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Short communication

Chemical constituents of *Cycas vespertilio*

Consolacion Yasaña Ragasa[a,b,*], Vincent Antonio Santos Ng[b], Esperanza Maribel Guiao Agoo[c], Chien-Chang Shen[d]

[a] *Chemistry Department, De La Salle University Science & Technology Complex, Leandro V. Locsin Campus, Binan City, Laguna, Philippines*
[b] *Chemistry Department, De La Salle University, Manila, Philippines*
[c] *Biology Department, De La Salle University, Manila, Philippines*
[d] *National Research Institute of Chinese Medicine, Taipei, Taiwan*

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ABSTRACT

Chemical investigation of *Cycas vespertilio* A. Lindstr. & K.D. Hill, Cycadaceae, a plant endemic to the Philippines, yielded pinoresinol (**1**), sesamin (**2**), paulownin (**3**), a mixture of β-sitosterol and stigmasterol, and triacylglycerols from the cone base; **1**, **3**, β-sitosterol, stigmasterol, triacylglycerols, and lariciresinol (**4**) from the cataphylls; β-sitosterol from the megasporophyll lamina; β-sitosterol and a mixture of *trans*-4-hydroxycinnamate fatty acid esters (**5**) and *cis*-4-hydroxycinnamate fatty acid esters (**6**) from the unripe sarcotesta; and β-sitosterol and triacylglycerols from the ripe sarcotesta. The structures **1**–**6** were elucidated by extensive 1D and 2D NMR spectroscopy.

Introduction

Cycas, the only currently known genus of the Family Cycadaceae, is considered as fossil plants though they may have evolved only about 12 million years ago (Nagalingum et al., 2011). 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 (Donaldson, 2003). They also grow on volcanic, limestone, ultramafic, sandy, or even water-logged soils in grassland and forest habitats (Madulid and Agoo, 2009).

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 (Madulid and Agoo, 2009; Lindstrom et al., 2008; Agoo and Madulid, 2012). All species, except for *C. edentata*, are endemic to the Philippines (Lindstrom et al., 2008).

This study is part of our research on the chemical constituents of *Cycas* species endemic to the Philippines. In an earlier study, we reported the isolation of sterols, triacylglycerols, and a diterpene from the different parts of *Cycas sancti-lasallei* (Ng et al., 2015). We report herein the isolation or fractionation and identification of pinoresinol (**1**), sesamin (**2**), paulownin (**3**), a mixture of β-sitosterol and stigmasterol and triacylglycerols from the cone base; **1**, **3**, triacylglycerols, and lariciresinol (**4**) from the cataphylls; β-sitosterol from the megasporophyll lamina; β-sitosterol and a mixture of *trans*-4-hydroxycinnamate fatty acid esters (**5**) and *cis*-4-hydroxycinnamate fatty acid esters (**6**) from the unripe sarcotesta; and β-sitosterol and triacylglycerols from the ripe sarcotesta. The structures **1**–**6** were elucidated by extensive 1D and 2D NMR spectroscopy. This is the first study on the chemical constituents of *C. vespertilio*.

* Corresponding author.
E-mail: consolacion.ragasa@dlsu.edu.ph (C.Y. Ragasa).

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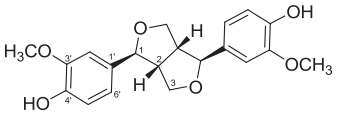
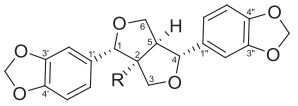


1



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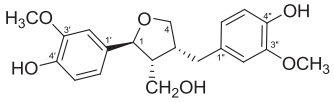

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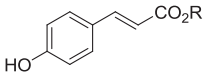
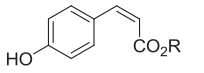


5 R=long chain fatty acids



6 R=long chain fatty acids

Materials and methods

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. Solvents were evaporated under vacuum using Heidolph WB2000. Column chromatography was performed with silica gel 60 (70–230 mesh). TLC 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.

Cycas vespertilio A. Lindstr. & K.D. Hill, Cycadaceae, cone base, cataphylls, megasporophyll lamina, unripe sarcotesta, and ripe sarcotesta were collected from Iloilo, Panay Island, Philippines in April 2013. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3112).

The air-dried cone base (100 g), cataphylls (123.5 g), megasporophyll lamina (92 g), and freeze-dried unripe sarcotesta (19 g) and ripe sarcotesta (51.6 g) of *C. vespertilio* were separately ground in a blender, soaked in CH_2Cl_2 at room temperature for three days and then filtered. The solvent was evaporated under vacuum to yield crude extracts of 1.1 g, 1.2 g, 0.5 g, 0.3 g, and 0.6 g for cone base, cataphylls, megasporophyll lamina, unripe sarcotesta, and ripe sarcotesta, respectively. These extracts were chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment.

The 10% acetone in CH_2Cl_2 fraction from the chromatography of the crude cone base was rechromatographed (3×) using 10% EtOAc in petroleum ether to yield triacylglycerols (8 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3×) using 15% EtOAc in petroleum ether to yield **2** (4 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether, followed by 20% EtOAc in petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were combined and rechromatographed using 15% EtOAc in petroleum ether to yield a mixture of β-sitosterol and stigmasterol (7 mg) after washing with petroleum ether. The fractions eluted with 20% EtOAc in petroleum ether were combined and rechromatographed (4×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume) to yield **3** (3 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed (5×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume) to yield **1** (5 mg) after washing with petroleum ether.

The 30% acetone in CH_2Cl_2 fraction from the chromatography of the crude cataphyll extract was rechromatographed using 15% EtOAc in petroleum ether, followed by 20% EtOAc in petroleum ether. The fractions eluted with 20% EtOAc in petroleum ether were combined and rechromatographed (5×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume) to yield **3** (3 mg) after washing with petroleum ether. The 70–80% acetone in CH_2Cl_2 fractions were combined and rechromatographed (4×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6 by volume) to yield **4** (3 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction from the chromatography of the crude unripe sarcotesta extract was rechromatographed (3×) using 15% EtOAc in petroleum ether to yield a mixture of **5** and **6** (3 mg).

*Pinoresinol (**1**)*

^{13}C NMR (150 MHz, $CDCl_3$): δ 85.86 (C-1), 54.15 (C-2), 71.66 (C-3), 132.90 (C-1′), 108.56 (C-2′), 146.68 (C-3′), 145.22 (C-4′), 114.24 (C-5′), 118.96 (C-6′), 55.95 (3′-OCH_3).

*Sesamin (**2**)*

^1H NMR (600 MHz, $CDCl_3$): δ 4.70 (2H, d, *J* = 4.2 Hz, H-1, H-4), 3.03 (2H, m, H-2, H-5), 3.85 (2H, dd, *J* = 3.6, 9.0 Hz, H-3, H-6), 6.83 (2H, d, *J* = 1.2 Hz, H-2,2′), 6.75–6.79 (4H, H-5′, H-5″, H-6′, H-6″), 5.93 (2× —OCH_2O—), 4.22 (2H, dd, *J* = 6.6, 9.0 Hz, H-3, H-6); ^{13}C NMR (150 MHz, $CDCl_3$): δ 85.76 (C-1, C-4), 54.31 (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—).

*Paulownin (**3**)*

^1H NMR (600 MHz, $CDCl_3$): δ 6.91 (2H, dd, *J* = 1.8, 16.8 Hz), 6.77–6.86 (m, 4H), 5.97 (s, 2H, —OCH_2O—), 5.94 (s, 2H, —OCH_2O—),4.80 (1H, s, H-1), 4.03 (1H, d, *J* = 9.0 Hz, H-3), 3.92 (1H, d, *J* = 9.6 Hz, H-3), 4.82 (1H, d, *J* = 4.8 Hz, H-4), 3.03 (1H, m H-5), 3.82 (1H, dd, *J* = 6.0, 9.0 Hz, H-6), 4.50 (1H, dd, *J* = 8.4, 9.0 Hz, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ 87.48 (C-1), 91.64 (C-2), 74.74 (C-3), 85.76 (C-4), 60.35 (C-5), 71.66 (C-6), 134.57 (C-1′), 129.09 (C-1″), 107.38, 106.90 (C-2′, C-2″), 148.12, 148.01 (C-3′, C-3″), 147.94, 147.28 (C-4′, C-4″), 108.61, 108.21 (C-5′, C-5″), 120.09 (C-6′), 119.81 (C-6″), 101.26, 101.11 (2× —OCH_2O—).

*Laricresinol (**4**)*

^{13}C NMR (150 MHz, $CDCl_3$): δ 82.81 (C-1), 52.60 (C-2), 42.40 (C-3), 72.89 (C-4), 33.33 (C-5), 60.94 (C-6), 134.76 (C-1′), 108.26 (C-2′), 146.61 (C-3′), 145.02 (C-4′), 114.24 (C-5′), 118.75 (C-6′), 132.26 (C-1″), 121.19 (C-2″), 146.50 (C-3″), 143.97 (C-4″), 114.39 (C-5″), 111.17 (C-6″), 55.93 (OCH_3).

trans-*4-Hydroxycinnamate fatty acid esters (**5**)*

^{13}C NMR (150 MHz, $CDCl_3$): δ 127.89 (C-1), 115.81 (C-2, C-6), 129.90 (C-3, C-5), 157.47 (C-4), 144.10 (C-7), 115.87 (C-8), 167.43 (C-9), 64.45/64.61 (C-1′), 28.24 (C-2′), 130.02, 130.23 (=CH), 27.20, 27.27 (allylic CH_2′), 22.68, 26.05, 29.02–29.76 ($(CH_2')_n$), 31.91 (CH_2′), 14.11/14.06 (CH_3′ terminal).

cis-*4-Hydroxycinnamate fatty acid esters (**6**)*

^{13}C NMR ($CDCl_3$): δ 127.11 (C-1), 114.89 (C-2, C-6), 132.33 (C-3, C-5), 156.48 (C-4), 143.02 (C-7), 117.42 (C-8), 166.59 (C-9), 64.45/64.61 (C-1′), 22.68, 26.05, 28.24 (C-2′), 130.02, 130.23 (=CH), 29.3–29.8 [29.27, 29.35, 29.51, 29.43, 29.52 29.58, 29.65, 29.69, 29.76 $(CH_2')_n$], 31.92 (CH_2′), 14.11/14.06 (CH_3′ terminal).

Results and discussion

The structures of **1**–**6** were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of their ^{13}C NMR data with those reported for pinoresinol (**1**) (Ragasa et al., 2000), sesamin (**2**) (Lee et al., 2002), paulownin (**3**) (Angle et al., 2008), lariciresinol (**4**) (Ragasa et al., 2000), *trans*-4-hydroxycinnamate fatty acid esters (**5**) (Ragasa and Alimboyoguen, 2013), and *cis*-4-hydroxycinnamate fatty acid esters (**6**) (Nishimura et al., 2009).

These results indicate that *Cycas vespertilio* shares similar chemical characteristics with other members of the family Cycadaceae: *Cycas beddomei* which contained pinoresinol (**1**) (Das et al., 2006); *Cycas circinalis* L. which yielded lariciresinol (**4**) (Ferreira et al., 2009); and *C. micronesica* K. D. Hill. which yielded β-sitosterol, and stigmasterol (Marler et al., 2006). To our knowledge, this is the first report on the occurrence of **2**–**3** and **5**–**6** in the genus *Cycas* and the family Cycadaceae. Thus, **2**–**3** and **5**–**6** may become chemotaxonomic markers for *Cycas vespertilio* and could be used to distinguish among *Cycas* species.

Authors' contributions

EMGA collected and identified the sample and wrote part of the introduction. VASN worked on the isolation and purification of the chemical constituents of *C. vespertilio*. CYR worked on the purification of the chemical constituents, conducted literature search, elucidated the structures of the compounds and wrote the manuscript. CCS obtained the NMR spectra, conducted literature search and reviewed the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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