Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

CHEMICAL CONSTITUENTS OF THE MOSS *Calyptothecium ramosii* BROTH.

(Juzuk Kimia bagi Lumut *Calyptothecium ramosii* Broth.)

Consolacion Y. Ragasa[1, 2], Virgilio C. Linis[3], Chien-Chang Shen[4], Mary Stephanie Carranza[1], Maria Carmen Tan[1]*

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

**Corresponding author: maria.carmen.tan@dlsu.edu.ph*

Received: 5 July 2021; Accepted: 10 August 2021; Published: 29 August 2021

Abstract

Calyptothecium ramosii Broth. of the moss family Pterobyaceae is a species indigenous to the Philippines. Chemical investigation of the dichloromethane extract of *C. ramosii* has led to the isolation of γ-polypodatetraene (**1**), cinnamic acid (**2**) and saturated long-chain hydrocarbons (**3**). The structure of **1** was elucidated by extensive 1D and 2D nuclear magnetic resonance spectroscopy (NMR) and confirmed by comparison of its NMR data with literature data. The structures of **2** and **3** were identified by comparison of their NMR data with literature data.

Keywords: *Calyptothecium ramosii* Broth., cinnamic acid, long-chain hydrocarbons, γ-polypodatetraene, Pterobyaceae

Abstract

Calyptothecium ramosii Broth. ialah lumut dari famili Pterobyaceace merupakan spesies asli bagi Filipina. Kajian juzuk kimia dilakukan ke atas ekstrak *C. ramosii* menggunakan diklorometana untuk memisahkan γ-polipodatetraen (**1**), asid sinamik (**2**) dan hidrokarbon tepu rantaian panjang (**3**). Struktur 1 telah dicirikan melalui spektroskopi nuklear magnetik resonan 1D dan 2D dan ditentusahkan melalui perbandingan data kajian literatur dan data NMR.

Kata kunci: *Calyptothecium ramosii* Broth., asid sinamik, hidrkarbon rantaian panjang, γ-polipodatetraen, Pterobyaceae

Introduction

Calyptothecium ramosii Broth. in the moss family Pterobryaceae, is a species indigenous to the Philippines having several gatherings from the islands of Luzon, Mindoro, and Samar [1]. The species is also documented in southern China [2]. Its genus, *Calyptothecium* Mitt.,

is a large group of Bryophytes characterized by having a primary axis in the form of a creeping, conspicuous stoloniform shoot attached to the substratum by rhizoids that arise in the leaf axils [3]. Diagnostic characteristics include secondary axes (stems) diverging from these stoloniform shoots, which are typically pinnately branched with crowded, apiculate leaves and small auricles forming at the leaf bases [4]. The most obvious and most easily seen feature in *C. ramosii* is its large and rugose auricles [5, 6]. Similar auricles may also occur in another species of Philippine *Calyptothecium*, the *C. urvilleanum* (Müll.Hal.) Broth. [7]. In *C. ramosii*, the stem leaves are observed to be smooth with sharply acuminate apices compared to the undulate appearance of the stem leaves of *C. urvilleanum*, with abruptly acute or obtuse leaf apices [5]. *Calyptothecium ramosii* is primarily considered a corticolous epiphyte but saxicolous populations have been documented [5]. Published literature has revealed the antioxidant characteristics of the bryophyte due to the major presence of the constituents: phytol, phytol acetate, 7,9-di-tert-butyl-1-oxaspiro-4,5-deca-6,9-diene-2,8-dione, and 4,8,12,16-tetramethylheptadecan-4-olide, detected by gas chromatography spectroscopy [8].

There are no reported studies on faster screening and larger surveying of the chemical constituents and biological activities of *C. ramosii*. Nuclear magnetic resonance (NMR) spectroscopy allows for the differentiation of optical and geometric isomers, as well as the presence of hydrocarbons with similar ions. We report herein the isolation of γ-polypodatetraene (**1**), cinnamic acid (**2**), and saturated long-chain hydrocarbons (**3**) from *C. ramosii*. The chemical structures of **1** and **2** are presented in Figure 1.

Materials and Methods

Sample collection

The moss species, *Calyptothecium ramosii* Broth. was collected and authenticated by one of the authors (Dr. Virgilio C. Linis, VCL). The moss was gathered from a large boulder inside a residual lowland forest along the lower slope of Pantingan peak at 400 m elevation on November 13, 2017. Collection locality is in the Municipality of Bagac, Province of Bataan in Luzon Island, Philippines, with coordinates 14° 33' 32.08" N 120° 27' 3.93" E.

Isolation of the Chemical Constituents of *C. ramosii*

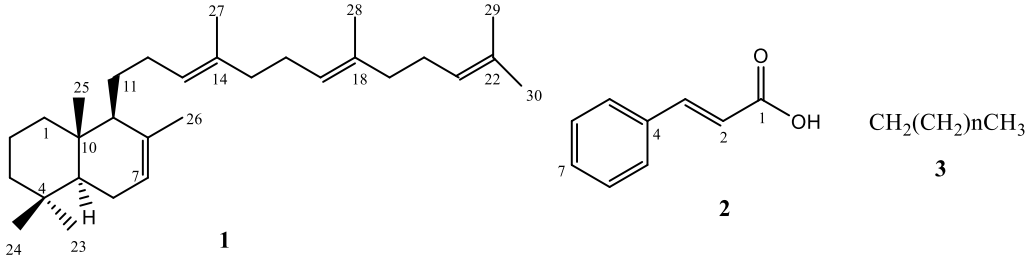
The freeze-dried moss (37.27 g) was ground in a blender and then soaked in dichloromethane (CH_2Cl_2) for 3 days and filtered. The filtrate was concentrated in vacuo to afford a crude extract (71.9 mg) which was chromatographed by gradient elution using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The CH_2Cl_2 fraction was re-chromatographed using petroleum ether. The less polar fractions were combined and re-chromatographed in petroleum ether to yield **3** (0.2 mg) after washing with petroleum ether. The more polar fractions were re-chromatographed (2×) using 1% ethyl acetate (EtOAc) in petroleum ether to afford **1** (2.2 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was re-chromatographed (3×) using acetonitrile (CH_3CN): diethyl ether (Et_2O): CH_2Cl_2 (0.5:0.5:9, v/v) to obtain **2** (1.0 mg) after washing with petroleum ether.

General isolation procedure

Pasteur pipette was used for the fractionation of the crude extract. Fractions of 1 mL volumes were collected and monitored by thin layer chromatography (TLC). Fractions containing spots with similar R_f values were combined and re-chromatographed using the appropriate solvent. TLC-pure isolates were combined, and after evaporation of the solvent, were subjected to NMR analysis.

General experimental procedure

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian VNMRS spectrometer in deuterated chloroform ($CDCl_3$) at 600 MHz for hydrogen proton magnetic resonance (1H NMR) and 150 MHz for carbon-13 nuclear magnetic resonance (13C 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 F254 and the plates were visualized by spraying with vanillin/ sulfuric acid (H_2SO_4) solution followed by warming.



1 **2** **3**

Figure 1. Chemical structures of γ-polypodatetraene (**1**), cinnamic acid (**2**) and saturated long-chain hydrocarbons (**3**) from *C. ramosii*

Results and Discussion

Silica gel chromatography of the dichloromethane extract of *C. ramosii* afforded γ-polypodatetraene (**1**), cinnamic acid (**2**) and saturated long-chain hydrocarbons (**3**).

γ-polypodatetraene (1)

^{1}H NMR (600 MHz, $CDCl_3$): δ 0.94, 1.84 (m, H_2-1), 1.42, 1.52 (m, H_2-2), 1.14, 1.40 (m, H_3-3), 1.16 (m, H-5), 1.84, 1.94 (m, H_2-6), 5.37 (br s, H-7), 1.61 (m, H-9), 1.20, 1.42 (m, H_2-11), 1.94, 2.15 (m, H_2-12), 5.12 (m, H-13), 1.98 (m, H_2-15), 2.06 (m, H_2-16), 5.10 (m, H-17), 1.98 (m, H_2-19), 2.06 (m, H_2-20), 5.09 (m, H-21), 0.83 (s, H_3-23), 0.85 (s, H_3-24), 0.72 (s, H_3-25), 1.69 (s, H_3-26), 1.58 (s, H_3-27, H_3-29), 1.59 (s, H_3-28), 1.66 (s, H_3-30); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 39.2 (C-1), 18.8 (C-2), 42.4 (C-3), 33.0 (C-4), 50.2 (C-5), 23.8 (C-6), 122.0 (C-7), 135.6 (C-8), 54.2 (C-9), 36.7 (C-10), 27.3 (C-11), 30.2 (C-12), 124.8 (C-13), 135.0 (C-14), 39.7 (C-15), 26.8 (C-16), 124.3 (C-17), 135.0 (C-18), 39.7 (C-19), 26.7 (C-20), 124.4 (C-21), 131.3 (C-22), 33.2 (C-23), 21.9 (C-24), 13.5 (C-25), 22.2 (C-26), 16.2 (C-27), 16.0 (C-28), 17.7 (C-29), 25.7 (C-30).

***Trans*-cinnamic acid (2)**

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.42 (d, *J* = 16.2 Hz, H-2), 7.65 (d, *J* = 16.2 Hz, H-3), 7.51 (m, H-5, H-9), 7.36 (m, H-6, H-7, H-8).

Hydrocarbons (3)

^{1}H NMR (600 MHz, $CDCl_3$): δ 1.23 (br s, -CH_2-), 0.86 (t, *J* = 7.2 Hz, terminal CH_3).

The ^{1}H NMR spectrum of **1** gave resonances for three methyl singlets at δ 0.72, 0.83 and 0.85; five allylic methyl singlets at δ 1.58 (2×), 1.59, 1.66, 1.69; four olefinic protons at δ 5.37 (1H, br s) and 5.08-5.12 (3H, m); allylic methylene protons (δ 1.81-2.15); and shielded methylene protons (δ 0.95-1.52). The ^{13}C NMR data of **1** indicated resonances for four protonated olefinic carbons at δ 122.04, 124.26, 124.40, 124.82, four non-protonated olefinic carbons at δ 131.25, 134.95 (2×), 135.62. The remaining twenty-two carbons were attributed to methyl, methylene and methine carbons in **1**. The structure of **1** was elucidated by 2D NMR spectroscopy (COSY, HSQC and HMBC). The COSY spectrum of **1** indicated five isolated spin systems as follows: H_2-1/H_2-2/H_2-3; H-5/H_2-6/H-7; H-9/H_2-11/H_2-12/H-13; H_2-15/H_2-16/H-17; H_2-19/H_2-20/H-21. Protons attached to carbons were assigned from HSQC data. The structure of **1** was determined by analysis of the HMBC correlations of H_3-23, H_3-24/C-3, C-4, C-5, H_3-25/C-1, C-5, C-9, C-10, H_3-26/C-7, C-8, C-9, H_3-27/C-13, C-14, C-15, H_3-28/C-17, C-18, C-19, H_3-29/C-21, C-22, C-30 and H_3-30/C-21, C-22, C-29. All long-range correlations are consistent with the structure of **1**, which was further confirmed by comparison of data collected with γ-polypodatetraene [9, 10].

γ-Polypodatetraene was the first example of a bicyclic triterpenoid hydrocarbon which was isolated from *Polystichum polyblepharum* leaflets [11]. Previous literature has demonstrated that the triterpene may be one of many triterpenes with cytotoxic activities against neoplastic cell lines and with anti-inflammatory

properties [12, 13]. Another study reported that **2** was also found in the moss *Floribundaria aurea* subsp. nipponica [14]. Cinnamic acid was found to exhibit antibacterial and are particularly cytotoxic for bacteria and cancer cells [15]. Published reports of compound **3** suggested that the hydrogen-carbon links are generally used as chief components of fuel and everyday products which may be very toxic in its handling [16]. The potential utilization of these compounds are not limited to their uses in a clinical setting, but as well as in industrial and manufacturing applications.

The NMR data of **2** and **3** were in accordance with the data reported in the literature for cinnamic acid [17] and saturated long-chain hydrocarbons [18], respectively.

Conclusion

Calyptothecium ramosii Broth., a moss endemic to the Philippines, was investigated for its chemical constituents. Fractions analyzed by NMR analysis revealed the presence of: (**1**) γ-polypodatetraene, (**2**) cinnamic acid, and (**3**) saturated long-chain hydrocarbons, isolated for the first time from *C. ramosii*.

Acknowledgement

A project endowment (Project Number: (10 IR U 2TAY16-3TAY17) from the De La Salle University Science Foundation *via* the University Research Coordination Office (URCO) is thankfully recognized.

References

1. Tan, B. C. and Iwatsuki, Z. (1991) A new annotated Philippine moss checklist. *Harvard Papers in Botany*, 1(3): 1-64.
2. Linis, V. C. (2006) A review of intra-Philippine distribution patterns of Philippine mosses based on recent evidence of tectonic evolution of the archipelago: Insights and problems. *Banwa*, 3(1&2): 31-64.
3. Mitten, W. (1868) A list of the Musci collected by the Rev. Thomas Powell in the Samoa or Navigator's Islands. *Journal of the Linnean Society*, 10: 166-195.
4. Noguchi, A. (1986) Notulae bryologicae, XII. *Journal of the Hattori Botanical Laboratory*, 60: 149-158.
5. Bartram, E. B. (1939). Mosses of the Philippines. *Philippine Journal of Science*, 68(1–4):1-437.
6. Brotherus, V. F. (1913). Contributions to the bryological flora of the Philippines IV. Philippine Journal of *Science*, 8:65-98.
7. Hyvönen, J. (1989). Bryophyte flora of the Huon Peninsula, Papua New Guinea. XXVI. Pterobryaceae (Musci). *Acta Botanica Fennica*, 137: 1-40.
8. Carranza, M.S., Linis, V., Ragasa, C. and Tan, M. (2019). Chemical constituents and antioxidant potentials of seven Philippine mosses. *Malaysian Journal of Analytical Sciences*, 23(6):950-962.
9. Shiojima, K., Arai, Y., Masuda, K., Kamada, T. and Ageta, H. (1983). Fern constituents: Polypodatetraenes, novel bicyclic triterpenoids, isolated from polypodiaceous and aspidiaceous plants. *Tetrahedron Letters*, 24(51): 5733-5736.
10. Kinoshita, M., Ohtsuka, M., Nakamura, D. and Akita, H. (2002). First synthesis of (+)-α- and (+)-γ-polypodatetraenes. *Chemical and Pharmaceutical Bulletin*, 50(7): 930-934.
11. Shiojima, K., Arai, Y., Masuda, K., Kamada, T., Suzuki, M. and Ageta, H. (1997). Chemotaxonomy of fern plants. (V). Japanese *Polystichum* species. *Nature Medicine*, 51(6): 523-527.
12. Chudzik, M., Korzonek-Szlacheta, I. and Krol, W. (2015). Triterpenes as potentially cytotoxic compounds. *Molecules*, 20: 1610-1625.
13. Kushiro, T., Ebizuka, Y. (2010). Chapter 1.18: Triterpenes. comprehensive natural products II. Reference module in chemistry, molecular sciences and chemical engineering. vol 1: pp. 673-708.
14. Toyota, M., Masuda, K. and Asakawa, Y. (1998). Triterpenoid constituents of the moss *Floribundaria aurea* subsp. *nipponica*. *Phytochemistry*, 48(2): 297-299.
15. Zolfaghari, B., Yazdiniapour, Z., Sadeghi, M., Akbari, M., Troiano, R. and Lanzotti, V. (2020). Cinnamic acid derivatives from welsh onion (*Allium fistulosum*) and their antibacterial and cytotoxic activities. *Phytochemical analysis*, 32(1): 84-90.
16. Curtis, J., Metheny, E., Sergeant, S.R. (2021). Hydrocarbon toxicity. *StatPearls*. Treasure Island (FL): StatPearls Publishing.

17. Ragasa, C. Y., Biona, K. and Shen, C.-C. (2014). Chemical constituents of *Sechium edule* (Jacq.) Swartz. *Der Pharma Chemica*, 6(5): 251-255.

18. Ragasa, C. Y., Medecilo, M. P. and Shen, C.-C. (2015). Chemical constituents of *Moringa oleifera* Lam. leaves. *Der Pharma Chemica*, 7(7): 395-399.