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Research Article

Lignans and a Furanone from *Cycas sancti-lasallei*

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ABSTRACT
Chemical investigation of the dichloromethane extracts of *Cycas sancti-lasallei*, a plant endemic to the Philippines, led to the isolation of sesamin (**1**), 4-hydroxymethyl-3,5-dimethyldihydro-2-furanone (**2**), squalene, and phytyl fatty acid ester from the petiole and rachis; and 8-hydroxypinoresinol (**3**) from the sclerotesta. The structures of **1**-**3** were elucidated by extensive 1D and 2D NMR spectroscopy. The relative stereochemistry of **3** was determined from NOESY.

Keywords: *Cycas sancti-lasallei*, Cycadaceae, sesamin, 4-hydroxymethyl-3,5-dimethyldihydro-2-furanone, 8-hydroxypinoresinol

INTRODUCTION

Cycas, the only currently known genus of the Family Cycadaceae, are considered as fossil plants though they may have evolved only about 12 million years ago[1]. The cycads resemble palms in morphology and are commonly called sago palm. These are widely distributed in the Tropics, with species found in Asia, Africa, Southeast Asia, Pacific, and Australia[2]. They also grow on volcanic, limestone, ultramafic, sandy, or even water-logged soils in grassland and forest habitats[3].

In the Philippines, there are eleven cycad species namely, *C. aenigma* K. D. Hill & Lindstrom, *C. curranii* (J. Schust.) K. D. Hill, *C.edentata* de Laub., *C. lacrimans* Lindstrom & K. D. Hill, *C. nitida* K. D. Hill & Lindstrom, *C. riuminiana* Porte ex Regel, *C. saxatilis* K. D. Hill & Lindstrom, *C. sancti-lasallei* Agoo & Madulid, *C. wadei* Merr., *C. vespertilio* Lindstrom & K. D. Hill, and *C. zambalensis* Madulid & Agoo[3-5]. All species, except for *C. edentata*, are endemic to the Philippines[4]. *C. revoluta*, a widely cultivated species, is an introduced species from Japan and Taiwan.

The demand of *Cycas* species for domestic and international horticultural trade, grassland and forest fires of anthropogenic origin, and conversion of their natural habitats to settlements and other land uses have threatened to varying degrees the wild populations of the genus[6]. Some of these species which have been assessed and evaluated in the 2010 IUCN Red List of Threatened Species are *C. curranii*[7], *C. wadei*[8] and *C. zambalensis* as Critically Endangered (CR)[7], *C. riuminiana* (E) as Endangered (E)[7], and *C. saxatilis* as Vulnerable (V)[9].

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 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, and triglycerides, and β-sitosteryl fatty acid esters from the sclerotesta; and β-sitosteryl fatty acid esters from the bark of *Cycas sancti-lasallei*[10]. 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[11]. Recently, we reported the isolation of β-sitosteryl fatty acid ester and a mixture of β-sitosterol and stigmasterol from the bark and sclerotesta of *C. edentata*. The bark also yielded 9αH-isopimara-7,15-diene[12].

We report herein the isolation and identification of sesamin (**1**), 4-hydroxymethyl-3,5-dimethyldihydro-2-furanone (**2**), squalene, and phytyl fatty acid ester from the petiole and rachis; and 8-hydroxypinoresinol (**3**) from the sclerotesta of *Cycas sancti-lasallei*. To the best of our knowledge this is the first report on the isolation of these compounds from *C. sancti-lasallei.*

EXPERIMENTAL

General Experimental Procedure

NMR spectra were recorded on a Varian VNMR Spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR

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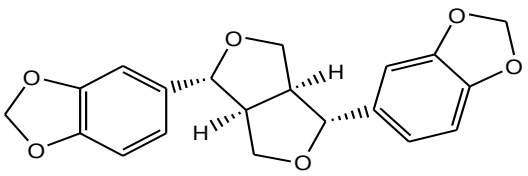

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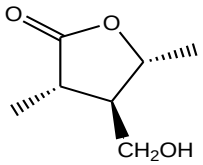

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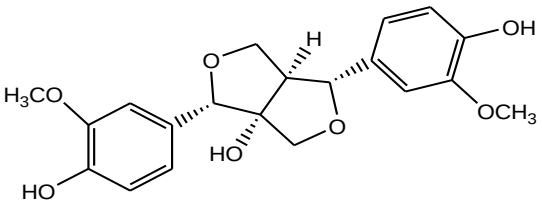

3

Chemical structures of sesamin (**1**), 4-hydroxymethyl-3,5-dimethyldihydro-2-furanone (**2**), and 8-hydroxypinoresinol (**3**)

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.

Sample Collection

Cycas sancti-lasallei petiole and rachis, and sclerotesta were collected from Malasag, Cugman, Cagayan de Oro, Misamis Oriental, Philippines on April 25, 2014. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3116).

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. Fifty 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. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of Chemical Constituents from Petiole and Rachis

The air-dried petiole and rachis of *C. sancti-lasallei* (47 g) was ground ina 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 squalene (7 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 7.5% EtOAc in petroleum ether to afford phytyl fatty acid ester (4 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 20% EtOAc in petroleum ether to afford **1** (5 mg) after washing with petroleum ether. The 70% acetone in CH_2Cl_2 fraction was rechromatographed (5 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6 by volume ratio) to afford **2** (6 mg).

Isolation of 8-Pinoresinol from Sclerotesta

The air-dried sclerotesta of *C. sancti-lasallei* (166 g) was 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 (0.2 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 70% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6 by volume ratio) to afford **3** (7 mg) after washing with petroleum ether.

Sesamin (**1**): ^1H NMR (600 MHz, $CDCl_3$): δ 4.70 (2H, d, *J* = 4.8 Hz, H-1, H-4), 3.03 (2H, m, H-2, H-5), 3.84 (2H, dd, *J* = 3.6, 9.0 Hz, H-3, H-6), 4.21 (2H, dd, *J* = 7.2, 9.0 Hz, H-3, H-6), 6.83 (2H, d, *J* = 1.8 Hz, H-2, H-2'), 6.75-6.79 (4H, H-5', H-5", H-6', H-6"), 5.93 (2× –OCH_2O–); ^{13}C NMR (150 MHz, $CDCl_3$): δ 85.78 (C-1, C-4), 54.32 (C-2, C-5), 71.70 (C-3, C-6), 135.03 (C-1', C-1"), 106.48 (C-2', C-2"), 147.96 (C-3', C-3"), 147.10 (C-4', C-4"), 108.18 (C-5', C-5"), 119.35 (C-6', C-6"), 101.06 (2× –OCH_2O–).

4-Hydroxymethyl-3,5-dimethyldihydro-2-furanone (**2**): ^1H NMR (600 MHz, $CDCl_3$): δ 1.27 (3 H, d, *J* = 6.6 Hz), 1.45 (3H, d, *J* = 6 Hz), 1.85 (1H, m), 2.59 (1H, dq, *J* = 11.4, 7.2 Hz), 3.75 (1H, dd, *J* = 10.8, 4.8 Hz), 3.81 (1H, dd, *J* = 10.8, 4.2 Hz), 4.37 (dq, 1 H, *J* = 9.0, 6.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 178.78 (C-2), 76.79 (C-5), 60.70 (C-8), 53.19 (C-4), 37.68 (C-3), 20.05 (C-6), 14.10 (C-7).

8-Hydroxypinoresinol (**3**): ^1H NMR (600 MHz, $CDCl_3$): δ 4.80 (s, H-1), 4.04 (1H, d, *J* = 9.0 Hz, H-3), 3.89 (1H, d, *J* = 9.6 Hz, H-3), 4.83 (1H, d, *J* = 4.8 Hz, H-4), 3.10 (1H, m, H-5), 3.82 (1H, dd, *J* = 6.0, 9.0 Hz, H-6), 4.50 (1H, d, *J* = 8.4, 9.0 Hz, H-6), 6.96 (1H, d, *J* = 1.8 Hz), 6.97 (d, *J* = 9.6 Hz), 6.94 (d, *J* = 7.8 Hz), 6.88 (d, *J* = 1.8 Hz), 3.91, 3.89 (s, 3'–OCH_3, 3"-OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 87.80 (C-1), 91.65 (C-2), 74.69 (C-3), 85.80 (C-4), 60.08 (C-5), 71.68 (C-6), 126.99 (C-1'), 132.38 (C-1"), 109.31 (C-2'), 109.00 (C-2"), 146.94 (C-3'), 146.64 (C-3"), 146.02

(C-4'), 145.44 (C-4"), 114.68 (C-5'), 114.26 (C-5"), 119.60 (C-6'), 119.68 (C-6"), 56.02 (3'–OCH_3), 55.94 (3"–OCH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the *Cycas sancti-lasallei* afforded sesamin (**1**)[13], and 4-hydroxymethyl-3,5-dimethyldihydro-2-furanone (**2**)[14,15], squalene[16], phytyl fatty acid ester[17] from the petiole and rachis; and 8-hydroxypinoresinol (**3**)[18] from the sclerotesta. The structures of **1**-**3** were elucidated by extensive 1D and 2D NMR spectroscopy. The relative stereochemistry of **3** was determined from NOESY.

Sesamin (**1**) was reported to exhibit antihypertensive effect in humans[19]. It exhibited antioxidant activity in the liver and regulated the transcription levels of hepatic metabolizing enzymes for lipids and alcohol[20]. It reduced serum and liver lipid levels and increased hepatic fatty acid oxidation[21,22]. Lignan **1** showed anti-angiogenic activity and has potential for preventing tumor angiogenesis[23]. It exhibited significant antifeedant activity and moderate growth inhibition towards 4th instar larvae of *Spilarctia oblique*[24]. Furthermore, it exerted anti-inflammatory effects in rats that were augmented by linseed oil[25]. On the other hand, the cell injury by 3-morpholinosydnonimine (SIN-1), a $ONOO^-$ generator was reported to be significantly reduced by 8-hydroxypinoresinol (**3**) and phillygenin, indicating antioxidant activities[26]. This lignan was also reported to exhibit powerful antioxidant and high vasorelaxant activities[27].

CONCLUSION

Sesamin (**1**), 4-hydroxymethyl-3,5-dimethyldihydro-2-furanone (**2**), and 8-hydroxypinoresinol (**3**) were isolated for the first time from *Cycas sancti-lasallei*. To the best of our knowledge, this is the first report on the isolation of these compounds from the genus *Cycas*, and the family Cycadaceae.

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