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Diterpenoids from the wood of *Podocarpus neriifolitus*

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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 - "Thông 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

^{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. 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-CH_2Cl_2-MeOH (increasing amounts of CH_2Cl_2 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 (325 mg) was further separated by column chromatograph on silica gel eluted with CH_2Cl_2-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 $[M + Na]^+$.

^{1}H-NMR (500 MHz, $CDCl_3$ & CD_3OD): δ_H 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*).

^{13}C-NMR (125 MHz, $CDCl_3$ & CD_3OD), see table 1.

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

^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 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*).

^{13}C-NMR (125 MHz, $CDCl_3$), see table 1.

Totarol-19-carboxylic acid (3):

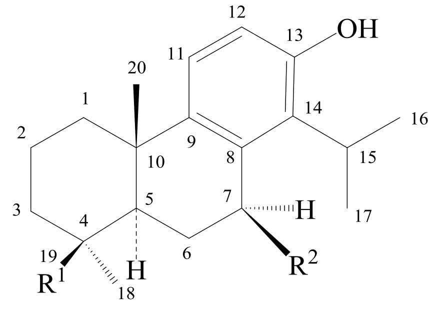
^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 6.99 (1H, *d*, *J* = 8.5 Hz), 6.52 (1H, *d*, *J* = 8.5 Hz), 3.31 – 3.25 (1H, *m*), 2.95 (1H, *dd*, *J* = 16.5, 4.5 Hz), 1.35 (1H, *d*, *J* = 7.0 Hz), 1.34 (1H, *d*, *J* = 7.0 Hz), 1.33 (1H, *s*), 1.12 (1H, *s*).

^{13}C-NMR (125 MHz, $CDCl_3$), 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 $C_{20}H_{28}O_4$.

The ^{1}H- and ^{13}C-NMR spectra of **1** (table 1) showed the presence of an isopropyl group [δ_C 28.10 (*d*), 20.64 (*q*), 20.68 (*q*) and δ_H 3.53-3.50 (*m*), 1.42 (*d*), 1.37 (*d*)], an aromatic ring [δ_C 154.36 (*s*), 140.35 (*s*), 134.24 (*s*), 133.39 (*s*), 124.21 (*d*), 117.13 (*d*)], two methyl carbons [δ_C 28.57 (*q*) and 22.51 (*q*)] attached to quaternary carbons, and a carboxyl group [δ_C 181.13 (*s*)]. These data and the molecular formula suggested that **1** was a diterpenoid. The HMBC correlations observed between H-12 (δ_H 6.68), H-16 (δ_H 1.37), H-17 (δ_H 1.42) and C-14 (δ_C 133.39), between H-15 (δ_H 3.53-3.50) and C-8 (δ_C 134.24); and between H-11 (δ_H 6.99), H-12 (δ_H 6.68), H-15 (δ_H 3.53-3.50) and C-13 (δ_C 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 (δ_H 1.97) and C-7 (δ_C 65.45) and between H-7 (δ_H 4.99) and C-5 (δ_C 45.38), C-8 (δ_C 134.24), C-9 (δ_C 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 ^{13}C-NMR data (in pyridine-d_5) 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].



1: R^1 = COOH R^2 = OH

2: R^1 = CH_3 R^2 = H

3: R^1 = COOH R^2 = H

Figure 1: The structure of compounds **1-3** isolated from *Podocarpus neriifolius* wood

^{1}H- and ^{13}C-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 ^{1}H- and ^{13}C-NMR spectra exhibited the presence of an isopropyl group [δ_C 27.15 (*d*), 20.34 (*q*), 20.34 (*q*) and δ_H 3.33-3.27 (*m*), 1.35 (d), 1.33 (*d*)], an aromatic ring [δ_C 151.97 (*s*), 143.19 (*s*), 134.01 (*s*), 130.97 (*s*), 122.97 (*d*), 114.27 (*d*)], three methyl carbons [δ_C33.23 (*q*), 25.17 (*q*) and 21.57 (*q*)] attached to quaternary carbons. In addition, it showed five methylene carbons (δ_C 41.56, 39.58, 28.75, 19.48, 19.34), a methine carbon (δ_C 49.55) and two quaternary carbons (δ_C 37.68, 33.25). ^{1}H- and ^{13}C-NMR data of **2** are identical with those of totarol in previous report [8]. So, compound **2** was determined as totarol.

Table 1: ^{13}C-NMR spectral data of compounds **1** (in $CDCl_3$ + CD_3OD) and **2-3** (in $CDCl_3$)

Position	**1**	**2** ($CDCl_3$)	**3** ($CDCl_3$)
1	40.09	41.56	40.11
2	20.44	19.47	20.07
3	37.72	39.58	37.22
4	43.5	33.25	43.75
5	45.38	49.55	52.08
6	31.15	19.34	21.09
7	65.45	28.75	30.01
8	134.24	134.01	134.26
9	140.35	143.19	140.98
10	38.92	37.68	38.52
11	124.21	122.97	124.14
12	117.13	114.27	114.58
13	154.36	151.97	152.05
14	133.39	130.97	130.89
15	28.10	27.15	27.26
16	20.64	20.34	20.31
17	20.68	20.34	20.41
18	28.57	33.23	28.62
19	181.13	21.57	183.91
20	22.51	25.17	23.20

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 totarol-19-carboxylic acid by comparison with reported data [9]. This compound was found in some *Podocarpus* species [7, 9].

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