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Research Article

Chemical Constituents of *Cycas curanii* (J.Schust.) K.D.Hill

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ABSTRACT
Chemical investigation of the dichloromethane extract of *Cycas curanii* led to the isolation of squalene (**1**), lutein (**2**), chlorophyll a (**3**), phytol (**4**), long chain 1-alkene (**5**), and triacylglycerols (**6**) from the leaflets; **1**, **6**, and β-sitosteryl fatty acid ester (**7**) from the sarcotesta; **6** and β-sitosterol (**8**) from the bark; **7** and a mixture of **8** and stigmasterol (**9**) from the lamina; **6** and **8** from the endotesta; **6**, β-sitosterone (**10**), a mixture of **7** and phytyl fatty acid ester (**11**), and a mixture of **8** and **9** from the petiole; **6** and **8** from the roots; and **8** from the sclerotesta. The structures of **1**-**11** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Cycas curanii*, Cycadaceae, squalene, lutein, chlorophyll a, phytol, β-sitosterol, β-sitosteryl fatty acid ester, β-sitosterone, phytyl fatty acid ester

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]. Furthermore, *C. vespertilio* male cone afforded **XI**, **XIV**, **III**, and fatty alcohols[11]. 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 alcohols from the leaflets; and **III**, **VI** and **VII** from the petiole and rachis of *Cycas aenigma*[12]. We report herein the isolation of squalene (**1**), lutein (**2**), chlorophyll a (**3**), phytol (**4**), long chain 1-alkene (**5**), and triacylglycerols (**6**) from the leaflets; **1**, **6**, and β-sitosteryl fatty acid ester (**7**) from the sarcotesta; **6** and β-sitosterol (**8**) from the bark; **7** and a mixture of **8** and stigmasterol (**9**) from the lamina; **6** and **8** from the endotesta; **6**, β-sitosterone (**10**), a mixture of **7** and phytyl fatty acid ester (**11**), and a mixture of **8** and **9** from the petiole; **6** and **8** from the roots; and **8** from the sclerotesta of *C. curanii*. To our knowledge, this is the first report on the isolation of **1**-**11** from *C. curanii.*

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

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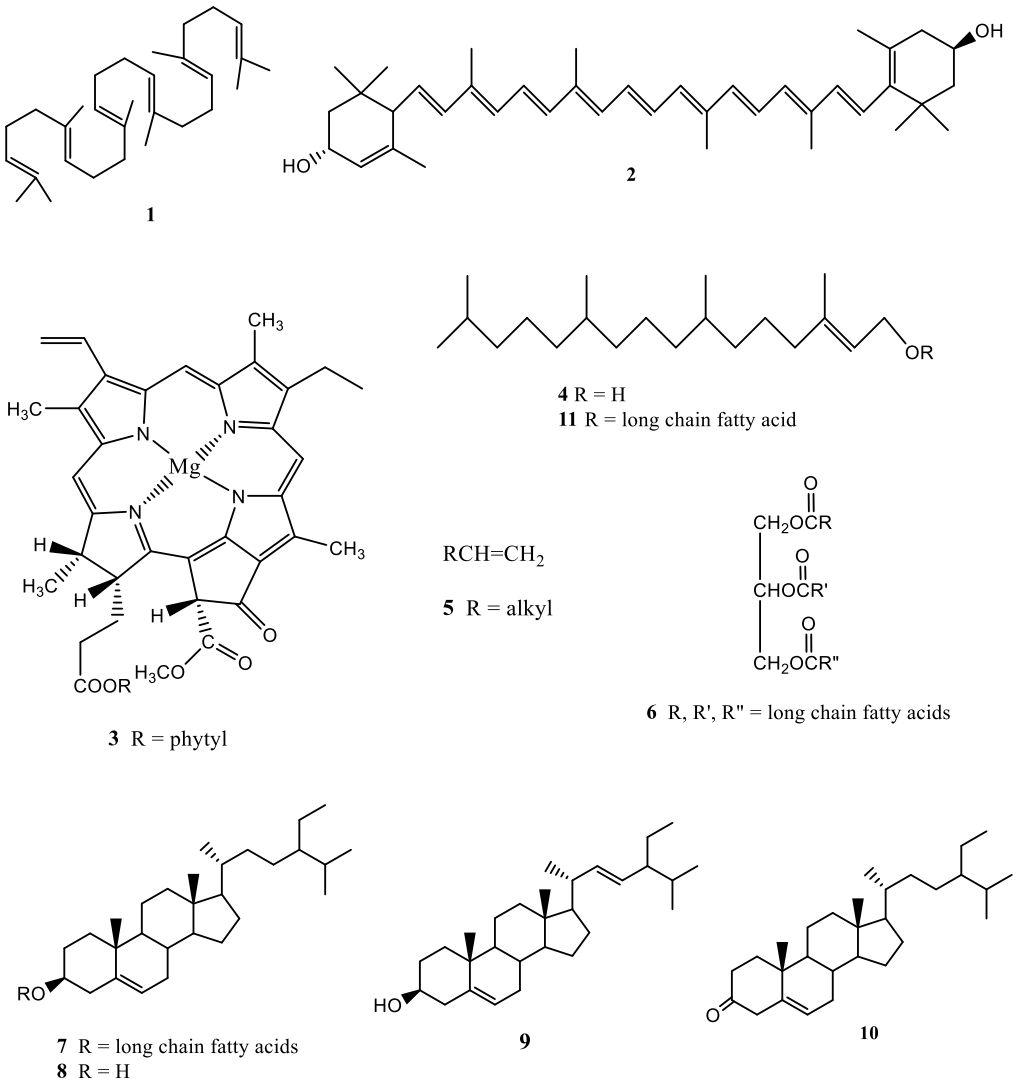

Chemical structures of squalene (**1**), lutein (**2**), chlorophyll a (**3**), phytol (**4**), long chain 1-alkene (**5**), triacylglycerols (**6**), β-sitosteryl fatty acid ester (**7**), β-sitosterol (**8**), stigmasterol (**9**), β-sitosterone (**10**), and phytyl fatty acid ester (**11**) from *C. curanii.*

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.

Sample Collection

Cycas curanii were collected in 2015. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH3113).

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the fractionation of the crude extracts. Ten milliliter fractions were collected. 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.

Isolation of chemical constituents from the leaflets

The air-dried leaflets of *C. curanii* (255.5 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (3.7 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to yield **1** (2 mg) and **5** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to afford **6** (5 mg). The more polar fractions were combined and rechromatographed (3 ×) using 10% EtOAc in petroleum ether to yield **4** (2 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 20% EtOAc in petroleum ether to provide **3** (5 mg) after washing with petroleum ether, followed by Et_2O. 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 yield **2** (5 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of chemical constituents from the sarcotesta

The air-dried sarcotesta of *C. curanii* (110.5 g) were ground in a blender, soaked in CH_2Cl_2 for 3 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 at 10% increment. The CH_2Cl_2 fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed (2 ×) using petroleum ether to yield **1** (2 mg). The more polar fractions were combined and rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **7** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to provide **6** (4 mg).

Isolation of chemical constituents from the bark

The air-dried bark of *C. curanii* (95.5 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.2 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to afford **6** (3 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_2Cl_2 to yield **8** (5 mg) after washing with petroleum ether.

Isolation of chemical constituents from the lamina

The air-dried lamina of *C. curanii* (33 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.4 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 2.5% EtOAc in petroleum ether to yield **7** (4 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_2Cl_2 to afford a mixture of **8** and stigmasterol (**9**) (5 mg) after washing with petroleum ether.

Isolation of chemical constituents from the endotesta

The air-dried endotesta of *C. lacrimans* (91 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.25 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to yield **6** (4 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 20% EtOAc in petroleum ether to afford **8** (5 mg) after washing with petroleum ether.

Isolation of chemical constituents from the petiole

The air-dried petiole of *C. curanii* (77 g) were ground in a blender, soaked in CH_2Cl_2 for 3 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 at 10% increment. The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 7.5% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (3 ×) using the same solvent to afford **6** (4 mg). The more polar fractions were combined and rechromatographed (3 ×) using 10% EtOAc in petroleum ether to yield a mixture of **7** and **11** (5 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford **10** (2 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to provide a mixture of **8** and **9** (7 mg) after washing with petroleum ether.

Isolation of chemical constituents from the roots

The air-dried roots of *C. curanii* (20.5 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.1 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to afford **6** (2 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to provide **8** (3 mg) after washing with petroleum ether.

Isolation of chemical constituents from the sclerotesta

The air-dried sclerotesta of *C. curanii* (65.5 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.1 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford **8** (2 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *C. curanii* led to the isolation of squalene (**1**)[13], lutein (**2**)[14], chlorophyll a (**3**)[15], phytol (**4**)[16], long chain 1-alkene (**5**)[17], and triacylglycerols (**6**)[13] from the leaflets; **1**, **6**, and β-sitosteryl fatty acid ester (**7**)[9] from the sarcotesta; **6** and β-sitosterol (**8**)[13] from the bark; **7** and a mixture of **8** and stigmasterol (**9**)[11] from the lamina; **6** and **8** from the endotesta; **6**, β-sitosterone (**10**)[18], a mixture of **7** and phytyl fatty acid ester (**11**)[9], and a mixture of **8** and **9** from the petiole; **6** and **8** from the roots; and **8** from the sclerotesta. The structures of **1**-**11** were identified by comparison of their NMR data with literature data.

These results indicate that *C. curanii* shares similar chemical characteristics with other members of the genus *Cycas* and the family Cycadaceae: *C. sancti-lasallei* which contained **1** and **7**-**10**[9]; *C. vespertilio*[10.11], *C. aenigma*[12], and *C. lacrimans*[8] which yielded **6**, **8**, and **9**[8]. To our knowledge, this is the first report on the isolation of **1**-**11** from *C. curanii.*

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