New Bibenzyl Cannabinoid from the New Zealand Liverwort *Radula marginata*

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The ether extract of the New Zealand liverwort *Radula marginata* afforded a new cannabinoid type bibenzyl compound named perrottetinenic acid, and two new bibenzyls, together with a known cannabinoid, perrottetinene. Their structures were established by two dimensional (2D) NMR spectral data. The structure of perrottetinenic acid was a similar to that of Δ^1-tetrahydrocannabinol, a known hallucinogen. Cannabinoid type bibenzyls have been isolated from liverwort *Radula perrottetii*, though have not previously been reported from the liverwort *R. marginata*.

Key words cannabinoid; bibenzyl; liverwort; *Radula*; chemosystematics

We are continuing the chemosystematic study of the liverworts by gas chromatography (GC)- and liquid chromatography (LC)-MS analysis, as resolution of the relationships between species of liverworts is difficult because of their small size, and ephemeral nature of the sporophytic generation, which yields many conventionally critical taxonomic characters. This is particularly true of the genus *Radula*. This investigation is part of a chemosystematic study devoted to assisting the resolution of infrageneric relationships within *Radula*. During our investigation of the genus of *Radula*, we found the cannabinoid type compound, perrottetinene in the Japanese liverwort, *Radula perrottetii*.[1] *Radula* species are very rich sources of aromatic compounds and are extremely rich in prenylated bibenzyls.[2—4] The ether extract of the New Zealand liverwort *R. marginata*, which have not yet been investigated phytochemically, afforded new cannabinoid type and prenylated bibenzyls.

The ether extract of *R. marginata* was chromatographed on silica gel to give three new aromatic compounds **1**, **3** and **4**, together with known **2**, **5**, **6** and δ-tocopherol (Fig. 1).

Perrottetinenic acid; Compound **1**, $[\alpha]_D$ −165.8°, showed absorption bands characteristic of a carboxyl group at 2800—3100 and 1615 cm^{-1}. The presence of a benzene ring is apparent from UV absorption at 257.4 nm and an intense aromatic absorption at 1566 cm^{-1} in the IR spectrum. The ^{1}H-NMR spectrum (Table 1) of **1** was similar to that of perrottetinene (**2**), except for the appearance of a shifted proton signal at δ 12.21 (1H, s,) and for the absence of an aromatic signal of **2**. Furthermore, five aromatic ring protons at δ 7.20 (1H, tt, J=1, 8 Hz, H-6″), 7.22 (2H, br d, J=8, H-4″, 8″) and 7.30 (2H, br t, J=8 Hz, H-5″, 7″) were observed in the ^{1}H-NMR spectrum of **1**, due to a 1-substituted aromatic ring. A fragment peak, tropylium cation ($C_7H_7^+$) at m/z 91 in the electron impact (El)-MS of **1** provided further evidence for this aromatic ring. The EI-MS spectrum of **1** showed a molecular ion peak at m/z 392, its constitution of $C_{25}H_{28}O_4$ was confirmed by its high resolution EI-MS measurement. Analysis of the heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra (important correlations are summarized in Fig. 2) supports the structural assignment. The nuclear Overhauser enhancement spectroscopy (NOESY spectrum of **1** (Fig. 3) showed correlations between H-3 and 4, confirming the stereochemistry of a *cis*-fused ring. Consideration of these spectral data led to the conclusion that the compound was perrottetinenic acid (**1**), although its absolute configuration remains to be clarified. It is noteworthy that in this investigation the GC-MS analysis of **1** showed a same retention time and mass spectrum as those of **2** because of decarboxylation of **1** in an injection at 250 °C of GC.

Compound **3** was isolated as a minor constituent of this species, the coloration of which showed dark blue with spray of $FeCl_3$ on a TLC. The presence of a phenolic hydroxyl group was anticipated. The structure of **3** was deduced by comparing its spectral data with those of **2**. Whereas the car-

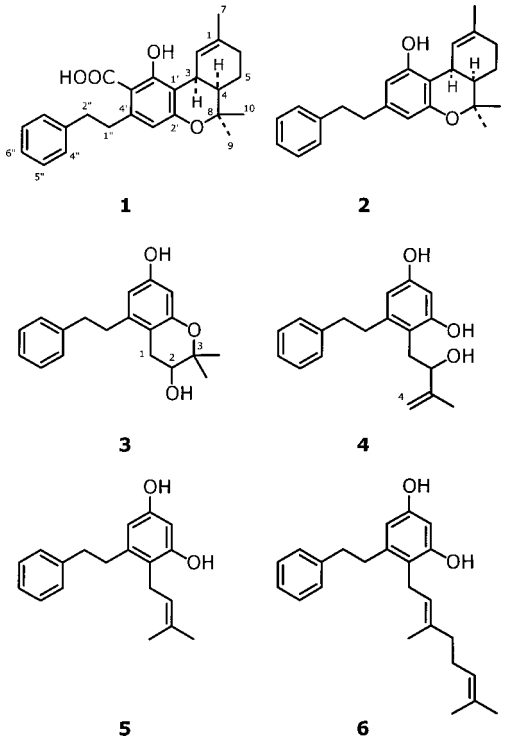

Fig. 1. Structures of Bibenzyls **1**—**6**, Isolated from *R. marginata*

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Table 1. NMR Data of Compounds **2**—**4** in $CDCl_3$

No.	^{1}H			^{13}C		
	2	**3**	**4**	**2**	**3**	**4**
1	—	2.49, 2.73 (each, dd, 5, 16)	2.77—2.85[a]	134.3	28.5	32.1
2	6.33 (br d, 3)	3.73 (br t, 5)	4.19 (dd, 4, 7)	121.8	69.8	79.0
3	3.60 (br s)	—	—	31.3	76.2	147.1
4	1.79 (ddd, 3, 6, 11)	1.25 (3H, s)[d]	4.90 (br t, 1, *cis*) 5.04 (s, *trans*)	39.9	24.5[d]	111.3
5	1.44 (m, axial), 1.95 (equatorial)[a]	1.31 (3H, s)[d]	1.83 (br s, 3H)	20.7	22.0[d]	17.9
6	1.97—2.02[a] (2H)	—	—	29.6		
7	1.70 (3H, s)	—	—	23.7		
8	—	—	—	77.8		
9	1.30 (3H, s)	—	—	25.3		
10	1.43 (3H, s)	—	—	25.7		
1′	—	6.21 (d, 2.5)	6.35 (d, 2.5)	110.8	102.0	102.8
2′	—	—	—	158.8	153.7	157.1
3′	6.27 (s)	—	—	112.6	109.6	117.0
4′	—	—	—	145.1	142.6	142.3
5′	—	6.34 (d, 2.5)	6.32 (d, 2.5)	102.6	108.8	108.8
6′	—	—	—	165.4	154.9	155.0
1″	3.14, 3.19 (each, ddd, 6, 11, 13)	2.78 (2H, dd, 5, 9)	2.77—2.85a	38.7	34.5	35.7
2″	2.87, 2.90 (each, ddd, 6, 11, 13)	2.86 (2H, dd, 5, 9)	2.77—2.85a	38.0	36.7	37.7
3″	—	—	—	142.2	141.5	141.5
4″	7.22 (br d, 8)[b]	7.15 (br d, 8)[a]	7.15 (br d, 8)[b]	128.4[a]	128.4[a]	128.4[a]
5″	7.30 (br t, 8)[c]	7.28 (br t, 8)[b]	7.29 (br t, 8)[c]	128.4[a]	128.5[b]	128.3[b]
6″	7.20 (tt, 1, 8)	7.20 (tt, 1, 8)	7.20 (br t, 8)	125.9	126.1	126.1
7″	7.30 (br t, 8)[c]	7.28 (br t, 8)[b]	7.29 (br t, 8)[c]	128.4[a]	128.5[b]	128.3[b]
8″	7.22 (br d, 8)[b]	7.15 (br d, 8)[a]	7.15 (br d, 8)[b]	128.4[a]	128.4[a]	128.4[a]
COOH		—		175.1		
OH	12.21 (s)	—				

a—*c*) Overlapped signals. *d*) Signal assignments may be interchangeable in each column.

boxylic acid absorption was missing in the IR spectrum of **3**, the presence of a hydroxyl group (3596, 3336 cm^{-1}) and an aromatic ring (1618, 1597 cm^{-1}) were confirmed. Further evidence for the presence of the aromatic ring was provided from the absorption band at 285.8 nm in the UV spectra of **3**. The EI-MS spectra of **3** exhibited a molecular ion peak at *m/z* 298 and tropylium ion peak also observed at *m/z* 91 same as that in the case of **2**. ^{1}H-NMR spectrum of **3** showed methylene protons at δ 2.49 and 2.73 (each dd, J=5, 16 Hz), which coupled with a methine proton at δ 3.73 bearing an oxygenated carbon. The NOESY spectrum of **3** showed a correlation between the methylene protons and benzyl methylene protons at δ 2.78, which further correlated with an aromatic proton at δ 6.34 (d, J=2.5 Hz, H-5′). Analysis of the HMQC and HMBC spectra of **3** supports the structural assignment. Accordingly, the structure of **3** was elucidated as shown in Fig. 1.

Compound **4** was isolated as a minor constituent from this species. The dark blue coloration on tlc with $FeCl_3$ showed the presence of a phenolic hydroxyl group. The IR spectrum of **4** showed the presence of the hydroxyl group at 3598 cm^{-1} and UV spectrum showed the absorption of the aromatic ring at 285.4 nm. The EI-MS spectrum showed a fragment peak at *m/z* 91 and a molecular ion at *m/z* 298. The ^{1}H-NMR spectrum of **4** attributed the signals at δ 6.32 and 6.35 (each 1H, d, J=2.5 Hz, H-5′, 1′), 7.15 (2H, br d, J=8 Hz, H-4″, 8″), 7.20 (1H, br t, J=8 Hz, H-6″) and 7.29 (2H, br t, J=8 Hz, H-5″, 7″) to the same partial structure as compounds **1**—**3**. Further resonance proton at δ_H 2.77—2.85 (6H, overlapping signals) correlated to three methylene carbons at δ_C 32.1, 35.7 and 37.7 in the HMQC spectrum of **4**. Correlation between

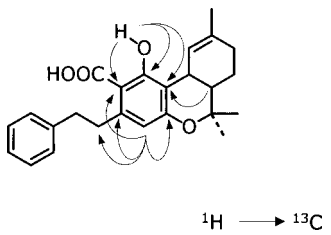

Fig. 2. HMBC Correlations for Perrottetinenic Acid (**2**)

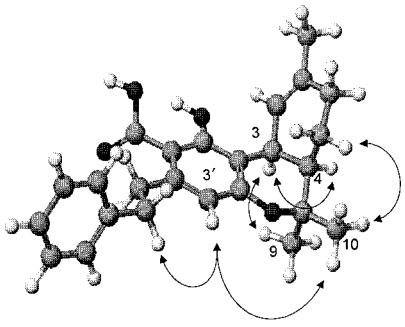

Fig. 3. NOE Correlations for Perrottetinenic Acid (**2**)

the signal of methine proton bearing an oxygenated carbon at δ 4.19 (1H, dd, J=4, 7 Hz, H-2) and two proton signals in the overlapping signals at δ 2.77—2.85 were observed in the ^{1}H–^{1}H COSY spectrum of **4**. Analysis of the HMQC and HMBC spectra supported the structural assignment.

The absolute configuration of compounds **1**—**4**, isolated from the present species, remains to be clarified. Attempts to

establish their absolute configuration were thwarted through lack of material. The prenylated bibenzyls **5** and **6** are the most significant chemical indicators for *Radula* species.[3,4] These chemicals serve to distinguish *Radula* chemosystematically from other related liverwort genera, such as *Lejeunea* species.

Experimental

General Experimental Procedures TLC was carried out on silica gel precoated glass plates with hexane–EtOAc (4 : 1 and 1 : 1 v/v). Detection was performed with Godin reagent.[5] For normal-phase column chromatography (CC), silica gel 60 (40—63 mm) was used. The mixture of CH_2Cl_2–MeOH (1 : 1) was used for column chromatography on Sephadex LH-20 as a solvent. Optical rotations were recorded on a JASCO DIP-1000 automatic digital polarimeter. NMR spectra were recorded at 150 MHz for ^{13}C-, and at 600 MHz for ^{1}H- on a Varian UNITY 600. The chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. UV spectra were recorded in spectroscopic-grade EtOH on a Shimadzu UV-1650PC. IR spectra were measured on a JASCO FT/IR-41. GC-MS spectra were recorded on a Hewlett-Packard HP-6890 gas chromatograph with a 5972A mass selective detector. The temperature programming for GC-MS analysis was performed from 50 °C to 250 °C at 8 °C/min, and isothermal at 250 °C for 5 min. The injection temperature 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. Helium was used as the carrier gas at 1 ml/min.

Extraction and Isolation of Compounds *Radula marginata* (Herbarium specimen No. YM2; dry wt. 4 g) was collected in May 2001 at Waitakere Range, Auckland in New Zealand. Voucher specimens are deposited at Faculty of Pharmaceutical Sciences, Tokushima Bunri University and Plant Systematics, School of Biological Sciences, University of Auckland.

R. marginata was ground mechanically and extracted with Et_2O for 2 weeks. The ether extract (0.31 g) was chromatographed on silica gel to divide into 9 fractions (I—IX). The fraction III (28.5 mg) was purified by HPLC [column; COSMOSIL 5SL-II, i.d. 10×250 mm, mobile phase; *n*-hexane–EtOAc (4 : 1 v/v)] to give perrottetinene (**2**) (22.5 mg; 7.3% of total extract) and δ-tocopherol (1.5 mg; 0.5%). The spectral data of **2** were identical to those of perrottetinene, which has been isolated from the liverwort *R. perrottetii*.[1] The fraction VI (21.3 mg) was rechromatographed on Sephadex LH-20 followed by HPLC to give perrottetinenic acid (**1**) (3.5 mg; 1.1%). Further purification of fraction VIII (80.1 mg) with HPLC gave compounds **3** (4.2 mg; 1.4%) and **4** (5.5 mg; 1.8%), together with known compounds **5** (44.5 mg; 14.5%) and **6** (6.9 mg; 2.2%). Compounds **5** and **6** were identified as known compounds by means of their spectral data.[1,3,4]

Perrottetinenic Acid (**1**): An oil, high resolution (HR) EI–MS *m/z*: 392.1985 (Calcd for $C_{25}H_{28}O_4$: 392.1988). EI–MS *m/z* (rel. int.): 392 $(M)^+$ (13), 374 $(M-H_2O)^+$ (21), 348 $(M-CO_2)^+$ (100), 333 (55), 305 (28), 265 (63), 257 (40), 105 (27), 91 (56). $[\alpha]_D^{22}$ −165.8° (c=0.35, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3500, 3100—2800 (broad), 1615, 1566, 1369, 1258, 1170, 1135, 893. λ_{max} (EtOH) nm (log ε): 301.4 (3.26), 257.4 (3.88), 224.6 (4.51).

3: An oil, EI–MS *m/z* (rel. int.): 298 $(M)^+$ (71), 227 (100), 207 (16), 149 (19), 123 (21), 91 (49). $[\alpha]_D^{22}$ +8.4° (c=0.38, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3596, 3336, 1618, 1597, 1453, 1133. λ_{max} (EtOH) nm (log ε): 285.8 (3.38), 205.8 (4.59).

4: An oil, EI–MS *m/z* (rel. int.): 298 $(M)^+$ (21), 280 (6), 228 (75), 227 (100), 123 (53), 91 (60). $[\alpha]_D^{22}$ 0° (c=0.37, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3598, 3321, 1623, 1594, 1496, 1453, 1141. λ_{max} (EtOH) nm (log ε): 285.4 (3.42), 205.8 (4.62).

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