Novel Prenyl Bibenzyls from the New Zealand Liverwort *Marsupidium epiphytum*

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The ether extract of the New Zealand liverwort *Marsupidium epiphytum* gave four new prenyl bibenzyl derivatives, along with a known prenyl bibenzyl derivative which has been isolated from the Ecuadorian liverwort *Lethocolea glossophylla*; their structures were determined by 2D-NMR spectrum. The chemical constituents of *Marsupidium epiphytum* are highly characteristic since they elaborate dihydrooxepin type compounds and prenyl type bibenzyls. These structures are closely related to those found in *Radula* spp. (Radulaceae), although bibenzyls with two prenyl groups have not been isolated from the *Radula* spp. Although *Marsupidium* spp. are different from *Radula* spp. morphologically, the constituents are closely related. This is the first example of isolation of prenyl bibenzyl derivatives from *M. epiphytum*, a species which has not previously been investigated phytochemically.

Key words liverwort; *Marsupidium epiphytum*; prenyl bibenzyl; chemosystematic

In the course of investigating bryophyte chemistry, we have isolated many unique compounds from liverworts.[1)] Liverworts are the most primitive terrestrial plants and occasionally produce unique terpenoids and aromatic compounds. Although the same compounds isolated from higher plants have also been isolated from liverworts, many of these, especially terpenoids, are found as enantiomeric isomers in liverworts. Thus, the chemical composition of bryophytes in natural product chemistry is quite interesting.

In general, the morphologic classification of liverworts is quite difficult, since the morphological characteristics of the gametophytes are small and unclear. Some species of liverworts have been reclassified. For instance, the species *Marsupidium epiphytum* also has the botanical designation of *Tylimanthus epiphytus*. Thus, there are two names for the same species. Most liverworts possess oil bodies where secondary metabolites accumulate in cells. Thus, it is important for chemosystematics to analyze liverworts phytochemically since oil body constituents are defining characteristics of the species of liverworts.

Seven species of *Marsupidium* are recognized in New Zealand.[2)] *Marsupidium epiphytum* Colenso belongs to the leafy liverwort family Acrobolbaceae. Another species of this family, *Lethocolea glossophylla*, has been investigated and new prenylated bibenzyl derivatives have been isolated.[3,4)] These structures are closely related to those found in the *Radula* spp. (Radulaceae), although bibenzyls with two prenyl groups have not been isolated from the *Radula* spp. Herein, we report the isolation and structural elucidation of four new prenyl bibenzyl derivatives from *M. epiphytum*, a species which has not yet been investigated phytochemically.

Results and Discussion

The ether extract of *Marsupidium epiphytum* was chromatographed on silica gel using hexane–EtOAc gradient, giving a mixture of compounds **1**—**4**, together with known compounds 1(10)-aristolene and β-barbatene. The mixture was rechromatographed on Sephadex LH-20 and purified by prep-HPLC on silica gel column using hexane to give four new compounds **1**—**4**.

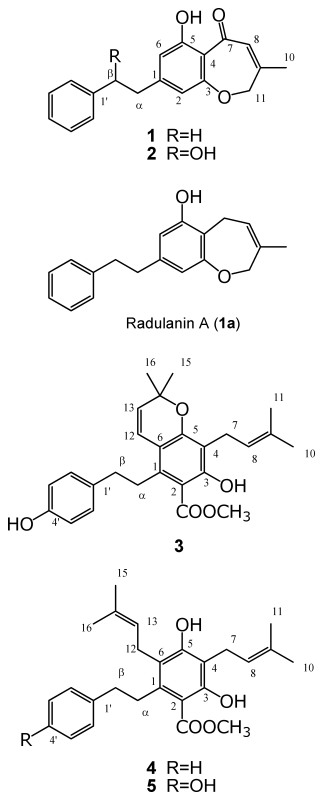

Fig. 1. Prenyl Bibenzyls Were Isolated from the New Zealand Liverwort *Marsupidium epiphytum*

The electron ionization (EI) mass spectrum of **1** gave a molecular ion peak at *m*/*z* 294 as the base peak, and high-resolution analysis showed a molecular formula $C_{19}H_{18}O_3$, confirming 11 degrees of unsaturation. Fourier transform (FT)-IR showed a hydroxyl group at 3588 and α,β-unsaturated carbonyl group at 1626 cm^{-1}. UV spectrum of **1** exhibited absorption bands for an aromatic ring at 296 and 258 nm. The ^{13}C-NMR spectrum of **1** (Table 1) contained 19 carbons, including 14 olefinic, a carbonyl, a methyl and three methylene carbons, one of which was oxygenated. The ^{1}H-NMR

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Table 1. NMR Data for Compounds **1**—**4** in $CDCl_3$

No.	**1**		**2**		**3**		**4**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1		152.0		148.3		137.8		141.1
2	6.37 (d 1.5)	110.7	6.41 (d 1.5)	111.6		105.4		105.5
3		160.3		160.3		161.7		160.1
4		112.3		112.7		115.4		112.3
5		164.9		164.9		156.3		158.5
6	6.57 (d 1.5)	112.9	6.61 (d 1.5)	113.9		113.0		119.4
7		193.1		193.2	3.34 (d 7.0)	22.1	3.45 (d 7.1)	22.5
8	6.29 (bs)	131.9	6.29 (bs)	131.9	5.23 (m)	122.3	5.26 (tq 1.4, 7.1)	121.6
9		154.4		154.6		131.2		135.0
10	2.08 (d 0.7)	22.9	2.08 (d 1.5)	22.9	1.68 (d 1.1)	25.8	1.76 (d 1.4)	25.9
11	4.56 (s)	72.4	4.55, 4.58 (each d, 16)	72.4	1.80 (d 0.5)	17.9	1.83 (s)	18.1
12					6.56 (d 10.2)	119.0	3.39 (d 6.3)	25.1
13					5.63 (d 10.2)	129.0	5.07 (tq 1.4, 6.3)	122.9
14						76.0		133.2
15					1.42 (s)	27.9	1.71 (d 1.4)	25.7
16					1.42 (s)	27.9	1.78 (d 0.8)	17.9
3-OH					11.66 (s)		11.64 (s)	
4′-OH					4.80 (s)			
5-OH	13.40 (s)		13.39 (s)				6.04 (s)	
α	2.85 (m)	38.0	2.94 (d 7.0)	46.0	3.15 (m)	32.6	3.17 (m)	33.4
β	2.91 (m)	36.5	4.94 (t 7.0)	74.6	2.73 (m)	36.4	2.81 (m)	37.4
1′		141.0		143.4		134.3		142.3
2′, 6′	7.18 (d 7.7)	128.3	7.37 (m)	125.8	7.09 (d 8.5)	129.2	7.23 (d 7.6)	128.1
3′, 5′	7.29 (t 7.7)	128.4	7.36 (m)	128.6	6.79 (d 8.5)	115.3	7.32 (d 7.6)	128.5
4′	7.21 (t 7.7)	126.1	7.30 (m)	127.9		153.8	7.32 (m)	126.0
$COOCH_3$						172.3		172.4
					3.95 (s)	52.1	3.96 (s)	52.1

Measured at 600 MHz for ^1H and 150 MHz for ^{13}C.

spectrum of **1** showed signals of aromatic ring protons at δ_H 7.18 (2H), 7.29 (2H) and 7.21 (1H), and benzyl methylene protons at δ 2.85 (2H) and 2.91 (2H). The heteronuclear multiple bond correlation (HMBC) spectrum of **1** (Fig. 2) showed a correlation between the benzyl methylene proton at 2.91 (H_2-β) and aromatic carbons at 128.3 (C-2′, 6′) and δ 141.0 (C-1′), demonstrating the presence of two benzyl groups in **1**. A fragment peak at *m/z* 91 (91% intensity tropylium ion $[C_7H_7]^+$) in the EI-MS of **1** provided further evidence for this partial structure. The ^1H-NMR of **1** further showed a downfield shifted hydroxy proton at δ 13.40, correlating with three aromatic carbons at δ 164.9 (C-5), 112.9 (C-6) and 112.3 (C-4) in the HMBC spectrum of **1**. ^1H–^1H spin coupling of the H-6 proton signal at δ 6.57 (J=1.5 Hz) between δ 6.37 (J=1.5 Hz, H-2), and $^3J_{CH}$ correlations between these two protons and C-α carbon at δ 38.0 were observed in the ^1H–^1H correlation spectroscopy (COSY) and HMBC spectra, respectively. The exchangeable hydroxy proton at δ 13.40 disappeared upon addition of D_2O, whose chemical shift exhibited hydrogen-bond formation between the α,β-unsaturated carbonyl in **1**. The ^1H- and ^{13}C-NMR spectra of **1** were similar to those of radulanin A (**1a**) that has been isolated from the epiphytic liverwort *Radula* species,[5,6] except for the absence of H_2-7 proton signal and the appearance of a downfield shifted vinyl methyl group at δ 2.08 of **1**. Correlations in the HMBC spectrum between the vinyl methyl group at δ 2.08 (d, J=0.7 Hz) and oxygenated methylene at δ 72.4 (C-11), 154.4 (C-9), and 131.9 (C-8) were observed; subsequently, a remaining partial structure of 3-methyl-oxepin-5(*2H*)-one of **1** was established. Accordingly, the structure of **1** was established as shown in Fig. 1.

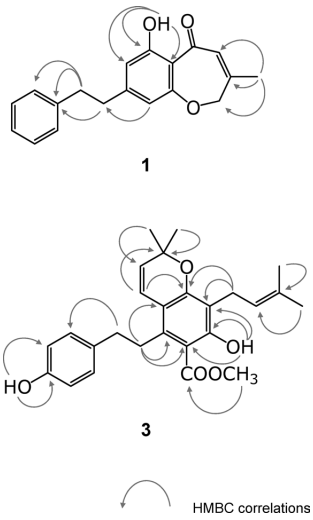

Fig. 2. Important HMBC Correlations for Compounds **1** and **3**

The FT-IR of **2** showed absorption bands at 3600 and 1627 cm^{-1}. UV spectrum exhibited the presence of an aromatic ring at 299 and 256 nm. The EI mass spectrum of **2** showed a molecular ion peak at *m/z* 310, and indicated a difference of 16 in molecular weight compared with that of **1**. The ^1H- and ^{13}C-NMR data of **2** was similar to that of **1**, except for the appearance of an oxygenated proton signal at δ 4.94 (t, J=7 Hz), and δ 74.6. Analysis of the heteronuclear multiple quantum coherence (HMQC) and HMBC spectra supports the structural assignment. In particular, the long range ^1H–^{13}C correlation of H_2-β with C-1′, C-2′, and C-6′

supports the positioning of the hydroxyl group at C-β. Thus, the structure of **2** was established as shown in Fig. 1 when compared with the spectral data of **1**, although its absolute configuration remains to be clarified.

Compound **3** was obtained as pale yellow oil. The FT-IR spectrum of **3** showed an absorption band for hydroxyl and carbonyl groups at 3598 and 1652 cm^{-1}. The UV spectrum exhibited absorption for the aromatic ring at 259 and 325 nm. The EI mass spectrum had a molecular ion peak at *m/z* 422. The ^{13}C-NMR spectrum of **3** (Table 1) contained 26 carbons including 16 olefinic, 1 carbonyl, 5 methyls and 1 quaternary sp^3 carbon, which was oxygenated. These carbon signals and the high-resolution mass data (*m/z* 422.2090) established the molecular formula as $C_{26}H_{30}O_5$, confirming 12 degrees of unsaturation. The ^{1}H-NMR spectrum of **3** showed an A_2B_2 doublet of aromatic protons at δ 6.79 and 7.09 (each 2H, d, J=8.5 Hz), which was evidence for the presence of a 1,4-disubstituted aromatic ring. The EI mass spectrum of **3** showed the expected fragment ion peak at *m/z* 107 (hydroxytropylium ion), providing further evidence for the above partial structure. Moreover, the ^{1}H-NMR spectrum showed an equivalent methyl signal at δ 1.42 (6H, s), two vinyl methyls at δ 1.68 and 1.80 and a methoxy group at δ 3.95. Analysis of the HMQC and HMBC spectra (summarized in Fig. 2) supports the structural assignment. In particular, the long range ^{1}H–^{13}C correlation of the equivalent methyl protons (H_3-15, 16) with a quaternary carbon at δ 76.0 (C-14) and sp^2 carbon at δ 129.0 (C-13) supports the positioning of the geminal dimethyl group at C-14. The ether linkage between C-14 and C-5 was apparent from the degree of unsaturation of **3**. Further correlations between two vinyl methyl groups (C-10, 11) and two sp^2 carbons at δ 131.2 (C-9) and 122.3 (C-8) were observed in the HMBC spectrum of **3**, while ^{1}H–^{1}H correlation between olefinic H-8 at δ 5.23 (m) proton and a methylene proton at δ 3.34 (2H, d, J=7 Hz) was observed in the COSY spectrum of **3**. The above data clearly demonstrates the presence of a prenyl group in the structure of **3**. The methoxy group proton was long range coupled to the carbonyl carbon at δ 172.3, indicating the presence of a carbomethoxyl group whose carbonyl formed a hydrogen-bond with the phenolic hydroxy proton, the most deshielded resonance at δ 11.66 (s) in the ^{1}H-NMR spectrum of **3**. The hydrogen-bonded proton was further correlated to three quaternary sp^2 carbons at δ 105.4 (C-2), δ 161.7 (C-3) and δ 115.4 (C-4) in the HMBC spectrum of **3**. The most downfield shifted C-3 was attached by the phenolic hydroxyl group. The methylene proton H-7 at δ_H 3.34 (2H, d, J=7 Hz) correlated to C-5 at δ 156.3, which further correlated with olefinic H-12. Furthermore, the correlation between δ_C 115.4 (C-4) and H-7 indicated the presence of the prenyl group at C4; accordingly, there is only one possible position, C-2, for the attachment of the carbomethoxyl group since the correlations between H_2-α at δ 3.15 (m) and C-1, 2 and C-6 were observed. Acquisition of a nuclear Overhauser enhancement spectroscopy (NOESY) spectrum confirmed the substitution pattern. Namely, correlation between the olefinic H-12 and H_2-α at δ 3.15 (m) and H_2-β at δ 2.73 (m) exhibited evidence for the position of the benzyl methylene at C-1. Accordingly, the structure of **3** was established as shown in Fig. 1.

The spectral data of **4** closely resembled that of compound **5**, which has been isolated from the Ecuadorian liverwort *Lethocolea glossophylla*.[3,4] The structure of **4** was deduced by comparing its spectral data with that of **5**. The mass spectrum of **4** showed a molecular ion peak at *m/z* 408, indicating the molecular weight was less than 16 mass units from **5**. The fragmentation of **4** displayed characteristics for a benzyl moiety at *m/z* 91 (base peak) as a stable tropylium ion, while a hydroxytropylium ion at *m/z* 107 was observed in the EI-mass spectrum of **5**. The ^{1}H-NMR spectral pattern of **4** was similar to that of **5**, except for the absence of the A_2B_2 signal for 1,4-disubstituted aromatic ring of **5** and for the appearance of five aromatic proton signals at δ 7.23 (2H) and 7.32 (3H). Consideration of these spectral data led to the structural determination of **4** as shown in Fig. 1.

The chemical constituents of *M. epiphytum* are highly characteristic since they elaborate dihydrooxepin type compounds **1**, **2** and prenyl bibenzyls **3**—**5**. These structures are closely related to those found in *Radula* species (Radulaceae), although bibenzyls with two prenyl groups have not been isolated from the latter species.

Experimental

General TLC was carried out on silica gel precoated glass plates with hexane–EtOAc (1 : 1 and 4 : 1). Detection was with Godin's reagent.[7] For normal phase column chromatography (CC), silica gel 60 (40—63 μm) was used. A mixture of CH_2Cl_2–MeOH (1 : 1) was used for CC on Sephadex LH-20 as solvent. NMR spectra were recorded at 150 MHz for ^{13}C and 600 MHz for ^{1}H in $CDCl_3$. EI-MS were measured at 70 eV. Temperature programming of GC-mass analysis was performed from 50 °C isothermal for 3 min, then 50—250 °C at 5 °C min^{-1}, and finally isothermal at 250 °C for 15 min. Injection temp was 250 °C. A fused silica column coated with DB-17 (30 m×0.25 mm i.d., film thickness 0.25 μm) was used. Semi-preparative HPLC was carried out with a JASCO LC-8A pump equipped with a Knauer RI detector using silica gel prepacked column (10×250 mm, 5 μm; Nacalai Tesque, Kyoto, Japan). Optical rotation was taken on a JASCO DIP-1000 polarimeter. IR spectral data were obtained on a Shimadzu FTIR-8400S spectrometer. The UV spectrum was acquired on a Shimadzu UV-1650PC spectrometer.

Plant Material *Marsupidium epiphytum* Colenso (Herbarium specimen Nz 132; dry weight 5.4 g) was collected in December 2000 at Mount Ruapehu on North Island, New Zealand. A voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and Isolation *M. epiphytum* (Nz 132) was dried for 1 week, impurities removed, and then ground mechanically and extracted with Et_2O for 2 weeks. The ether extract (0.36 g; 6.7% yield of the dry weight) was chromatographed on silica gel using hexane–EtOAc gradient, giving 5 fractions (I—V). 1(10)-Aristolene and β-barbatene were detected by GC-MS analysis of fr. I. Fr. III (30.3 mg) and fr. V (40.5 mg) were rechromatographed on Sephadex LH-20 to afford a mixture of prenyl bibenzyls **1** from fr. III and **2** from fr. V. Then, the mixture of **1** was purified by preparative HPLC on silica gel using hexane–EtOAc (9 : 1 v/v) to afford **1** (2.7 mg; 0.8% yield of the extract). Further purification of the mixture of **2** by preparative HPLC on silica gel using hexane–EtOAc (8 : 2 v/v) gave **2** (2.4 mg; 0.7%) from fr. V. Fr. II (25.1 mg) and IV (20.2 mg) were rechromatographed on Sephadex LH-20 and gave a mixture of **4** from fr. II, and a mixture of **3** and **5** from fr. IV, respectively. The mixtures were further purified by preparative HPLC on silica gel using hexane–EtOAc (8 : 2 v/v) to afford **3** (8.7 mg; 2.4%), **4** (3.4 mg; 0.94%) and **5** (1.8 mg; 0.5%), respectively. The spectral data of **5** was identical to that obtained from *Lethocolea glossophylla*.[3,4]

Compound **1**: Colorless oil; FT-IR ($CHCl_3$) cm^{-1}: 3588, 1626 (C=O), 1575, 1206. UV (EtOH) λ_{nm} (log ε): 296 (3.96), 258 (3.83), 201 (4.54). EI-MS *m/z* (rel. int.): 294 [M^+] (100), 280 (11), 265 (66), 91 (91). High resolution (HR)-EI-MS *m/z* 294.1258 [$M]^+$, (Calcd for $C_{19}H_{18}O_3$, 294.1256).

Compound **2**: Colorless oil; $[\alpha]_D^{22}$ 2644 (c=0.24, $CHCl_3$). FT-IR ($CHCl_3$) cm^{-1}: 3600, 1627 (C=O), 1576, 1206. UV (EtOH) λ_{nm} (log ε): 299 (3.91), 256 (3.79), 201 (4.47). EI-MS *m/z* (rel. int.): 310 [M^+] (17), 204 (100), 107 (19), 79 (19), 77 (14). HR-EI-MS *m/z* 310.1207 [$M]^+$, (Calcd for $C_{19}H_{18}O_4$, 310.1205).

Compound **3**: Pale yellow oil; FT-IR ($CHCl_3$) cm^{-1}: 3598, 3340, 1652 (C=O), 1594, 1514, 1297. UV (EtOH) λ_{nm} (log ε): 325 (3.62), 259 (4.50), 227 (4.34). EI-MS *m/z* (rel. int.): 422 [M^+] (49), 407 (100), 375 (31), 107

(37). HR-EI-MS *m*/*z* 422.2090 $[M]^+$, (Calcd for $C_{26}H_{30}O_5$, 422.2093).

Compound **4**: Colorless oil; FT-IR ($CHCl_3$) cm^{-1}: 3399, 1650 (C=O), 1604, 1496, 1270, 1205. UV (EtOH) λ_{nm} (log ε): 314 (3.71), 263 (4.00), 225 (4.45). EI-MS *m*/*z* (rel. int.): 408 $[M^+]$ (93), 364 (70), 362 (47), 333 (55), 321 (51), 277 (65), 91 (100). HR-EI-MS *m*/*z* 408.2302 $[M]^+$, (Calcd for $C_{26}H_{32}O_4$, 408.2301).

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