Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(6):65-68

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

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

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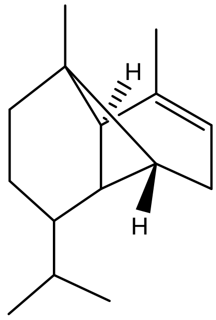
INTRODUCTION

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 repellant 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.*



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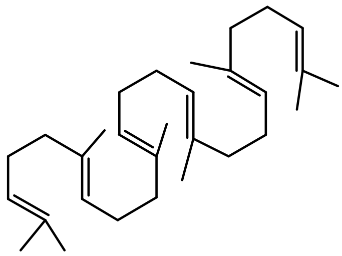

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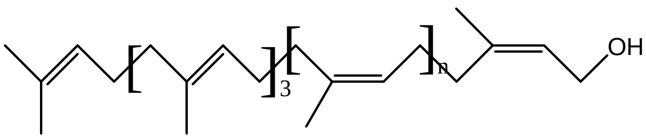

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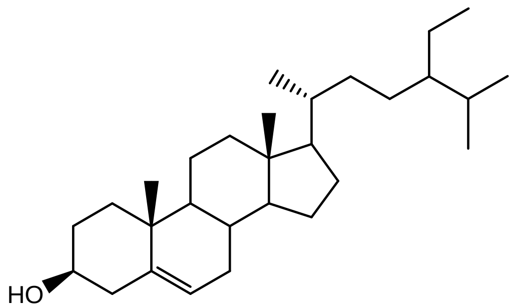

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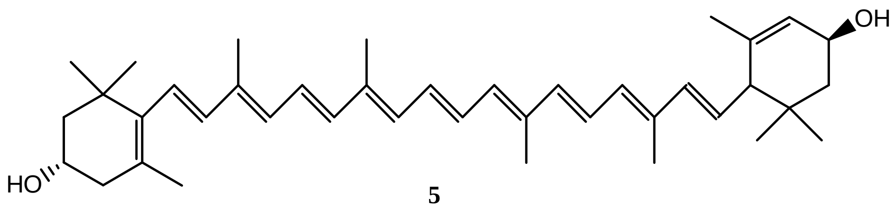

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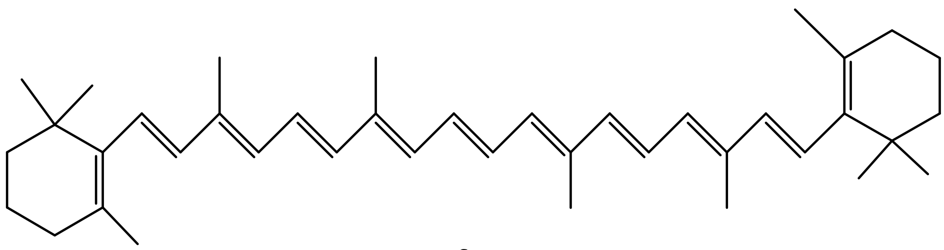

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Fig. 1. Chemical Constituents of Aphanamixis polystachya: α-copaene (1), squalene (2), polyprenol (3), β-sitosterol (4), lutein (5), and β-carotene (6)

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 F_{254}. The plates were visualized with vanillin-H_2SO_4 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 *Rf* 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 CH_2Cl_2 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 CH_2Cl_2 at 10 % increments by volume as eluents. The CH_2Cl_2 fraction was rechromatographed using petroleum ether (5×) as eluent to afford **1** (35 mg). The 10 % acetone in CH_2Cl_2 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 CH_2Cl_2 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 CH_2Cl_2 fractions were combined and rechromatographed (2×) in $CH_3CN:Et_2O:CH_2Cl_2$ (0.5:0.5:9 by volume) to afford **5** (28 mg) after washing with petroleum ether, followed by diethyl ether.

α-Copaene (**1**): ^{1}H NMR (150 MHz, $CDCl_3$): δ 2.08 (H-2), 5.17 (H-4, brt, *J* = 1.8 Hz), 2.15 (H_2-5), 1.55 (H-6), 1.68 (H-7), 1.58 (H-8), 1.48, 1.60 (H_2-9), 1.62, 1.74 (H_2-10), 1.52 (H-11), 0.80 (H_3-12, d, *J* = 6.5 Hz), 0.82 (H_3-13, d, *J* = 6.5 Hz), 0.77 (H_3-14, s), 1.63 (H_3-15, brs). ^{13}C NMR (150 MHz, $CDCl_3$): 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 $C_{15}H_{24}$ $[M]^+$).

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 ^{13}C 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 ^{1}H and/or ^{13}C NMR data with those reported in the literature.

Acknowledgement

A research grant from the Commission on Higher Education (CHED) is gratefully acknowledged.

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