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Chemical Constituents of *Alstonia scholaris* (L.) R. Br.

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ABSTRACT

*Chemical investigation of the dichloromethane extract of the leaves of Alstonia scholaris (L.) R. Br. has yielded mixtures of erythrodiol (**1a**), uvaol (**1b**), and betulin (**1c**) in a 1:1:1 ratio, oleanolic acid and ursolic acid in a 2:1 ratio, β-amyrin acetate (**3b**) and α-amyrin acetate (**3b**) in a 1:4 ratio, and β-sitosterol (**4a**) and stigmasterol (**4b**) in a 3:2 ratio; squalene (**5**), β-sitosteryl-3β-glucopyranoside-6′′-O-fatty acid esters (**6**), and chlorophyll a (**7**). The structures of **1**–**7** were identified by comparison of their NMR data with literature data.*

Keywords: *Alstonia scholaris*, Apocynaceae, erythrodiol, uvaol, erythrodiol, betulin, oleanolic acid, ursolic acid, β-amyrin acetate, α-amyrin acetate, β-sitosterol, stigmasterol, squalene, β-sitosteryl-3β-glucopyranoside-6′′-*O*-fatty acid esters, chlorophyll a

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INTRODUCTION

Alstonia scholaris (L.) R. Br., locally known as dita, is used for the treatment of fever, chronic diarrhea, dysentery, beri-beri, congestion of the liver, and ulcers [1]. Previous studies reported the isolation of 2,3-secofernane triterpenoids, alstonic acids A and B [2]; 3β-acetate-24-nor-urs-4,12-diene ester triterpene, 3β-hydroxy-24-nor-urs-4,12,28-triene triterpene, 3,28-β-diacetoxy-5-olea-triterpene, α-amyrin acetate, and ursolic acid [3,4] lupeol acetate [5]; flavonoids [6]; monoterpene [7], triterpene [8-10]; iridoids [11]; megastigmane-3β,4α,9-triol, 7-megastigmene-3,6,9-triol and C13-norisoprenoid [12] from *Alstonia scholaris*. The essential oil of the flowers of *A. scholaris* yielded 2-dodecyloxirane (31.83%), 1,2-dimethoxy-4-(2-propenyl)benzene (8.49%), spinacene (6.09%), 1,54-dibromotetrapentacontane (5.13%), 2,6,10,15-tetramethylheptadecane (4.91%), terpinyl acetate (3.74%), linalool (2.22%), tritetracontane (2.17%), 2-(3-methyl-1,3-butadienyl)-1,3,3-trimethyl-1-cyclohexanol (1.78%), 9-methyl-5-methylene-8-decen-2-one (1.58%) as the main constituents [13]. The ethanolic extract of the flowers of *A. scholaris* afforded alstoprenyol, 3-β-hydroxy-28-β-acetoxy-5-olea, alstoprenylene 3β-acetate-24-nor-urs-4,12,2'-triene ester, α-amyrin acetate, α-amyrin, lupeol acetate, and 3β-hydroxy-24-nor-urs-4,12,28-triene [14]. Many studies reported the isolation of alkaloids from *A. scholaris* [15–24].

In an earlier study, we reported the isolation of cycloeucalenol, cycloartanol, lupeol, lupeol acetate, and betulin from the leaves of *A. scholaris* [25]. Recently, we obtained mixtures of α-amyrin acetate, β-amyrin acetate and lupeol

acetate; and α-amyrin fatty acid esters, β-amyrin fatty acid esters, lupeol fatty acid esters and phytyl fatty acid esters from the flowers of *A. scholaris* [26].

In this study, the leaves of *A. scholaris* afforded mixtures of erythrodiol (**1a**), uvaol (**1b**), and betulin (**1c**); oleanolic acid (**2a**) and ursolic (**2b**); β-amyrin acetate (**3b**) and α-amyrin acetate (**3b**); β-sitosterol (**4a**) and stigmasterol (**4b**); squalene (**5**); β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**6**); and chlorophyll a (**7**). The structures of **1**–**7** are presented in Fig. 1.

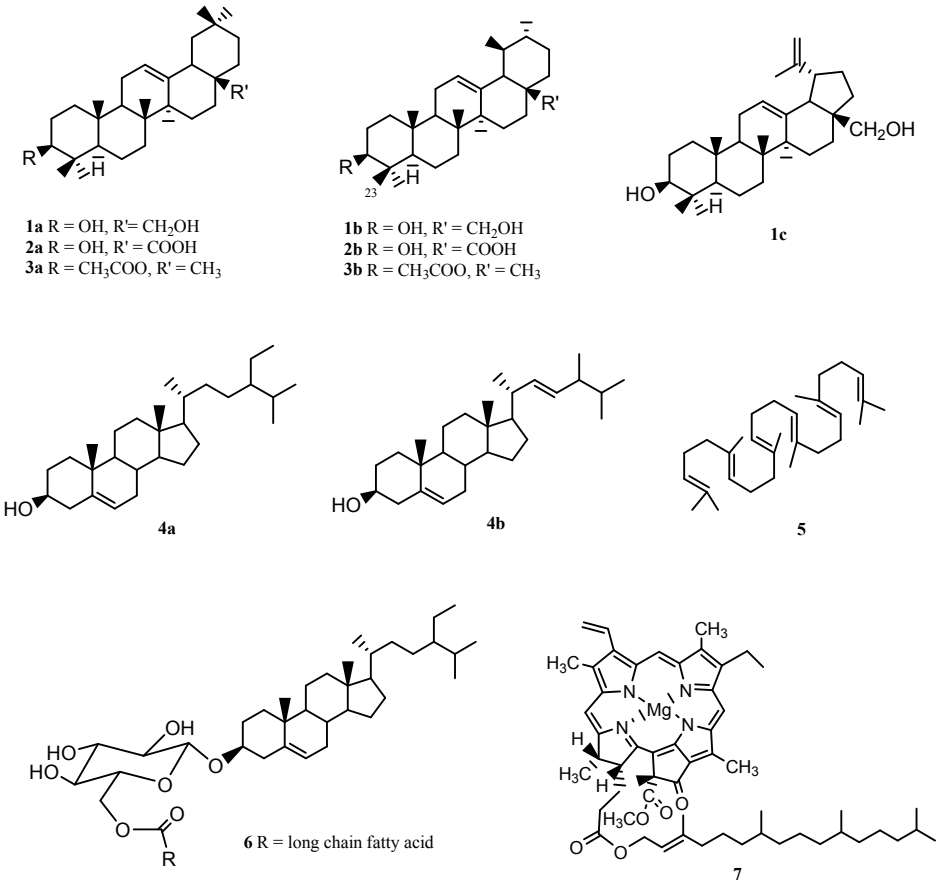

Fig. 1. Chemical structures of erythrodiol (1a), uvaol (1b), betulin (1c), oleanolic acid (2a), ursolic (2b), β-amyrin acetate (3b), α-amyrin acetate (3b), β-sitosterol (4a), stigmasterol (4b), squalene (5), β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (6), chlorophyll a (7) from the leaves of *A. scholaris*

MATERIALS AND METHODS

General Experimental Procedure

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Ascend 400 in $CDCl_3$ at 400 MHz. Column chromatography was performed with silica gel 60 (70-230 mesh, Merck). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} (Merck) and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming. All solvents used are analytical grade.

Sample Collection

Samples of *Alstonia scholaris* (L.) R. Br. leaves were collected from the De La Salle University – Manila campus in October 2015. The samples were authenticated at the Botany Division, Philippine National Museum.

General Isolation Procedure

A glass column 12 inches in height and 0.5 inch internal diameter was used for the chromatography. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume as eluents. Five 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. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the Chemical Constituents from the Leaves of Alstonia scholaris

The air-dried *A. scholaris* leaves (113.21 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.22 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 in 10% increments by volume.

The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×**)** using petroleum ether to yield **5** (4 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×**)** using petroleum ether to afford a mixture of **3a** and **3b** (4 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×**)** using 10% EtOAc in petroleum ether to yield **7** (3 mg) after washing with petroleum ether, followed by Et_2O. The 40% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether, followed by 20% EtOAc in petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were combined and rechromatographed using the same solvent to afford a mixture of **4a** and **4b** (3 mg) after washing with petroleum ether. The fractions eluted with 20% EtOAc in petroleum ether were combined and rechromatographed using the same solvent to yield a mixture of **1a**, **1b** and **1c** (5 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×**)** using 20% EtOAc in petroleum ether to yield a mixture of **2a** and **2b** (3 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v). Fractions from this column were combined and rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (1.5:1.5:7, v/v) to afford **6** (3 mg) after trituration with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the leaves of ***A.*** *scholaris* yielded **1**–**7**. The NMR spectra of **1a** are in accordance with data reported in the literature for erythrodiol [27]; **1b** for uvaol [28]; **1c** for betulin [28]; **2a** for oleanolic acid [29]; **2b** for ursolic acid [29]; **3a** for β-amyrin acetate [30]; **3b** for α-amyrin acetate [30]; **4a** for β-sitosterol [31]; **4b** for stigmasterol [31]; **5** for squalene [31]; **6** for β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters [32]; and **7** for chlorophyll a [33].

The 1:1:1 ratio of the mixture of (**1a**):(**1b**):(**1c**) was deduced from the integrations and intensities of the ^{1}H NMR resonances for the olefinic protons of **1a** at δ 5.17 (t, *J* = 3.6 Hz, H-12) [27], **1b** at δ 5.12 (t, *J* = 3.6 Hz, H-12) [28], and **1c** at δ 4.66 (br d, *J* = 2 Hz, H_a-29) and 4.56 (br s, H_b-29) [28]. The 2:1 ratio of the mixture of oleanolic acid (**2a**):ursolic acid (**2b**) was deduced from the integrations and intensities of the ^{1}H NMR resonances for the olefinic protons of **2a** at δ 5.27 (t, *J* = 3.6 Hz, H-3) [29] and **2b** at δ 5.24 (t, *J* = 3.2 Hz, H-3) [29]. The 1:4 ratio of the mixture of β-amyrin acetate (**3a**):α-amyrin acetate (**3b**) was deduced from the integrations and intensities of the ^{1}H NMR resonances for the olefinic protons of **3a** at δ 5.15 (t, *J* = 3.2 Hz, H-3) [30] and **3b** at δ 5.10 (t, *J* = 3.6 Hz, H-3) [30]. The presence of the acetate functional groups was deduced from the appearance of methyl singlets at δ 2.02 and 2.03. The integrations of the ^{1}H NMR resonances for the olefinic protons of **4a** at δ 5.33 (brd, *J* = 5.2 Hz, H-6) [31] and **4b** at δ 5.33 (brd, *J* = 5.2 Hz, H-6), 5.13 (dd, *J* = 8.4, 15.2 Hz, H-22) and 5.00 (dd, *J* = 8.8, 15.2 Hz, H-23) [31] suggested that the ratio of **4a** and **4b** is about 3:2.

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