOH 12 : 1) to give 9 fractions. Fraction 2 (2.0 g) was subjected to silica column (petroleum ether-acetone 8 : 1, 10 : 1, petroleum ether-CHCl₃ 1 : 1, 3 : 2, 2 : 1) to afford **1** (8 mg), **2** (8 mg) and **3** (10 mg) and fraction 6 (650 mg) was purified by repeated silica gel column (petroleum ether-acetone 3 : 1, 4 : 1) to yield **4** (6 mg), respectively.

(+)-Parryin-15,16-*O*-isopropylidene (**1**), viscous oil; $[\alpha]_D^{26} +57.08^\circ$ (c 0.44, CHCl₃); IR bands (KBr): 3455, 2928, 1689, 1462, 1380, 1300, 1260, 1209, 1162, 999, 859 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.51 (1H, dd, J = 7.8, 7.1 Hz, H-15), 4.45 (1H, s, OH), 3.96 (1H, dd, J = 7.8, 7.1 Hz, H-16a), 3.62 (1H, dd, J = 7.8, 7.8 Hz, H-16b), 2.85 (1H, ddd, J = 14.3, 12.8, 1.8 Hz, H-7a), 2.56 (1H, s, H-14), 2.38 (1H, ddd, J = 14.3, 6.9, 1.2 Hz, H-7b), 2.09 (1H, brd, J = 10.3 Hz, H-5), 1.94, 1.92 (1H, m, H-12a, 12b), 1.84, 1.59 (1H, m, H-11a, 11b), 1.79, 1.34 (1H, m, H-6a, 6b), 1.66, 1.41 (1H, m, H-1a, 1b), 1.47 (2H, m, H-2), 1.44, 1.26 (3H each, s, H-1', 3'), 1.38, 1.31 (1H, m, H-3a, 3b), 1.14 (3H, s, H-17), 0.93 (3H, s, H-18), 0.91 (3H, s, H-20), 0.80 (3H, s, H-19); ¹³C NMR (100 MHz, CDCl₃) δ 34.6 (t, C-1), 19.0 (t, C-2), 41.7 (t, C-3), 34.5 (s, C-4), 48.4 (s, C-5), 20.5 (t, C-6), 45.4 (t, C-7), 213.6 (s, C-8), 85.3 (s, C-9), 42.6 (s, C-10), 34.6 (t, C-11), 30.0 (t, C-12), 46.6 (s, C-13), 64.7 (d, C-14), 77.7 (d, C-15), 66.2 (t, C-16), 28.1 (q, C-17), 34.0 (q, C-18), 21.7 (q, C-19), 15.9 (q, C-20), 24.7 (q, C-1'), 108.9 (s, C-2'), 26.2 (q, C-3'); EIMS m/z (rel. int.): 378 [M]⁺ (2), 363 (3), 345 (6), 320 (24), 302 (58), 287 (51), 277 (23), 259 (67), 241 (13), 227 (10), 195 (100), 182 (38), 177 (27), 164 (64), 149 (32), 135 (28), 121 (50), 109 (42), 95 (53), 81 (44), 69 (51); HRESIMS m/z 379.2866 (calcd for C₂₃H₃₉O₄ 379.2848).

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