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NEW PRENYLATED BIBENZYLS FROM BORNEAN LIVERWORT *ACROBOLBUS SACCATUS*

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Abstract – Liverworts are the most primitive terrestrial plant which known to produce unique and wide variety of compounds. Ethyl acetate crude extract was subjected to several chromatographic techniques for purification. Three new compounds, saccatenes A-C (**1**-**3**) were isolated together with two known prenyl bibenzyl derivative, 2,2-dimethyl-5-hydroxy-6-carboxy-7-(2-phenylethyl) chromene (**4**) and radulanin A-5-one (**5**) from the Bornean liverwort *Acrobolbus saccatus* (Hook.) Trevis collected from Mountain Trus Madi, Sabah, Malaysia. The structures of the three new metabolites were established by analyses of the spectroscopic data (1D NMR, 2D NMR, HRESIMS and IR), and the antibacterial activity against eight selected human pathogenic species of bacteria were tested.

Liverworts are primitive terrestrial plant known to produce unique and wide variety of structurally interesting compounds.[1,2] But, the morphological classification of liverwort is not an easy task due to its high varieties and small morphological gametophyte. Previously, the liverwort family Acrobolbaceae was reported to consists of three genera: *Acrobolbus*, *Marsupidium* and *Tylimanthus*. However, recent molecular and morphological studies have suggested that those three genera are to be grouped into a single genus *Acrobolbus*.[3-5] Chemosystematics has been proven to be a valuable tool to resolve taxonomic classification uncertainties especially in liverwort.[6] Bornean liverworts have showed diverse structural

attribute with the presence of interesting novel metabolites.[7-9] Our continuous interest in the chemical composition of Bornean liverwort *Acrobolbus saccatus* resulted in the isolation of three new compounds, saccatenes A-C (**1-3**) along with two known prenyl bibenzyl derivatives, 2,2-dimethyl-5-hydroxy-6-carboxy-7-(2-phenylethyl) chromene (**4**) and radulanin A-5-one (**5**),[10,11] as shown in Chart 1. Herein, the isolation, structure elucidation, chemosystematics and antibacterial activities of these compounds are discussed.

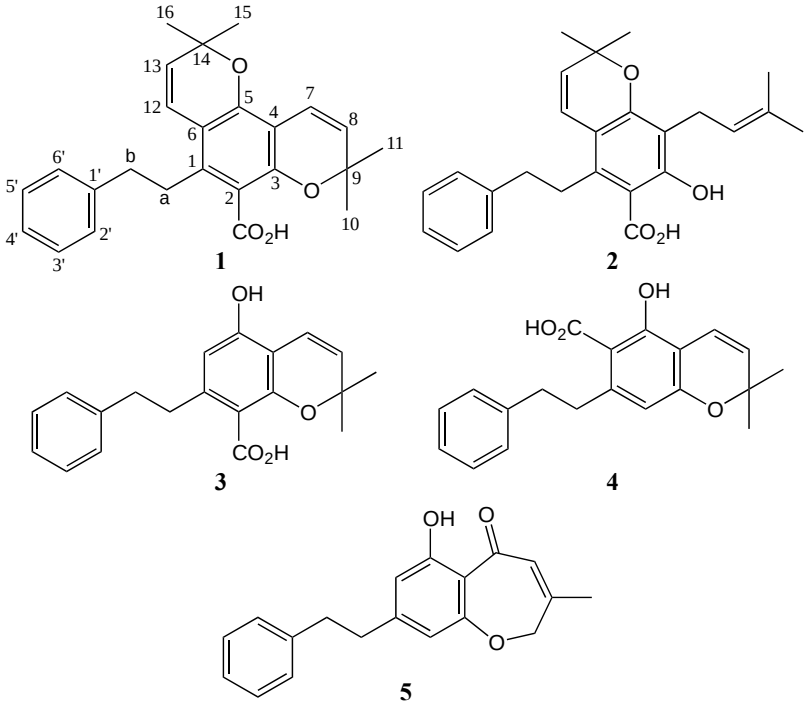

Chart 1. Chemical structures of **1-5**

Saccatene A (**1**) was isolated as colorless oil. The molecular formula was established as $C_{25}H_{26}O_4$ by HRESIMS $[M + H]^+$ ion at *m/z* 391.1894 (calculated for $C_{25}H_{27}O_4$, 391.1904). The IR absorption at 3528 and 1690 cm^{-1} indicated the presence of hydroxyl and carbonyl groups in the molecule. Compound **1** correspond to 25 carbon atoms based on HRESIMS data, however its ^{13}C-NMR spectrum (Table 1) showed 21 signals with various intensities due to overlapped carbon signals at δ_C 129.1 and 129.2, as well as the carbon signals resonance at near similar chemical shift δ_C 129.0 to 129.2 has increased the difficulty for the structure elucidation during proton-carbon one bond assignment through HSQC correlations and substructures connection by HMBC correlations. The ^{1}H-NMR spectrum (Table 2) contained less proton signals (δ_H 7.32, 7.29, 7.19, 6.69, 6.57, 5.65, 3.26, 2.85 and 1.53) than expected which limited the information provided by HMBC experiment.

Table 1. ^{13}C-NMR data of **1**-**3** (recorded at 150 MHz in $CDCl_3$, δ in ppm)

No.	1	2	3
1	142.0	139.9	148.5
2	111.6	113.9	104.1
3	153.0	163.3	161.3
4	109.2	116.0	108.3
5	152.3	157.6	159.2
6	115.4	113.9	112.5
7	117.1	22.7	116.9
8	129.0	123.1	128.3
9	80.0	131.9	78.3
10	28.4	18.6	29.0
11	28.4	26.5	29.0
12	119.3	119.7	
13	130.2	129.6	
14	77.4	76.8	
15	28.7	28.7	
16	28.7	28.7	
α	32.8	32.7	39.6
β	37.7	37.9	38.7
1’	142.7	142.8	142.6
2’	129.1	129.1	129.1
3’	129.2	129.0	129.0
4’	126.6	126.0	126.6
5’	129.2	129.0	129.0
6’	129.1	129.1	129.1
CO_2H	167.0	176.7	176.0

Table 2. ^{1}H-NMR data of **1**-**3** (recorded at 600 MHz, δ in ppm, J in Hz)

No.	1	2	3
2			
6			6.24 (s)
7	6.69 (d, J = 9.6)	2.05 (s) 3.34 (d, J = 7.6)	6.70 (d, J = 9.6)
8	5.67 (d, J = 9.6)	5.23 (t, J = 7.6)	5.54 (d, J = 9.6)
10	1.53 (s)	1.80 (s)	1.44 (s)
11	1.53 (s)	1.67 (s)	1.44 (s)
12	6.57 (d, J = 10.3)	6.56 (d, J = 10.3)	

13	5.65 (d, *J* = 10.3)	5.62 (d, *J* = 10.3)	
15	1.44 (s)	1.42 (s)	
16	1.44 (s)	1.42 (s)	
α	3.26 (m)	3.27 (m)	3.19 (m)
β	2.85 (m)	2.81 (m)	2.88 (m)
2’	7.29 (d, *J* = 8.3)	7.24 (d, *J* = 7.6)	7.20 (d, *J* = 8.3)
3’	7.32 (t, *J* = 6.9)	7.21 (t, *J* = 7.6)	7.28 (t, *J* = 7.6)
4’	7.19 (t, *J* = 6.9)	7.16 (t, *J* = 7.6)	7.19 (t, *J* = 8.9)
5’	7.32 (t, *J* = 6.9)	7.21 (t, *J* = 7.6)	7.28 (t, *J* = 7.6)
6’	7.29 (d, *J* = 8.3)	7.24 (d, *J* = 7.6)	7.20 (d, *J* = 8.3)

The overlapped signals at δ_H 7.32 and 7.29; δ_C 129.1 and 129.2 highly indicated a phenyl moiety when the integration of latter protons with signal at δ_H 7.19 was 2:1 ratio. Therefore, a phenylethyl unit was confirmed upon comparison of chemical shifts with its analogue **5**. This part was further confirmed by the NMR data such as ^{1}H-^{1}H COSY, HMBC and proton-proton vicinal coupling constants between the aromatic methines (H-4’, H-5’ and H-6’). Subsequently, the substructure of phenylethyl unit has extended to another aromatic ring based on HMBC cross peak of H-α to three sp^2 quaternary carbons C-1, C-2 and C-6 as shown in Figure 1. The three-bond HMBC correlations of H_3-15 and H_3-16 to respective carbons C-15 and C-16, as well as their correlations to both C-13 and C-14, allowed the placement of *gem*-dimethyl group at C-14 and establishment of another six-membered heteroatom ring through ethereal linkage. In this context, a structural fragment at H-12/H-13 determined by ^{1}H-^{1}H COSY and HMBC correlations of H-12 to C-1 and C-5; and H-13 to C-6 along with the previous HMBC correlations from *gem*-dimethyl unit at C-14 have constructed a sequence of carbon chain C-5-C-6-C-12-C-13-C-14 that formed a six-membered heteroatom monocyclic through ethereal linkage between C-5 and C-14. These findings, in addition to vicinal proton coupling constant between H-12 and H-13 ($^3J_{12\text{-}13}$ = 10.3 Hz), as well as NOE correlation between H-12 and H-13, deduced a *cis* configuration double bond at C-12/C-13 and six-membered ring was connected by an ethereal linkage across C-5 and C-14 based on comparison of chemical shifts with those of reported analogues.[10,12] The *gem*-dimethylpyran unit was confirmed fused at C-5/C-6 due to a NOE cross peak between H-β to H-12.

Another *gem*-dimethylpyran unit was also determined by the similar way as discussed above, and fused at C-3/C-4 with ethereal linkage between C-3 and C-9, the fused position of this unit was consistent with those of similar analogues and biogenetic pathway further supported this fused position.[10,12-14] From the required of 13 degrees of unsaturation, the nine degrees of unsaturation were attributed by eight pairs of olefins and one carbonyl group, hence the remaining four degrees of unsaturation must be four cyclic ring.

The existence of these two dimethylpyran units permitted the establishment of another benzyl ring (C-1 to C-6) attached to phenylethyl moiety. These connections leaving a carboxylic acid group (δ_C 167.0) attached to an unoccupied sp^2 quaternary carbon at C-2. In fact, this bibenzyls with two prenyl groups was considered first time isolated from liverwort. This compound was believed biosynthesized from cyclization of **2** and unnamed prenyl bibenzyl derivative (**3**).[10] Thus, the relative structure of **1** was determined unambiguously as shown in Chart 1.

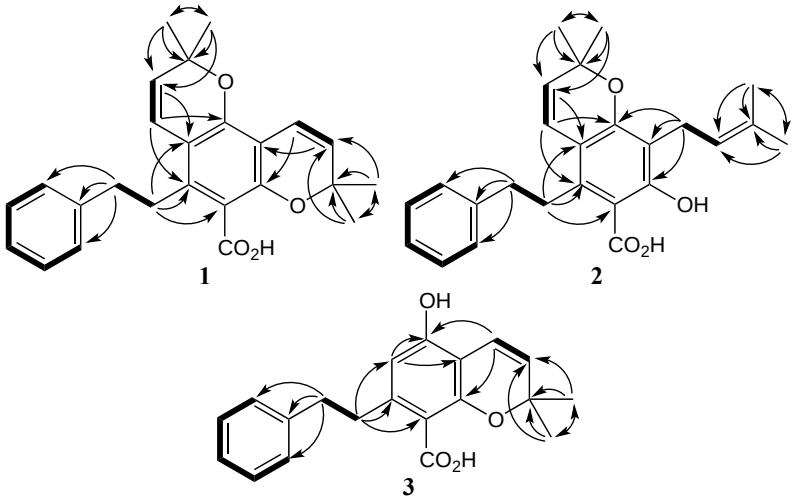

Figure 1. ^{1}H-^{1}H COSY and key HMBC correlations of **1**-**3**

Saccatene B (**2**) was isolated as colorless oil. The molecular formula was determined as $C_{25}H_{28}O_4$ on the basis of HRESIMS $[M + H]^+$ at *m/z* 393.2041 (calculated for $C_{25}H_{29}O_4$, 393.2060). The IR spectrum showed absorptions at 3429 (hydroxyl) and 1670 cm^{-1} (carboxyl). Upon comparison of NMR data (Tables 1 and 2) of **2** with those of similar unnamed analogue (**3**) revealed their structure was resembled except the replacement of hydroxyl group at C-4' and methyl ester at C-2 in unnamed prenyl bibenzyl derivative (**3**) by methine at C-4' and carboxyl group at C-2 in **2**.[10] This finding was consistent with the HRESIMS data.

Saccatene C (**3**) was isolated as colorless oil. The molecular formula of $C_{20}H_{20}O_4$ was deduced from HRESIMS $[M + H]^+$ ion at *m/z* 325.1413 (calculated for $C_{20}H_{21}O_4$, 325.1408). The IR spectrum showed absorptions for hydroxyl and carbonyl groups at 3427 and 1658 cm^{-1}. Comparison of NMR data revealed structure of **3** was similar to 2,2-dimethyl-5-hydroxy-6-carboxy-7-(2-phenylethyl) chromene (**4**) and aglaiabbrevin B.[11,12] The structures of **3** and **4** was very similar except $-CO_2H$ group at different position.[11] The NMR data of **3** and **4** are different, in addition, structure **4** was fixed by methylation,[11] therefore the ether linkage in **3** must be between C-3 and C-9. Otherwise, the ether linkage between C-5 and C-9 would make **3** identical to those of **4** which was not plausible, unless they possessed similar

NMR data or atropisomer, although them clearly have different NMR data and free rotation of phenylethyl function exclude this possibility. While, compound **3** differed from aglaiabbrevin B was additional of carboxyl unit at C-2 and replacement of hydroxyl moiety by methine proton.[12] This is confirmed by the HRESIMS spectrum.

The genus Radula is rich source of bibenzyls and prenyl bibenzyl types of chemical structures.[2] The chemical constituents of *Marsupidium epiphytum* are quite similar to those found in *Radula* spp., except the absence of bibenzyls with two prenyl groups from the latter species.[10] The Bornean *A. saccatus* elaborated highly characteristic dihydrooxepin-type compound **5**, and prenylated bibenzyls derivatives **1**-**4**. These types of compound were exactly similar to those isolated from *M. epiphytum*. The results of our chemical analysis were in good agreement with previous molecular and morphological studies.[3-5] Hence, the genera of *Acrobolbus*, *Marsupidium* and *Tylimanthus* should be grouped into a single genus from chemotaxonomical point of view. To the best of our knowledge, this is a first isolation of bibenzyl with two fused of pyran units from liverwort as shown in compound **1**. To date, the bibenzyls from liverworts were either prenylated or formed a pyran unit but never associated with two pyran units.[3,12] Antibacterial properties of **1**-**5** were evaluated against *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *S. typhimurium*, *Staphylococcus aureus* and *Yersinia enterocolitica*. However, only **1**, **2** and **5** showed weak antibacterial activity against *L. monocytogenes*, *S. enteritidis* and *Y. enterocolitica* at an MIC value of 25 µg/mL, and showed negligible activities with MIC > 250 µg/mL with the rest of the bacterial strains. Other compounds did not show any activity against these bacteria.

EXPERIMENTAL

Optical rotations were measured on an AUTOPOL IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, USA). IR spectra were obtained on a Thermo Nicolet Avatar FTIR spectrophotometer (Thermo, Tokyo, Japan). The NMR spectral data were recorded on JEOL ECA 600 instrument (JEOL, Tokyo, Japan) with TMS as an internal standard. The HRESIMS data were obtained on a LCMS-IT-TOF (Shimadzu, Kyoto, Japan). Preparative TLC was performed with silica gel plate (Merck, Frankfurt, Germany; Kieselgel 60 F_{254}). Column chromatography was performed on silica gel (70–230 mesh; Merck, Frankfurt, Germany).

Plant Materials. Specimens of *A. saccatus* (Hook.) Trevis was collected from Mount Trus Madi (05º33’33.9’’N, 116º29’56.8’’E), Sabah, Malaysia on August 2015. The voucher specimen (BORHB0021) is deposited in the BORNEENSIS Herbarium of Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

Extraction and Isolation. Air dried specimens (48 g) were extracted with methanol (MeOH) at room

temperature (1.0 L x 3 each for one week). The crude extract was suspended in water (H_2O) and partitioned with ethyl acetate (EtOAc). After the removal of the organic solvent, the EtOAc fraction (1.2 g) was chromatographed on Si gel column using hexane (Hex) and EtOAc system as eluent with increasing polarity (Hex/EtOAc: 9:1, 8:2, 7:3, 6:4, 1:1 100% EtOAc) to yield six fractions 1-6. Fraction 2 (60 mg) was subjected to preparative TLC with Hex/EtOAc (7:3) to yield **5** (6.7 mg). Fraction 5 (60 mg) was subjected to preparative TLC with Hex/EtOAc (2:1) to give **1** (7.6 mg), **2** (3.1 mg), and **4** (1.1 mg). Fraction 6 (60 mg) was subjected to preparative TLC with Hex/EtOAc (1:1) to yield **3** (11.5 mg).

Saccatene A (1): colorless oil; IR ν_{max} (cm^{-1}): 3528, 1690, 1582, 1517 and 1180; ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2; HRESIMS *m/z* 391.1894 $[M + H]^+$ (calculated for $C_{25}H_{27}O_4$, 391.1904).

Saccatene B (2): colorless oil; IR ν_{max} (cm^{-1}): 3429, 1670, 1591, 1514 and 1130; ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2; HRESIMS *m/z* 393.2041 $[M + H]^+$ (calculated for $C_{25}H_{29}O_4$, 393.2060).

Saccatene C (3): colorless oil; IR ν_{max} (cm^{-1}): 3427, 1658, 1590, 1510 and 1120; ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2; HRESIMS *m/z* 325.1413 $[M + H]^+$ (calculated for $C_{20}H_{21}O_4$, 325.1408).

Antibacterial Bioassay. Isolated pure compounds were subjected to antibacterial bioassay using eight aforementioned strains of clinical bacteria obtained from Queen Elizabeth Hospital (Kota Kinabalu, Sabah, Malaysia). Bacterial assay was conducted according to known procedure.[15]

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