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Research Article

Chemical Constituents of *Cycas edentata*

Consolacion Y. Ragasa[a,b*], Vincent Antonio S. Ng[b], Esperanza Maribel G. Agoo[c], Chien-Chang Shen[d]
[a]Chemistry Dept, De La Salle University Science & Technology Complex, Leandro V. Locsin Campus, Binan City, Laguna, Philippines.
[b]Chemistry Department, De La Salle University 2401 Taft Avenue, Manila, Philippines.
[c]Biology Department, De La Salle University 2401 Taft Avenue, Manila, Philippines.
[d]National Research Institute of Chinese Medicine, Ministry of Health and Welfare, 155-1, Li-Nong St., Sec. 2, Taipei, Taiwan.
***Corresponding author's E-mail:** consolacion.ragasa@dlsu.edu.ph

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ABSTRACT

Chemical investigation of the dichloromethane extracts of *Cycas edentata* led to the isolation of 9αH-isopimara-7,15-diene (**1**), β-sitosteryl fatty acid ester (**2**), and a mixture of β-sitosterol (**3a**) and stigmasterol (**3b**) from the bark; and **2** and a mixture of **3a** and **3b** from the sclerotesta. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy, while those of **2-3b** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Cycas edentata*, Cycadaceae, 9αH-isopimara-7,15-diene, β-sitosteryl fatty acid ester, β-sitosterol, stigmasterol

INTRODUCTION

Cycas edentata of the Cycadaceae family is classified as near threatened by the IUCN.[1] This species which occurs along shorelines is declining due to loss of coastal habitat. *C. edentata* is native to Indonesia, Malaysia, Myanmar, Philippines, Thailand and Vietnam.[1] There are no reported chemical and biological activity studies on *C. edentata.*

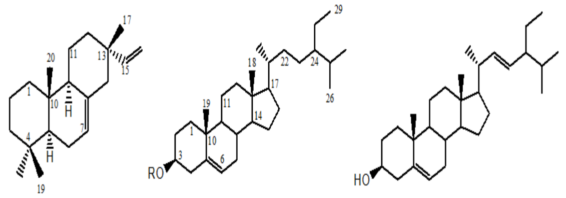
This study was conducted as part of our research on the chemical constituents of *Cycas* species that are endemic and native to the Philippines.

We recently reported the isolation of squalene, β-sitosterol, stigmasterol, and triglycerides from the sarcotesta; β-sitosterol, stigmasterol, triglycerides and phytyl fatty acid esters from the endotesta; β-sitosterol, stigmasterol, triglycerides, and β-sitosteryl fatty acid esters from the sclerotesta; and β-sitosteryl fatty acid esters from the bark of *Cycas sancti-lasallei*.[2]

Another *Cycas* species, *C. lacrimans* yielded isopimaran-19-ol from the megasporophyll lamina; 9αH-isopimara-7,15-diene and triacylglycerols from the bark; triacylglycerols, oleic acid, and 1,2-dioleylglycerol from the leaflets; triacylglycerols, β-sitosterol, and stigmasterol from the petiole and rachis; β-sitosterol from the roots; and triacylglycerols and β-sitosterol from the endotesta and sclerotesta.[3]

We report herein the isolation of 9αH-isopimara-7,15-diene (**1**), β-sitosteryl fatty acid ester (**2**), and a mixture of β-sitosterol (**3a**) and stigmasterol (**3b**) in about 3.5:1 ratio from the bark; and **2** and a mixture of **3a** and **3b** in about 3.5:1 ratio from the sclerotesta of *C. edentata*.

To the best of our knowledge this is the first report on the isolation of **1-3b** from the plant.



1 2 R = long chain fatty acids 3a R = OH 3b

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 edenta bark and sclerotesta were collected in November 2014 and authenticated by one of the authors (EMGA). Voucher specimens were deposited in the De La Salle University-Manila Herbarium (DLSUH 3114).

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 Bark

The air-dried bark of *C. edentata* (144 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 (0.7 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** (3 mg) after washing with petroleum ether.

The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) in 5% EtOAc in petroleum ether to yield **2** (2 mg) after washing with petroleum ether.

The 30% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in $CH_3CN:Et_2O:CH_2Cl_2$ (0.5:0.5:9 by volume) to yield a mixture of **3a** and **3b** (4 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Sclerotesta

The air-dried sclerotesta of *C. edentata* (48 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 (0.1 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment.

The CH_2Cl_2 fraction was rechromatographed (3 ×) in 5% EtOAc in petroleum ether to yield **2** (2 mg) after washing with petroleum ether.

The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) in 20% EtOAc in petroleum ether to yield a mixture of **3a** and **3b** (7 mg) after washing with petroleum ether.

9αH-Isopimara-7,15-diene (**1**): ^1H NMR (600 MHz, $CDCl_3$): δ 0.85 (6H, s, H-17, H-20), 0.86 (3H, s, H-18); 0.90 (3H, s, H-19), 4.85 (d, *J*=10.8 Hz, H-16), 4.92 (d, *J*=17.4 Hz, H-16), 5.34 (brs, H-7), 5.80 (dd, *J*=17.4, 10.8 Hz, H-15). ^{13}CNMR (150 MHz, $CDCl_3$): δ 14.94 (C-20), 18.85 (C-2), 20.19 (C-11), 21.46 (C-17), 22.69 (C-19), 23.46 (C-6), 32.82 (C-4), 33.62 (C-18), 35.43 (C-10), 36.22 (C-12), 37.38 (C-13), 39.87 (C-1), 42.28 (C-3), 46.18 (C-14), 50.38 (C-5), 52.00 (C-9), 109.13 (C-16), 121.68 (C-7), 135.52 (C-8), 150.50 (C-15).

β-Sitosteryl Fatty Acid Esters (**2**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 36.99 (C-1), 31.52 (C-2), 73.68 (C-3), 42.30 (C-4), 139.71 (C-5), 122.58 (C-6), 32.19, 31.92 (C-7, C-8), 50.01 (C-9), 36.15 (C-10), 21.02 (C-11), 39.71(C-12), 42.30 (C-13), 56.67 (C-14), 24.28 (C-15), 28.24 (C-16), 56.01(C-17), 11.84 (C-18), 19.32 (C-19), 36.59 (C-20), 19.02 (C-21), 33.92 (C-22), 29.13 (C-23), 45.82 (C-24), 26.04 (C-25), 18.76 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C-29), 173.30 (C-1'), 34.70 (C-2'), 29.76, 29.70, 29.65, 29.59, 29.52, 29.47, 29.36, 29.34, 29.32, 29.27, 29.25, 29.16, 29.13, 29.10, 29.08, 27.80, 27.21, 27.19, 27.16, 26.39, 26.04, 25.62, 25.06, 25.04, 24.28, 23.42, 23.05, 22.69, 22.57 (CH_2), 130.21, 130.06, 129.98, 129.76 (CH=), 14.12, 14.07 (terminal CH_3).

β-Sitosterol (**3a**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.81 (C-3), 42.31 (C-4), 140.74 (C-5), 121.72 (C-6), 31.89, 31.90 (C-7, C-8), 50.14 (C-9), 36.49 (C-10), 21.07 (C-11), 39.76 (C-12), 42.20 (C-13), 56.75 (C-14), 24.35 (C-15), 28.24 (C-16), 56.03 (C-17), 11.97 (C-18), 19.39 (C-19), 36.14 (C-20), 18.77 (C-21), 33.93 (C-22), 26.04 (C-23), 45.82 (C-24), 29.13 (C-25), 19.02 (C-26), 19.81 (C-27), 23.05 (C-28), 11.85 (C-29).

Stigmasterol (**3b**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.81 (C-3), 42.29 (C-4), 140.74 (C-5), 121.72 (C-6), 31.89 (C-7, C-8), 50.11 (C-9), 36.49 (C-10), 21.09 (C-11), 39.67 (C-12), 42.20 (C-13), 56.85 (C-14), 24.35 (C-15), 28.92 (C-16), 55.93 (C-17), 12.04 (C-18), 19.39 (C-19), 40.49 (C-20), 21.09 (C-21), 138.31 (C-22), 129.25 (C-23), 51.23 (C-24), 31.89 (C-25), 21.20 (C-26), 18.97 (C-27), 25.40 (C-28), 12.25 (C-29).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *C. edentata* yielded **1**-**3b** from the bark; and **2**-**3b** from the sclerotesta.

The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its NMR data with those reported in the literature for 9αH-isopimara-7,15-diene.[2]

Compounds **2**-**3b** were identified by comparison of their NMR data with those reported in the literature for β-sitosteryl fatty acid ester (**2**),[3] β-sitosterol (**3a**),[4] and stigmasterol (**3b**).[4]

The mixture of **3a** and **3b** in about 3.5:1 ratio was deduced from the ^1H NMR resonances for the olefinic protons of **3a** at δ 5.33 (dd, *J*=1.8, 5.4 Hz, H-6) and **3b** at δ 5.33 (dd, *J*=1.8, 5.4 Hz, H-6), 5.13 (dd, *J*=9.0, 15.0 Hz, H-22) and 5.00 (dd, *J*=9.0, 15.0 Hz, H-23).[5]

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Conflict of Interest: None.