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Diterpenoids from *Fokienia hodginsii*

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Abstract

In continuous research on the chemical constituents of the twigs and leaves of *Fokienia hodginsii* (Dunn) A. Henry et Thomas growing in Highland, Lam Dong province 4 diterpenoids, including 3-oxo-totarol (totarolone, **1**), 3*β*-hydroxytotarol (**2**), 15-*nor*-labda-8(17),12*E*-diene-14-carboxaldehyde-19-oic acid (**3**) and 13-oxo-15,16-dinorlabda-8(17),11*E*-diene-19-oic acid (**4**) were isolated. Their structures were elucidated by the spectroscopic methods and comparison with reported data. This is the first report on the isolation of compounds **1**, **2** and **3** from this plant.

Keywords. *Fokienia hodginsii*; totarane; *nor*-labdane diterpenoid.

1. INTRODUCTION

Fokienia hodginsii (Dunn) A. Henry et Thomas (synonym *Cupressus hodginsii* Dunn)-"local name Pơ mu"- belongs to family Cupressaceae. It is a big tree, 20-30 m tall, mainly distributed in China and Vietnam [1]. Po mu tree is known for its beauty, fragrance and high-value wood. Previous investigations of this plant have been mainly focused on the chemical composition of the essential oil [2]. Hitherto, there is only one report on the chemical constituents of this species growing in China. Its main components included diterpenoids: isopimarane, labdane, and icetexane [2]. In our previous chemical investigation, from the ethyl acetate extract of twigs and leaves of this plant growing in Highland, Lam Dong province there yielded two megastigmanes, namely drummondol and vomivoliol [3]. In our continued work on the hexane extract of this plant, 4 diterpenoids as 3-oxo-totarol (totarolone, **1**), 3*β*-hydroxytotarol (**2**), 15-*nor*-labda-8(17),11*E*-diene-14-carboxaldehyde-19-oic acid (**3**) and 13-oxo-15,16-*dinor*-labda-8(17),11*E*-diene-19-oic acid (**4**) were isolated and structurally determined.

2. EXPERIMENTAL

2.1. General

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) were taken on a Bruker Avance AM500 spectrometer using TMS as internal standard for ^{1}H and solvent signal for ^{13}C. ESI-MS was taken on an Agilent 1100 LC-MSD Trap spectrometer. TLC aluminum sheets of silica gel Merck 60 F254 (layer thickness 0.2 mm) were used. Column chromatography (CC) was carried out on silica gel Merck 60 (0.040-0.063 mm) and Sephadex LH-20.

2.2. Plant Material

F. hodginsii was collected in Highland, Lam Dong province, Vietnam in August, 2012 and identified by Dr. Nguyen Tien Hiep. A voucher specimen (No. VNMN. B000005002) is deposited in the Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam.

2.3. Extraction and Isolation

The dried and powdered mixture of twigs and leaves of *F. hodginsii* (900 g) was extracted ultrasonically with methanol - water (90:10, v/v) at 40 ^{o}C, three times, each 4 h. After concentration under reduced pressure, the crude extract was suspended in water and sequentially partitioned in *n*-hexane, ethyl acetate and *n*-butanol. The organic solvents were evaporated to yield the corresponding extracts (14.0,

18.3 and 13.2 g, respectively). The hexane extract (14.0 g) was subjected to silica gel column chromatography with gradient elution of *n*-hexane - EtOAc mixtures (from 100:1 to 70:30) to give twenty fractions (F1-F20). Fraction F10 was separated by a Sephadex LH-20 column with MeOH as eluent to give totarolone (**1,** 20 mg). Fraction F16 was further purified on a silica gel column eluted with hexane-EtOAc (95:5) to yield 3*β*-hydroxy-totarol (**2,** 10 mg). Fractions F16 and F12 were repurified on a Sephadex LH-20 column using methanol as eluent to give 13-oxo-15,16-dinorlabda-8(17),11*E*-diene-19-oic acid (**3,** 10 mg) and 15-*nor*-labda-8(17),12*E*-diene-14-carboxaldehyde-19-oic acid (**4,** 10 mg), respectively.

Totarolone (3-oxototarol, 1): ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 6.95 (1H, d, *J* = 8.5 Hz, H-11), 6.58 (1H, d, *J* = 8.5 Hz, H-12), 3.27 (1H, br s, H-15), 3.02 (1H, dd, *J* = 17.0, 5.5 Hz, H-7a), 2.72 (1H, m, H-7b), 2.68 (1H, m, H-2a), 2.58 (1H, m, H-2b), 1.34, 1.33 (each 3H, d, *J* = 6.5 Hz, H-15&H-16), 1.29 (3H, s, H-20), 1.17 (3H, s, H-19), 1.14 (3H, s, H-18); ^{13}C-NMR (500 MHz, CD_3OD) δ_C: 220.56 (C-3), 155.12 (C-13), 140.11 (C-9), 134.93 (C-8), 132.05 (C-14), 124.81 (C-11), 115.58 (C-12), 51.52 (C-5), 48.32 (C-4), 39.62 (C-1), 38.39 (C-10), 35.79 (C-2), 30.14 (C-7), 28.85 (C-15), 27.30 (C-18), 25.07 (C-20), 21.68 (C-6), 21.47 (C-19), 20.58, 20.51 (C-16 & C-17).

3*β*-Hydroxytotarol (2): ESI-MS *m/z*: 285.1 $[M-H_2O+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 6.92 (1H, d, *J* = 8.5 Hz, H-11), 6.53 (1H, d, *J* = 8.5 Hz, H-12), 3.24 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 2.96 (1H, dd, *J* = 16.5, 6.0 Hz, H-7a), 2.71 (1H, m, H-7b), 1.33, 1.32 (each 3H, d, *J* = 6.5 Hz, H-15 & H-16), 1.17 (3H, s, H-20), 1.07 (3H, s, H-19), 0.89 (3H, s, H-18); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 155.32 (C-13), 142.29 (C-9), 134.89 (C-8), 132.07 (C-14), 123.78 (C-11), 115.26 (C-12), 79.58 (C-3), 50.93 (C-5), 39.87 (C-4), 39.27 (C-1), 38.56 (C-10), 30.29 (C-7), 28.96 (C-2), 28.76 (C-19), 25.61 (C-20), 20.56 (C-16&C-17), 20.43 (C-6), 16.08 (C-18).

13-Oxo-15,16-*dinor*-labda-8(17),11*E*-diene-19-oic acid (3): ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 6.98 (1H, dd, *J* = 16.0, 10.5, H-11), 6.12 (1H, d, *J* = 16.0, H-12), 4.83 (1H, d, *J* = 1.5, H-17a), 4.45 (1H, d, *J* = 1.5, H-17b), 2.30 (3H, s, H-14), 1.24 (3H, s, H-18), 0.87 (3H, s, H-20); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 200.88 (C-13), 180.12 (C-19), 149.89 (C-8), 148.67 (C-11), 134.58 (C-12), 108.87 (C-17), 61.35 (C-9), 56.38 (C-5), 45.08 (C-4), 42.13 (C-1), 40.95 (C-10), 39.31 (C-3), 38.24 (C-7), 29.42 (C-18), 27.11 (C-14), 26.33 (C-6), 20.86 (C-2), 14.14 (C-20).

15-*Nor*-labda-8(17),12*E*-diene-14-carboxaldehyde-19-oic acid (4): ESI-MS *m/z*: 303.1 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 9.31 (1H, s, H-14), 6.57 (1H, td, *J* = 6.0, 1.5 Hz, H-12), 4.87 (1H, s, H-17a), 4.44 (1H, s, H-17b), 1.77 (3H, br s, H-16), 1.24 (3H, s, H-18), 0.74 (3H, s, H-20). ^{13}C-NMR (500 MHz, CD_3OD) δ_C: 197.07 (C-14), 181.13 (C-19), 158.69 (C-12), 149.57 (C-8), 140.01 (C-13), 108.03 (C-17), 57.25, 57.15 (C-5 & C-9), 45.15 (C-4), 41.38 (C-10), 40.55 (C-1), 39.42, 39.26 (C-3 & C-7), 29.54 (C-18), 27.24 (C-6), 25.73 (C-11), 21.14 (C-2), 13.37 (C-20), 9.29 (C-16).

3. RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of **1** revealed two aromatic *ortho*-coupled protons at δ_H 6.95 (1H, d, *J* = 8.5 Hz, H-11), 6.58 (1H, d, *J* = 8.5 Hz, H-12), three singlet methyl groups at δ_H 1.29 (3H, s, H-20), 1.17 (3H, s, H-19), 1.14 (3H, s, H-18), an isopropyl group attached to a benzene ring by two doublet methyl signals at δ_H 1.34, 1.33 (each 3H, *J* = 6.5 Hz) and one methine proton at δ_H 3.27 (1H, br s, H-15), and aliphatic protons in the range from δ_H 1.73 to 3.03 ppm. Its ^{13}C-NMR and DEPT spectra showed signals of 20 carbon atoms including a ketone group, 5xCH_3, 4xCH_2, 4xCH, and 6xCq. The presence of an aromatic ring was confirmed by two methine signals at δ_C 124.81 (C-11), 115.58 (C-12); one quaternary carbon bonded to an oxygen atom at δ_C 155.12 (C-13) and three other quaternary carbons at δ_C 140.11 (C-9), 134.93 (C-8), 132.05 (C-14). The HMBC correlations observed between H-16, H-17 and C-14; H-11 and the C-13 indicated that the isopropyl group was at C-14, and the phenolic hydroxyl group at C-13. Thus, the structure of **1** was concluded to be 13-hydroxy-8,11,13-totaratrien-3-one (3-oxototarol, 3-ketototarol). This compound showed the strong activity against some gram-positive and gram-negative bacteria as well as against some human pathogenic fungi [4].

The positive ESI-MS of **2** gave a molecular ion peak at *m/z* 285.1 $[M+H-H_2O]^+$. The spectral data and the molecular formula suggested that **2** had the same carbon skeleton as **1**. The NMR spectra of compounds **2** were similar with those of **1** except some changes at C-3. The replacement of ketone group by a hydroxyl in the structure of **2** is indicated by the absence of the ketone group and the presence of a hydroxyl signal [δ_H 3.24 (dd, *J* = 11.5, 5.0 Hz, H-3)/δ_C79.58 (C-3)], in its NMR spectra. Thus, compound **2** was elucidated as 3*β*-hydroxytotarol (totaradiol) by comparison with data in the literature [5].

The molecular formula of **3** was determined to be $C_{18}H_{26}O_3$ based on ion molecular peak at *m/z* 289.1 $[M-H]^-$ in the negative ESI-MS spectrum. In the ^{1}H- and ^{13}C-NMR spectra, characteristic signals for one

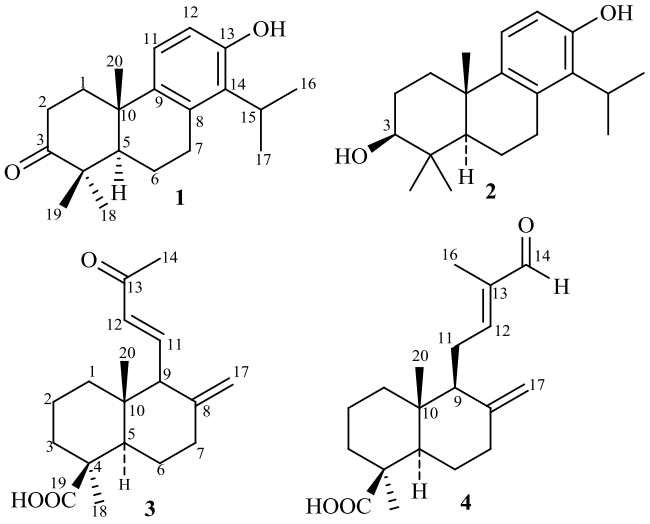

Figure 1: The structure of compounds **1-4**

ketone group (δ_C 200.88), one carboxylic acid (δ_C 180.12), a *trans*-disubstituted double bond [δ_H 6.98 (1H, dd, *J* = 16.0, 10.5 Hz, H-11)/δ_C 148.67; δ_H 6.12 (1H, d, *J* = 16.0 Hz, H-12)/δ_C 134.58], an exocyclic methylene group [δ_H 4.83 (s), 4.45 (s), δ_C 108.87 (t), 149.89 (s)], three tertiary methyl groups, six methylene groups, two methine groups were found. These data suggested that compound **3** is a *nor*-labdane diterpenoid. The long-range correlations in the HMBC spectrum between H-18 and C-4, C-19; H-17 and C-7, C-9; H-11, H-12, H-14 and C-13 were suggestive of that a carboxylic acid, an exocyclic methylene and a keton group must be located at C-4, C-8 and C-13, respectively. The NOEs correlations between H-9 and the methyl protons H-18 and H-5 implied that these protons were *α*-oriented. Combined its ^{1}H-, ^{13}C-NMR and 2DNMR spectral data, compound **3** was determined as 13-oxo-15,16-dinorlabda-8(17),11*E*-diene-19-oic acid [6].

The molecular formula of **4** was determined to be $C_{19}H_{28}O_3$ based on a molecular ion peak at *m/z* 303.1 $[M-H]^-$ in the negative ESI-MS and combination with NMR data. The spectral data and the molecular formula suggested that **4** had the same carbon skeleton as **3** with a difference of a moiety side-chain at C-9. The ^{1}H- and ^{13}C-NMR spectra of **4** showed the characteristic signals for the presence of a 3-methyl-4-oxo-2*E*-butenyl side chain, which were observed at δ_H 9.31 (1H, s, H-14), 6.57 (1H, td, *J* = 6.0, 1.5 Hz, H-12), 1.77 (3H, br s, H-16); δ_C 197.07 (d, C-14), 158.69 (d, C-12), 140.01 (s, C-13), 9.29 (q, C-16). By comparison of its ^{1}H- and ^{13}C-NMR spectral data with those of 15-*nor*-labda-8(17),12*E*-diene-14-carboxaldehyde-19-oic acid methyl ester [7], compound **4** was determined as 15-norlabda-8(17),12*E*-dien-14-carboxaldehyde-19-oic acid. Recently, this compound was also isolated from the twigs and leaves of *Fokienia hodginsii* growing in China [2].

Except **4**, the remaining compounds **1-3** were isolated from this plant for the first time.

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REFERENCES

1. Wenlong He, Bainian Sun, Yu-Sheng (Christopher) Liu. *Fokienia shengxianensis* sp. *nov. (Cupressaceae) from the late Miocene of eastern China and its paleoecological implications*, Review of Palaeobotany and Palynology, **176-177**, 24-34 (2012).
2. Xing-De Wu, Juan He, Xing-Yao Li,Liao-Bin Dong, Xun Gong, Liu-Dong Song, Yan Li, Li-Yan Peng, and Qin-Shi Zhao. *Diterpenoids from the twigs and leaves of Fokienia hodginsii*, J. Nat. Prod., **76(6)**, 1032-1038 (2013).
3. Dang Viet Hau, Nguyen Thanh Tam, Dao Đuc Thien, Tran Duc Quan, Tran Van Loc, Tran Van Sung, Trinh Thi Thuy. *Megastigmanes from Fokienia hodginsii collected in Highland*, Vietnam Journal of Chemistry, **54(6e2)**, 44-47 (2016).
4. Milena P. Popova, Ioanna B. Chinou, Ilko N. Marekov, Vassya S. Bankova. *Terpenes with antimicrobial activity from Cretan propolis*. Phytochemistry, **70**, 1262-1271 (2009).
5. Jayr De Paiva Campello, Sebastiao Ferreira Fonseca. *Terpenes of Podocarpus lambertius*, Phytochemistry, **14**, 243-248 (1975).

6. T. Katoh, R. Tanaka, M. Takeo, K. Nishide, M. Node. *A new synthesis of a potent cancer chemopreventive agent, 13-oxo-15,16-dinorlabda-8(17),11E-dien-19-oic acid from trans-communic acid*, Chem. Pharm. Bull., **50(12)**, 1625-1629 (2002).

7. M. Kobayashi, K. Ishida, S. Terabayashi, H. Mitsuhashi. *10-Hydroxypheophytins and a new norlabdane diterpene from the leaves of Cupressus funebris ENDL*, Chem. Pharm. Bull., **39(12)**, 3348-3349 (1991).

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