Diterpenoids from the wood of *Podocarpus neriifolius*

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Abstract

Using combined chromatographic methods, three known diterpenoids, inumakiol D (1), totarol (2) and totarol-19-carboxylic acid (3) along with β-sitosterol and β-sitosterol glucoside were isolated from the ethyl acetate extract of the wood of *Podocarpus neriifolius* collected in Lam Dong province, Vietnam. Their structures were determined by MS, 1D-, 2D-NMR data analysis and comparison with published references. This is the first report of compounds 1-3 from this plant.

Keywords: *Podocarpus neriifolius*, diterpenoid, inumakiol D, totarol, totarol-19-carboxylic.

1. INTRODUCTION

*Podocarpus neriifolius* D. Don - “Thong tre lá dài”- (Podocarpaceae) is tree with straight and round trunk, growing up to 20 - 25m high. It was sparsely distributed in primary forest areas in northern Vietnam as Nghean, Hatinh, Yenbai, Tuyenquang provinces ... and found at an altitude of 2300 m in the Bidoup Nui Ba National Park, Lamdong province. A decoction of the leaves of this plant is used in Vietnamese traditional medicine for the treatment of rheumatism and joint pain [1]. There have been some reports on the presence of flavonoids, diterpenoids in this plant [2-4]. In our research on the chemical constituents of Pinales species, three diterpenoids, inumakiol D (1), totarol (2) and totarol-19-carboxylic acid (3) and along with β-sitosterol and β-sitosterol glucoside were isolated from the ethyl acetate extract of the wood of *P. neriifolius*.

2. EXPERIMENTAL

2.1. General

¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) were taken on a Bruker Avance AM500 spectrometer using TMS as internal standard for ¹H and solvent signal for ¹³C. ESI-MS was taken on an Agilent 1100 LC-MSD Trap spectrometer. Merck TLC aluminum sheets with silica gel 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

*Podocarpus neriifolius* was collected in the Bidoup Nui Ba National Park in Lam Dong province, Vietnam in January, 2013 and identified by Dr. Nguyen Tien Hiep. A voucher specimen 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 wood of *Podocarpus neriifolius* (1.4 kg) was extracted with methanol: water (95:5 w/w) for 24 h, three times. After concentration under reduced pressure, the crude extract was suspended in water and sequentially partitioned with *n*-hexane, ethyl acetate and *n*-butanol. The organic solvents were evaporated to
yield the corresponding extracts (0.45 g, 15.0 g and 13.2 g), respectively.

The ethyl acetate residue (15.0 g) was subjected to silica gel column, eluting with solvent system n-hexane-CH₂Cl₂-MeOH (increasing amounts of CH₂Cl₂ from 70 to 100 % and MeOH from 0 to 35%) to yield 18 fractions (F1-F18). The fraction F3 (350 mg) was rechromatographed over a flash silica gel column using n-hexane-EtOAc (gradient from 99:1 to 85:15) as eluant to give compound 2 (33 mg). The fraction F9 (350 mg) was rechromatographed on silica gel column, eluted with hexane-EtOAc (8:2) to give 3 (15 mg). The fraction F14.3 (325 mg) was further separated by column chromatography on silica gel eluted with CH₂Cl₂-MeOH to afford compound 7 (35 mg) was rechromatographed on Sephadex LH-20 column eluting with MeOH to afford compound 7 (9 mg). The fraction F7 (419 mg) was rechromatographed on silica gel column, eluted with hexane-EtOAc (9:1) to give β-sitosterol (25 mg). The fraction F14.3 (325 mg) was further separated by column chromatography on silica gel eluted with CH₂Cl₂-MeOH (gradient from 100:1 to 80:20) to give seven subfractions (F14.1-F14.7). The subfraction F14.3 was purified on Sephadex LH-20 column eluting with MeOH to afford compound 1 (9 mg). The fraction F7 (419 mg) was rechromatographed on silica gel column, eluted with hexane-EtOAc (9:1) to give β-sitosterol (25 mg). The fraction F18 was repeatedly separated on Sephadex LH-20 column, eluted with MeOH to yield β-sitosterol glucoside (12 mg). NMR spectral data of β-sitosterol and β-sitosterol glucoside are in good agreement with those of published data [5, 6].

Inumakiol D (1): Yellow solid. ESI-MS m/z 355.3 [M + Na]⁺.

1H-NMR (500 MHz, CDCl₃ & CD₃OD): δH 6.99 (1H, d, J = 8.5 Hz), 6.68 (1H, d, J = 8.5 Hz), 4.99 (1H, br s), 3.53-3.50 (1H, m), 1.42 (3H, d, J = 7.0 Hz), 1.37 (3H, d, J = 7.0 Hz), 1.31 (3H, s), 1.07 (3H, s).

13C-NMR (125 MHz, CDCl₃ & CD₃OD), see table 1.

Totarol (2): Colorless solid. ESI-MS m/z 287.5 [M+H]⁺.

1H-NMR (500 MHz, CDCl₃): δH 6.98 (1H, d, J = 8.5 Hz), 6.49 (1H, d, J = 8.5 Hz), 4.51 (1H, s), 3.30-3.27 (1H, m), 2.93 (1H, dd, J = 17.0, 6.5 Hz), 1.35 (1H, d, J = 7.0 Hz), 1.33 (1H, d, J = 7.0 Hz), 1.17 (3H, s), 0.94 (3H, s), 0.91 (3H, s).

13C-NMR (125 MHz, CDCl₃), see table 1.

Totarol-19-carboxylic acid (3):

1H-NMR (500 MHz, CDCl₃): δH 6.99 (1H, d, J = 8.5 Hz), 6.52 (1H, d, J = 8.5 Hz), 3.31 – 3.25 (1H, m), 2.95 (1H, dd, J = 16.5, 4.5 Hz), 1.35 (1H, d, J = 7.0 Hz), 1.34 (1H, d, J = 7.0 Hz), 1.33 (1H, s), 1.12 (1H, s).

13C-NMR (125 MHz, CDCl₃), see table 1.

3. RESULTS AND DISCUSSION

Compound 1 was obtained as yellow solid. The positive ESI-MS of 1 gave a molecular ion peak at m/z 355.3 [M+Na]⁺, corresponding to the molecular formula C₂₀H₂₉O₄.

The 1H- and 13C-NMR spectra of 1 (table 1) showed the presence of an isopropyl group [δC 28.10 (d), 20.64 (q), 20.68 (q) and δH 3.53-3.50 (m), 1.42 (d), 1.37 (d)], an aromatic ring [δC 154.36 (s), 140.35 (s), 134.24 (s), 133.39 (s), 124.21 (d), 117.13 (d)], two methyl carbons [δC 28.57 (q) and 22.51 (q)] attached to quaternary carbons, and a carboxyl group [δC 181.13 (s)]. These data and the molecular formula suggested that 1 was a diterpenoid. The HMBC correlations observed between H-12 (δH 6.68), H-16 (δH 1.37), H-17 (δH 1.42) and C-14 (δC 133.39), between H-15 (δH 3.53-3.50) and C-8 (δC 134.24); and between H-11 (δH 6.99), H-12 (δH 6.68), H-15 (δH 3.53-3.50) and C-13 (δC 154.36) indicated that the isopropyl group was attached at C-14 and the phenolic hydroxyl group was at C-13. Other HMBC correlations noted between H-5 (δH 1.97) and C-7 (δC 65.45) and between H-7 (δH 4.99) and C-5 (δC 45.38), C-8 (δC 134.24), C-9 (δC 140.35) showed that the hydroxyl group was located at C-7. From above spectral data, the structure of 1 was determined as inumakiol D. The 13C-NMR data (in pyridine-d₅) of 1 were in good agreement with those of inumakiol D in [7]. This compound was found for the first time in Podocarpus macrophyllus [7].

Figure 1: The structure of compounds 1-3 isolated from Podocarpus nerifolius wood.

1H- and 13C-NMR spectral data of compound 2 were similar with those of 1 except for the presence of an additional methyl group and the absence of one carbonyl and one secondary hydroxyl. Its 1H- and 13C-NMR spectra exhibited the presence of an
isopropyl group [\(\delta_c 27.15 \ (d), \ 20.34 \ (q), \ 20.34 \ (q)\) and \(\delta_{\text{H}} 3.33-3.27 \ (m), \ 1.35 \ (d), \ 1.33 \ (d)\)], an aromatic ring [\(\delta_c 151.97 \ (s), \ 143.19 \ (s), \ 134.01 \ (s), \ 130.97 \ (s), \ 122.97 \ (d), \ 114.27 \ (d)\)], three methyl carbons [\(\delta_c 33.23 \ (q), \ 25.17 \ (q)\) and \(21.57 \ (q)\)] attached to quaternary carbons. In addition, it showed five methylene carbons [\(\delta_c 41.56, \ 39.58, \ 28.75, \ 19.48, \ 19.34\)], a methine carbon [\(\delta_c 37.68, \ 33.25\)], \(1^H\) and \(1^C\)-NMR data of 2 are identical with those of totarol in previous report [8]. So, compound 2 was determined as totarol-19-carboxylic acid by comparison with reported data [9]. This compound was found in some Podocarpus species [7, 9].

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**REFERENCES**


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**Table 1:** \(^1^C\)-NMR spectral data of compounds 1 (in CDCl\(_3\) + CD\(_3\)OD) and 2-3 (in CDCl\(_3\))

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</table>

1D-NMR spectra of compound 3 are also similar to those of compound 1 except for the absence of a hydroxyl group at C-7. Thus, 3 was elucidated as

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