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Chemical constituents of *Cycas riuminiana*

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ABSTRACT

*Chemical investigation of the dichlroromethane extracts of Cycas riuminiana, a plant native to the Philippines, affordedα-tocopherol (**1**), phytyl fatty acid ester (**2**),squalene(**3**),lutein (**4**), chlorophyll a (**5**), long chain 1-alkene, and linoleic acid from the leaflets; a mixture of β-sitosterol (**6**) and stigmasterol (**7**) from the petiole and rachis, and roots;**6** andtriacylglycerol (**8**) from thesarcotesta; and **6**,**8**, anda mixture of methyl fatty acid ester (**9**) and β-sitosteryl fatty acid ester (**10**)from the endotesta. The structure of **1**was elucidated by extensive 1D and 2D NMR spectroscopy, while those of **2**-**10**were identified by comparison of their NMR data with literature data.*

Keywords: *Cycas riuminiana*, Cycadaceae, α-tocopherol,phytyl fatty acid ester, squalene, lutein, chlorophyll a, β-sitosterol, stigmasterol, triacylglycerol, fatty acid methyl 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].

There are no reported chemical and biological activity studies on *C. riuminiana*. However, a number of studies have been reported on the chemical constituents of other indigenous Philippine *Cycas*. We earlier reported the chemical constituents of the different parts of *C. sancti-lasallei*[8-11], *C. vespertilio*[12, 13], *C. zambalensis*[14], *C. lacrimans*[15-17], *C. aenigma*[18,19], and *C. edentata* [20,21].

We report herein the isolation of α-tocopherol(**1**), phytyl fatty acid ester (**2**),squalene (**3**),lutein (**4**), and chlorophyll a (**5**) from the leaflets; a mixture of β-sitosterol (**6**) and stigmasterol (**7**) from the petiole and rachis, and roots;**6**

andtriacylglycerol (**8**) from the sarcotesta; and **6**, **8**, anda mixture of methyl fatty acid ester (**9**) and β-sitosteryl fatty acid ester (**10**) from the endotesta. The strictures of **1**-**10**are presented in Fig. 1.

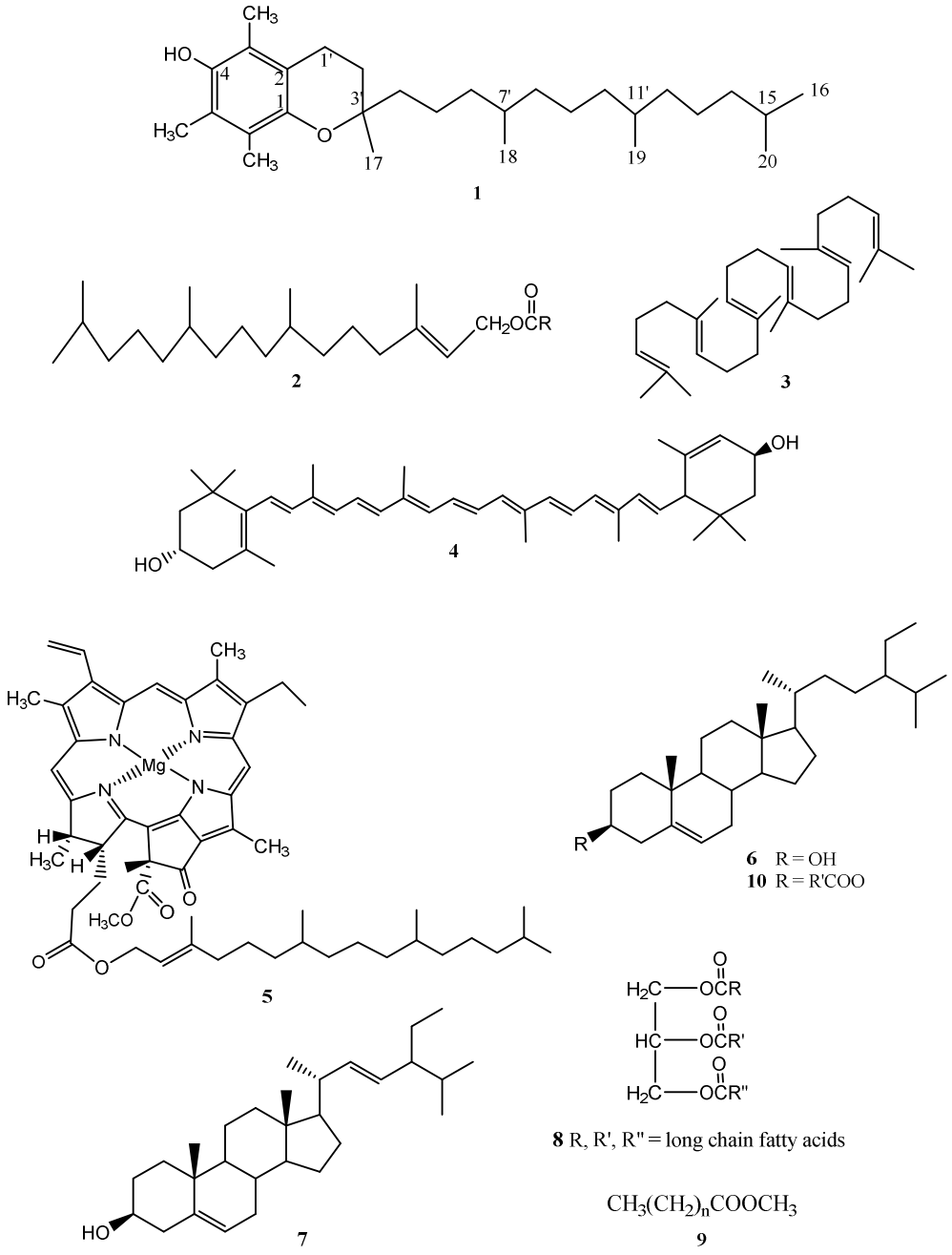

Fig. 1. Chemical structures of α-tocopherol (1), phytyl fatty acid ester (2),squalene (3),lutein (4), chlorophyll a (5), β-sitosterol (6), stigmasterol (7),triacylglycerol (8), methyl fatty acid ester (9) and β-sitosteryl fatty acid ester (10) from *Cycas riuminiana*.

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 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. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Plant material

Cycas riuminiana leaflets, petiole and rachis,roots, and seeds were collected from Magalang, Pampanga in December 2014. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3117).

Isolation of the Chemical Constituents of the Leaflets

The air-dried leaflets (218 g) of *C. riuminiana*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 (6.2 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2fraction was rechromatographed (2 ×) using petroleum ether to afford **3** (4 mg) and long chain 1-alkene (2 mg). The 10% acetone in CH_2Cl_2fraction was rechromatographed (3 ×) using 1% EtOAc in petroleum ether to yield **2** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to yield **1**(3 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_2Cl_2 to afford **5**(8 mg) after washing with petroleum ether, followed by Et_2O. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yieldlinoleic acid(7 mg). The 60% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford **4**(7 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of the Chemical Constituents of the Petiole and Rachis

The air-dried petiole and rachis (75 g) of *C. riuminiana*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 (0.7 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford a mixture of **6** and **7**(9 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Roots

The air-dried roots (44.2 g) of *C. riuminiana*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 (0.3 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents.The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford a mixture of **6** and **7**(5 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Sarcotesta

The air-dried sarcotesta (144.5g) of *C. riuminiana*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 (1.6g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 2.5% EtOAc in petroleum ether to afford **8**(11 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 15% EtOAc in petroleum ether to afford **6**(5 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Endotesta

The air-dried endotesta (156.5 g) of *C. riuminiana*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 (1.2 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2fraction was rechromatographed (2 ×) using 1% EtOAc in petroleum ether to yield a mixture of **9** and **10** (3

mg). The20% acetone inCH_2Cl_2fraction was rechromatographed (3 ×) using7.5% EtOAc in petroleum ether to afford **8**(10 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2×) using 15% EtOAc in petroleum ether to afford **6**(5 mg) after washing with petroleum ether.

α-Tocopherol (**1**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.09 (s, 3-CH_3), 2.14 (s, 5-CH_3), 2.09 (s, 6-CH_3), 2.58 (t, *J* = 6.6 Hz, H_2-1'), 1.75, 1.80(H_2-2'),1.52, 1.50 (H_2-4'), 1.22, 1.24 (H_2-5'),1.05, 1.25 (H_2-6'),1.36 (H-7'),1.05, 1.25 (H_2-8'),1.36 (H-9'),1.05, 1.25 (H_2-10'),1.36 (H-11'),1.05, 1.25 (H_2-12'),1.30, 1.18 (H-13'), 1.12, 1.12 (H_2-14'), 1.50 (H-15), 0.84 (d, *J* = 6.6 Hz, CH_3-16/CH_3-20), 0.85 (d, *J* = 6.6 Hz, CH_3-16/CH_3-20), 1.21 (s, CH_3-17),0.82 (d, *J* = 6.6 Hz, CH_3-18/ CH_3-19), 0.83 (d, *J* = 6.6 Hz, CH_3-18/ CH_3-19), 4.15 (4-OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 145.53 (C-1), 117.36 (C-2), 118.43 (C-3), 144.51 (C-4), 120.98(C-5), 122.61(C-6),11.77 (Me, C-3), 12.21 (Me, C-5), 11.28 (Me, C-6), 20.75 (C-1'), 31.53(C-2'), 74.52 (C-3'), 39.79 (C-4'),21.03 (C-5'), 37.46 (C-6'), 32.79 (C-7'), 37.44 (C-8'), 24.44 (C-9'), 37.27 (C-10'), 32.70 (C-11'), 37.41 (C-12'), 24.80 (C-13'), 39.36 (C-14'), 27.98 (C-15'), 22.71 (C-16'), 23.79 (C-17'), 19.65, 19.74 (C-18', C-19'), 22.62 (C-20').

RESULTS AND DISCUSSION

Silica gel chromatography of the CH_2Cl_2 extracts of *Cycas riuminiana*yielded α-tocopherol (**1**) [22],phytyl fatty acid ester (**2**) [23], squalene (**3**) [24],lutein (**4**) [25], chlorophyll a (**5**) [26], long-chain 1-alkene [27], and linoleic acid [28] from the leaflets; a mixture of β-sitosterol (**6**) [29, 30] and stigmasterol (**7**) [30] from the petiole and rachis, and roots; **6** andtriacylglycerol (**8**)[31] from the sarcotesta; **6**, **8**, anda mixture of methyl fatty acid ester (**9**) [32] and β-sitosteryl fatty acid ester (**10**) [33]in about 5:3 ratio from the endotesta.The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy, while those of **2**-**10** were identified by comparison of their NMR data with literature data. The ratios of β-sitosterol (**4**) and stigmasterol (**5**)from the petiole and rachis, and roots are 4:1 and 7:1, respectively. This ratio was deduced from the integrations of the ^{1}H NMR resonances for the olefinic protons of **5** at δ 5.13 (dd, *J* = 8.4, 15 Hz), 5.00 (dd, *J* = 8.4, 15 Hz), and 5.33 (dd, *J* = 1.8, 3.6 Hz) and the olefinic protons of **4** at 5.33 (dd, *J* = 1.8, 3.6 Hz) [29, 30].

Vitamin E consists of a mixture of tocopherols and tocotrienols that are synthesized by plants from homogenestic acid [34]. The most abundant form in nature [35] and most biologically active is α-tocopherol (**1**) [36]. α-Tocopherol (**1**)was reported to exhibit gene regulatory activity [34]; regulates phosphorylation cascades [35]; modulates the *in vitro* expression of some significant proteins/enzymes in various cell types involved in atherogenesis [37]; decreases protein kinase C activity [38]; controls expression of the α-tropomyosin gene [39]; inhibits smooth muscle cell proliferation [40]; increases phosphoprotein phosphatase 2A activity [41]; and decreases proinflammatory cytokine IL-1β release via the inhibition of the 5-lipoxygenase pathway [42]. α-Tocopherol also inhibits PKC activity which was shown to have a key role on vascular smooth muscle cell [41, 43], monocytes, macrophages, neutrophils, fibroblasts and mesangial cells [44-47]growth arrest.

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