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Research Article

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

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ABSTRACT

Chemical investigation of the dichloromethane extracts of *Andrographis paniculata* (Burm.f.) Nees led to the isolation of andrographolide (**1**), 14-deoxyandrographolide (**2**), 14-deoxy-12-hydroxyandrographolide (**3**), a mixture of β-sitosterol (**4a**) and stigmasterol (**4b**) in a 3:1 ratio, and chlorophyll a (**5**) from the leaves; a mixture of **4a** and **4b** in a 3:2 ratio, 5,2'-dihydroxy-7,8-dimethoxyflavone or skullcapflavone I (**6**), and a mixture of long chain *trans*-cinnamate esters (**7a)** and β-sitosteryl fatty acid esters (**7b**) from the roots; **4a**, monogalactosyl diacylglycerols (**8**), lupeol (**9**), and triacylglycerols (**10**) from the pods; and **2** from the stems. The structures of **1**-**3** and **6** were elucidated by extensive 1D and 2D NMR spectroscopy, while the structures of **4**, **5** and **7**-**10** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Andrographis paniculata*, Acanthaceae, andrographolide, 14-deoxy andrographolide, 14-deoxy-12-hydroxyandrographolide, β-sitosterol, stigmasterol, chlorophyll a, 5,2'-dihydroxy-7,8-dimethoxyflavone, skullcapflavone I, long chain *trans*-cinnamate esters, β-sitosteryl fatty acid esters, diacyl monogalactosylglycerol, lupeol, triacylglycerols

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 other main diterpenoids are deoxyandrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographide and isoandrographolide[3,4]. From the $EtOA_C$-soluble fraction of the ethanol or methanol extract, 5-hydroxy-7,8-dimethoxyflavone, 5-hydroxy-7,8,2',5'-tetramethoxy flavone, 5-hydroxy-7,8,2',3'-tetramethoxyflavone, 5-hydroxy-7,8,2'-trimethoxyflavone, 7-*O*-methylwogonin and 2'-methyl ether were isolated as the main flavonoids[5-7]. Twenty flavonoids: 5,5'-dihydroxy-7,8,2'-trimetroxyflavone, 5-hydroxy-7,8,2',6'-tetramethoxyflavone, 5,3'-dihydroxy-7,8,4'-trimethoxyflavone, 2'-hydroxy-5,7,8-trimethoxyflavone, 5-hydroxy-7,8,2',3',4'-pentamethoxyflavone, wightin, 5,2',6'-trihydroxy-7-methoxyflavone 2'-*O*-β-D-glucopyranoside, 5,7,8,2'-tetramethoxyflavone, 5-hydroxy-7,8-dimethoxyflavanone, 5-hydroxy-7,8-dimethoxyflavone, 5,2'-dihydroxy-7,8-dimethoxyflavone, 5-hydroxy-7,8,2',5'-tetramethoxyflavone, 5-hydroxy-7,8,2',3'-tetramethoxyflavone, 5-hydroxy-7,8,2'-trimethoxyflavone, 5,4'-dihydroxy-7,8,2',3'-tetramethoxyflavone, dihydroneobaicalein, andrographidine A, andrographidine B, andrographidine C and 5,2'-dihydroxy-7,8-dimethoxyflavone 2'-*O*-β-D-glucopyranoside; three diterpenoids: andrograpanin, neoandrographolide and andrographolide; two phenylpropanoids: *trans*-cinnamic acid and 4-hydroxy-2-methoxycinnamaldehyde; and oleanolic acid, β-sitosterol and β-daucosterol were isolated from the roots of *A paniculata*[8]. 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*[9]. We report herein the isolation of andrographolide (**1**), 14-deoxyandrographolide (**2**), 14-deoxy-12-hydroxyandrographolide (**3**), β-sitosterol (**4a**), stigmasterol (**4b**) and chlorophyll a (**5**) from the leaves; **4a**, **4b**, 5,2'-dihydroxy-7,8-dimethoxyflavone (**6**), long chain *trans*-cinnamate esters (**7a)** and β-sitosteryl fatty acid esters (**7b**) from the roots; **4a**, monogalactosyl diacylglycerols (**8**), lupeol (**9**), and triacylglycerols (**10**) from the pods; and **2** from the stems of *A. paniculata*. The chemical structures of **1**-**10** are presented in Fig. 1. To the best of our knowledge this is the first report on the isolation of **7a**-**10** from *A. paniculata*.

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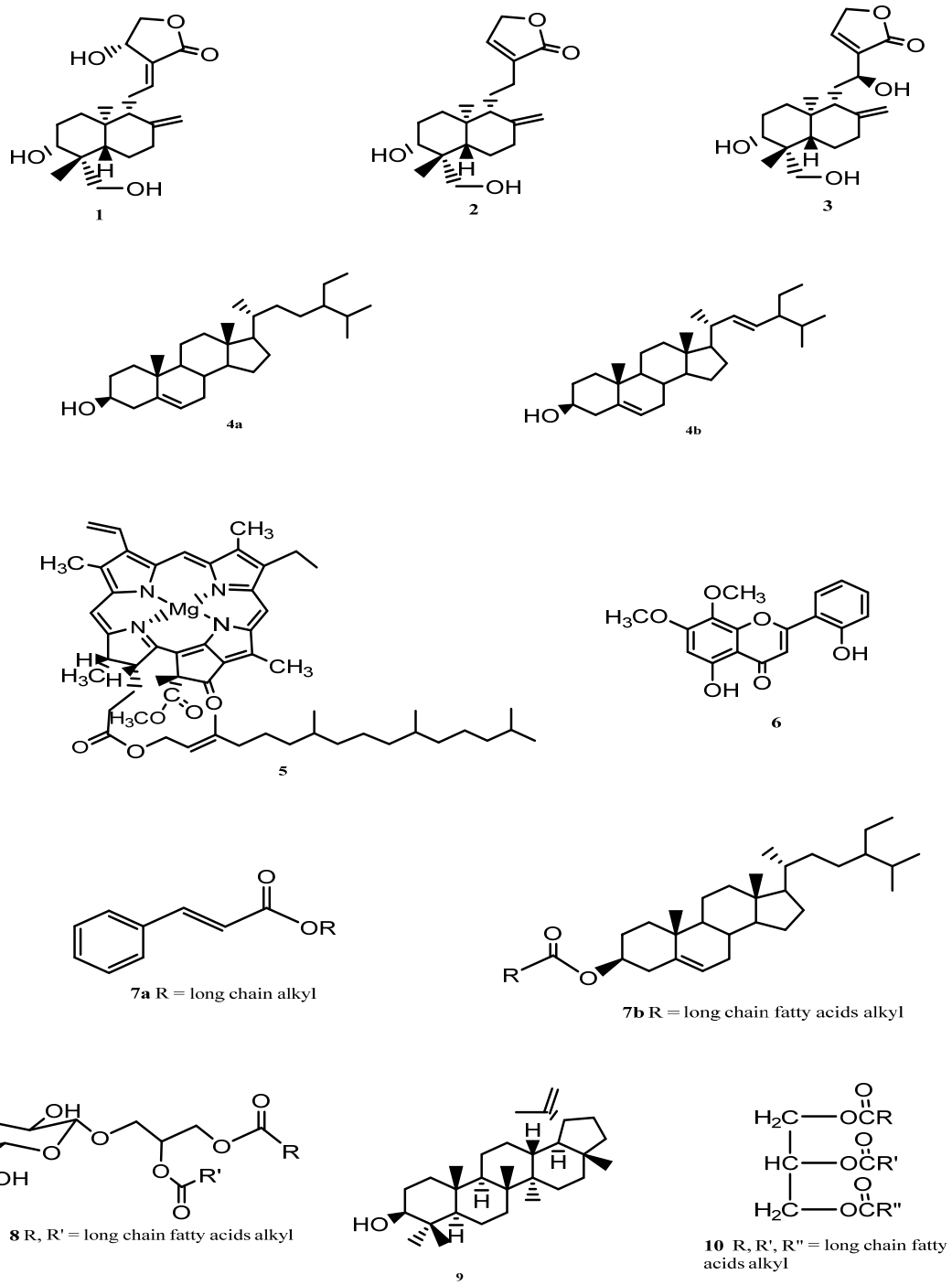

Figure 1: Chemical structures of andrographolide (**1**), 14-deoxyandrographolide (**2**), 14-deoxy-12-hydroxy andrographolide (**3**), β-sitosterol (**4a**), stigmasterol (**4b**), chlorophyll a (**5**), 5,2'-dihydroxy-7,8-dimethoxyflavone (**6**), long chain *trans*-cinnamate esters (**7a**), β-sitosteryl fatty acid esters (**7b**), monogalactosyl diacylglycerols (**8**), lupeol (**9**), and triacylglycerols (**10**) from *Andrographis paniculata.*

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H 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, roots, stems and pods 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 20% and 30% acetone in CH_2Cl_2 fractions were combined and rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford **5** (9 mg) after washing with petroleum ether, followed by Et_2O. The 50% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to yield a mixture of **4a** and **4b** (6 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to yield **2** (7 mg) after trituration with petroleum ether. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1.5:1.5:7, v/v) to yield **1** (7 mg) and **3** (5 mg) after trituration with petroleum ether.

Isolation of the Chemical Constituents of the Roots

The freeze-dried *A. paniculata* leaves (61.4 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 (0.84 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 2.5% EtOAc in petroleum ether to afford a mixture of **7a** and **7b** (5 mg) after washing with petroleum ether. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 10% EtOAc in petroleum ether to yield a mixture of **4a** and **4b** (8 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to yield **6** (4 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of the Chemical Constituents of the Pods

The freeze-dried *A. paniculata* leaves (66.5 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 (3.2 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 10% acetone in CH_2Cl_2 fraction was rechromatographed using (2 ×) 10% EtOAc in petroleum ether to afford **10** (9 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to afford **9** (4 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to yield **4a** (8 mg) after washing with petroleum ether. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1.5:1.5:7, v/v) to yield **8** (5 mg).

Isolation of the Chemical Constituents of the Stems

The freeze-dried *A. paniculata* leaves (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 50% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to yield **2** (4 mg) after trituration with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the different parts of *Andrographis paniculata* yielded **1**–**10**. The structures of **1**-**3** and **6** were elucidated by extensive 1D and 2D NMR spectroscopy. The NMR spectra of **1** are in accordance with data reported in the literature for andrographolide[10]; **2** for 14-deoxyandrographolide[11], **3** for 14-deoxy-12-hydroxy andrographolide[9], **4a** for β-sitosterol[12,13], **4b** for stigmasterol[12,13], **5** for chlorophyll a[14], **6** for 5,2'-dihydroxy-7,8-dimethoxyflavone or skullcapflavone I[15], **7a** for long chain *trans*-cinnamate esters[16], **7b** for β-sitosteryl fatty acid esters[17], **8** for monogalactosyl diacylglycerols[18], **9** for lupeol[19], and **10** for triacylglycerols[20].

Earlier studies reported that andrographolide (**1**) exhibited anti-diabetic[11-23], anti-retroviral[24], cardioprotective[25] and anti-inflammatory[26-29], antiproliferative and proapoptotic[30,31], anti-angiogenic[32], anti-thrombotic[33], anti-urothelial[34], anti-leishmaniasis[35], hepatoprotective[36,37], protective activity against alcohol-induced hepatic and renal toxicity[38], and anticancer[39-47]. 14-Deoxyandrographolide (**2**) was reported to exhibit immunomodulatory and anti-atherosclerotic[1], vasorelaxation *in vitro* and *in vivo*[48,49], and apoptotic[50]. Furthermore, **2** showed enhanced proliferation and interleukin-2 (IL-2) induction in human peripheral blood lymphocytes[51]. On the other hand, 14-deoxy-12-hydroxy andrographolide (**3**) was reported to be cytotoxic to human lung carcinoma (A549) with an IC_{50} value of 20 µg/mL and showed slight antimicrobial activities[9].

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