



## Ethyl Haematommate from *Stereocaulon graminosum* Schaer.: Isolation and Crystal Structure

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**Abstract** – Herein, we reported the phytochemical investigation of whole thallus Sumatran lichen, *Stereocaulon graminosum* Schaer, and isolated a mono aromatic compound, ethyl haematommate (**1**). The structure of compound **1** have been established based on spectroscopic data and confirmed by single crystal X-ray structure analysis.

**Keywords** – *Stereocaulon*, Lichen, Mono aromatic, Crystal structure

### Introduction

The genus *Stereocaulon* includes fruticose lichens distributed worldwide, from polar to tropical zones. This genus is usually found on plateaus with low temperature and on rocky surfaces exposed to direct sunlight.<sup>1</sup> To best of our knowledge, only a few phytochemical studies have been conducted on this genus. Previously, we isolated lobarin, a diphenyl ether compound, from *S. halei* and stictic acid derivatives from *S. montagneanum*.<sup>2-3</sup> Following our continuous interest in the phytochemical composition of this genus, herein we focused on one of the Sumatran species from this genus, namely *S. graminosum* Schaer. that was harvested from Mount Talang (altitude ~2.300 m above sea level), West Sumatra, Indonesia.

This paper aimed to describe the isolation and elucidate the structure of the monoaromatic compound ethyl haematommate (**1**) obtained from the ethyl acetate extract of *S. graminosum*. Compound **1** is known to be an intermediate product in depside biosynthesis.<sup>4,5</sup> This compound has been reported to have antioxidant,<sup>6</sup> antiglycation,<sup>7</sup> and antimicrobial<sup>8,9</sup> properties. The chemical structure of

compound **1** was established by spectroscopic analysis (UV, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and HMBC). The structure was also confirmed by single crystal X-ray crystallographic analysis.

### Experimental

**General experimental procedures** – Melting point was measured on a Fisher Melting Point apparatus. UV spectra were performed on a Shimadzu 1700 spectrophotometer. FTIR spectra were run on a PerkinElmer FTIR spectrometer. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded at 500 and 125 MHz, respectively, on a Jeol 500 MHz NMR spectrometer using CDCl<sub>3</sub> as solvents. Chromatographic separation was performed using vacuum liquid chromatography on silica gel (Merck 35 - 70 µm). TLC analytic (Merck silica gel 60F254) were eluted using two standard solvent systems<sup>9</sup>: toluene/acetic acid (85:15) (C); toluene/EtOAc/formic acid (139:83:8) (G). Visualization of plates was carried out under UV light (254 and 365 nm) and using anisaldehyde H<sub>2</sub>SO<sub>4</sub> (v/v) reagent then heating.

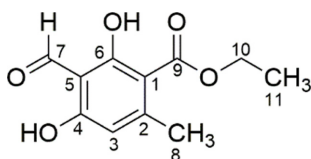
**Lichen Material** – *S. graminosum* was collected on Mount Talang, Solok, West Sumatra (altitude ~2.300 m above sea level). The voucher specimens were identified by Harrie Sipman (Berlin Botanical Museum) and deposited at Biota Sumatran Laboratory, Andalas University, West Sumatra (Indonesia) under the reference number FS13.

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**Fig. 1.** Chemical structure of compound **1** isolated from lichen *S. graminosum*.

**Table 1.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of ethyl haematommate (**1**) in  $\text{CDCl}_3$  ( $\delta$  in ppm, 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ )<sup>a</sup>

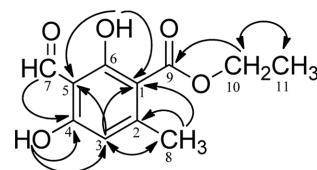
Position	Compound <b>1</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	-	104.4
2	-	152.6
3	6.27 (1H, s)	112.2
4	12.39 (1H, s)	166.7
5	-	108.6
6	12.97 (1H, s)	168.5
CHO-7	10.33 (1H, s)	194.4
CH <sub>3</sub> -8	2.53 (3H, d, $J = 7.15$ )	25.4
CO-9	-	171.7
OCH <sub>2</sub> -10	4.43 (2H, bd, $J = 7.10$ )	62.1
CH <sub>3</sub> -11	1.42 (3H, t, $J = 7.1$ )	14.3

<sup>a</sup>  $J$  value are in parentheses and reported in Hz

**Extraction and isolation** – Three kilograms of air-dried whole thalli of *S. graminosum* were macerated with *n*-hexane, ethyl acetate (EtOAc), acetone, and methanol successively. Each filtrate was concentrated *in vacuo* to obtain *n*-hexane, EtOAc, acetone, and methanol extracts. Twenty-two grams of ethyl acetate extract was chromatographed by vacuum liquid chromatography on silica gel (35 - 70  $\mu\text{m}$ , 350 g) with a solvent gradient consisting of *n*-hexane/EtOAc (100:0  $\rightarrow$  0:100) to yield 10 subfractions. The subfraction 1 - 2 (7 g) was selected for further chromatography using a silica gel column (35 - 70  $\mu\text{m}$ , 140 g) and eluted employing *n*-hexane/EtOAc (80:20 v/v). Eight fractions were subsequently obtained and 130 mg of compound **1** was purified by recrystallization in *n*-hexane/EtOAc (1:1) (v/v).

**Ethyl haematommate (1)** – colorless block powder crystals (EtOAc), mp 106 - 107  $^{\circ}\text{C}$ ; IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 2923, 1729, 1628, 1416, 1303, 1253; UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  nm (log  $\epsilon$ ): 246 (4.41), 289 (4.06), 349 (3.51);  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 500 MHz) and  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 125 MHz); see Table 1.

**Single-crystal X-ray diffraction and refinement** – Single-crystal X-ray diffraction patterns were recorded at 93 K in  $\omega$ -scan mode (R-Axis RAPID II, Rigaku, Japan) using a Cu K $\alpha$  X-ray source (50 kV, 100 mA) and a graphite monochromator. The integrated and scaled data were empirically corrected for absorption effects using



**Fig. 2.** Key HMBC correlations of compound **1**.

ABSCOR<sup>10</sup> and the initial structure was solved with SIR 2014<sup>11</sup> using the direct method and refined on  $F_o^2$  with SHELXL 2014.<sup>12</sup> All non-hydrogen atoms were anisotropically refined, and hydrogen atom positions were calculated geometrically and included in the calculation using the riding model. Hydrogen atoms attached to oxygen atoms were located using a differential Fourier map. All hydrogen atoms were freely refined, and molecular graphics were produced using Mercury 3.7 software.<sup>13</sup>

## Result and Discussion

Compound **1** was obtained as colorless block powder crystals. The UV spectrum of compound **1** with absorption maxima at 246, 289, and 349 nm suggested that it has a monoaromatic type structure.<sup>14</sup> The IR spectrum showed bands for hydroxyl phenolic, carbonyl, and aromatic groups at 2923, 1729, and 1628  $\text{cm}^{-1}$ , respectively.

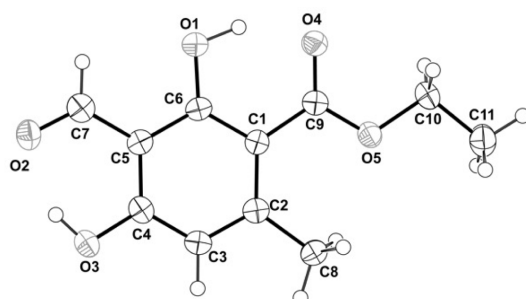
Table 1 shows the  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra of compound **1**. The  $^1\text{H}$ -NMR spectral data of **1** indicated the presence of signals for aromatic protons at  $\delta_{\text{H}}$  6.27 (1H, s), two hydroxyl groups at  $\delta_{\text{H}}$  12.39 (1H, s) and  $\delta_{\text{H}}$  12.97 (1H, s), one carbonyl group at  $\delta_{\text{H}}$  10.33 (1H, s), one ethoxy group at  $\delta_{\text{H}}$  4.43 (bd,  $J = 7.10$  Hz), and two methyl groups at  $\delta_{\text{H}}$  2.53 (d,  $J = 7.15$  Hz) and 1.42 (t,  $J = 7.10$  Hz). The  $^{13}\text{C}$ -NMR spectrum exhibited 11 carbon signals comprising two carbonyl carbon atom groups (aldehyde and ester) at  $\delta_{\text{C}}$  194.1 and  $\delta_{\text{C}}$  177.7, one aromatic carbon signal at  $\delta_{\text{C}}$  168.5, 166.7, 152.6, 112.2, 108.6, 104.1), one ethoxyl at  $\delta_{\text{C}}$  62.1, and two methyl carbon signals at  $\delta_{\text{C}}$  25.4 and  $\delta_{\text{C}}$  14.3.

In the HMBC spectra (Fig. 2), the complete structure is described with the following connectivity: aromatic proton H-3 at  $\delta_{\text{H}}$  6.27 with CH<sub>3</sub>-8 ( $\delta_{\text{C}}$  25.4), C-1 ( $\delta_{\text{C}}$  104.1) and C-5 ( $\delta_{\text{C}}$  108.6), aldehyde CHO-7 at  $\delta_{\text{H}}$  10.33 with C-4 ( $\delta_{\text{C}}$  166.7), ethoxy proton OCH<sub>2</sub>-10 at  $\delta_{\text{H}}$  4.43 with CO-9 and CH<sub>3</sub>-11 ( $\delta_{\text{C}}$  177.7 and 14.3, respectively). Therefore, the chemical structure of compound **1** was identified as ethyl haematommate according to the NMR data as well as its comparison to the literature.<sup>9</sup>

Single crystal X-ray crystallography is one of the most advanced methods for structural determination of many

**Table 2.** Crystallographic data of compound **1**

Chemical formula	C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>
$M_r$	224.21
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	93
$a, b, c$ (Å)	6.7257 (4), 12.1568 (7), 12.3739 (8)
$T_{\min}, T_{\max}$	0.455, 0.905
No. of measured, independent, and observed [ $I > 2\sigma(I)$ ] reflections	11356, 1848, 1490
$R_{\text{int}}$	0.079
$(\sin \theta/\lambda)_{\text{max}}$ (Å <sup>-1</sup> )	0.602
$T_{\min}, T_{\max}$	0.455, 0.905
$R[F^2 > 2\sigma(F^2)], wR(F^2), S$	0.043, 0.101, 0.96
No. of reflections	1848
No. of parameters	159

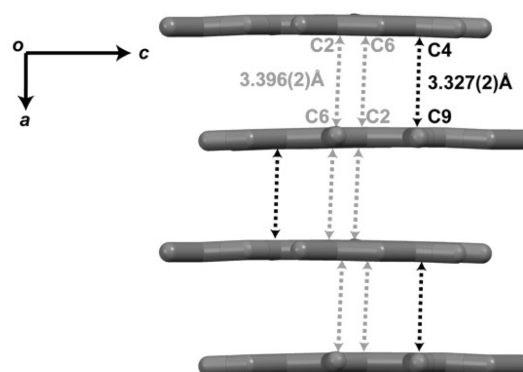
**Fig. 3.** Thermal ellipsoid structures of compound **1** with atom labelling drawn at a 50% probability level. The asymmetric unit contained one molecule of compound **1**.

compounds including the natural products.<sup>15-17</sup> To determine the crystal structure of compound **1** through single crystal X-ray crystallography, the isolate was recrystallized. Compound **1** was dissolved in *n*-hexane:EtOAc (1:1, v/v) solvent mixture and the solution was maintained under ambient conditions until colorless block-shaped single crystals were obtained. A suitable large single crystal ( $0.646 \times 0.121 \times 0.100$  mm<sup>3</sup>) was selected for single crystal X-ray diffraction. The analysis of single crystal X-ray crystallography revealed that compound **1** is crystallized in the orthorhombic crystal system and the  $P2_12_12_1$  space group. The crystallographic data and thermal ellipsoid drawing for compound **1** are shown in Table 2 and Fig. 3, respectively.

Compound **1** expectedly adopts a planar conformation. All bond distances, angles, and dihedral angles appear within normal range except C8–C2–C1 ( $124.8(2)^\circ$ ) and C8–C2–C3 ( $115.7(2)^\circ$ ) compared with the mean value reported in Cambridge Structural Database (CSD, version 5.38, updated July 2017).<sup>18</sup> The deviation in the bond angle may be a result of the strong intramolecular O1–H1...O4 interaction. Interestingly, only the intramolecular

**Table 3.** Hydrogen-bond geometry of compound **1**

$D-H\cdots A$	$D-H$ (Å)	$H\cdots A$ (Å)	$D\cdots A$ (Å)	$D-H\cdots A$ (°)
O1–H...O4	0.98 (3)	1.63 (4)	2.509 (2)	146 (3)
O3–H...O2	0.90 (3)	1.76 (3)	2.581 (3)	151 (3)

**Fig. 4.** The  $\pi$ - $\pi$  interactions in compound **1** create a layered architecture propagating in the (001) plane. Hydrogen atoms are omitted for clarity.

hydrogen bonds were observed in the crystal. The numerical details of the hydrogen bond in the crystal structure of compound **1** are listed in Table 3. The two hydroxyl-carbonyl intramolecular hydrogen bonds of O1–H...O4 and O3–H...O2 enclose  $S_1^1$  (6) loops. The intermolecular interactions between compound **1** molecules are mainly composed by  $\pi$ - $\pi$  interaction in which the perpendicular distances between two rings are between  $3.327(2)$  Å and  $3.396(2)$  Å for C4...C9 and C2...C6, respectively. Together, this  $\pi$ - $\pi$  interaction forms a layered architecture propagating in the (001) plane (Fig. 4).

In summary, we have succeeded to isolate compound **1** from *S. graminosum* and analyze its chemical structure by spectroscopy. In addition, the crystal structure of com-

pound **1** was determined for the first time by single crystal X-ray structure analysis. Our current study is underway to identify other isolates from *S. graminosum* and other species from the same genus. This paper is expected to provide an additional phytochemical contribution of the poorly understood *Stereocaulon* genus.

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### Supplementary Materials

The online version of this article contains supplementary materials: experimental procedures and characterizations of compound **1**. CCDC 1585998 contains the supplementary crystallographic data presented in this paper. These data can be obtained free of charge at [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif), by e-mailing [data\\_request@ccdc.cam.ac.uk](mailto:data_request@ccdc.cam.ac.uk), or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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