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Research Article

# Chemical Constituents of *Cycas lacrimans*

Vincent Antonio S. Ng[1], Esperanza Maribel G. Agoo[2], Chien-Chang Shen[3], Consolacion Y. Ragasa[1,4*]

[1]*Chemistry Department, De La Salle University 2401 Taft Avenue, Manila 1004, Philippines,*
[2]*Biology Department, De La Salle University 2401 Taft Avenue, Manila 1004, Philippines,*
[3]*National Research Institute of Chinese Medicine, 155-1, Li-Nong St., Sec. 2, Taipei 112, Taiwan*
[4]*Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Binan City, Laguna 4024, Philippines*

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**ABSTRACT**
Chemical investigation of *Cycas lacrimans*, a plant endemic to the Philippines, led to the isolation of isopimaran-19-ol (**1**) from the megasporophyll lamina; 9αH-isopimara-7,15-diene (**2**) and triacylglycerols (**3**) from the bark; **3**, oleic acid (**4**), and 1,2-dioleylglycerol (**5**) from the leaflets; **3**, β-sitosterol (**6a**), and stigmasterol (**6b**) from the petiole and rachis; **6a** from the roots; and **3** and **6a** from the endotesta and sclerotesta. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy, while those of **2-6b** were identified by comparison of their $^{1}$H and/or $^{13}$C NMR data with literature data.

**Keywords***: Cycas lacrimans*, Cycadaceae, isopimaran-19-ol, 9αH-isopimara-7,15-diene, β-sitosterol, stigmasterol, triacylglycerols

## INTRODUCTION

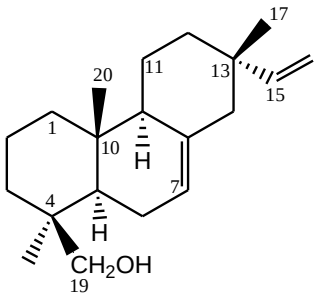
*Cycas* resemble palms in morphology and are commonly called sago palm. They are considered as fossil plants though they may have evolved only about 12 million years ago[1]. They are widely distributed in the Tropics[2] where they grow on volcanic, limestone, ultramafic, sandy, or even water-logged soils 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. lacrimans*. However, some *Cycas* species have been studied for their chemical constituents and biological activities. Cycasin, a carcinogenic toxin was isolated from the most studied *Cycas* species, *C. revoluta* Thunb. and *C. circinalis* L.[8,9]. Biflavonoids, lignans, flavan-3-ols, flavone-C-glucosides, nor-isoprenoids, and a flavanone were obtained from the methanolic extract of the leaflets of *C. circinalis* L. and the chloroform extract of *C. revoluta* Thunb. Three of the biflavonoids exhibited moderate activity against *S. aureus* and methicillin-resistant *S. aureus*[10]. Moreover, the leaves of *C. revoluta* Thunb. and *C. circinalis* L. yielded lariciresinol, naringenin and biflavonoids[11]. β-Sitosterol β-D-glucoside, stigmasterol β-D-glucoside, β-sitosterol, and stigmasterol were obtained from the seeds of *C. micronesica* K. D. Hill[12], while *C. beddomei* afforded a new biflavonoid, along with pinoresinol, hinokiflavone, and amento flavones[13,14]. The leaves of *C. panzhihuaensis* yielded a new flavone, along with 2,3-dihydrohinokiflavone, a biflavone, vanillic acid, sitosterol and daucosterol[15]. Chavicol β-rutinoside, amentoflavone, podocarpus flavone A, a biflavone, β-sitosterol, daucosterol and palmitic acid were isolated from the methanolic extracts of the stems, flowers and seeds of *C. panzhihuaensis* L.[16]. This study is part of our research on the chemical constituents of the genus *Cycas.* We earlier reported the isolation of squalene (**I**), β-sitosterol (**IIa**), stigmasterol (**IIb**), and triglycerides (**III**) from the sarcotesta; **IIa**, **IIb**, **III**, and phytyl fatty acid esters (**IV**) from the endotesta; **IIa**, **IIb**, **III**, and β-sitosteryl fatty acid esters (**V**) from the sclerotesta; and **III** and **V** from the bark of *Cycas sancti-lasallei*[17]. We report herein the isolation of isopimaran-19-ol (**1**) from the megasporophyll lamina; 9αH-isopimara-7,15-diene (**2**) and triacylglycerols (**3**) from the bark; **3**, oleic acid (**4**), and 1,2-dioleylglycerol (**5**) from the leaflets; **3** and a mixture of β-sitosterol (**6a**) and stigmasterol (**6b**) in a 4:1 ratio from the petiole and rachis; **6a** from the roots; and **3** and **6a** from the endotesta and sclerotesta of *C. lacrimans***.** To the best of our knowledge this is the first report on the isolation of these compounds from the plant.

## MATERIALS AND METHODS

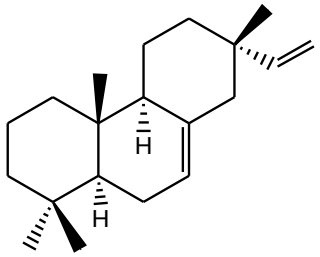
*General Experimental Procedure*

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for $^{1}$H NMR and 150 MHz for $^{13}$C NMR spectra or on a Varian Unity Inova

**Author for Correspondence*




**1**

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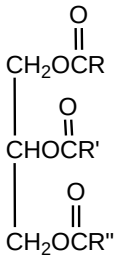
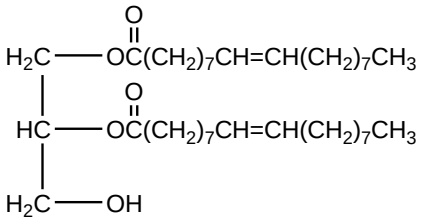


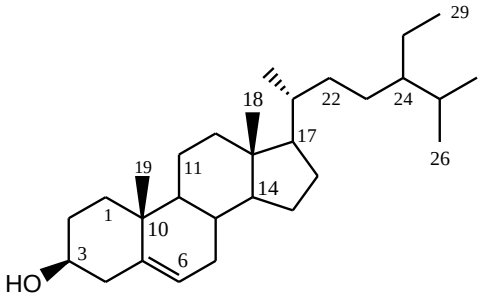

**3** R, R', R" = long chain fatty acids

$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$

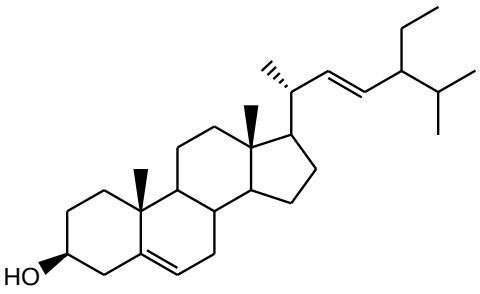
**4**




**5**




**6a**




**6b**

Fig. 1. Chemical structures of the constituents from *Cycas lacrimans*: isopimaran-19-ol (**1**), 9αH-isopimara-7,15-diene (**2**), triacylglycerols (**3**), oleic acid (**4**), 1,2-dioleylglycerol (**5**), β-sitosterol (**6a**), and stigmasterol (**6b**).

spectrometer in $CDCl_3$ at 500 MHz for $^1H$ NMR and 125 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 lacrimans* leaflets, petiole and rachis, megasporophyll lamina, bark, roots, endotesta, and sclerotesta were collected in 2013. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH3113).

*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 Megasporophyll Lamina*

The air-dried megasporophyll lamina of *C. lacrimans* (26 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 (0.20 g) which was chromatographed using increasing proportions of acetone in $CH_2Cl_2$ at 20% increment. The 20% acetone in $CH_2Cl_2$ fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford **1** (3 mg) after washing with petroleum ether.

*Isolation of the Chemical Constituents of the Bark*

The air-dried bark of *C. lacrimans* (46 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 (0.15 g) which was chromatographed using increasing proportions of acetone in $CH_2Cl_2$ at 20% increment. The $CH_2Cl_2$ fraction was rechromatographed (2 ×) using 2.5% EtOAc in petroleum ether to afford **2** (2 mg). The 40% acetone in $CH_2Cl_2$ fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to yield **3** (5 mg).

*Isolation of the Chemical Constituents of the Leaflets*

The air-dried leaflets of *C. lacrimans* (87.5 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 (3 g) which was chromatographed using increasing proportions of acetone in $CH_2Cl_2$ at 10% increment. The 40% acetone in $CH_2Cl_2$ fraction was rechromatographed (3 ×) using 15% EtOAc using petroleum ether to yield **3** (15 mg) after washing with petroleum ether. The 50% acetone in $CH_2Cl_2$ fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to yield **4** (10 mg) after washing with petroleum ether. The 60% acetone in $CH_2Cl_2$ fraction was rechromatographed (3 ×) using $CH_3CN$:$Et_2O$:$CH_2Cl_2$ (1:1:8, v/v) to yield **5** (12 mg).

*Isolation of the Chemical Constituents of the Petiole and Rachis*

The air-dried petiole and rachis of *C. lacrimans* (68 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 (0.3 g) which was chromatographed using increasing proportions of acetone in $CH_2Cl_2$ at 10% increment. The $CH_2Cl_2$ fraction was rechromatographed (4 ×) using 7.5% EtOAc in petroleum ether to yield **3** (8 mg). The 40% acetone in $CH_2Cl_2$ fraction was rechromatographed using 15% EtOAc in petroleum ether. The more polar fractions were combined and rechromatographed (3 ×) using $CH_2Cl_2$ to yield a mixture of **6a** and **6b** in a 4:1 ratio (4 mg) after washing with petroleum ether. The less polar fractions were combined and rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford **7** (6 mg).

*Isolation of the Chemical Constituents of the Root*

The air-dried root of *C. lacrimans* (3.2 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 40% acetone in $CH_2Cl_2$ fraction was rechromatographed (4 ×) using $CH_2Cl_2$ to afford **6a** (4 mg).

*Isolation of the Chemical Constituents of Endotesta*

The freeze-dried endotesta of *C. lacrimans* (198 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 (0.9 g) which was chromatographed using increasing proportions of acetone in $CH_2Cl_2$ at 10% increment. The 20% acetone in $CH_2Cl_2$ fraction was rechromatographed (3 ×) using 1% EtOAc in petroleum ether to yield **3** (6 mg). The 40% acetone in $CH_2Cl_2$ fraction was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to yield **6a** (7 mg) after washing with petroleum ether.

*Isolation of the Chemical Constituents of Sclerotesta*

The freeze-dried ripe sclerotesta of *C. lacrimans* (117.2 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 (0.15 g) which was chromatographed using increasing proportions of acetone in $CH_2Cl_2$ at 10% increment. The 20% acetone in $CH_2Cl_2$ fraction was rechromatographed (3 ×) using 1% EtOAc in petroleum ether to yield **3** (4 mg). The 40% acetone in $CH_2Cl_2$ fraction was rechromatographed (2 ×) using $CH_2Cl_2$ yield **6a** (3 mg).

***Isopimaran-19-ol*** (**1**): $^{13}$C NMR (150 MHz): δ 39.8 (C-1), 18.5 (C-2), 35.3 (C-3), 37.9 (C-4), 46.1 (C-5), 22.9 (C-6), 121.6 (C-7), 135.6 (C-8), 52.1 (C-9), 35.3 (C-10), 20.4 (C-11), 36.2 (C-12), 36.9 (C-13), 46.1 (C-14), 150.4 (C-15), 109.2 (C-16), 21.5 (C-17), 26.9 (C-18), 65.2 (C-19), 16.1 (C-20).

***9αH-Isopimara-7,15-diene*** (**2**): $^1$H NMR (600 MHz): δ 0.85 (6H, s, H-17, H-20), 0.86 (3H, s, H-18); 0.90 (3H, s, H-19), 0.98 (H-1a), 1.11 (H-5), 1.15 (H-3a), 1.34 (2H, H-11a, H-12a), 1.40 (H-3b), 1.42 (H-2a), 1.46 (H-12b), 1.50 (H-2b), 1.63 (H-9), 1.65 (H-11b), 1.80 (H-1b), 1.88 (H-14a), 1.90 (2H, H-6), 1.95 (H-14b), 4.85 (d, *J* = 11 Hz, H-16), 4.92 (d, *J* = 18 Hz, H-16), 5.33 (brs, H-7), 5.80 (dd, *J* = 18, 11 Hz, H-15).

***Triacylglycerols*** (**3**): $^1$H NMR (600 MHz, $CDCl_3$): δ 4.28 (2H, dd, *J* = 4.2, 12.0 Hz, glyceryl $CH_2O$), 4.12 (2H, dd, *J* = 6.0, 12.0 Hz, glyceryl $CH_2O$), 5.32 (1H, m, glyceryl CHO), 2.31 (6H, t, *J* = 7.5 Hz, α-$CH_2$), 5.33 (m, olefinic H), 2.75 (double allylic $CH_2$), 1.98-2.05 (allylic, $CH_2$), 1.23-1.35 ($CH_2$), 0.87 (t, *J* = 6.6 Hz, $CH_3$); $^{13}$C NMR (150 MHz, $CDCl_3$): δ 62.09 (glyceryl $CH_2$), 68.87 (glyceryl CH), 173.26, 173.30 (C=O α), 172.84 (C=O β), 34.02, 34.05, 34.19 (C-2), 24.83, 24.86 (C-3), 29.05, 29.08, 29.12 (C-4), 29.18, 29.20, 29.27 (C-5), 29.48 (C-6), 22.57, 22.69 (C-8), 130.23, 130.01, 129.70 (C-9), 127.89, 128.06, 129.68 (C-10), 25.62, 27.17, 27.19, 27.22, 29.32, 29.34, 29.36, 29.52, 29.62, 29.66, 29.70, 29.76 ($CH_2$), 31.52, 31.90, 31.92 ($CH_2$), 14.07, 14.12 (terminal $CH_3$).

***Oleic acid*** (**4**): $^1$H NMR (500 MHz, $CDCl_3$): δ 5.33 (m, =CH), 2.33 (t, *J* = 7.5 Hz, α-$CH_2$), 1.97-2.01 (m, allylic $CH_2$), 1.60 (m, β-$CH_2$), 1.24-1.32 ($CH_2$), 0.86 (t, *J* = 7.0 Hz).

***1,2-Dioleylglycerol*** (**5**): $^1$H NMR (500 MHz, $CDCl_3$): δ 5.33 (4H, m), 5.06 (1H, m, glyceryl CHO), 4.28 (1H, dd, *J* = 4.5, 11.5 Hz, glyceryl $CH_2O$), 4.12 (1H, dd, *J* = 5.5, 12.0 Hz, glyceryl $CH_2O$), 3.71 (2H, brs, glyceryl $CH_2OH$), 2.32 (t, *J* = 6.0 Hz, α-$CH_2$), 1.97-2.04 (allylic $CH_2$), 1.60 (m, β-$CH_2$), 1.22-1.28 ($CH_2$), 0.86 (t, *J* = 6.0 Hz, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 173.1 (C=O α), 172.8 (C=O β), 130.03 (C-9), 129.69 (C-10), 72.10 (glyceryl CHO), 61.96 (glyceryl $CH_2OH$), 61.55 (glyceryl $CH_2O$), 34.26, 34.10, 34.08, 31.91, 31.90, 29.76, 29.69, 29.65, 29.61, 29.52, 29.46, 29.35, 29.31, 29.26, 29.22, 29.17, 29.10, 29.08, 29.05, 27.21, 27.16, 24.91, 24.88, 24.86, 22.67, 22.65 ($CH_2$), 14.11 (terminal $CH_3$).

***β-Sitosterol*** (**6a**): $^{13}$C NMR (150 MHz, $CDCl_3$): δ 37.2 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5),121.7

(C-6), 31.9 (C-8), 31.90 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 12.0 (C-18), 19.4 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.0 (C-23), 45.8 (C-24), 29.1 (C-25), 19.0 (C-26), 19.8 (C-27), 23.0 (C-28), 11.9 (C-29).

***Stigmasterol*** (**6b**): $^{13}$C NMR (125 MHz, $CDCl_3$): δ 37.2 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 29.1 (C-16), 56.0 (C-17), 12.0 (C-18), 19.4 (C-19), 40.5 (C-20), 21.1 (C-21), 138.3 (C-22), 129.3 (C-23), 51.2 (C-24), 31.9 (C-25), 21.1 (C-26), 19.0 (C-27), 25.4 (C-28), 12.1 (C-29).

## RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *Cycas lacrimans* led to the isolation of isopimaran-19-ol (**1**)[18] from the megasporophyll lamina; 9αH-isopimara-7,15-diene (**2**)[17] and triacylglycerols (**3**)[19] from the bark; **3**, oleic acid (**4**)[20], and 1,2-dioleylglycerol (**5**)[20] from the leaflets; **3**, and a mixture of β-sitosterol (**6a**)[21] and stigmasterol (**6b**)[21] in a 4:1 ratio from the petiole and rachis; **6a** from the roots; and **3** and **6a** from the endotesta and sclerotesta. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy, while those of **2-6b** were identified by comparison of their $^{1}$H and/or $^{13}$C NMR data with literature data.

Isopimaran-19-ol (**1**) was first reported as a constituent of *Fritillaria thunbergii* Miq. (Liliaceae)[18]. Other sources of this diterpene are *Calceolaria peteoalaris* Cav. (Scrophulariaceae)[22] and fungus rice pathogen, *Gibberella fujikuroi* (Nectriaceae)[23]. This diterpene, also known as 7,15-isopimaradien-19-ol and akhdarenol was reported to exhibit antimicrobial activity with MIC values of 3.90 μg/ml against *S. aureus* and of 7.81 μg/ml against *Enterococcus hirae*[24]. It also showed antifeedant effects on *Leptinotarsa decemlineata*, was cytotoxic to insect Sf9 cells, and also affected mammalian Chinese Hamster Ovary cells[25].

## ACKNOWLEDGEMENT

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