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TERPENOIDS FROM THE LEAVES AND STEMS OF *DYSOXYLUM TPONGENSE*[#]

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Abstract. Study on chemical constituents from the leaves and stems of *Dysoxylum tpongense* Pierre resulted in the isolation of six known compounds (**1**–**6**). The chemical structures of isolated compounds were identified as cabraleahydroxylactone (**1**), cabraleahydroxylactone-3-acetate (**2**), (+)-spathulenol (**3**), *β*-sitosterol (**4**), stigmasterol (**5**), and stigmast-4-en-3-one (**6**) by comparison of the physicochemical parameters, the NMR and mass spectral data with those reported in the literature.

Keywords: Dysoxylum tpongense Pierre, Meliaceae, cabraleahydroxylactone, phytosterol.

Classification numbers: 1.1.1, 1.1.6

1. INTRODUCTION

The genus *Dysoxylum*, belonging to the family Meliaceae, comprises about 75 species, growing widely in South and South-eastern Asia such as Malaysia, India, Indonesia, China, New Zealand and Australia [1]. In Vietnam, 14 species distributed throughout the country have been described [2]. Study of several species of this genus such as *Dysoxylum kuskususense* [3], *D. richii*, *D. hainanense*, *D. acutangulum* [4], *D. cauliflorum* [5], *D. variabile* [6], *D. spectabile* [7], etc led to the isolation and structural elucidation of different types of triterpenoids [5, 8], diterpenoids [7], sesquiterpenoids [6] and limonoids [7] with many biological activities including osteoclast differentiation inhibitory activity [9], acetylcholinesterase inhibitory activity [10] and cytotoxic effects against different cancer cell lines such as KB cells [6], multidrug-

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resistant KB cell line KB-C2 (colchicine resistant) [11], leukemia cell line HL-60 [11], liver cancer cell line SMMC-7721 [11] and hepatocarcinoma cell HepG2 [8], breast cancer MCF-7 [11], leukemia K-562 and lung cancer NCI-H522 [3].

Dysoxylum tpongense Pierre (*D. tpongense*), local name Huynh dan bap, is a tree (3–10 m height) with oblong leaflets and 14 x 8 mm capsules, distributed commonly in the mountainous areas 1000–1100 m higher than sea level. So far, no chemical studies on this species have been reported. In the present paper, we described the isolation and structural elucidation of six known compounds, cabraleahydroxylactone (**1**), cabraleahydroxylactone-3-acetate (**2**), (+)-spathulenol (**3**), β-sitosterol (**4**), stigmasterol (**5**), and stigmast-4-en-3-one (**6**) from the methanol extract of the leaves and stems of *D. tpongense*.

2. MATERIALS AND METHODS

2.1. General experimental procedures

NMR spectra were measured on a Bruker AVANCE 600 MHz spectrometer and Varian Unity Inova 500 MHz spectrometer. APCI-MS (+) spectra was determined by Agilent 1260 Series Single Quadrupole LC/MS systems. Column chromatography (C.C) was carried out on silica gel (Si 60 F_{254}, 230-400 mesh, Merck). All solvents were distilled before use. Precoated plates of silica gel 60 F_{254} were used for analytical purposes. Compounds were visualized under UV radiation (254, 365 nm) and by spraying plates with 10 % H_2SO_4 followed by heating with a heat gun. HPLC was carried out using a Water system with a UV detector and an YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size, YMC Co., Ltd., Japan) and HPLC solvents were from Burdick & Jackson, USA.

2.2. Plant material

The leaves and stems of *Dysoxylum tpongense* Pierre were collected in Mai Chau, Hoa Binh province, Viet Nam. The scientific name of the plant was identified by ethno-botanist Ngo Van Trai (National Institute of Medicinal Materials, NIMM). A voucher specimen (C-400) was deposited in the herbarium of the Institute of Natural Products Chemistry, VAST, Ha Noi, Viet Nam.

2.3. Extraction and isolation

Dried powdered leaves and stems of *D. tpongense* (1.6 kg) were extracted with MeOH under reflux for three times (each 3 L) and then concentrated under decreased pressure to yield a black crude MeOH extract (80.0 g). The crude MeOH extract was then suspended in hot MeOH-water (1:1, v/v) and successively partitioned with dichloromethane (DCM) and ethyl acetate (EtOAc). The resulting fractions were concentrated under decreased pressure to give the corresponding solvent-soluble fractions DCM (20.0 g), EtOAc (2.4 g), and water layer (90 mL).

The DCM fraction (20.0 g) was subjected to C.C on flash silica gel column (400–630 mesh) with gradient solvents of hexane–acetone (1:0 to 0:1, v/v) to afford 7 fractions (Fr. D1 to D7).

Fraction D2 was further subjected on a silica gel C.C, eluting with an isocratic solvent mixture of hexane–DCM–EtOAc (2:1:0.2, v/v/v), to obtain 6 sub-fractions (D2.1 to D2.6). The

sub-fraction D2.2 was chromatographed on a YMC RP-18 column, eluting with methanol-water (5:1, v/v) to yield compound **3** (20.3 mg).

Fraction D3 was chromatographed on a silica gel C.C using a solvent mixture of hexane–DCM–methanol (7:1:0.1, v/v/v) to produce three fractions (D3.1 to D3.3). Fraction D3.2 was further chromatographed over a silica gel column using *n*-hexane–acetone (6:1, v/v) to obtain 4 sub-fractions (D3.2.1 to D3.2.4). The sub-fraction D3.2.1 was purified on an YMC C_{18} column using acetone–water (3:1, v/v) as mobile phase to yield compounds **4** (20.0 mg), **5** (12.5 mg), and **6** (15.0 mg).

Fraction D4 was chromatographed on a silica gel column, eluting with *n*-hexane–acetone (5:1, v/v) to obtain 6 fractions (D4.1 to D4.6). Fraction D4.5 was further chromatographed on a silica gel column using DCM–hexane–EtOAc (3:1:0.6, v/v/v) to produce 5 sub-fractions (D4.5.1 to D4.5.5). The sub-fraction D4.5.1 was purified on a silica gel C.C by using gradient solvents hexane–DCM–EtOAc (3:1:0.2, v/v/v) to get compound **1** (4.0 mg). The sub-fraction D4.5.5 was subjected to C.C. over silica gel eluting with hexane–acetone (8:1 to 2:1, v/v) to yield compound **2** (16 mg).

Cabraleahydroxylactone (**1**): amorphous powder; $C_{27}H_{44}O_3$ (M = 416); m.p: 241–242 ^{0}C; $[\alpha]_D^{22}$ = +18.0 (*c* 0.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ_H (ppm): 1.25 (1H, m, H-1a), 1.39 (1H, m, H-1b), 1.81 (1H, m, H-2a), 1.93 (1H, m, H-2b), 3.38 (1H, br s, H-3), 1.24 (1H, m, H-5), 1.40 (2H, m, H-6), 1.24 (1H, m, H-7a), 1.58 (1H, m, H-7b), 1.42 (1H, m, H-9), 1.17 (1H, m, H-11a), 1.52 (1H, m, H-11b), 1.59 (1H, m, H-13), 1.09 (1H, m, H-15a), 1.48 (1H, m, H-15b), 1.82 (1H, m, H-16a), 1.94 (1H, m, H-16b), 1.98 (1H, m, H-17), 0.94 (3H, s, CH_3-18), 0.83 (3H, s, CH_3-19), 1.35 (3H, s, CH_3-21), 1.91 (1H, m, H-22a), 2.10 (1H, m, H-22b), 2.53 (1H, m, H-23a), 2.63 (1H, m, H-23b), 0.93 (3H, s, CH_3-25), 0.82 (3H, s, CH_3-26), 0.88 (3H, s, CH_3-27); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ_C (ppm): 33.6 (C-1), 25.3 (C-2), 76.2 (C-3), 37.6 (C-4), 49.4 (C-5), 18.2 (C-6), 35.0 (C-7), 40.5 (C-8), 50.3 (C-9), 37.2 (C-10), 21.2 (C-11), 26.8 (C-12), 43.1 (C-13), 50.3 (C-14), 31.1 (C-15), 49.3 (C-16), 15.5 (C-17), 16.0 (C-18), 25.3 (C-19), 90.2 (C-20), 25.4 (C-21), 31.2 (C-22), 29.2 (C-23), 176.8 (C-24), 28.3 (C-25), 22.1 (C-26), 16.3 (C-27).

Cabraleahydroxylactone-3-acetate (**2**): white powder; $C_{29}H_{46}O_4$ (M = 458); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ_H (ppm): 1.45 (1H, m, H-1a), 1.15 (1H, m, H-1b), 1.87 (1H, m, H-2a), 1.61 (1H, m, H-2b), 4.62 (1H, t, *J* = 2.4 Hz, H-3), 1.23 (1H, m, H-5), 1.43 (2H, m, H-6), 1.28 (1H, m, H-7a), 1.59 (1H, m, H-7b), 1.43 (1H, m, H-9), 1.50 (1H, m, H-11a), 1.19 (1H, m, H-11b), 1.75 (1H, m, H-12a), 1.25 (1H, m, H-12b), 1.59 (1H, m, H-13), 1.93 (1H, m, H-15a), 1.51 (1H, m, H-15b), 1.85 (1H, m, H-16a), 1.26 (1H, m, H-16b), 2.00 (1H, m, H-17), 0.97 (3H, s, CH_3-18), 0.86 (3H, s, CH_3-19), 1.37 (3H, s, CH_3-21), 2.11 (1H, m, H-22a), 1.14 (1H, m, H-22b), 2.64 (1H, m, H-23a), 2.56 (1H, m, H-23b), 0.84 (3H, s, CH_3-25), 0.88 (3H, s, CH_3-26), 0.93 (3H, s, CH_3-27), 2.08 (3H, s, CO$\underline{CH_3}$); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ_C (ppm): 34.2 (C-1), 22.9 (C-2), 78.3 (C-3), 36.7 (C-4), 50.7 (C-5), 18.0 (C-6), 35.1 (C-7), 40.5 (C-8), 50.3 (C-9), 37.2 (C-10), 21.2 (C-11), 26.8 (C-12), 43.1 (C-13), 50.3 (C-14), 31.1 (C-15), 25.0 (C-16), 49.3 (C-17), 15.5 (C-18), 16.0 (C-19), 90.2 (C-20), 25.4 (C-21), 31.2 (C-22), 29.2 (C-23), 176.8 (C-24), 27.9 (C-25), 21.7 (C-26), 16.4 (C-27), 170.8 ($\underline{C}OCH_3$), 21.2 (CO$\underline{C}H_3$).

(+)-spathulenol (**3**): colorless oil; $C_{15}H_{24}O$ (M = 220); $[\alpha]_D^{22}$ = +57.3 (*c* 0.21, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ_H (ppm): 0.47 (1H, dd, *J* = 9.7, 11.6 Hz, H-1), 0.72 (1H, ddd, *J* = 5.5, 8.9, 11.6 Hz, H-2), 1.01 (1H, d, *J* = 16.4 Hz, H-3a), 1.98 (1H, m, H-3b), 2.04 (1H, t, *J* = 13.1 Hz, H-4a), 2.42 (1H, dd, *J* = 6.2, 13.1 Hz, H-4b), 2.21 (1H, dt, *J* = 9.7, 6.2 Hz, H-6), 1.90 (1H, m, H-7a), 1.64 (1H, m, H-7b), 1.78 (1H, m, H-8a), 1.59 (1H, m, H-8b), 1.31 (1H, t, *J* = 9.7 Hz, H-10),

1.04 (3H, s, CH_3-12), 1.06 (3H, s, CH_3-13), 4.69 (1H, s, H-14a), 4.67 (1H, s, H-14b), 1.28 (3H, s, CH_3-15); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ_C (ppm): 29.9 (C-1), 27.5 (C-2), 24.8 (C-3), 38.9 (C-4), 153.4 (C-5), 53.4 (C-6), 26.7 (C-7), 41.7 (C-8), 81.0 (C-9), 54.3 (C-10), 20.3 (C-11), 26.1 (C-12), 28.7 (C-13), 106.3 (C-14), 16.3 (C-15).

***β*-sitosterol** (**4**): white powder; $C_{29}H_{50}O$ (M = 414); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (ppm): 5.34 (1H, m, H-6), 3.51 (1H, m, H-3), 0.70 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 0.94 (3H, d, *J* = 6.5 Hz, CH_3-21), 0.83 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.85 (3H, d, *J* = 6.5 Hz, CH_3-27), 0.88 (3H, t, *J* = 7.0 Hz, CH_3-29); ^{13}C-NMR (125 MHz, $CDCl_3$), δ_C (ppm): 140.8 (C-5), 33.9 (C-22), 26.1 (C-23), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 55.9 (C-17), 51.2 (C-9), 50.1 (C-24), 42.3 (C-4), 42.2 (C-12), 40.4 (C-20), 37.2 (C-7), 36.1 (C-1), 42.3 (C-13), 33.9 (C-25), 31.8 (C-8), 31.6 (C-13), 31.6 (C-16), 29.1 (C-2), 28.2 (C-28), 26.0 (C-15), 24.2 (C-11), 21.1 (C-26), 21.0 (C-21), 19.3 (C-27), 18.7 (C-19), 12.2 (C-29), 12.0 (C-18).

Stigmasterol (**5**): colorless needles; $C_{29}H_{48}O$ (M=412); m.p: 166–167 ^{0}C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (ppm): 5.35 (1H, m, H-6), 3.52 (1H, m, H-3), 0.70 (3H, s, CH_3-18), 0.96 (3H, s, CH_3-19), 0.92 (3H, d, *J* = 6.5 Hz, CH_3-21), 5.16 (1H, dd, *J* = 15.0, 8.5 Hz, H-22), 5.03 (1H, dd, *J* = 15.0, 8.5 Hz, H-23), 0.80 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.85 (3H, d, *J* = 7.0 Hz, CH_3-27), 0.80 (3H, t, *J* = 7.0 Hz, CH_3-29); ^{13}C-NMR (125 MHz, $CDCl_3$), δ_C (ppm): 140.7 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 56.0 (C-17), 50.2 (C-9), 51.2 (C-24), 42.3 (C-4), 39.8 (C-12), 40.5 (C-20), 31.9 (C-7), 37.2 (C-1), 42.2 (C-13), 29.1 (C-25), 31.9 (C-8), 28.2 (C-16), 31.6 (C-2), 23.0 (C-28), 24.3 (C-15), 21.1 (C-11), 19.0 (C-26), 18.7 (C-21), 19.8 (C-27), 19.4 (C-19), 12.2 (C-29), 12.0 (C-18).

Stigmast-4-en-3-one (**6**): amorphous powder; m.p: 95.0–96.0 ^{o}C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 5.71 (1H, s, H-4), 0.70 (3H, s, CH_3-18), 1.17 (3H, s, CH_3-19), 0.91 (d, *J* = 6.6 Hz, CH_3-21), 0.82 (3H, d, *J* = 6.6 Hz, CH_3-26), 0.80 (3H, d, *J* = 6.6 Hz, CH_3-27), 0.84 (3H, t, *J* = 6.8, CH_3-29) ; ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 35.5 (C-1), 33.8 (C-2), 199.7 (C-3), 123.7 (C-4), 171.7 (C-5), 32.9 (C-6), 32.0 (C-7), 35.6 (C-8), 53.8 (C-9), 38.6 (C-10), 21.0 (C-11), 39.6 (C-12), 42.3 (C-13), 55.8 (C-14), 24.2 (C-15), 28.2 (C-16), 55.9 (C-17), 11.9 (C-18), 17.4 (C-19), 36.1 (C-20), 18.7 (C-21), 34.0 (C-22), 26.0 (C-23), 45.7 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.0 (C-28), 11.9 (C-29).

3. RESULTS AND DISSCUSSION

Repeated column chromatography of the DCM-soluble fraction from the leaves and stems of *D. tpongense* resulted in the isolation of six known compounds (**1**–**6**). The chemical structures of six compounds were identified as cabraleahydroxylactone (**1**), cabraleahydroxylactone-3-acetate (**2**), (+)-spathulenol (**3**), *β*-sitosterol (**4**), stigmasterol (**5**) and stigmast-4-en-3-one (**6**) by comparison of the physicochemical and spectroscopic data with that reported in the literature.

Compound **1** was isolated as amorphous powder with the molecular formula $C_{27}H_{44}O_{13}$. The ^{1}H-NMR spectrum of **1** showed signals for six tertiary methyl groups [δ_H 0.94 (3H, s, CH_3-18), 0.83 (3H, s, CH_3-19), 0.93 (3H, s, CH_3-25), 1.35 (3H, s, CH_3-21), 0.82 (3H, s, CH_3-26), and 0.88 (3H, s, CH_3-27)] and an oxymethine proton at δ_H 3.38 (1H, br s, H-3). The ^{13}C-NMR spectrum showed signals corresponding to 27 carbons, including a carboxylic carbon at δ_C 176.8 (C-24), a quaternary oxygenated carbon at δ_C 90.2 (C-20), and six tertiary methyl carbons [δ_C 16.0 (C-18), 25.3 (C-19), 25.4 (C-21), 28.3 (C-25), 22.1 (C-26), and 16.3 (C-27)]. The above proton and carbon data suggested that **1** were assignable to a dammarane-type nortriterpenoid

[12]. Thus, the structure of **1** was determined to be cabraleahydroxylactone by comparison with the published data [13].

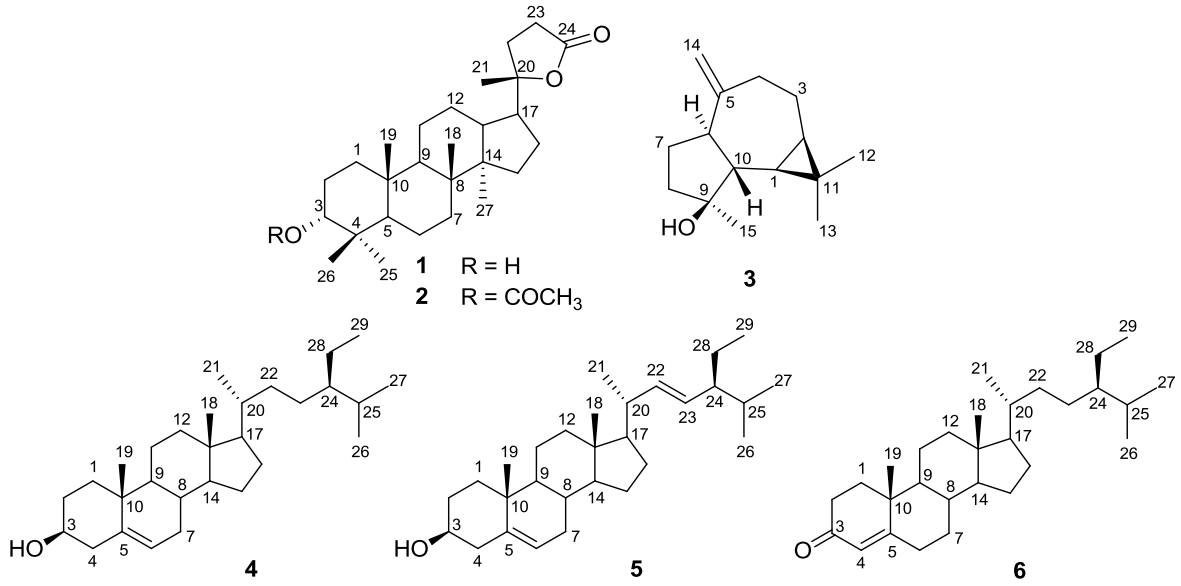

Figure 1. Structures of compounds isolated from *Dysoxylum tpongense.*

Compound **2** was obtained as white powder with the molecular formula $C_{29}H_{46}O_4$. The ^{1}H- and ^{13}C-NMR spectra of **2** are similar to those of cabraleahydroxylactone (**1**). However, the hydroxyl group located at C-3 of compound **1** was replaced by an acetate group in **2** [δ_H 2.08 (COC$\underline{H}_3$), δ_C 21.3 (CO$\underline{C}H_3$), and 170.8 ($\underline{C}OCH_3$)]. Based on this analysis and by comparison with the published data, the structure of compound **2** was determined to be a derivative of compound **1** and named cabraleahydroxylactone-3-acetate [14].

Compound **3** was isolated as colorless oil and its mass spectral data suggested the molecular formula as $C_{15}H_{24}O$. Its optical rotation had a value of +57.3 (*c* 0.21, $CHCl_3$). The ^{1}H-NMR spectrum of **3** showed signals for three tertiary methyl groups [δ_H 1.04 (CH_3-12), 1.06 (CH_3-13), and 1.28 (CH_3-15)], four methine proton signals [δ_H 0.47 (H-1), 0.72 (H-2), 2.21 (H-6), and 1.31 (H-10)], as well as signals for five methylene groups [δ_H 1.01 (H-3a), 1.98 (H-3b), 2.04 (H-4a), 2.42 (H-4b), 1.90 (H-7a), 1.64 (H-7b), 1.78 (H-8a), 1.59 (H-8b), 4.69 (H-14a), and 4.67 (H-14b)]. The ^{13}C-NMR spectrum of **3** showed signals corresponding to 15 carbons, including three quaternary carbons [δ_C 153.4 (C-5), 81.0 (C-9), and 20.3 (C-11)], three tertiary methyl carbons [δ_C 26.1 (C-12), 28.7 (C-13), and 16.3 (C-15)], four methine carbons [δ_C 29.9 (C-1), 27.5 (C-2), 53.4 (C-6), and 54.3 (C-10)] and five methylene carbons [δ_C 24.8 (C-3), 38.9 (C-2), 26.7 (C-7), 41.7 (C-8) and 106.3 (C-14)]. These above proton and carbon data suggested that **3** were assignable to a sesquiterpene lactone and thus, compound **3** was determined to be (+)-spathulenol by comparison with the published data [15].

Compound **4** was isolated as white powder and its mass spectral data suggested the molecular formula as $C_{29}H_{50}O$. The ^{1}H-NMR spectrum of **4** showed the presence of six methyl signals that appeared as two methyl singlet [δ_H 0.70 (3H, s, CH_3-18), and 1.01 (3H, s, CH_3-19)], three methyl

doublets that appeared [δ_H 0.94 (3H, d, J = 6.5 Hz, CH_3-21), 0.83 (3H, d, J = 6.5 Hz, CH_3-26), and 0.85 (3H, d, J = 6.5 Hz, CH_3-27)] and a methyl triplet at δ_H 0.88 (3H, t, J = 7.0 Hz, CH_3-29). The $^{1H\text{-}NMR}$ spectra of **4** also showed one olefinic proton at δ_H 5.34 (1H, m, H-6) and a proton corresponding to the proton connected to the C-3 hydroxyl group at δ_H 3.51 (1H, m, H-3). The ^{13}C-NMR spectrum showed signals corresponding to 29 carbons, including six methyls, eleven methylene, ten methines and three quaternary carbons [(C-5, C-10, C-13)]. Based on these evidences, the structure of **4** was assigned as β-sitosterol by comparison with the published data [16].

Compound **5** was obtained as colorless needles with the molecular formula $C_{29}H_{48}O$. The ^{1}H- and ^{13}C-NMR spectra of **5** are similar to those of β-sitosterol (**4**). However, two olefinic proton signals [δ_H 5.16 (1H, dd, J = 15.0, 8.5 Hz, H-22), 5.03 (1H, dd, J = 15.0, 8.5 Hz, H-23) and the corresponding carbon signals were found [δ_C 138.3 (C-22), and 129.3 (C-23)] in **5**, respectively. Thus, the structure of compound **5** was determined as stigmasterol [16].

The ^{1}H- and ^{13}C-NMR spectra of **6** are also similar to those of β-sitosterol (**4**). However, the hydroxyl group located at C-3 of **4** was replaced by a ketone group [δ_C 199.7 (C=O)] in **6**. The ^{1}H- and ^{13}C-NMR spectra of **6** also showed signals for one olefinic group [(δ_H 5.71 (1H, s, H-4), δ_C 123.7 (C-4), and 171.7 (C-5)]. Based on the above analysis, the structure of compound **6** was determined to be stigmast-4-en-3-one by comparison with the published data [17].

4. CONCLUSION

Chemical investigation of *Dysoxylum tpongense* Pierre resulted in the isolation of six known compounds (**1**–**6**), namely cabraleahydroxylactone (**1**), cabraleahydroxylactone-3-acetate (**2**), (+)-spathulenol (**3**), β-sitosterol (**4**), stigmasterol (**5**), and stigmast-4-en-3-one (**6**). Their structures were elucidated by the interpretation of NMR spectral data, mass spectra as well as comparison with those from the literature.

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