



Terpenoids and sterol from *Aphanamixis polystachya*

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ABSTRACT

The dichloromethane extract of the air-dried leaves of *Aphanamixis polystachya* (Wall.) Parker afforded α -copaene (1), squalene (2), polyprenol (3), β -sitosterol (4), lutein (5), and β -carotene (6). The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry.

Key words: *Aphanamixis polystachya*, Meliaceae, α -copaene, squalene, polyprenol, β -sitosterol, lutein, β -carotene

INTRODUCTION

Aphanamixis polystachya (Wall.) Parker of the family Meliaceae is a native of Indonesia, Malaysia, Singapore, Taiwan, and the Province of China [1]. The bark of *A. polystachya* was reported to exhibit antioxidant [2], antitumor [3], and radioprotective [4] properties. Furthermore, the bark showed antifeedant and repellent properties as well as toxicity against the red flour beetle [5]. *A. polystachya* leaf extracts exhibited antimicrobial, cytotoxic and antioxidant activities [6]. A number of studies have been conducted on the chemical constituents of the different parts of *A. polystachya* which reported the isolation of diterpenes [7], limonoids [8-15], lignans [16], flavonoid glycosides and a chromone [17], triterpenes [18, 19], sesquiterpenes [20], and alkaloids [21].

We report herein the isolation and structure elucidation of α -copaene (1), and the isolation and identification of squalene (2), polyprenol (3), β -sitosterol (4), lutein (5), and β -carotene (6) (Fig. 1) from the dichloromethane extract of the leaves of *A. polystachya*. This is the first report on the isolation of 1 from *A. polystachya*.

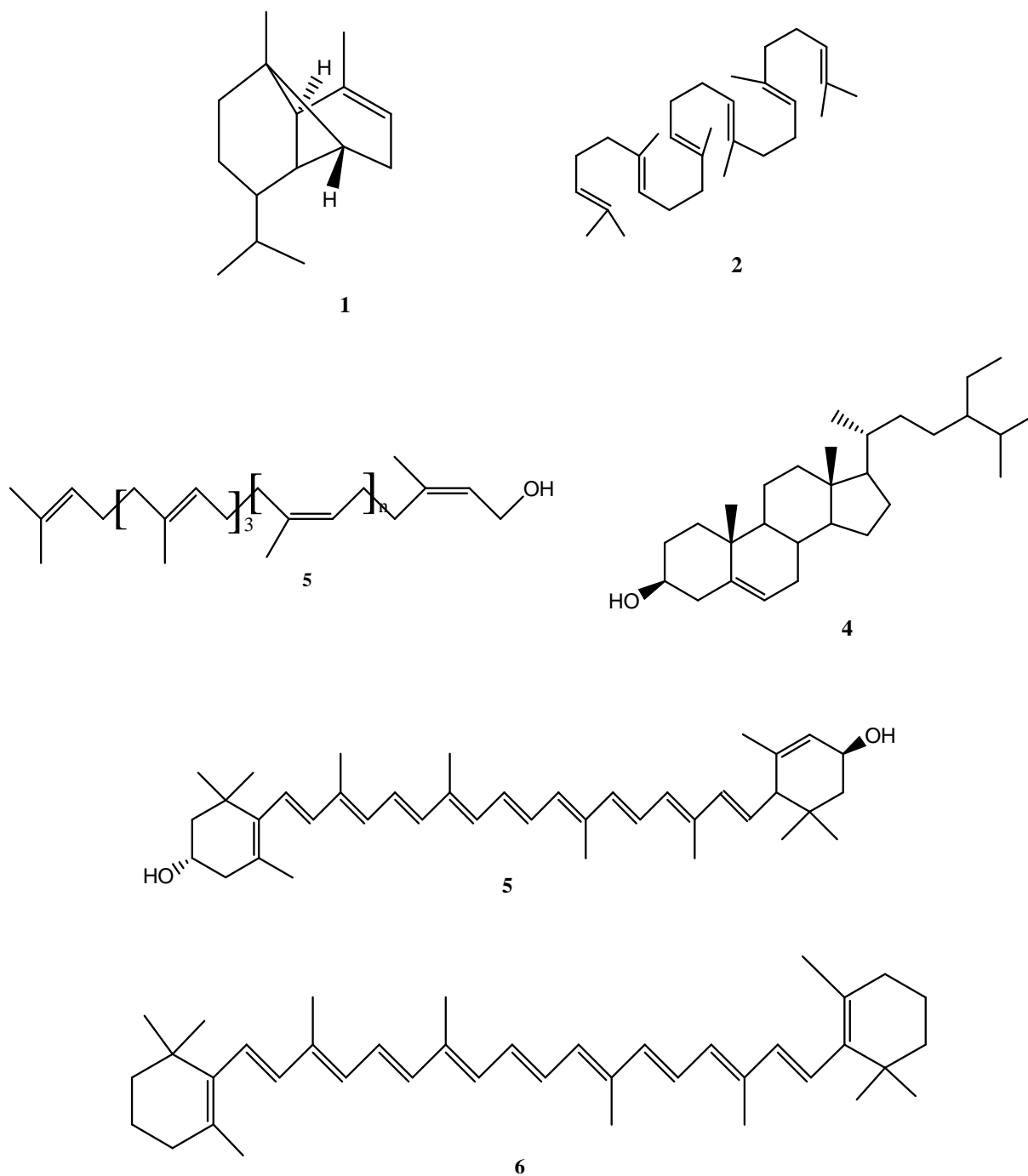


Fig. 1. Chemical Constituents of *Aphanamixis polystachya*: α -copaene (1), squalene (2), polyprenol (3), β -sitosterol (4), lutein (5), and β -carotene (6)

EXPERIMENTAL SECTION

General Experimental Procedures

HREIMS was obtained on a Finnigan MAT 95S mass spectrometer. NMR spectra were recorded on a Varian Unity Inova spectrometer in CDCl_3 at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR spectra. Column chromatography

was performed with silica gel 60 (70-230 mesh), while the TLC was performed with plastic-backed plates coated with silica gel F₂₅₄. The plates were visualized with vanillin-H₂SO₄ and warming.

A glass column (18 inches in height and 1.0 inch internal diameter) was packed with silica gel. The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10 % increments) as eluents. 100 mL fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *R_f* values were combined and rechromatographed. A glass column (12 inches in height and 0.5 inch internal diameter) was used for the rechromatography. 5 mL fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. 1 mL fractions were collected.

Sample Collection

Leaf samples of *Aphanamixis polystachya* were collected from Bacnotan, La Union, Philippines in March 2013. It was authenticated at the Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman, Quezon City, Philippines where a voucher specimen was deposited with accession number 14669.

Isolation of Constituents from the Leaves of *A. polystachya*

The air-dried leaves (1 kg) of *A. polystachya* were soaked in CH₂Cl₂ for three days, and then filtered. The filtrate was concentrated under vacuum to afford the crude extract (49 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10 % increments by volume as eluents. The CH₂Cl₂ fraction was rechromatographed using petroleum ether (5×) as eluent to afford **1** (35 mg). The 10 % acetone in CH₂Cl₂ fraction from the chromatography of the crude extract was rechromatographed (3×) in petroleum ether to afford **2** (15 mg) and **6** (12 mg) after washing with petroleum ether. The 20 % acetone in CH₂Cl₂ fraction was rechromatographed (4×) in 10 % EtOAc in petroleum ether to afford **3** (10 mg). The 30 % acetone in dichloromethane fraction was rechromatographed (3×) in 15 % EtOAc in petroleum ether to afford **4** (5 mg) after washing with petroleum ether. The 40 % to 60 % acetone in CH₂Cl₂ fractions were combined and rechromatographed (2×) in CH₃CN:Et₂O:CH₂Cl₂ (0.5:0.5:9 by volume) to afford **5** (28 mg) after washing with petroleum ether, followed by diethyl ether.

α-Copaene (**1**): ¹H NMR (150 MHz, CDCl₃): δ 2.08 (H-2), 5.17 (H-4, brt, *J* = 1.8 Hz), 2.15 (H₂-5), 1.55 (H-6), 1.68 (H-7), 1.58 (H-8), 1.48, 1.60 (H₂-9), 1.62, 1.74 (H₂-10), 1.52 (H-11), 0.80 (H₃-12, d, *J* = 6.5 Hz), 0.82 (H₃-13, d, *J* = 6.5 Hz), 0.77 (H₃-14, s), 1.63 (H₃-15, brs). ¹³C NMR (150 MHz, CDCl₃): δ 39.38 (C-1), 36.90 (C-2), 143.95 (C-3), 116.06 (C-4), 30.04 (C-5), 54.23 (C-6), 44.29 (C-7), 44.73 (C-8), 22.34 (C-9), 36.22 (C-10), 32.21 (C-11), 19.93 (C-12), 19.66 (C-13), 19.25 (C-14), 23.08 (C-15). HRMS: *m/z* = 204.1878 (Calcd. 204.1873 for C₁₅H₂₄ [M]⁺).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *Aphanamixis polystachya* (Wall.) Parker afforded **α-copaene** (**1**), **squalene** (**2**), **polyprenol** (**3**), **β-sitosterol** (**4**), **lutein** (**5**), and **β-carotene** (**6**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry. Furthermore, **1** gave similar ¹³C NMR resonances with those reported in the literature for **α-copaene** [22]. The structures of **squalene** [23], **polyprenol** [24], **β-sitosterol** [25], **lutein** [26], and **β-carotene** [25] were identified by comparison of their ¹H and/or ¹³C NMR data with those reported in the literature.

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