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New Pimarane-Type Diterpenoid and *ent*-Eudesmane-Type Sesquiterpenoid from Bornean Liverwort *Mastigophora diclados*

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Abstract: Three populations of *Mastigophora diclados* were collected from Mount Kinabalu, Mount Alab, and Mount Trus Madi. Each population yielded a total of four, four, and five secondary metabolites, respectively. A new compound, dicladoic acid (**1**) and the enantiomer of the known chlorantene G (**8**), along with six known compounds (**2**-**7**) were isolated from the MeOH extract of Bornean liverwort *Mastigophora diclados*. The structures of the novel metabolites were established by analyses of the spectroscopic data, including 1D NMR, 2D NMR, HRESIMS and IR. Isolated compounds shed some light into the chemosystematics of secondary metabolites in *M. diclados*. Herbertane-type sesquiterpenes were identified as the major metabolites in all three populations in Borneo and could be regarded as a suitable chemotaxonomical marker.

Keywords: Pimarane; diterpenoid; *Mastigophora diclados*; liverwort; Borneo Island. © 2017 ACG Publications. All rights reserved.

1. Introduction

The liverwort *Mastigophora diclados* (Bird.) Nees is a primitive plant, which has been classified into the family Lepicoleaceae, subfamily Mastigophoroideae [1]. *Mastigophora* is closely related to *Herbertus* (Herbertaceae) in its chemical composition, both having the herbertane-type sesquiterpenoids as chemical markers [2]. The liverwort *M. diclados* can be found in Western Europe, Southeast Africa as well as Southeast Asia regions and it is common in highlands of Malaysia. The phytochemical investigation of *M. diclados* from Malaysia, Japan, Taiwan, Tahiti and Madagascar has shown the presence of monomers and dimers of herbertane, macrocyclic bisbibenzyls, *ent*-trachylobane and *ent*-pimarane diterpenoids [3-10]. These results showed occurrence of chemotype variations depending on geographical differences. Hence, present investigation delves upon the chemical constituents of Bornean *M. diclados* collected from three different localities. A new pimarane-type diterpenoid (**1**) and a new *ent*-eudesmane-type sesquiterpenoid (**8**), together with six known compounds (**2**-**7**) have been isolated. The isolation, structure elucidation and chemosystematic of the compounds are discussed.

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2. Materials and Methods

2.1. Biological Materials

Three specimens (**A1-A3**) of *M. diclados* originated from natural populations of three mountain regions were collected from Mount Kinabalu (**A1**) (6°1'52.7''N, 116°32'59.7''E), Mount Alab (**A2**) (5°40'11.3''N, 116°15'12.04''E) and Mount Trus Madi (**A3**) (5°33'13.1''N, 116°30'41.9''E) in October 2013, December 2014 and August 2015, respectively. Voucher specimens **A1-A3** (BORB0002, BORB0022 and BORB0023) are deposited in the BORNEENSIS Herbarium of Institute for Tropical Biology and Conservation (BORH), Universiti Malaysia Sabah.

2.2. Extraction and Isolation

Air dried plant material of each specimen [**A1** (80 g), **A2** (40 g) and **A3** (74 g)] were extracted with methanol (MeOH) at room temperature (1.0 L x 3 each for one week). The crude extract of each specimen was suspended in distilled water (150 ml) and partitioned with ethyl acetate (EtOAc) (50 ml x 3). The combined organic layers were dried over sodium sulfate (anhydrous) and concentrated *in vacuo* to afford EtOAc extracts 2.3 g (**P1**), 1.2 g (**P2**) and 2.0 g (**P3**), respectively. Each EtOAc extracts **P1-P3** (1 g) were chromatographed on a Si gel column using n-hexane (Hex) and EtOAc solvent system as eluent with increasing polarity (Hex/EtOAc: 9:1, 8:2, 7:3, 6:4, 1:1, 100 % EtOAc, and $CHCl_3$/MeOH/H_2O: 65:25:4) to yield seven fractions, 1-7.

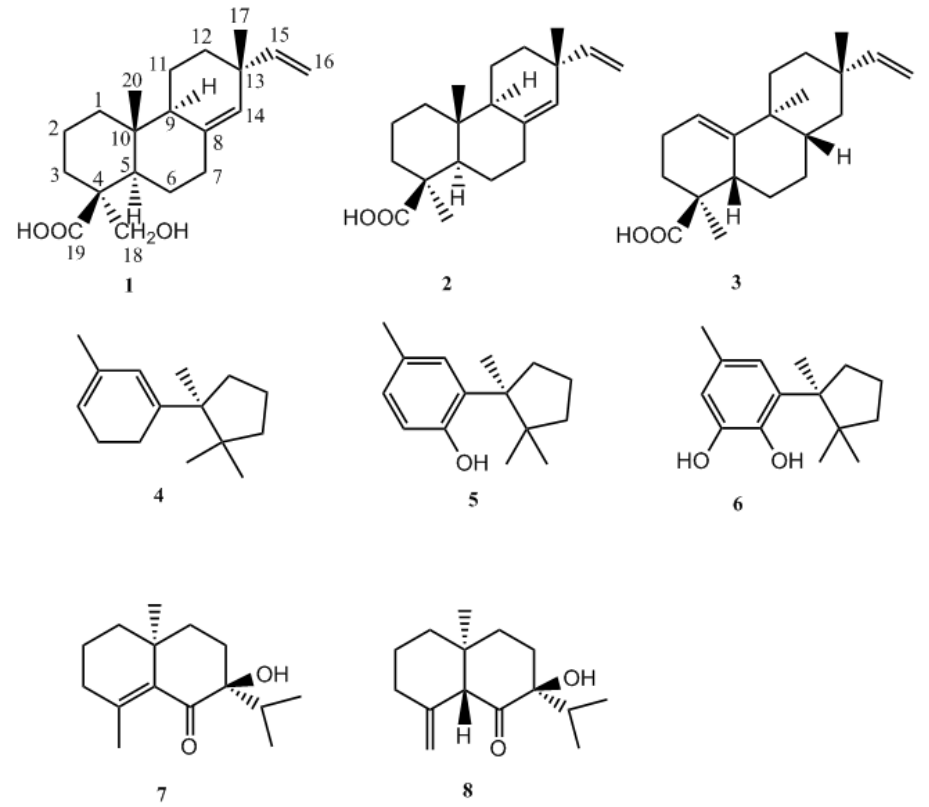

Figure 1. Structures of compounds **1-8**.

P1 fraction 1 (348.0 mg) was subjected to PTLC (100% Tol) to obtain **4** (15.6 mg) (1.6 % yield) and **5** (12.4 mg) (1.2 % yield). Fraction 2 (452.7 mg) yielded **6** (452.7 mg) (45.3 % yield) right

after the open column chromatography. Compound **3** (1.9 mg) (0.2 % yield) was isolated from fraction 3 (29.8 mg) by PTLC (Hex/EtOAc: 3:1).

P2 fraction 2 (210.1 mg) was subjected to PTLC (100% Tol and Hex/EtOAc: 95:5) to yield **4** (10.9 mg) (1.1 % yield), **7** (6.4 mg) (0.6 % yield) and **8** (6.6 mg) (0.7 % yield). Fraction 3 (125.8 mg) was subjected to PTLC (Hex/EtOAc: 8:2) to give **6** (4.0 mg) (0.4 % yield).

P3 fraction 1 (26.2 mg) was subjected to preparative TLC (100% Hex) to yield **4** (2.4 mg) (0.2 % yield). Compounds **2** (1.2 mg) (0.1 % yield), **5** (6.4 mg) (0.6 % yield), and **6** (15.0 mg) (1.5 % yield) were isolated from fraction 2 (528.6 mg) by PTLC (100 % Tol and Hex/EtOAc: 9:1). Fraction 6 (88.0 mg) was subjected to PTLC ($CHCl_3$/MeOH: 96:4) and afforded **1** (4.5 mg) (0.5 % yield).

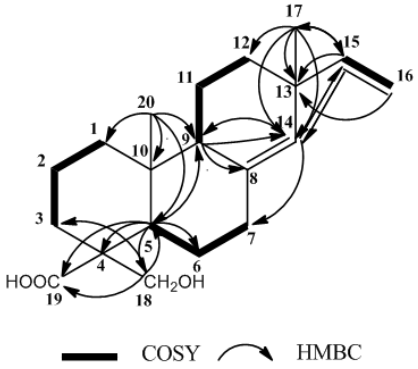

Figure 2. ^{1}H-^{1}H COSY correlations and the selected HMBC correlations of compound **1**.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as colourless oil $[\alpha]_D^{24}$ + 4.4 (*c* 0.36, $CHCl_3$). The molecular formula of **1** was determined to be $C_{20}H_{30}O_3$ with six degrees of unsaturation in the molecule by HR-ESI-MS (*m/z* 319.2284 $[M + H]^+$, calcd. 319.2268) and NMR data (Table 1). The IR spectra showed the absorptions for OH group (3390 cm^{-1}) and carbonyl group (1715 cm^{-1}). The ^{13}C NMR spectrum of **1** displayed twenty signals corresponding to two methyls, nine methylenes (one being oxygenated), four methines and five quaternary carbon atoms, including a carbonyl carbon at δ181.7 (C-19). The downfield chemical shift value of the methylene group at δ72.3 (C-18) indicated the attachment of the hydroxyl moiety. Structural assignments were carried out based on ^{1}H-^{1}H COSY and HMBC spectral data. ^{1}H-^{1}H COSY experiment revealed the sequences of the correlations depicted by the bold lines in Figure 2. In the HMBC experiment, the three-bond correlations of H_3-17 to (C-12), (C-13), (C-14) and (C-15) confirmed the placement of the methyl group at (C-13). The other methyl group at (C-10) was confirmed by HMBC correlations between H_3-20 to (C-1), (C-5), (C-9) and (C-10). Placement of the hydroxymethyl and carbonyl group at (C-4) was confirmed by the HMBC correlations of H-3 and H-5 to (C-18) as well as H-5 to (C-4), (C-6), (C-18) and (C-19). The remaining connection of the double bond (C-8) was confirmed by the HMBC correlations of H-14 to (C-7) and (C-9). The presence of one carbonyl and two double-bond groups can explain three degrees of unsaturation, and the remaining three indicated the presence of a tricyclic ring. Based on these findings, the planar structure of **1** was established as shown in Figure 2.

The relative stereochemistry of **1** was deduced from the NOESY experiment. The absence of NOE correlations between H-5/H_3-20 revealed the *trans* configuration of the decalin junction. NOE correlations observed between H-5/H_2-18 showed the placement of (C-18). Furthermore, the expected conformational rigidity of the *trans* decalin system, coupled with ^{1}H-NMR chemical shifts, and on same biosynthetic pathway, the α configuration of (C-18) as well as the β configuration of (C-19)

were proposed to be identical to the co-occurring compound **2** [11]. The relative stereochemistry of H-9 was deduced based on the cross peak observed between H-5/H-9 in the NOESY experiment. Therefore, compound **1** was determined to be pimara-8(14),15-dien-18-ol-19-oic acid, namely dicladoic acid (Figure 1).

A total of six known compounds have been isolated, including 4-*epi*-sandaracopimaric acid (**2**) [11], rosa-1(10),15-dien-18-oic acid (**3**) [12], herbertene (**4**) [13], α-herbertenol (**5**) [13], herbertene-1,2-diol (**6**) [13], and *ent*-7-hydroxyeudesm-4-en-6-one (**7**) [14]. Furthermore, an enantiomer of eudesmane, *ent*-chlorantene G (**8**) was also isolated. The optical rotation of **8** {$[\alpha]_D^{24}$ -173.3 (*c* 0.12, $CHCl_3$)} was opposite to that of the reported chlorantene G {$[\alpha]_D^{20}$ +135.0} [15]. The structures of these compounds were determined by comparison with reference spectral data. To the best of our knowledge as well as supported by results from SciFinder, no record of isolation of compound **3** from lower plant has been reported. Hence, this study represents the first report of the isolation of compound **3** from lower plant, *Mastigophora diclados*.

Table 1. ^{1}H and ^{13}C NMR data for compound **1***.

Position	1	
	^{13}C	^{1}H
1α	39.5 (CH_2)	1.06 (1H, *td*, *J* = 4.1, 13.8)
1β		1.82 (1H, *brd*, *J* = 13.8)
2α	19.6 (CH_2)	1.55-1.60 (1H, *m*)
2β		1.88 (1H, *dt*, *J* = 4.1, 13.8)
3α	33.0 (CH_2)	1.12 (1H, *td*, *J* = 4.1, 13.1)
3β		2.38 (1H, *brd*, *J* = 13.1)
4	50.7 (C)	
5	51.1 (CH)	1.38 (1H, *td*, *J* =4.1, 13.8)
6α	25.0 (CH_2)	1.74-1.79 (1H, *m*)
6β		1.65-1.69 (1H, *m*)
7α	36.9 (CH_2)	1.97 (1H, *td*, *J* = 4.8, 13.8)
7β		2.26 (1H, *dq*, *J* = 2.8, 4.1, 13.8)
8	136.9 (C)	
9	50.3 (CH)	1.72 (1H, *t*, *J* = 7.6)
10	39.6 (C)	
11α	19.6 (CH_2)	1.48-1.54 (1H, *m*)
11β		1.55-1.60 (1H, *m*)
12α	35.1 (CH_2)	1.32-1.38 (1H, *m*)
12β		1.42-1.47 (1H, *m*)
13	38.0 (C)	
14	129.6 (CH)	5.23 (1H, *brs*)
15	149.4 (CH)	5.76 (1H, *dd*, *J* = 10.3, 17.2)
16α	111.0 (CH_2)	4.90 (1H, *dd*, *J* = 1.4, 17.2)
16β		4.88 (1H, *dd*, *J* = 1.4, 10.3)
17	26.8 (CH_3)	1.02 (3H, *s*)
18α	72.3 (CH_2)	3.42 (1H, *d*, *J* = 10.3)
18β		4.05 (1H, *d*, *J* = 10.3)
19	181.7 (C)	
20	15.1 (CH_3)	0.75 (3H, *s*)

* 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR in $CDCl_3$ δ in ppm, *J* in Hz

3.2. Chemosystematic of Bornean Mastigophora diclados

Phytochemical contents of *M. diclados* collected from three different localities (Mount Kinabalu, Mount Alab and Mount Trus Madi) in Borneo Island were investigated. The occurrence of different chemotypes was detected depending on variation of localities (Table 2). Pimarane-type diterpenes (**1**-**3**) were detected in specimens from both Mount Kinabalu and Mount Trus Madi. The eudesmane-type sesquiterpenes (**7**-**8**) were detected only from Mount Alab's specimen. In addition, herbertane-type sesquiterpenes (**4**-**6**) were found as major metabolites in specimens collected from all three localities. These herbertane-type metabolites (**4**-**6**) were known to be the chemotaxonomical markers for *M. diclados*. These results suggested that the evolutionary relationship between Mount Kinabalu and Mount Trus Madi is more related compared to Mount Alab. Previous study reported the detection of Mastigophorenes [4] in specimen from Borneo Island but absent in our present study. This could be explained by the variability of *M. diclados*. Morphological study of *M. diclados* showed three different types of leave appearances which indicated the existence of three *M. diclados* varieties [16]. Microscopic investigation of specimens from Mount Kinabalu and Mount Trus Madi showed similar morphological traits whereas specimen from Mount Alab showed different leave appearance (data not shown). Our results are in consistent with previous study [16] suggesting there are three chemotypes from Bornean *M. diclados*. In conclusion, combination of both morphological traits studies and chemosystematics studies could increase the accuracy of species identification and help to resolve taxonomic problems.

Table 2. Chemotype variations of *Mastigophora diclados* from Borneo Island

Specimen Location	**Compounds**		
	Herbertane	**Pimarane**	**Eudesmane**
Mount Kinabalu	+	+	-
Mount Alab	+	-	+
Mount Trus Madi	+	+	-

+; -: Presence (+) and absence (-) in the specimen.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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