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Chemical Composition of *Andrographis paniculata* (Burm.f.) Nees.

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ABSTRACT

The dichloromethane extracts of the leaves of Andrographis paniculata led to the isolation of neoandrographolide (1), 1,5-dimethyl-1,5-cyclooctadiene (2) and 2-hydroxyethyl benzoate (3), and squalene (4) from the leaves, while the stems yielded 1 and 2. The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy.

Keywords: Andrographis paniculata, Acanthaceae, neoandrographolide, 1,5-dimethyl-1,5-cyclooctadiene, 2-hydroxyethyl benzoate.

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INTRODUCTION

Andrographis paniculata (Burm.f.) Nees is a medicinal herb with extremely bitter taste. It has been used for centuries to treat respiratory infections, fever, herpes, sore throat and a variety of other chronic and infectious diseases [1]. Its major constituents are diterpenoids, flavonoids and polyphenols [2]. The major diterpenoid in A. paniculata is andrographolide which makes up about 4%, 0.8~1.2% and 0.5~6% in dried whole plant, stem and leaf extracts, respectively [3,4]. The isolation of deoxyandrographolide, neoandrographolide, 14-deoxy-11,12-didehydro andrographide and isoandrographolide [3,4] have also been reported [3,4]. Andrograpanin, neoandrographolide and andrographolide were also isolated from the roots of A. paniculata [5]. A review on the chemical constituents and pharmacological activities of A. paniculata has been provided [2].

We earlier reported the isolation of 14-deoxy-12-hydroxyandrographolide, 14-deoxyandrographolide and 14-deoxy-11,12-dihydroandrographolide from the leaves of A. paniculata [6]. We also reported the isolation of andrographolide, 14-deoxyandrographolide, 14-deoxy-12-hydroxyandrographolide, β-sitosterol, stigmasterol and chlorophyll a from the leaves; β-sitosterol, stigmasterol, 5,2'-dihydroxy-7,8-dimethoxyflavone, long chain trans cinnamate esters and β-sitosteryl fatty acid esters from the roots; β-sitosterol, monogalactosyl diacylglycerols, lupeol, and triacylglycerols from the pods; and 14-deoxyandrographolide from the stems of A. paniculata [7]. Recently, we reported the isolation of squalene, polyprenol, lutein, chlorophyll a, β-sitosterol, and stigmasterol from the stems; and α-amyrin acetate, triacylglycerols, lupeol, α-amyrin, and β-amyrin from the leaves of A. paniculata [8].

This study reports on the isolation of neoandrographolide (**1**), 1,5-dimethyl-1,5-cyclooctadiene (**2**), 2-hydroxyethyl benzoate (**3**), and squalene (**4**) from the leaves; **1** and **2** from the stems of A. paniculata. The chemical structures of **1**-**4** are presented in Fig. 1.

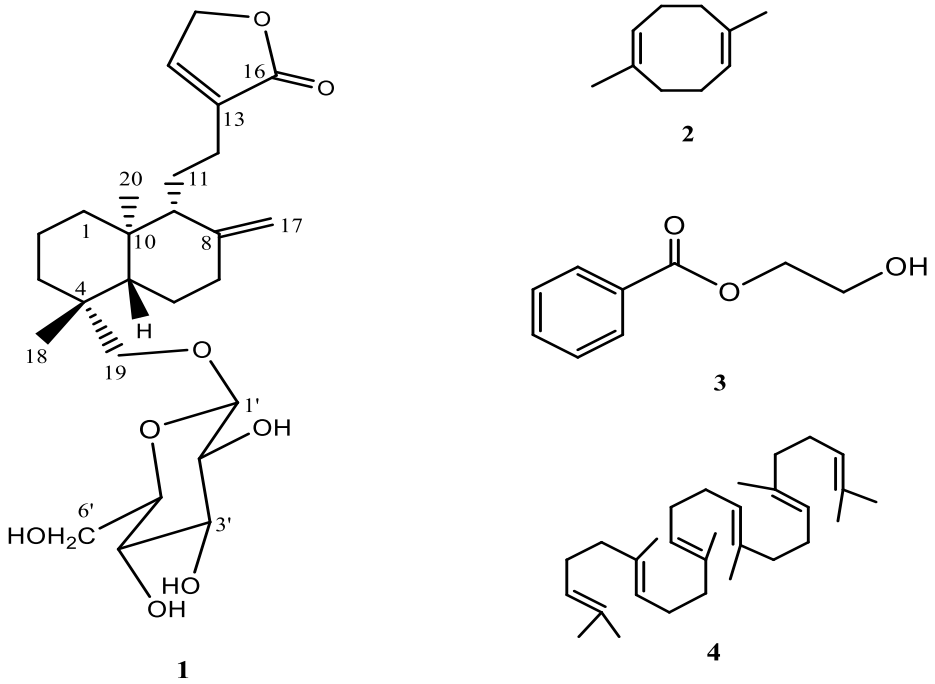

Fig. 1. Chemical structures of neoandrographolide (1), 1,5-dimethyl-1,5-cyclooctadiene (2), 2-hydroxy ethylbenzoate (3), and squalene (4) from *A. paniculata.*

MATERIALS AND METHODS

General Experimental Procedure:

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin

layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

General Isolation Procedure:

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. Twenty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Plant material:

The Andrographis paniculata (Burm.f.) Nees leaves and stems were collected from Abukay, Bataan in September 2015. The plant was authenticated at the Botany Division, Philippine National Museum.

Isolation of the Chemical Constituents of the Leaves:

The freeze-dried *A. paniculata* leaves (125.8 g) were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (4.9 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 and 10% acetone in CH_2Cl_2 fractions were combined and rechromatographed (3 ×) using 1% EtOAc in petroleum ether to afford **2** (4 mg) and **4** (3 mg). The 50% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield **3** (2 mg) after washing with petroleum ether. The acetone and 90% acetone in CH_2Cl_2 fractions were combined and rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6, v/v) to afford **1** (5 mg) after trituration with petroleum ether.

Isolation of the Chemical Constituent of the Stems:

The freeze-dried *A. paniculata* stems (159 g) were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (2.5 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed (2 ×) using 1% EtOAc in petroleum ether to afford **2** (3 mg). The 90% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6, v/v) to afford **1** (2 mg) after trituration with petroleum ether.

Neoandrographolide:

^{1}H-NMR (600 MHz, $CDC1_3$): δ 1.04, 1.77 (H_2-1), 1.48 (H_2-2), 0.98, 1.85 (H_2-3), 1.23 (H-5), 1.60, 1.82 (H_2-6), 1.94, 2.40 (H_2-7), 1.62 (H-9), 1.60, 1.75 (H_2-11), 2.12, 2.44 (H_2-12), 7.08 (br s, H-14), 4.754 (br s, H-15a), 4.751 (br s, H-15b), 4.57 (br s, H-17a), 4.85 (br s, H-17b), 0.98 (s, H_3-18), 3.18 (d, *J* = 9.6 Hz, H-19a), 4.00 (d, *J* = 9.6 Hz, H-19b), 0.63 (s, H_3-20), 4.20 (d, *J* = 7.8 Hz, H-1'), 3.32 (dd, *J* = 7.8, 9.0 Hz, H-2'), 3.54 (t, *J* = 9 Hz, H-3'), 3.58 (t, *J* = 9 Hz, H-4'), 3.35 (m, H-5'), 3.80 (dd, *J* = 4.8, 12 Hz, H-6a'), 3.00 (dd, *J* = 3.6, 12 Hz, H-6b'); ^{13}C-NMR (150 MHz, $CDC1_3$): δ 38.87 (C-1), 18.91 (C-2), 35.95 (C-3), 38.18 (C-4), 56.17 (C-5), 24.55 (C-6), 38.52 (C-7),147.26 (C-8), 56.42 (C-9), 39.54 (C-10), 21.71 (C-11), 24.51 (C-12), 134.85 (C-13), 143.84 (C-14), 70.10 (C-15), 174.35 (C-16), 109.65 (C-17), 27.77 (C-18), 72.70 (C-19), 15.39 (C-20), 103.11 (C-1'), 73.99 (C-2'), 76.21 (C-3'), 70.59 (C-4'), 75.08 (C-5'), 62.48 (C-6'),

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *A. paniculata* led to the isolation of **1**-**4**. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy. The NMR spectra of **2** are in accordance with data reported in the literature for 1,5-dimethyl-1,5-cyclooctadiene [9]; **3** for 2-hydroxyethyl benzoate [10], and squalene (**4**) [11].

Earlier studies reported that neoandrographolide (**1**) exhibited significant anti-inflammatory effects [12-14], anti-infective property [14], anti-allergic activity [15], protective effects on hepatotoxicity [14,16], potent hypolipidemic effects and cardiovascular protection without significant liver damage [17]. Furthermore, **1** was reported as a chemosensitizer in S-Jurkat and X chromosome-linked inhibitor of apoptosis protein (XIAP)-overexpressing Jurkat cells [18]. Another study reported that **1** did not show cytotoxic activity against KB and P388 cells [19].

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