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Chemical constituents of *Cycas mindanaensis*

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ABSTRACT

*Chemical investigation of the dichlroromethane extracts of Cycas mindanaensis, a plant endemic to the Philippines afforded α-tocopherol (**1**),squalene (**2**), and long chain 1-alkenes (**3**) from the leaflets;**2**, β-sitosterol (**4**) and β-sitosteryl fatty acid esters(**5**) from the bark; **2**, triacylglycerols (**6**), and hydrocarbons (**7**)from the roots; **5**, **6**, anda mixture of **4** and stigmasterol (**8**) from the petiole and rachis; and **6**,fatty alcohols (**9**) and a mixture of **4** and **8**from the megasporophyll lamina. The structures of **1**-**9**were identified by comparison of their NMR data with literature data.*

Keywords: *Cycas mindanaensis*, Cycadaceae, α-tocopherol,squalene, 1-alkenes, β-sitosterol, β-sitosteryl fatty acid ester, stigmasterol, triacylglycerols, fatty alcohols, hydrocarbons

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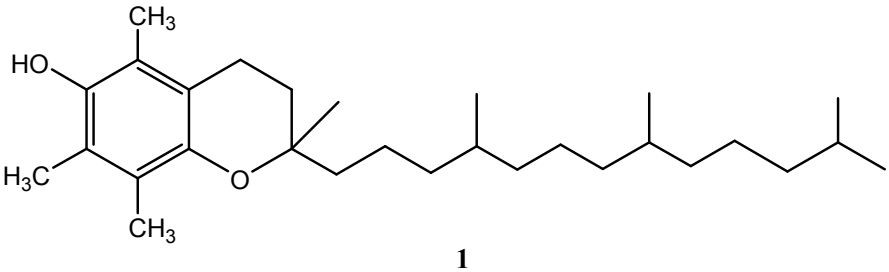
INTRODUCTION

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].

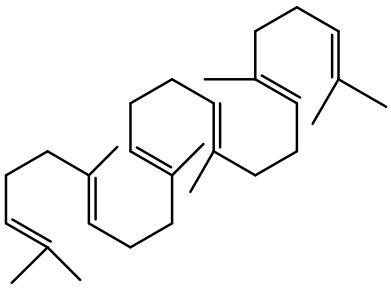
There are no reported chemical and biological activity studies on *C. mindanaensis*. However, a number of studies have been reported on the chemical constituents of other indigenous Philippine *Cycas.* We earlier reported the chemical constituents of the different parts of *C. sancti-lasallei* [8-11], *C. vespertilio* [12, 13], *C. zambalensis* [14], *C. lacrimans* [15-17], *C. aenigma* [18, 19], *C. riuminiana* [20], *C. nitida* [21], *C. wadei* [22],and *C. edentata* [23, 24].

We report herein the isolation of α-tocopherol(**1**),squalene (**2**), and long chain 1-alkenes (**3**) from the leaves; **2**, β-sitosterol (**4**) and β-sitosteryl fatty acid esters (**5**) from the bark; **2**, triacylglycerols (**6**), and hydrocarbons(**7**)from the roots; **5**, **6**, anda mixture of **4** and stigmasterol (**8**) from the petiole and rachis; and **6**,fatty alcohols (**9**) and a mixture of **4** and **8** from the megasporophyll lamina of*Cycas mindanaensis*The structures of **1**-**9**are presented in Fig. 1.

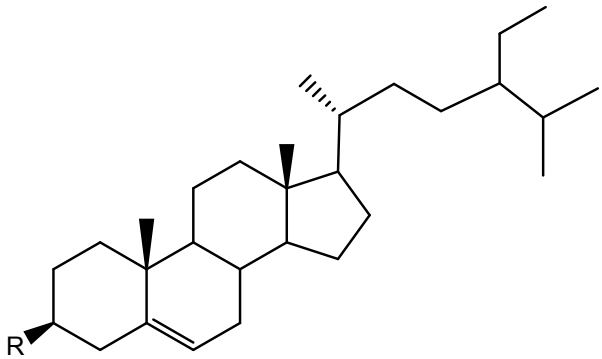


1

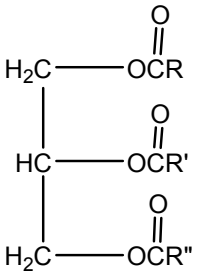
3 $RCH=CH_2$
7 $CH_3(CH_2)nCH_3$
9 RCH_2OH



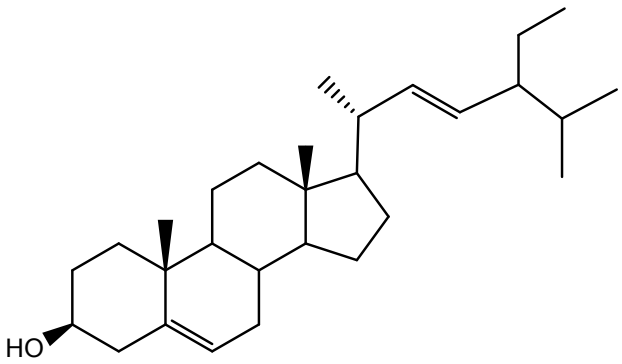
2



4 R = OH
5 R = R'COO



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



8

Fig. 1. Chemical structures of α-tocopherol(1),squalene (2), long chain 1-alkenes (3), β-sitosterol (4), β-sitosteryl fatty acid esters (5), triacylglycerols (6), hydrocarbons (7), stigmasterol (8),and fatty alcohols (9) from *C. mindanaensis*

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.

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 mindanaensis leaflets, bark, roots, petiole and rachis, and megasporophyll lamina were collected from Mati, Davao Oriental, Philippines on June 14, 2015. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3121).

Isolation of the Chemical Constituents of the Leaflets

The air-dried leaflets (176 g) of *C. mindanaensis* 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 (2.9 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford**2** (4 mg) and**3** (2 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using % EtOAc in petroleum ether to yield **1**(3 mg).

Isolation of the Chemical Constituents of the Bark

The air-dried bark (47.0 g) of *C. mindanaensis*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 (0.4 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford**2** (3 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 2.5% EtOAc in petroleum ether to yield **5**(3 mg). The 60% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford **4** (7 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of the Chemical Constituents of the Roots

The air-dried roots (129.8 g) of *C. mindanaensis*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 (0.4 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed using petroleum ether to afford**7** (3 mg). The more polar fractions were combined and rechromatographed using petroleum ether to yield **2** (1 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 5% EtOAc in petroleum ether to afford **6**(5 mg).

Isolation of the Chemical Constituents of the Petiole and Rachis

The air-dried petiole and rachis (129.2 g) of *C. mindanaensis*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 CH_2Cl_2 fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **5**(4 mg). The 30% acetonein CH_2Cl_2 fraction was rechromatographed (2 ×) using 2.5% EtOAc in petroleum ether to afford **6**(7 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford a mixture of **4** and **8**(8 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of the Chemical Constituents of the Megasporophyll Lamina

The air-dried megasporophyll lamina (56.4 g) of *C. mindanaensis*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 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether, followed by 5% EtOAc in petroleum ether. The fractions eluted with 2.5% EtOAc in petroleum ether were combined and rechromatographed (2 ×) using 5% EtOAc in petroleum etherto afford **6**(5 mg). The fractions eluted with 5% EtOAc in petroleum ether were combined and rechromatographed (2 ×) using 7.5% EtOAc in petroleum etherto afford **9**(4 mg).The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford a mixture of **4** and **8** (6 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the CH_2Cl_2 extracts of *Cycas mindanaensis* yielded α-tocopherol (**1**) [20], squalene (**2**) [25],andlong-chain 1-alkene (**3**) [26] from the leaflets; β-sitosterol (**4**) [27] and β-sitosteryl fatty acid esters (**5**) [8] from the bark; **2**, triacylglycerols (**6**) [28], and hydrocarbons (**7**)[29] from the roots; **5**, **6**, anda mixture of **4** and stigmasterol (**8**) [30] from the petiole and rachis; and**6**,fatty alcohols (**9**) [27] and a mixture of **4** and **8** from the megasporophyll lamina. The structures of **1**-**9**were identified by comparison of their NMR data with literature data.

Cycas mindanaensis shares similar chemical characteristics with other species of the genus *Cycas.* Compounds **1**-**6** and **8**were also isolated from *C. riuminiana* [20]. The commonly isolated compounds from the different parts of the other *Cycas* speciesare β-sitosteroland triacylglycerols [8-24], while **1** was only found in *C. riuminiana* [20] and *C. mindanaensis*.An analogue of **1**, δ-tocopherol was isolated from *C. wadei* [22]. To our knowledge, this is the first report on the isolation of **1**-**9**from *C. mindanaensisi.*

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