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Research Article

An Isoflavonoid Phtoalexin and a Sesquiterpene from *Cycas wadei*

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ABSTRACT
Chemical investigation of the dichloromethane extract of the roots of *Cycas wadei*, a plant endemic to the Philippines, led to the isolation of a new isoflavonoid phtoalexin, wadeiol (**1**) and selin-4(5)-en-1β,11-diol (**2**). The structures of these compounds were elucidated by extensive 1D and 2D NMR spectroscopy.

Keywords: *Cycas wadei,* Cycadaceae, isoflavonoid phtoalexin, wadeiol, sesquiterpene, selin-4(5)-en-1β,11-diol

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]. Ten out of the eleven cycad species in the Philippines are endemic. The most studied *Cycas* are *Cycas revoluta* and *C. circinalis* which contain the carcinogenic toxin cycasin[4,5]. The methanolic extract of the leaflets of *C. circinalis* L. and the chloroform extract of *C. revoluta* yielded biflavonoids, lignans, flavan-3-ols, flavone-C-glucosides, nor-isoprenoids, and a flavanone. Three of the biflavonoids exhibited moderate activity against *S. aureus* and methicillin-resistant *S. aureus*[6]. Further studies on the chemical constituents of the leaves of *C. revoluta* and *C. circinalis* afforded lariciresinol, naringenin and biflavonoids which are derivatives of amentoflavone and hinokiflavone[7]. A number of studies have been reported on the chemical constituents of 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. edentata*[20,21], *C. riumimiana*[22], *C. curranii*[23], *C. nitida*[24,25], *C. mindanaensis*[26], *C, flabellata*[27], and *C. saxatilis*[28]. We recently reported the isolation of δ-tocopherol, β-sitosterol, β-sitosteryl fatty acid ester, and triacylglycerol from the megasporophyll lamina; β-sitosterol, trilinolein, a mixture of linoleic acid and oleic acid, and hydrocarbons from the sarcotesta; β-sitosterol, chlorophyll a, fatty alcohol, and hydrocarbons from the leaflets; a mixture of β-sitosterol and stigmasterol, squalene, and fatty alcohol from the petiole and rachis; β-sitosterol and triacylglycerol from the endotesta; β-sitosteryl fatty acid ester and triacylglycerol from the sclerotesta; and a mixture of β-sitosterol and stigmasterol, squalene and fatty alcohol from the roots of *Cycas wadei*[29]. We report herein the isolation of a new isoflavonoid phtoalexin, wadeiol (**1**) and selin-4(5)-en-1β,11-diol (**2**) from the roots of *C. wadei*. The structures of **1** and **2** are presented in Fig. 1. This is the first report on the isolation of **1** and **2** from *C. wadei*, the genus *Cycas*, and the family Cycadaceae.

MATERIALS AND METHODS

General Isolation 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). The HRESIMS spectrum for **1** was obtained on an AB Sciex QSTAR XL high resolution electrospray mass spectrometer. 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

The roots of *Cycas wadei* were collected in 2014. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3115).

Isolation of the Chemical Constituents of the Roots

The air-dried roots of *C. wadei* (15.5 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The

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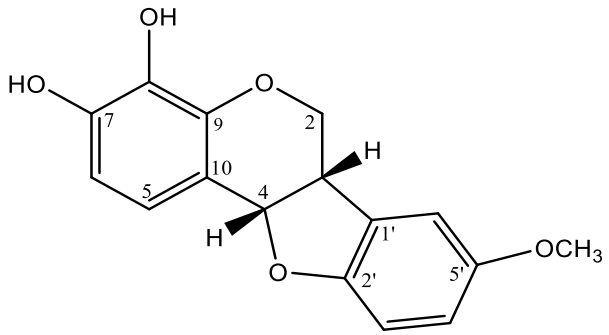

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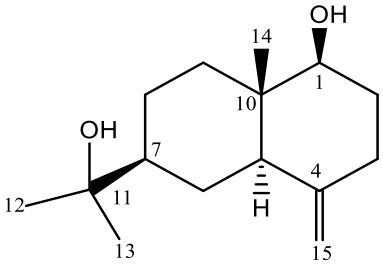

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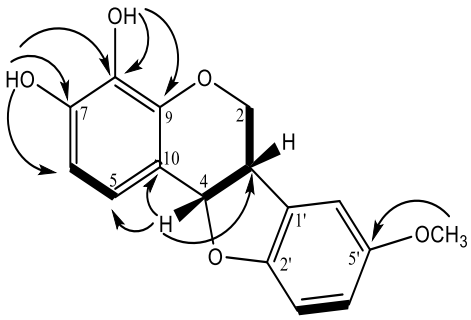
Figure 1: Chemical structures of wadeiol (**1**) and selin-4(5)-en-1β,11-diol (**2**) from *C. wadei*.



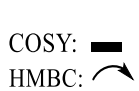


Figure 2: ^{1}H-^{1}H COSY and key ^{1}H-^{13}C long-range correlations of **1**.

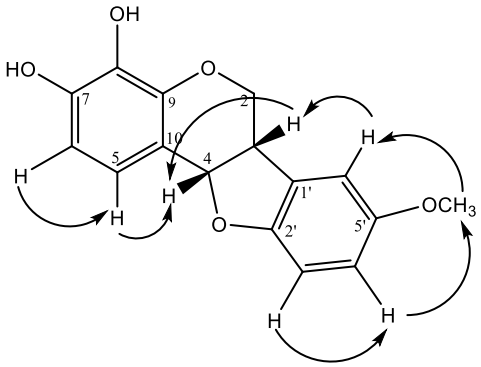


Figure 3: Key NOESY correlations of **1**.

solvent was evaporated under vacuum to afford a crude extract (0.45 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 80% acetone in CH_2Cl_2 fraction was rechromatographed using CH_2Cl_2. The less polar fractions were combined and rechromatographed (2 ×) using CH_2Cl_2 to afford **1** (5 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1.5:1.5:7 by volume ratio) to yield **2** (3 mg) after washing with petroleum ether.

Wadeiol (1): ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.68 (dd, *J* = 3.0, 10.8 Hz, H-2), 4.33 (dd, *J* = 4.8, 10.8 Hz, H-2), 3.57 (m, H-3), 5.52 (d, *J* = 7.2 Hz, H-4), 6.99 (d, *J* = 8.4 Hz, H-5), 6.66 (d, *J* = 8.4 Hz, H-6), 7.11 (*J* = Hz, H-3'), 6.44 (H-4'), 6.45 (s, H-6'), 3.75 (s, OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 67.04 (C-2), 39.65 (C-3), 78.73 (C-4), 121.82 (C-5), 109.53 (C-6), 144.37 (C-7), 131.51 (C-8), 143.06 (C-9), 112.53 (C-10), 118.63 (C-1'), 160.71 (C-2'), 124.71 C-3'), 96.94 (C-4'), 161.24 (C-5'), 106.51 (C-6'), 55.50 (OCH_3). HRESIMS *m/z* 285.0764 $[M-H]^-$ ($C_{16}H_{13}O$ requires 285.0763).

Selen-4(15)-en-1β-11-diol (2): ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.39 (dd, *J* = 4.2, 11.4 Hz, H-1), 1.56, 1.80 (H_2-2), 2.10, 2.30 (H_2-3), 1.72 (H-5), 1.20. 1.68 (H_2-6), 1.35 (H-7), 1.24, 1.70 (H_2-8), 1.18, 1.97 (H_2-9), 1.20 (s, H_3-12 and H_3-13), 0.66 (s, H_3-14), 4.51 (d, *J* = 1.8 Hz, H-15), 4.75 (d, *J* = 1.8 Hz, H-15); ^{13}C NMR (150 MHz, $CDCl_3$): δ 79.34 (C-1), 31.14 (C-2), 34.17 (C-3), 148.88 (C-4), 47.46 (C-5), 24.58 (C-6), 48.91 (C-7), 22.15 (C-8), 36.92 (C-9), 40.15 (C-10), 72.01 (C-11), 27.09, 27.31 (C-12, C-13), 10.21 (C-14), 106.87 (C-15).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the roots of *Cycas wadei*, a plant endemic to the Philippines, led to the isolation of wadeiol (**1**) and selin-4(5)-en-1β,11-diol (**2**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy as follows. The ^{1}H NMR and COSY spectra of **1** indicated resonances for aromatic proton doublets at δ 6.66 and 6.99 which were ortho coupled to each other by 8.4 Hz and another aromatic proton doublets at δ 6.44 and 7.11 which were ortho coupled to each other by 7.8 Hz. Another set of coupled protons was detected at δ 3.68 which was geminally coupled to the proton at δ 4.33 by 10.8 Hz. These oxymethylene protons were also coupled to the methine proton at δ 3.57 by 3.0 and 4.8 Hz, respectively. This methine proton was also coupled to the oxymethine proton at δ 5.52 by 7.2 Hz. An aromatic proton singlet was detected at δ 6.45, while two hydroxyl protons were deduced from the resonances at δ 5.35 and 5.30. The ^{13}C NMR spectrum gave resonances for five protonated aromatic carbons at δ 96.94, 106.51, 109.53, 124.71 and 121.82; two non-protonated aromatic carbons at δ 112.53 and 118.63; four oxygenated aromatic carbons at δ 131.51, 143.06, 144.37, 160.71 and 161.24; an oxymethylene carbon at δ 67.04; an oxymethine carbon at δ 78.73; a methine carbon at δ 39.65; and a methoxy carbon at δ 55.50. These resonances indicate an isoflavonoid

phtoalexin[30] with two hydroxyl and a methoxy substituents. The protons attached to carbon atoms were assigned from HSQC 2D NMR data (see experimental), and the structure of **1** was elucidated by analysis of the HMBC 2D NMR data. Key HMBC correlations are shown in Fig. 1. Thus, one of the hydroxyls at δ 5.30 was attached to C-8 based on long-range correlations between this hydroxyl and C-7, C-8 and C-9. The second hydroxyl at δ 5.35 was attached to C-7 since long-range correlations were observed between this hydroxyl and and C-6, C-7, and C-8. The methoxy at δ 3.75 was attached to C-5' due to long-range correlations between these protons and C-5'. Long-range correlations were also observed between H-4 and C-2, C-5, C-9, and C-10. All long-range correlations observed are consistent with the structure of **1**. The relative stereochemistry of **1** was deduced from the NOESY spectrum as follows. H-5 was close in space to H-6; H-4 to H-5; H-3 to H-6'; H-3' to H-4'; and H_2-2 to H-3 to H-4. The NOESY correlations and the relative stereochemistry of **1** are shown in Fig. 2. The structure of **1** was confirmed by negative HRESIMS which gave a pseudo molecular ion *m/z* = 285.0764 $[M-H]^-$ corresponding to a molecular formula of $C_{16}H_{13}O_5$. The structure of **2** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its ^{13}C NMR data with those reported in the literature[31].

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