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Secondary Metabolites from *Cycas aenigma.*

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ABSTRACT

Chemical investigation of *Cycas aenigma*, a plant endemic to the Philippines, led to the isolation of squalene (**1**), a mixture of β-sitosterol (**2a**) and stigmasterol (**2b**) from the microsporophyll lamina; and a mixture of **2a** and **2b,** triacylglycerols (**3**), and a mixture of phytyl fatty acid ester (**4a**) and β-sitosteryl fatty acid ester (**4b**) from the roots of *Cycas aenigma*. The structures of **1-4b** were identified by comparison of their NMR data with literature data.

Keywords: *Cycas aenigma,* Cycadaceae, squalene, β-sitosterol, phytyl fatty acid ester, β-sitosteryl fatty acid ester

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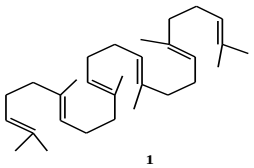
INTRODUCTION

Cycas resemble palms in morphology and are commonly called sago palm. They are considered as fossil plants though they may have evolved only about 12 million years ago [1]. They are widely distributed in the Tropics [2] where they grow on volcanic, limestone, ultramafic, sandy, or even water-logged soils in grassland and forest habitats [3].

The demand of *Cycas* species for domestic and international horticultural trade, grassland and forest fires, and conversion of their natural habitats to settlements and other land uses have threatened to varying degrees the wild populations of the genus [4]. Some of these threatened species are *C.* curranii [5]*, C. wadei* [6] and *C. zambalensis* as Critically Endangered (CR) [5], *C. riuminiana* as Endangered (E) [5], and *C. saxatilis* as Vulnerable (V) [7].

This study is part of our research on the chemical constituents of the genus *Cycas.* We earlier reported the isolation of isopimaran-19-ol (**I**) from the megasporophyll lamina; 9αH-isopimara-7,15-diene (**II**) and triacylglycerols (**III**) from the bark; **III**, oleic acid (**IV**), and 1,2-dioleylglycerol (**V**) from the leaflets; **III**, β-sitosterol (**VI**), and stigmasterol (**VII**) from the petiole and rachis; **VI** from the roots; and **III** and **VI** from the endotesta and sclerotesta of *C. lacrimans* [8]. In another study, we reported the isolation of **III**, **VI**, **VII**, and squalene (**VIII**) from the sarcotesta; **III**, **VI**, **VII**, and phytyl fatty acid esters (**IX**) from the endotesta; **III**, **VI**, **VII**, and β-sitosteryl fatty acid esters (**X**) from the sclerotesta; and **III** and **X** from the bark of *C. sancti-lasallei* [9]. Another *Cycas* species, *C. vespertilio* yielded **III**, a mixture of **VI** and **VII**, pinoresinol (**XI**), sesamin (**XII**), and paulownin (**XIII**) from the cone base; **III**, **VI**, **VII**, **XI**, **XIII**, and lariciresinol (**XIV**) from the cataphylls; **VI** from the megasporophyll lamina; **VI** and a mixture of *trans*-4-hydroxycinnamate fatty acid esters (**XV**) and *cis*-4-hydroxycinnamate fatty acid esters (**XVI**) from the unripe sarcotesta; and **III** and **VI** from the ripe sarcotesta [10]. Recently, we reported the isolation of 2-[2-hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol (**XVII**), **XI**, and fatty alcohol from the leaflets; and **III**, **VI** and **VII** from the petiole and rachis of *Cycas aenigma* [11].

We report herein the isolation of squalene (**1**), a mixture of β-sitosterol (**2a**) and stigmasterol (**2b**) from the microsporophyll lamina; and a mixture of **2a** and **2b,** triacylglycerols (**3**), and a mixture of phytyl fatty acid ester (**4a**) and β-sitosteryl fatty acid ester (**4b**) from the roots of *C. aenigma*. To our knowledge, this is the first report on the isolation of **1**, **4a** and **4b** from this plant.



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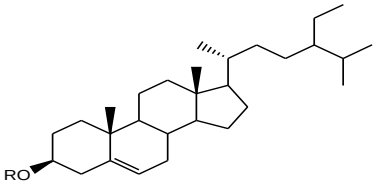
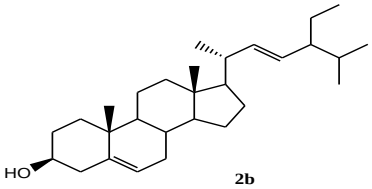
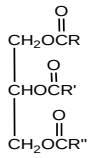


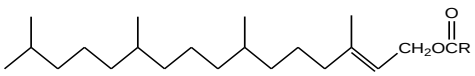
2a R = H
4b R = long chain fatty acid



2b



3 R, R', R" = long chain fatty acids



4a R = long chain fatty acid

Chemical structures of squalene (**1**), β-sitosterol (**2a**), stigmasterol (**2b**), triacylglycerols (**3**), phytyl fatty acid ester (**4a**), and β-sitosteryl fatty acid ester (**4b**) from *Cycas aenigma*.

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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.

Plant Material

Cycas aenigma roots and microsporophyll lamina were collected in 2013. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH3118).

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the chromatography of the crude extracts. 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 of smaller fractions from the first column. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the Chemical Constituents of the Microsporophyll Lamina

The air-dried microsporophyll lamina of *C. aenigma* (317.3 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 (2.3 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (3 ×) using petroleum ether to afford **1** (2 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_2Cl_2 to afford a mixture of **2a** and **2b** (4 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Roots

The air-dried roots of *C. aenigma* (24.5 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 (0.3 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to yield a mixture of **4a** and **4b** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 10% EtOAc in petroleum ether to afford **3** (4 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_2Cl_2 to afford a mixture of **2a** and **2b** (2 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *C. aenigma* yielded **1**-**2b** from the microsporophyll lamina; and **2a**-**4b** from the roots. Compounds **1**-**4b** were identified by comparison of their NMR data with those reported in the literature for squalene (**1**) [9], β-sitosterol (**2a**) [12], stigmasterol (**2b**) [12], triacylgycerols (**3**) [12], phytyl fatty acid ester (**4a**) [13], and β-sitosteryl fatty acid ester (**4b**) [9].

β-sitosterol (**2a**) and stigmasterol (**2b**) isolated from the microsporophyll lamina were also present in the roots and petiole and rachis (10) of *C. aenigma*, while triacylglycerols (**3**) obtained from the roots were also found in the petiole and rachis. Results of this study indicate that *C. aenigma* shares similar chemical characteristics with *C. sancti-lasallei* which contained squalene (**1**), phytyl fatty acid esters (**4b**) and β-sitosteryl fatty acid ester (**4b**) [9].

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