

# Novel Acyclic Diterpeneoid from Bornean Local Red Chilli Pepper *Capsicum frutescens* L.

Kazuki Tani <sup>1</sup>, Norhidayah Tumiran <sup>1</sup>, Rolinus Paulous <sup>1</sup> and  
Charles S. Vairappan <sup>1\*</sup>

<sup>1</sup>Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, 88400 Kota Kinabalu,  
Sabah, Malaysia

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**Abstract:** An unknown acyclic diterpene (3*S*, 6*E*, 10*E*, 14*Z*)-3-hydroxy-3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraene acid (**1**) along with four known secondary metabolites (3*S*, 6*E*, 10*E*, 14*Z*)-20-hydroxygeranylinalool (**2**), *trans*-capsaicin (**3**), nordihydrocapsaicin (**4**) and capsidiol (**5**) were isolated from the Bornean red chilli pepper *Capsicum frutescens* L. The structures of the secondary metabolites were determined based on spectroscopic data analysis such as NMR, HRESIMS, and IR data. The new compound **1** is a carboxylic acid precursor that would condensate with vanillylamine in the phenylpropanoid pathway in the biosynthesis of capsaicinoids. Discovery of this compound is an important milestone in our understanding of the capsaicinoids biosynthesis.

**Keywords:** *Capsicum frutescens* L; red chilli peppers; acyclic; diterpene. © 2022 ACG Publications. All rights reserved.

## 1. Introduction

Red chili pepper (*Capsicum frutescens* L.), which belongs to the genus *Capsicum* in the Solanaceae family, is one of the common chilies used in Malaysia, Indonesia, and Thailand [1,2]. This chili is also known as bird chili pepper and widely consumed as a food seasoning, coloring, additive in the food industry, and in traditional medicine [3,4]. This variety is also known as a source of nutrients such as vitamins A, C, and E, high mineral content, and antioxidant compounds such as carotenoids, flavonoids, polyphenols, capsaicinoids, and essential oils [5,6]. This variety is widely planted in Malaysia, with an annual production of approximately 38,000 Mt [2]. The secondary metabolites of *C. frutescens* L. have been shown to exhibit potent biological activities, such as anti-inflammatory, antioxidant, antifungal, and antibacterial properties [7-10].

This study aimed to identify new secondary metabolites from *C. frutescens* L. cultivated in the highlands of Sabah, Borneo, Malaysia. A new secondary metabolite (3*S*, 6*E*, 10*E*, 14*Z*)-3-hydroxy-3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraene acid (**1**) and four known secondary metabolites (**2–5**) were obtained from the Bornean *C. frutescens* L. variety (Figure 1). Here, we report the isolation and structural elucidation of the secondary metabolites isolated from this cultivar.

\* Corresponding author: E-Mail: [csv@ums.edu.my](mailto:csv@ums.edu.my)

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A novel acyclic diterpenoid from *Capsicum frutescens* L**2. Materials and Methods***2.1. General Experimental Procedures*

<sup>1</sup>H Nuclear Magnetic Resonance (NMR) (600 MHz) and <sup>13</sup>C NMR (150 MHz) spectra were recorded on a JEOL ECA 600 NMR spectrometer using CDCl<sub>3</sub> with TMS as an internal standard. The high-resolution mass spectrum was acquired *via* LCMS-IT-TOF (Shimadzu). The Optical rotation values were measured using an AUTOPOL IV automatic polarimeter (Rudolph Research Analytic) in chloroform at 25 °C and the Infrared spectroscopy (IR) spectra recorded on Fourier Transform Infrared spectroscopy (FTIR) (Agilent Technologies). Preparative Thin Layer Chromatography (TLC) was performed using silica gel glass plates (Merck, Kieselgel 60 F<sub>254</sub>), and Column Chromatography (CC) was conducted with silica gel (Merck, Kieselgel 60, 70-230 mesh). Semi preparative High Performance Liquid Chromatography (HPLC) (Shimadzu, LC-10 AT) was further performed using a Shimadzu HPLC system with a Luna<sup>®</sup> 5 μm phenyl-hexyl 100 Å LC column (Phenomenex, 250 × 4.60 mm) to isolate pure compounds.

*2.2. Plants Materials*

Specimens of *C. frutescens* L. were collected from Tuaran, North Borneo (6°10'37"N, 116°14'02"E), on November in 2019. The specimen was identified by Mr. Julius Kulip (Botanist, Taxonomist) in comparison with previous literature based on the flower, leaves and its morphological features [11]. The voucher specimen (BORH201901208) was deposited at the BORNEENSIS Collection of the Institute for Tropical Biology and Conservation, University Malaysia Sabah, Malaysia.

*2.3. Extraction and Isolation*

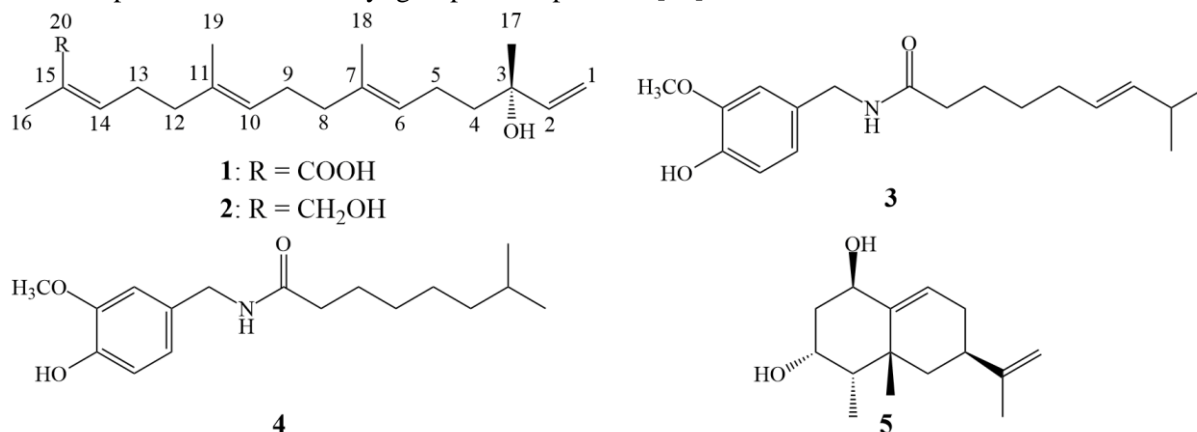
The dried red chilli (20.0 g) was extracted in ethanol (EtOH) at 27 °C for 7 days. The crude extract obtained was concentrated *in vacuo* and were partitioned between ethyl acetate (EtOAc)/distilled water (H<sub>2</sub>O). The EtOAc fraction (1.12 g) was subjected to silica gel CC eluted with a gradient solvent system consisting of *n*-hexane/EtOAc with an increasing polarity (9:1, 8:2, 7:3, 5:5, and 0:10) to yield five fractions 1-5. Fraction 3 was purified using preparative TLC in *n*-hexane-EtOAc (3:1) solvent system to give compound **2** (15.3 mg). The fraction 5 was separated by preparative TLC eluting in *n*-hexane-EtOAc (1:1) to yield compound **1** (8.6 mg). Two other fractions of fraction 5 was further purified by preparative HPLC eluting with acetonitrile (MeCN)/H<sub>2</sub>O to yield compounds **3** (9.0 mg) and **4** (5.0 mg). Finally, these compounds were purified using a phenyl-hexyl 100 Å LC column detected at 210 nm under gradient solvent elution of 50-100% MeCN/H<sub>2</sub>O. The remaining fraction 5 was further purified by preparative TLC eluted in toluene-EtOAc (1:1) to yield compound **5** (4.1 mg).

(3*S*, 6*E*, 10*E*, 14*Z*)-3-Hydroxy-3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraene acid (**1**): Red color oil; [α]<sub>D</sub><sup>25</sup> + 4.5 (*c* 0.1, CHCl<sub>3</sub>); IR (KBr): ν<sub>max</sub> 1695, 2925, and 3357 cm<sup>-1</sup>; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) spectral data are shown in Table 1; HRESIMS: *m/z* 343.2244 [M + Na]<sup>+</sup> (calcd. for C<sub>20</sub>H<sub>32</sub>O<sub>3</sub>Na, 343.2233).

**3. Results and Discussion***3.1. Structure Elucidation*

(3*S*, 6*E*, 10*E*, 14*Z*)-3-Hydroxy-3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraene acid (**1**) was isolated as a red oil and exhibited [α]<sub>D</sub><sup>25</sup> + 4.5 (*c* 0.1, CHCl<sub>3</sub>). The molecular formula C<sub>20</sub>H<sub>32</sub>O<sub>3</sub> was assigned based on High-Resolution Electron Spray Ionization Mass Spectroscopy (HRESIMS) [M + Na]<sup>+</sup> ion at *m/z* 343.2244 (calculated for C<sub>20</sub>H<sub>32</sub>O<sub>3</sub>Na, 343.2233). Therefore, five degrees of unsaturation were deduced for compound **1**. The IR spectrum revealed absorptions for carbonyl (1695 cm<sup>-1</sup>) and hydroxyl (3357 cm<sup>-1</sup>) functionalities. The <sup>1</sup>H NMR spectrum of compound **1** revealed the presence of four olefinic methine moiety signals at δ<sub>H</sub> 5.11 (1H, m, H-10), 5.12 (1H, m, H-6), 5.91 (1H, dd, *J* = 11.0, 17.9 Hz, H-

2), and 6.86 (1H, t,  $J = 6.9$  Hz, H-14), three allyl methylene signals at  $\delta_{\text{H}}$  2.02-2.07 (2H, m, H<sub>2</sub>-5), 2.09-2.12 (2H, m, H<sub>2</sub>-9), and 2.29 (2H, dd,  $J = 6.9, 14.4$  Hz, H<sub>2</sub>-13), three methylene signals at  $\delta_{\text{H}}$  1.55-1.63 (2H, m, H<sub>2</sub>-4), 2.00-2.02 (2H, m, H<sub>2</sub>-8), and 2.10-2.12 (2H, m, H<sub>2</sub>-12) and three vinyl methyl signals at  $\delta_{\text{H}}$  1.59 (3H, s, H<sub>3</sub>-18), 1.60 (3H, s, H<sub>3</sub>-19), 1.83 (3H, s, H<sub>3</sub>-16), and a methyl at  $\delta_{\text{H}}$  1.28 (3H, s, H<sub>3</sub>-17) attached to an oxycarbon at C-3 position. Terminal vinyl methylene moiety signals were also found at  $\delta_{\text{H}}$  5.06 (2H, d,  $J = 11.0$  Hz, H<sub>2</sub>-1), 5.22 (2H, d,  $J = 17.9$  Hz, H<sub>2</sub>-1), and 5.91 (1H, dd,  $J = 11.0, 17.9$  Hz, H-2) (Table 1). Additionally, the <sup>13</sup>C NMR spectrum indicated the presence of 20 carbons, which in conjunction with the Distortionless Enhancement Polarization Transfer (DEPT) and Heteronuclear Single Quantum Coherence (HSQC) measurements, were confirmed to be a carboxyl group ( $\delta_{\text{C}}$  172.5, C-20), terminal vinyl methylene carbon ( $\delta_{\text{C}}$  144.9, C-2 and 111.7, C-1), four methyl, six sp<sup>3</sup> methylene, four sp<sup>2</sup> methines, and four quaternary carbons (Table 1). Furthermore, the oxygenated-quaternary carbon ( $\delta_{\text{C}}$  73.7, C-3) signal indicated the presence of a hydroxyl group. These results were additionally confirmed *via* HRESIMS and FTIR spectroscopy data. Based on the obtained spectrum, compound **1** was assigned as an acyclic aliphatic diterpenoid with three olefinic bonds, one hydroxyl, one carboxyl, and one exomethylene group. Additionally, a careful comparison of compound **1** with previous reports of compound **2** further supported that the structures of compounds **1** and **2** were replaced with a hydroxyl group at C-20 in compound **2** and a carboxyl group in compound **1** [12].



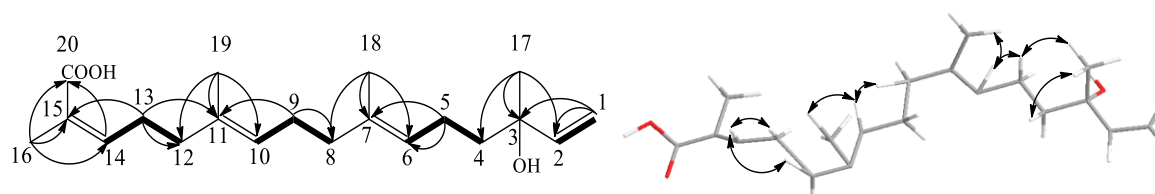
**Figure 1.** The chemical structures of the compounds **1–5** isolated from *C. frutescens* L.

The <sup>1</sup>H-<sup>1</sup>H Correlation Spectroscopy (COSY) spectra showed four short-spin systems (Figure 2): H<sub>2</sub>-1/H-2, H<sub>2</sub>-4/H<sub>2</sub>-5/H-6, H<sub>2</sub>-8/H<sub>2</sub>-9/H-10, and H<sub>2</sub>-12/H<sub>2</sub>-13/H-14. The structure of compound **1** was also established as an acyclic diterpene from the <sup>1</sup>H-<sup>13</sup>C Heteronuclear Multiple Bond Correlation spectroscopy (HMBC) correlation experiments (Figure 2) of H<sub>3</sub>-16 to C-14, C-15, and C-20; H<sub>3</sub>-17 to C-2, C-3, and C-4; H<sub>3</sub>-18 to C-6, C-7, and C-8; H<sub>3</sub>-19 to C-10, C-11, and C-12; H<sub>2</sub>-1 to C-2 and C-3; H<sub>2</sub>-5 to C-6 and C-7; H<sub>2</sub>-9 to C-8 and C-11; H<sub>2</sub>-13 to C-11, C-12, and C-15; and H-14 to C-20. The carboxyl group attached at C-15 was revealed by HMBC between H-14 and C-20, and between H<sub>3</sub>-16 and C-14, C-15, and C-20. The HMBC correlations between H<sub>2</sub>-1 and C-2 and C-3, and between H<sub>3</sub>-17 and C-2, C-3, and C-4 were important for confirming the position of terminal vinyl methylene and olefinic groups between C-1 and C-2, respectively. An attempt was made to confirm the absolute conformation; however, the Mosher method failed to establish the overall absolute configuration of compound **1** due to an unsuccessful reaction. However, the overall relative stereochemistry of compound **1** was elucidated using Nuclear Overhauser Effect Spectroscopy (NOESY) experimental data (Figure 2), NMR chemical shifts, and interpretation of coupling constants. H-14 showed NOE correlations with H<sub>2</sub>-12 and H<sub>2</sub>-13, suggesting *Z*-type H<sub>3</sub>-16. Additionally, the geometry of C-C for H<sub>3</sub>-18 and H<sub>3</sub>-19 was deduced as *E*-type based on the NOE observed between H<sub>2</sub>-5/H-6 and H-6/H<sub>3</sub>-18 (for H<sub>3</sub>-18), and H<sub>2</sub>-8/H<sub>10</sub> and H-10/H<sub>3</sub>-19 (for H<sub>3</sub>-19). Other NOE correlations between H<sub>2</sub>-4/H<sub>3</sub>-17 and H<sub>2</sub>-5/H<sub>3</sub>-17 suggest the  $\beta$  position of C-17. Furthermore, the key NOE correlations and coupling constant interpretation in compound **1** were consistent with those of compound **2** [12], supporting this inference. Hence, these 2-dimension NMR spectra confirmed the gross structure of compound **1**. The conformation of the secondary hydroxyl group was determined to be *S* based on data comparison with that of compound **2**, as described by Wallin *et al.* 1980 [12].

A novel acyclic diterpenoid from *Capsicum frutescens* L**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$ -NMR (600 and 150 MHz,  $\text{CDCl}_3$ ) data for compounds **1** and **2** ( $\delta$  in ppm,  $J$  in Hz)

Position	<b>1</b>		<b>2</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (Mult. $J$ )	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (Mult. $J$ )
1	111.7	5.06(2H, <i>d</i> , $J = 11.0$ Hz) 5.22 (2H, <i>d</i> , $J = 17.9$ Hz)	111.7	5.06 (2H, <i>d</i> , $J = 11.0$ Hz) 5.21 (2H, <i>d</i> , $J = 18.0$ Hz)
2	144.9	5.91 (1H, <i>dd</i> , $J = 11.0, 17.9$ Hz)	144.9	5.91 (1H, <i>dd</i> , $J = 11.0, 17.9$ Hz)
3	73.7	-	73.7	-
4	41.9	1.55-1.63 (2H, <i>m</i> ) 1.55-1.63 (2H, <i>m</i> )	42.0	1.53-1.62 (2H, <i>m</i> ) 1.53-1.62 (2H, <i>m</i> )
5	22.6	2.02-2.07 (2H, <i>m</i> ) 2.02-2.07 (2H, <i>m</i> )	22.7	1.97-1.99 (2H, <i>m</i> ) 1.97-1.99 (2H, <i>m</i> )
6	124.4	5.12 (1H, <i>m</i> )	124.3	5.08 (1H, <i>t</i> , $J = 6.9$ Hz)
7	135.2	-	135.4	-
8	39.4	2.00-2.02 (2H, <i>m</i> ) 2.00-2.02 (2H, <i>m</i> )	39.6	1.97-1.99 (2H, <i>m</i> ) 1.97-1.99 (2H, <i>m</i> )
9	26.2	2.09-2.12 (2H, <i>m</i> ) 2.09-2.12 (2H, <i>m</i> )	26.3	2.05-2.09 (2H, <i>m</i> ) 2.05-2.09 (2H, <i>m</i> )
10	125.2	5.11 (1H, <i>m</i> )	124.5	5.12 (1H, <i>t</i> , $J = 6.9$ Hz)
11	133.6	-	134.3	-
12	38.0	2.10-2.12 (2H, <i>m</i> ) 2.10-2.12 (2H, <i>m</i> )	39.8	2.05-2.09 (2H, <i>m</i> ) 2.05-2.09 (2H, <i>m</i> )
13	27.2	2.29 (2H, <i>dd</i> , $J = 6.9, 14.4$ Hz) 2.29 (2H, <i>dd</i> , $J = 6.9, 14.4$ Hz)	26.2	2.12 (2H, <i>dd</i> , $J = 6.9, 14.4$ Hz) 2.12 (2H, <i>dd</i> , $J = 6.9, 14.4$ Hz)
14	144.7	6.86 (1H, <i>t</i> , $J = 6.9$ Hz)	128.2	5.27 (1H, <i>t</i> , $J = 6.9$ Hz)
15	126.9	-	134.6	-
16	12.1	1.83 (3H, <i>s</i> )	21.3	1.78 (3H, <i>s</i> )
17	27.8	1.28 (3H, <i>s</i> )	27.7	1.27 (3H, <i>s</i> )
18	15.9	1.59 (3H, <i>s</i> )	16.1	1.58 (3H, <i>s</i> )
19	15.9	1.60 (3H, <i>s</i> )	16.0	1.58 (3H, <i>s</i> )
20	172.5	-	61.5	4.10 (1H, <i>s</i> ) 4.10 (1H, <i>s</i> )

Notably, the structures of compounds **2–5** were also reported from *Capsicum* sp. [13-16], and identified as (3*S*, 6*E*, 10*E*, 14*Z*)-20-hydroxygeranylinalool (**2**) [12], trans-capsaicin (**3**) [14], nordihydrocapsaicin (**4**) [15], and capsidiol (**5**) [16], based on a comparison of their (NMR and HRESIMS) spectroscopic data with literature.



**Figure 2.** The  $^1\text{H}$ - $^1\text{H}$  COSY (bold lines), key HMBC (arrows), and NOESY (double head arrows) correlations of compound **1**

Based on the existing biosynthetic knowledge, it is reasonable to propose that compound **1** is a precursor or an intermediate in the biosynthetic pathway of capsaicin. Capsaicinoids are primarily biosynthesized in the placenta of chili, where the highest concentration of vanillylamine is stored [17]. Biosynthesis of various capsaicinoids occurs *via* condensation of vanillylamine with a carboxylic acid precursor in the phenylpropanoid pathway [18]. An acyl precursor with a carbon range from C-9 to C-11 chains is usually synthesized by a series of enzymatic reactions from amino acids, which involves several basic steps, such as transamination, decarboxylation, elongation, and reduction. The elongation process is usually promoted by malonyl-CoA, and the chain length is determined by the number of cycles in the

elongation process. The final stage comprises ketone-to-alcohol reduction and dehydration, yielding a double bond to the fatty acid chain. Therefore, the presence of (3*S*, 6*E*, 10*E*, 14*Z*)-3-hydroxy-3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraene acid (**1**) is an important discovery, although we were unable to elucidate the final product between the condensation of compound **1** and vanillylamine. The production of the condensation product between these two precursors could be dictated by the fruit ripening or other environmental factors.

#### 4. Conclusion

To date, research on chili peppers is mainly conducted for the genus *Capsicum*. Despite the flow of consistent reports pertaining to chilli chemistry, there is still a shortage of information on chemical constituents of *C. frutescens* L., especially from the island of Borneo. In this investigation, a new compound (3*S*, 6*E*, 10*E*, 14*Z*)-3-hydroxy-3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraene acid (**1**), was isolated as the major metabolite from Bornean red chilli pepper *C. frutescens* L. Its structure is an analogue of a previously reported (3*S*, 6*E*, 10*E*, 14*Z*)-20-hydroxygeranyllinalool (**2**) [12]. Therefore, this study is an important discovery in the chemical assessment of chilli pepper.

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#### Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

#### ORCID

Kazuki Tani: [0000-0002-2760-3771](https://orcid.org/0000-0002-2760-3771)

Norhidayah Tumiran: [0000-0003-0225-3776](https://orcid.org/0000-0003-0225-3776)

Rolinus Paulous: [0000-0003-0057-738X](https://orcid.org/0000-0003-0057-738X)

Charles S. Vairappan: [0000-0001-7453-1718](https://orcid.org/0000-0001-7453-1718)

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