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Diterpenes from *Cycas nitida*

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ABSTRACT

*Chemical investigation of the dichlroromethane extracts of Cycas nitida afforded labda-8(17),13(16),14-trien-18-ol (**1**) from the petiole and rachis;18-hydroxy-isopimara-7,15-diene (**2**) from the megasporophyll lamina; and a mixture of abietatriene (**3**)and squalenefrom the roots. The structures of **1**-**3**were elucidated by extensive 1D and 2D NMR spectroscopy.*

Keywords: *Cycas nitida*, Cycadaceae, labda-8(17),13(16),14-trien-18-ol, 18-hydroxy-isopimara-7,15-diene, abietatriene, squalene

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INTRODUCTION

*Cycas*are gymnosperms which resemble palms in habit 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].

This study is part of our research on the chemical constituents of *Cycas* species endemic to the Philippines. 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. riuminiana* [20], *C. mindanaensis* [21],*C. wadei* [22],and *C. edentata* [23, 24].

We recentlyreported the isolation of triacylglycerol, squalene, fatty acid methyl esters, a mixture of β-sitosterol and stigmasterol, and fatty alcohol from the bark;triacylglycerol, β-sitosterol, and a mixture of fatty acid methyl esters and β-sitosteryl fatty acid ester rom the sarcotesta; squalene and chlorophyll a from the leaflets; squalene, a mixture ofβ-sitosterol and stigmasterol from the roots; triacylglycerol and β-sitosterol from the endotesta; β-sitosterol and

stigmasterol from the petiole and rachis; triacylglycerolsfrom the megasporophyll lamina; and squalene from the sclerotesta of *C. nitida* [25].

We report herein the isolation and structure elucidation of labda-8(17), 13(16),14-trien-18-ol (**1**) from the petiole and rachis; 18-hydroxy-isopimara-7,15-diene (**2**) from the megasporophyll lamina; anda mixture of abietatriene (**3**)and squalenefrom the roots of *Cycas nitida.*The structures of **1**-**3** are presented in Fig. 1. To the best of our knowledge this is the first report on the isolation of **1**-**3** from *C. nitida.*

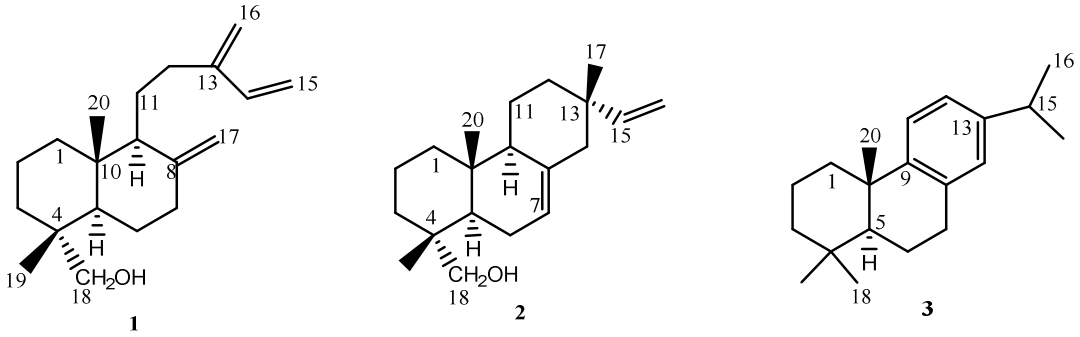

Fig. 1. Chemical structures of the diterpenes from *Cycas nitida:* labda-8(17),13(16),14-trien-18-ol (1), 18-hydroxy-isopimara-7,15-diene (2), and abietatriene (3)

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 withsilica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254}andthe plates were visualized by spraying with vanillin/H_2SO_4solution followed by warming.

Sample Collection

*Cycas nitida*petiole and rachis, megasporophyll lamina and roots were collected in 2014. Voucher specimens were collected from Rapu-Rapu Island, Albay and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3120).

Isolation of the Chemical Constituents of the Petiole and Rachis

The air-dried petiole and rachis (73.3 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.5 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 20% acetone in CH_2Cl_2fraction was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to afford a mixture of **1** (3 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Megasporophyll Lamina

The air-dried megasporophyll lamina (65 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.6 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to afford **2**(7 mg).

Isolation of the Chemical Constituents of the Roots

The air-dried roots (93 g) of C. nitida were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.7 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford a mixture of **3** and squalene (9 mg).

Labda-8(17),13(16),14-trien-18-ol (1): ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.00, 1.76 (H-1), 1.56 (H_2-2), 1.27, 1.42 (H_2-3), 1.42 (H-5). 1.33, 1.62 (H_2-6), 2.00, 2.38 (H_2-7), 1.70 (H-9), 1.50, 1.70 (H_2-11),1.98, 2.36 (H_2-12), 6.35 (dd, *J*= 10.8, 17.4 Hz, H-14), 5.02 (d, *J* = 10.8 Hz, H-15), 5.19 (d, *J* = 17.4 Hz, H-15'), 4.95 (br s, H-16), 4.97 (br s, H-16'), 4.54 (d, *J*=1.2 Hz, H-17), 4.83 (d, *J*= 1.2 Hz, H-17), 3.09 (d, *J*=10.8 Hz, H-18), 3.40 (d, *J*=10.8 Hz, H-18'), 0.73 (s, H_3-19), 0.70 (s, H_3-20). ^{13}C NMR (150 MHz, $CDCl_3$): δ 38.46(C-1), 18.68 (C-2), 35.41 (C-3), 37.94 (C-4), 48.51 (C-5), 24.19 (C-6), 38.08 (C-7), 148.38 (C-8), 56.52 (C-9), 39.47 (C-10), 22.30 (C-11), 30.31 (C-12), 147.10 (C-13), 139.07 (C-14), 113.18 (C-15), 115.51 (C-16), 106.43 (C-17), 72.11 (C-18), 17.59 (C-19), 14.98 (C-20).

18-Hydroxy-isopimara-7,15-diene (**2**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.00, 1.82(H_2-1), 1.50, 1.55 (H_2-2), 1.34, 1.38 (H_2-3), 1.40 (H-5), 1.84, 1.90 (H_2-6), 5.32 (dd, *J* = 2.4, 5.4 Hz, H-7), 1.67 (H-9), 1.36, 1.36(H_2-11), 1.46, 1.46 (H_2-12), 1.88, 1.94 (H_2-14), 5.78 (dd, *J* = 10.8, 17.4 Hz, H-15), 4.84 (dd, *J* = 1.2, 10.2 Hz, H-16), 4.90 (dd, *J* = 1.2, 17.4 Hz, H-16'), 0.86 (s, H_3-17), 3.10 (d, *J* = 10.8 Hz, H-18), 3.40 (d, *J* = 10.8 Hz, H-18'), 0.88 (s, H_3-19), 0.90 (s, H_3-20);^{13}C NMR (150 MHz, $CDCl_3$): δ 39.4 (C-1), 18.1 (C-2), 35.6 (C-3), 37.4 (C-4), 43.7 (C-5), 23.3 (C-6), 121.2 (C-7), 135.7 (C-8), 51.8 (C-9), 35.2 (C-10), 20.2 (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), 72.3 (C-18), 18.2 (C-19), 16.0 (C-20).

Abietatriene **(3)**:^{1}H NMR (600 MHz, $CDCl_3$): δ 2.22, 1.38 (H_2-1), 1.50, 1.55 (H_2-2), 1.16, 1.42 (H_2-3), 1.32 (H-5), 1.78, 1.82 (H_2-6), 2.80, 2.85 (H-7),7.12 (d, d, *J* = 7.8 Hz, H-11), 6.95 (dd,*J* = 1.2, 7.8 Hz,H-12), 6.86 (d,*J* = 1.2 Hz,H-14), 2.80 (H-15), 1.20 (d, *J*= 6.6 Hz, H-16), 1.20 (d, *J*= 6.6 Hz, H-17), 0.92 (s, H-18), 0.90 (s, H_3-19), 1.15 (s, H_3-20);^{13}C NMR (150 MHz, $CDCl_3$): δ 38.8 (C-1), 19.3 (C-2), 41.7 (C-3), 33.4 (C-4), 50.4 (C-5), 19.3 (C-6), 30.5 (C-7), 135.1 (C-8), 147.6 (C-9), 37.5 (C-10), 124.4(11), 124.3 (C-12), 145.4 (C-13), 126.8 (C-14), 33.4 (C-15), 23.4 (C-16), 23.4 (C-17), 33.3 (C-18), 21.6(C-19), 25.6 (C-20).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extractsof *Cycas nitida* yielded labda-8(17),13(16),14-trien-18-ol (**1**) from the petiole and rachis;18-hydroxy-isopimara-7,15-diene (**2**)from the leaflets; and a mixture of abietatriene (**3**)and squalenefrom the roots. The structures of **1**-**3** were elucidated by extensive 1D and 2D NMR spectroscopy.

The ^{1}H NMR spectrum of **1** indicated resonances for exomethylene protons at δ 4.54 and 4.83 which were coupled to each other by 1.2 Hz;olefinic methylene protons at δ 5.02 and δ 5.19 which were coupled to the olefinic methine proton at δ 6.35 by 10.8 and 17.4 Hz, respectively; olefinic methylene protons at δ 4.95 and 4.97 which appeared as broad singlets; two methylene hydroxyl protons at δ 3.40 and 3.49 which were coupled to each other by 10.8 Hz; and two methyl singlets at δ 0.70 and 0.73. The coupled protons were supported by the COSY spectrum which indicated four isolated spin systems as follows: H_2-1/H_2-2/H_2-3; H-5/H_2-6/H_2-7/H_2-17//H-9/H_2-11/H_2-12/H-14/H_2-15; H_2-16; and H_2-18 (Fig 2).

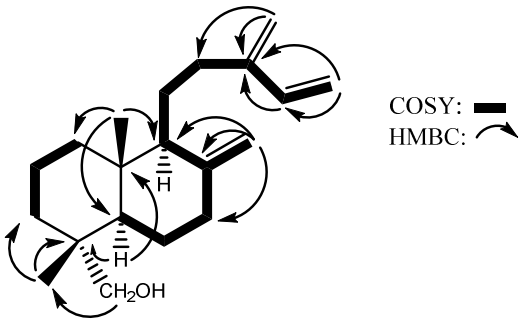

Fig. 2. ^{1}H-^{1}H COSY and key ^{1}H-^{13}C long-range correlations of 1

The ^{13}C NMR spectrum gave resonances for twenty carbons with the following functionalities: olefinic methylene carbons at δ 106.43, 113.18 and 115.51; olefinic methine carbon at δ 139.07; non protonated olefinic carbon at δ 147.10 and 148.38; methyl carbons at δ14.98 and 17.59; methylene carbons at δ 18.68, 22.30, 24.19, 30.31, 35.41, 38.08, and 38.46; oxy methane carbon at δ 72.11; methine carbons at δ 48.51 and 56.52; and quaternary carbons at δ 37.99 and 39.47. These resonances indicated a diterpene with three olefins and an alcohol functionalities.

Protons attached to carbons were assigned (see experimental part) from HSQC 2D NMR data and the structure of **1** was elucidated by analysis of the HMBC 2D NMR data: key HMBC correlations are shown in Fig. 2.Thus, the exocyclic methylene was attributed to C-17 based on long–range correlations between these protons and C-7, C-8 and C-9. The two other olefins were assigned to C-13 and C-14 on the basis of long-range correlations between H-14 and C-12, C-13, C-15 and C-16.The methylene hydroxyl was attached to C-4 due to long-range correlations between these protons and C-3, C-4, C-5 and C-19. The two methyls were assigned to C-19 and C-20 based on long-range correlations between H-5 and these carbons. All long-range correlations observed are consistent with the structure of **1**.

The relative stereochemistry of **1** (Fig. 3) was deduced from the NOESY spectrum as follows. The two methyl singlets (H_3-19 and H_3-20) were close to each other in space. On the opposite face of **1**,the methylene hydroxyl protons (H_2-18) were close to the methine proton (H-5), which was in turn close to the methine proton (H-9).

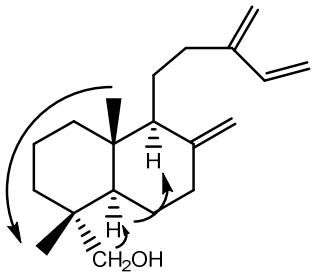

Fig. 3. Key NOESY correlations of 1

Literature search revealed that **1**has similar ^{1}H NMR resonances tolabda-8(17),13(16),14-trien-19-ol [26]. The differences are the resonances for the methyl protons at δ0.73 (s, H-19) and the methylene hydroxyl protons at δ 3.09 (d, *J* = 10.8 Hz, H-18) and 3.40 (d, *J* = 10.8 Hz, H-18') in**1** which wereshifted to δ 0.92 (s, H-18), 3.29 (d, *J* = 11 Hz, H-19) and 3.63 (d, *J* = 11 Hz, H-19'), respectively in labda-8(17),13(16),14-trien-19-ol [26]. The shielding of the methyl protons (H-18)in **1** may be due to the shielding effect of the methyl protons(H-20) which is on the same face of the molecule.

The structure of **2** was elucidated by extensive 1D and 2D NMR spectroscopy and identified as 18-hydroxy-isopimara-7,15-diene. Diterpene **2** gave similar resonances to 2α,18-dihydroxy-9-isopimara-7,15-diene [27], except for the resonances of the protons and carbons close to C-2 where their structures differ.The structure of **3**was elucidated by extensive 1D and 2D NMR spectroscopy and identified as abietatriene [28]. Squalene was identified by comparison of its ^{1}H NMR[8] and ^{13}C NMR [29] data with those reported in the literature.

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