ABSTRACT

Chemical investigation of the dichloromethane extract of the flowers of Alstonia scholaris (L.) R. Br. afforded mixtures of α-amyrin acetate (1a), β-amyrin acetate (1b) and lupeol acetate (1c); and α-amyrin fatty acid esters (2a), β-amyrin fatty acid esters (2b), lupeol fatty acid esters (2c) and phytyl fatty acid esters (2d). The structures of 1a-2d were identified by comparison of their NMR data with literature data.

Keywords: Alstonia scholaris, α-amyrin acetate, β-amyrin acetate, lupeol acetate, α-amyrin fatty acid esters, β-amyrin fatty acid esters, lupeol fatty acid esters, phytyl fatty acid esters

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-secoferane 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-megastigmatene-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%), 1,5,4-dibromotetrapentacontane (5.13%), 2,6,10,15-tetramethylheptadecane (4.91%), terpinyl acetate (3.74%), linalool (2.22%), trietracontane (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, 3β-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, cycloarctanol, lupeol, lupeol acetate, and betulin from the leaves of A. scholaris [25]. In this study, we obtained mixtures of α-amyrin acetate (1a), β-amyrin acetate (1b) and lupeol acetate (1c); and α-amyrin fatty acid esters (2a), β-amyrin fatty acid esters (2b) and lupeol fatty acid esters (2c).
esters (2c) and phytol fatty acid esters (2d) from the flowers of *A. scholaris*. The structures of 1a-2d are presented in Fig. 1.

**Fig. 1.** Chemical structures of α-amyrin acetate (1a), β-amyrin acetate (1b), lupeol acetate (1c), α-amyrin fatty acid esters (2a), β-amyrin fatty acid esters (2b), lupeol fatty acid esters (2c) and phytol fatty acid esters (2d) from the flowers of *A. scholaris*.

**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$_2$SO$_4$ solution followed by warming.

**Sample Collection**

Samples of *Alstonia scholaris* (L.) R. Br. flowers 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$_2$Cl$_2$ 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 Flowers of Alstonia scholaris

The air-dried A. scholaris flowers (265.8 g) were ground in a blender, soaked in CH\(_2\)Cl\(_2\) for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.59 g) which was chromatographed using increasing proportions of acetone in CH\(_2\)Cl\(_2\)in 10% increments by volume. The CH\(_2\)Cl\(_2\) fraction was rechromatographed (3 x) using 5% EtOAc in petroleum ether to afford a mixture of \(2a-2d\) (7 mg) after washing with petroleum ether. The 10% acetone in CH\(_2\)Cl\(_2\) fraction was rechromatographed (2 x) using 5% EtOAc in petroleum ether to afford a mixture of \(1a-1e\) (10 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the flowers of A. scholaris yielded \(1a-2d\). The NMR spectra of \(1a\) are in accordance with data reported in the literature for \(\alpha\)-amyrin acetate [26]; \(1b\) for \(\beta\)-amyrin acetate [27]; and \(1c\) for lupeol acetate [26]; \(2a\) for \(\alpha\)-amyrin fatty acid esters [28]; \(2b\) for \(\beta\)-amyrin fatty acid esters [28], \(2c\) for lupeol fatty acid esters [29]; and \(2d\) for phytol fatty acid esters [30].

The 3:1:1 ratio of the mixture of \(\alpha\)-amyrin acetate (\(1a\)):\(\beta\)-amyrin acetate (\(1b\)) : lupeol acetate (\(1c\)) was deduced from the integrations and intensities of the \(^1H\) NMR resonances for the olefinic protons of \(1a\) at \(\delta 5.10\) (t, \(J = 3.6\) Hz, H-12) [31], \(1b\) at \(\delta 5.15\) (t, \(J = 3.6\) Hz, H-12) [31], and \(1c\) at \(\delta 4.66\) (brd, \(J = 1.8\) Hz, H-29) and \(4.55\) (brd, \(J = 1.8\) Hz, H-29) [32]. The 3:1:1:1:1.5:1.5 ratio of the mixture of \(\alpha\)-amyrin fatty acid esters (\(2a\)) : \(\beta\)-amyrin fatty acid esters (\(2b\)) : lupeol fatty acid esters (\(2c\)) : phytol fatty acid esters (\(2d\)) was deduced from the integrations and intensities of the \(^1H\) NMR resonances for the olefinic protons of \(2a\) at \(\delta 5.11\) (t, \(J = 3.6\) Hz, H-3) [31], \(2b\) at \(\delta 5.16\) (t, \(J = 3.6\) Hz, H-3) [31], \(2c\) at \(\delta 4.67\) (br d, \(J = 1.8\) Hz, H-29) and \(4.55\) (br d, \(J = 1.2\) Hz, H-29) [32], and \(2d\) at \(\delta 4.57\) (d, \(J = 7.2\) Hz, H-1') [30]. The acetates of \(1a-1c\) were deduced from the methyl singlets at \(\delta 2.023, 2.014\) and \(2.006\) [31, 32], while long-chain fatty acid methylenes were indicated by the high intensity broad resonances centered at \(\delta 1.24\) [30-32].

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REFERENCES