

A New *Seco*-Clerodane-Type Diterpenoid from Bornean Liverwort *Schistochila acuminata*

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A new compound, schistochilic acid D (**1**) and two known compounds (**2** and **3**) were isolated from MeOH extract of Bornean liverwort *Schistochila acuminata* collected from Mount Trus Madi, Sabah. The structure of the new metabolite was established based on spectroscopic (1D NMR, 2D NMR, and IR) and HRESIMS data. In addition, another population of *S. acuminata* collected from Mount Alab (Sabah) yielded four known compounds, **2**, **3**, **4** and **5**. These compounds were tested for their biological potential against the B16-F10 cell line. Compounds **4** and **5** exhibited weak cytotoxic activity.

Keywords: Chemotype, Diterpenoid, Cytotoxic, *Schistochila acuminata*, Liverwort, Borneo.

Liverwort in the family Schistochilaceae are only be found in restricted places in New Zealand, Taiwan and Southeast Asia. Globally, 54 species have been recorded and five different chemotypes have been classified for this genus [1]. In Borneo, there are seven species of this genus available [2], but only one (*Schistochila aligera*) has been chemically investigated so far [3]. In this investigation, two populations of Bornean *S. acuminata* were analyzed where *seco*-clerodane-, clerodane- as well as dolabellane-type diterpenoids were isolated. A population from Mount Trus Madi gave one new *seco*-clerodane (**1**) and two known clerodanes (**2**, **3**), while population from Mount Alab gave three known clerodanes (**2**, **3**, **4**) and one dolabellane-type compound (**5**), as shown in Figure 1. Here we report the spectral evidence for the new compound and discuss the chemosystematic significance of these findings.

Compound **1** was isolated as optically active colorless oil, $[\alpha]_D^{27} +157.3$ (*c* 0.90, CHCl₃). The molecular formula of **1**, C₂₀H₂₈O₂ (corresponding to 7 degrees of unsaturation), was deduced from HR-ESI-MS measurements, *m/z* 301.2254 [M+H]⁺ (calcd for C₂₀H₂₉O₂, 301.2162). The IR absorption at 3390 cm⁻¹ and 1718 cm⁻¹ indicated the presence of a carboxyl group. HSQC and ¹³C-DEPT experiments coupled with ¹³C- and ¹H-NMR signals of compound **1** showed the presence of a conjugated carboxylic acid at δ_C 172.0 (C), two pairs of olefinic carbons at δ_C 144.2 (CH), δ_H 7.40 (1H, d), δ_C 137.0 (C), δ_C 128.7 (CH), δ_H 5.41 (1H, td), δ_C 127.9 (CH), δ_H 5.93 (1H, d), three vinylidenes at δ_C 147.9 (C), δ_C 116.3 (CH₂), δ_H 5.00 (2H, br s), δ_C 145.5 (C), δ_C 118.8 (CH₂), δ_H 5.09/4.86 (2H, br s), δ_C 139.7 (CH), δ_H 6.38 (1H, dd), δ_C 113.7, δ_H 5.23/5.06 (2H, d), two methyls at δ_C 19.2 (CH₃), δ_H 0.73 (3H, s), δ_C 14.5 (CH₃), δ_H 0.76 (3H, s), and five methylenes (Table 1). Thus, degrees of unsaturation could be attributed to five double bonds, one carboxylic acid and a monocyclic ring. The ¹H-NMR spectrum of **1** resembled the schistochilic acid A [4]. Therefore, compound **1** was suggested to be a 10-membered *seco*-clerodane diterpene.

Analysis of the ¹H-¹H COSY experiment revealed the sequences of correlations depicted by the bold lines in Figure 2. The HMBC correlations of H₃-20 with C-8, C-9, C10 and C-11 allowed us to attach C-20 to C-9. The exomethylene group attached between C-12

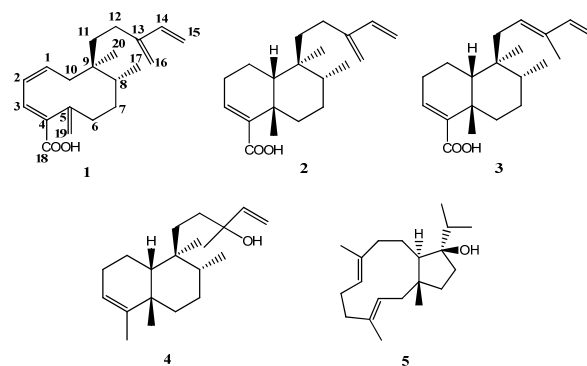


Figure 1: Structure of compounds 1-5.

Table 1: ¹H- and ¹³C-NMR data (600 and 150 MHz, CDCl₃) for **1** (δ in ppm, *J* in Hz).

Position	δ _C	Compound 1	
		δ _H (mult., <i>J</i> in Hz)	
1	128.7 (d)	5.41(td, <i>J</i> = 11.7, 4.1)	
2	127.9 (d)	5.93 (d, <i>J</i> = 11.7)	
3	144.2 (d)	7.40 (s)	
4	137.0 (s)		
5	145.5 (s)		
6α		2.68 (br d, <i>J</i> = 13.8)	
6β	34.5 (t)	2.10 (td, <i>J</i> = 13.8, 2.1)	
7α		1.56 (br t, <i>J</i> = 13.8)	
7β	29.9 (t)	0.82-0.86 (m)	
8	36.5 (d)	1.32-1.38 (m)	
9	38.7 (s)		
10α		2.21 (dd, <i>J</i> = 13.8, 5.2)	
10β	36.5 (t)	1.84 (br d, <i>J</i> = 13.8)	
11α		1.44-1.50 (m)	
11β	36.9 (t)	1.33-1.39 (m)	
12	26.6 (t)	2.14-2.22 (m)	
13	147.9 (s)		
14	139.7 (d)	6.38 (dd, <i>J</i> = 17.2, 11.0)	
15	113.7 (t)	5.23 (d, <i>J</i> = 17.2), 5.06 (d, <i>J</i> = 11.0)	
16	116.3 (t)	5.00 (br s)	
17	14.5 (q)	0.76 (d, <i>J</i> = 6.9)	
18	172.0 (s)		
19	118.8 (t)	5.09 (br s), 4.86 (br s)	
20	19.2 (q)	0.73 (s)	

and C-14 was confirmed by HMBC correlations of H-16 to C-12, C-13 and C-14. The other exomethylene group attached between C-4 and C-6 was confirmed by HMBC correlations of H₂-19 to C-4, C-5 and C-6. The carboxylic group attached to C-4 was confirmed

by correlations between H-3 to C-4 and C-18. Based on the foregoing evidence, the gross structure of **1** was determined as shown in Figure 2. The relative stereochemistry of **1** was deduced from the NOESY experiment. The NOESY correlations observed between H-7 α /H-10 α , H-10 α /H₃-17, H-7 α /H₃-17 and H₃-17/H₃-20, as well as the same biosynthetic pathway with the co-occurring compounds **2-4**, proposed the α configuration of methyl groups at C-8 and C-9. Thus, compound **1** was identified as schistochilic acid D. The other known diterpenoids were identified as (-)-3,13(16),14-*cis*-clerodatrien-18-oic acid (**2**) [5], (-)-3,12*E*,14-*cis*-clerodatrien-18-oic acid (**3**) [5], *cis*-3,14-clerodadien-13-ol (**4**) [6] and 12 β -hydroxydolabella-(3*E*,7*E*)-diene (**5**) [7].

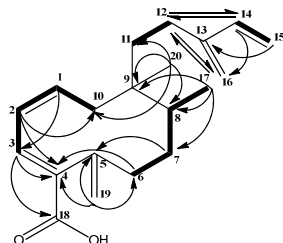


Figure 2: Key HMBC and ¹H-¹H COSY correlations for **1**.

Liverwort populations of this species collected from different localities showed chemical differences. In our study, compounds **1**, **2** and **3** were detected from *S. acuminata* collected from Mount Trus Madi (2000 m a.s.l.). However, compounds **2**, **3**, **4** and **5** were detected from *S. acuminata* collected from Mount Alab (1800 m a.s.l.). With a narrow range of elevation difference (200 m a.s.l.), the condition of a microhabitat may have affected the phytochemical content [8]. A slight differences of environmental factors have been recorded between Mount Trus Madi (temperature: 17.1 °C; relative humidity: 80.2 %; light intensity: 3211 lx; ultraviolet intensity: 0.120 MW/cm²) and Mount Alab (temperature: 18.7 °C; relative humidity: 79.5 %; light intensity: 4019 lx; ultraviolet intensity: 0.185 MW/cm²). The chemical differences are probably due to different environmental exposures such as light intensity, ultraviolet (UV) exposure, temperature, humidity, canopy coverage, soil pH and availability of moisture for which further research needs to be undertaken. We here add one more chemistry which including a dolabellane-type together with *seco*-clerodane- and clerodane-type to the list of Bornean *S. acuminata*.

All the isolated compounds were tested for their cytotoxic potential against *Mus musculus* skin melanoma (B16-F10) cancer cell line. Compounds **1-3** did not show inhibition, whereas compounds **4** and **5** showed weak cytotoxic inhibition against the B16-F10 cell line with LC₅₀ values of 40 and 62 μ g/mL⁻¹, respectively.

References

- Asakawa Y. (2004) Chemosystematics of the Hepaticae. *Phytochemistry*, **65**, 623-669.
- Chuah-Petiot MS. (2011) A checklist of Hepaticae and Anthocerotae of Malaysia. *Polish Botanical Journal*, **56**, 1-44.
- Nagashima F, Tori M, Asakawa Y. (1991) Diterpenoids from the east Malaysian liverwort *Schistochila aligera*. *Phytochemistry*, **30**, 849-851.
- Tori M, Masuya T, Asakawa Y. (1993) Three clerodane-diterpenoids from the liverwort *Schistochila nobilis*. *Phytochemistry*, **32**, 1229-1233.
- Chen JA, Huang CD, Wu CL. (1992) Clerodane-type diterpenoids from the Taiwanese liverwort *Schistochila acuminata*. *Journal of Chinese Chemical Society*, **39**, 263-266.
- Nagashima F, Suzuki M, Takaoka S, Asakawa Y. (2001) Sesqui- and diterpenoids from the Japanese liverwort *Jungermania infusca*. *Journal of Natural Products*, **64**, 1309-1317.
- Matsuo A, Kamio KI, Uohama K, Yoshida KI, Connolly JD, Sim GA. (1988) Dolabellane diterpenoids from the liverwort *Odontoschisma denudatum*. *Phytochemistry*, **27**, 1153-1159.
- Ghasemzadeh A, Jaafar HZ, Rahmat A, Wahab PE, Halim MR. (2010) Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zinger officinale* Roscoe). *International Journal of Molecular Science*, **11**, 3885-3897.
- Kim KN, Ahn G, Heo SJ, Kang SM, Kang MC, Yang HM, Kim D, Roh SW, Kim SK, Jeon BT, Park PJ, Jung WK, Jeon YJ. (2013) Inhibition of tumor growth *in vitro* and *in vivo* by fucoxanthin against melanoma B16-F10 cells. *Environmental Toxicology and Pharmacology*, **35**, 39-46.

Experimental

General: IR, Nexus, Thermo, USA; Optical Rotation, Rudolph, AUTOPOL V, USA; HRESIMS, Shimadzu, Japan; HPLC, Prominent, Shimadzu, Japan; NMR, JEOL ECA 600 MHz, Japan.

Plant material: *Schistochila acuminata* Stephani was collected from Mount Trus Madi (N5°33'37.98'' E116°27'52.56'') and Mount Alab (N5°49'17.34'' E116°20'33.24'') (Sabah, Malaysia). The voucher specimens (BORHB0013) were deposited in the BORNEENSIS Herbarium of the Institute for Tropical Biology and Conservation (BORH), Universiti Malaysia Sabah.

Extraction and isolation: Air dried specimens (50 g) were extracted with methanol (MeOH) at room temperature (2.0 L x 3). Each resulting MeOH extract was concentrated *in vacuo* and the concentrate was partitioned between ethyl acetate (EtOAc) and water (H₂O). The Mount Trus Madi EtOAc extract was then fractionated using silica gel CC with a step gradient of *n*-Hex and EtOAc with the ratios 9:1, 8:2, 7:3, 6:4, 1:1 and chloroform:MeOH:H₂O (65: 25: 4) to yield 6 fractions. Fraction 5 was treated by PTLC using Hex:EtOAc (2:1) to obtain **1** (14.0 mg). Fraction 4 was subjected to HPLC (A: 50% acetonitrile (ACN); B: 100% ACN, flow rate 2 mL min⁻¹, wavelength 210 nm, column C-18) to obtain **2** (3.0 mg) and **3** (5.2 mg). The Mount Alab EtOAc extract was similarly processed to yield **2** (3.6 mg) and **3** (5.9 mg) from fraction 4, whereas fraction 3 gave compounds **4** (24.4 mg) and **5** (14.3 mg).

Cytotoxic activity: Cancer cell line (B16-F10) was cultured and tested against the isolated compounds based on the method described by Kim *et al.* [9].

Schistochilic acid D

Colorless oil.

[α]_D²⁷: +157.3 (*c* 0.90, CHCl₃).

IR (KBr): 3390, 1718 cm⁻¹.

¹H- and ¹³C-NMR: Table 1.

HR-ESI-MS: *m/z* [M+H]⁺ calcd for C₂₀H₂₉O₂: 301.2162; found: 301.2254.

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