Bazzanane Sesquiterpenoids from the New Zealand Liverwort *Frullania falciloba*

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Two new bazzanane-type sesquiterpenoids have been isolated from the New Zealand liverwort *Frullania falciloba*. Their structures were confirmed by NMR and CD spectroscopy and chemical reactivity.

Key words liverwort; *Frullania falciloba*; bazzanane-type; sesquiterpenoid

Liverworts, which contain many different kinds of terpenoids and aromatic compounds, are distributed widely throughout the world.[1,2] The southern hemisphere, especially New Zealand, has many more endemic liverworts than the northern hemisphere.[3] We have previously reported several peculiar terpenoids and aromatic compounds from some liverworts endemic to New Zealand.[4—8] These terpenoids and aromatic compounds are chemosystematically specific to liverworts and are used as genetic markers for the species.

Approximately 30 species of *Frullania* species have been recorded in New Zealand.[3,9] In general, *Frullania* species contain elaborate sesquiterpene lactones, such as eudesmanolides and eremophilanolides, that cause potent allergenic contact dermatitis and/or contain bibenzyl derivatives.[1,2,10] We have now chemically analyzed *Frullania falciloba* TAYL. ex LEHM. The fractionation of the ether extract of *F. falciloba* resulted in the isolation of two new bazzanane-type sesquiterpenoids **1** and **2**. Their structures were determined by spectroscopic and chemical means.

The IR spectrum of **1** established the presence of a conjugated carbonyl group (1685 cm^{-1}) and chemical-ionization mass spectrometry (CI-MS) confirmed a quasimolecular ion at *m/z* 219 $[M+H]^+$. Its ^{1}H-NMR spectrum (Table 1) contained three methyls (δ 0.99, 1.07, 1.78), an *exo*-methylene (δ 4.81, 5.04) and an olefinic proton (δ 6.59). The ^{13}C-NMR spectrum (Table 2) exhibited 15 carbons. In combination with the distortionless enhancement by polarization transfer (DEPT) spectrum, we detected the presence of an *exo*-methylene (δ 158.6 C, 108.0 CH_2), trisubstituted olefinic carbons (δ 143.7 CH, 134.2 C) and a ketone carbonyl carbon (δ 200.7), as well as three methyls, five methylenes and two quaternary carbons. Based on the above spectral evidence, a molecular formula of $C_{15}H_{22}O$ was suggested which indicated a bicyclic sesquiterpenoid structure for compound **1**. Analysis of the ^{1}H–^{1}H correlation (^{1}H–^{1}H COSY) and the heteronuclear multiple bond correlation (HMBC) spectra as shown in Fig. 1 supported the structure of **1** to be a bazzanane-type sesquiterpene ketone. The stereochemistry of **1** was determined to be the same as bazzanenol (**3**)[11—13] by nuclear Overhauser and exchange spectroscopy (NOESY). To confirm the stereochemistry of **1**, bazzanenol (**3**), already isolated from *Bazzania pompeana*, was oxidized with tetrapropylammonium perruthenate (TPAP). The stereochemistry at C-2 of **3** was reconfirmed to be *S* by the circular dichroism (CD) spectrum of its *p*-bromobenzoate derivative (**4**). The spectral and optical data of the oxidized product from **3** was completely identical to that of **1**. Accordingly, the structure of **1**, named 2-oxobazzanene, was established as shown in Fig. 1.

The IR spectrum of **2** showed the presence of a carbonyl group (1703 cm^{-1}) and its molecular formula was determined to be $C_{15}H_{22}O_2$ (Calcd for 234.1620) by high-resolution electron impact mass spectrometry (HR-EI-MS). The ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2) showed the presence of an *exo*-methylene (δ_H 4.95, 5.05; δ_C 109.0 CH_2, 156.7 C), two methines associated with an oxygen atom (δ_H 3.50, 4.18; δ_C 75.3, 79.9), and a ketone carbonyl carbon (δ_C 211.9), as well as a secondary methyl, two tertiary methyls, a methine, four methylene, and two quaternary carbons. The above spectral data suggested that compound **2** was a tricyclic sesquiterpene ketone. The presence of an ether linkage in the molecule was supported by the results of the IR spectrum that lacked the characteristic absorption of a hydroxy group and the ^{13}C-NMR spectrum that showed two oxygenated methines (δ_C 75.3, 79.9). The ^{1}H–^{1}H COSY spectrum of **2** exhibited the presence of three partial segments: i)

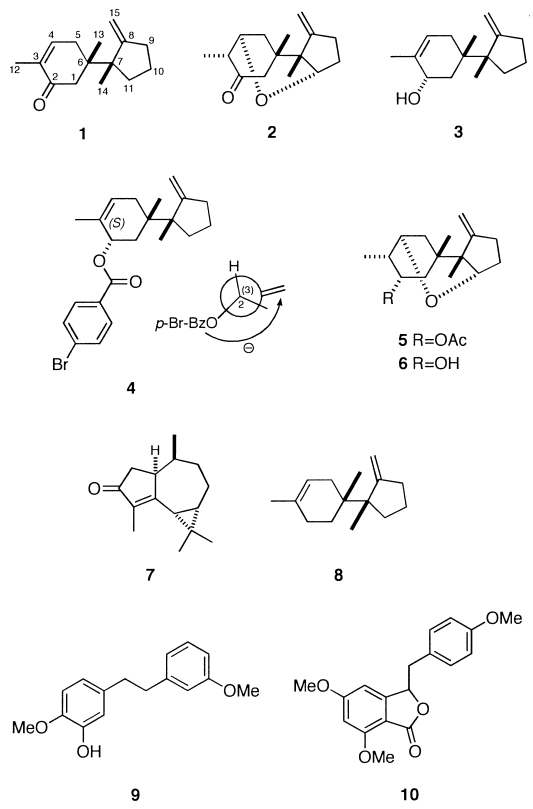

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Table 1. ^{1}H-NMR of **1**, **2**, **5**, and **6** (600 MHz, $CDCl_3$)

H	1	2	5	6
1	2.40 dd (15.7, 1.9)[a] α	2.85 dd (15.7, 3.8) α	2.23 br d (16.2) α	2.27 m α
	2.46 dd (15.7, 1.1) β	2.06 dd (15.7, 1.9) β	1.47 dd (16.2, 6.0) β	1.39 dd (15.7, 5.8) β
2			5.13 t (6.0)	3.94 t (5.8)
3		2.33 m	1.80 ddddd (7.1, 3.3, 3.3, 3.3, 3.3)	1.69 m
4	6.59 d sext. (6.0, 1.4)	4.18 ddd (3.8, 3.8, 1.9)	3.78 br s	3.79 br s
5	2.68 br d (18.7) α	1.61 dd (13.5, 1.9) α	1.74 ddd (13.2, 4.1, 3.0) α	1.75 ddd (13.2, 4.4, 3.0) α
	2.07 ddd (18.7, 6.3, 1.6) β	2.08 dt (13.5, 3.8) β	1.12 dd (13.2, 1.9) β	1.09 dd (13.2, 1.9) β
9	2.20 m α	2.77 m α	2.28 br d (13.2)	2.79 m
	2.34 br dd (14.6, 6.0) β	2.35 m β	2.80 m	2.28 m
10	1.70 m α	1.58 dd (12.9, 7.7) α	1.63—1.68 2H, m	1.62 m
	1.45 m β	1.68 dddd (12.9, 12.9, 7.7, 3.6) β		1.66 m
11	1.42 m α	3.50 d (3.6)	4.46 br s	4.56 d (3.8)
	1.87 m β			
12	1.78 quint. (1.4)	1.22 d (6.9)	1.02 d (7.4)	1.12 d (7.1)
13	0.99 s	1.27 s	1.09 s	1.08 s
14	1.07 s	0.96 s	0.96 s	1.09 s
15	4.81 d (2.7)	4.95 d (2.5)	4.79 d (2.7)	4.79 d (3.0)
	5.04 d (1.9)	5.05 s	5.10 br s	5.06 s
$OCO\underline{CH_3}$			2.09 s	

a) Coupling constants (*J* in Hz) are given in parentheses.

Table 2. ^{13}C-NMR of **1**, **2**, **5** and **6** (100 MHz, $CDCl_3$)

C	1	2	5	6
1	45.7	53.4	40.4	42.8
2	200.7	211.9	69.0	67.4
3	134.2	48.2	37.1	38.6
4	143.7	75.3	71.2	71.5
5	33.7	38.5	38.9	39.1
6	42.2	39.1	31.3	31.4
7	50.0	50.7	49.5	49.7
8	158.6	156.7	158.9	159.2
9	38.7	33.8	35.8	35.5
10	23.3	30.0	31.7	31.0
11	37.0	79.9	80.4	80.8
12	15.4	10.9	13.4	13.4
13	19.0	26.4	28.7	28.6
14	23.7	21.9	23.2	23.2
15	108.0	109.0	109.7	109.0
$-\underline{C}OCH_3$			170.5	
$-CO\underline{CH_3}$			21.4	

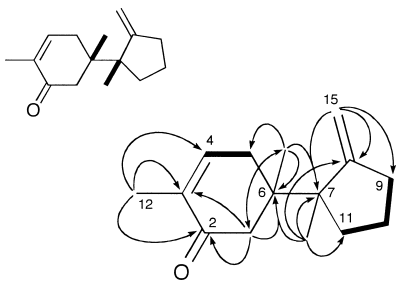

Fig. 1. ^{1}H–^{1}H COSY (Bold Lines) and Long-Range ^{1}H–^{13}C (Arrows) Correlations of **1**

CH_3–CH–CH(O–)–CH_2–, ii) –CH_2–, and iii) –CH_2–CH_2–CH(O–)–. The correlations of each partial segment were confirmed by the HMBC spectrum (Fig. 2). The NOESY spectrum clarified the stereochemistry of **2** as shown in Fig. 3. To obtain further confirmation of the structure, compound **2** was reduced with lithium aluminium hydride ($LiAlH_4$), followed by acetylation with pyridine and acetic anhydride to give a

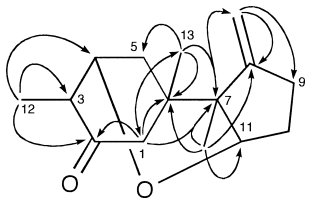

Fig. 2. Long-Range ^{1}H–^{13}C Correlations of **2**

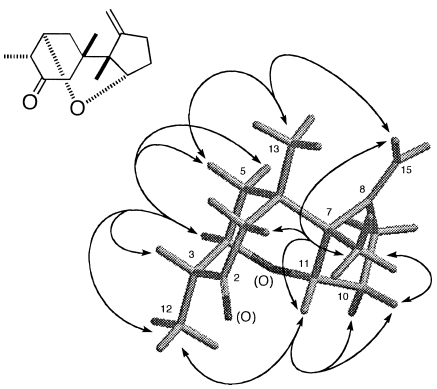

Fig. 3. NOE Correlations of **2**

monoacetate **5** and unreacted monoalcohol **6**.

The IR, ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2) of **5** ($C_{17}H_{26}O_3$ Calcd for 278.1882) showed the presence of an acetoxy group (1740, 1240 cm^{-1}; δ_H 2.09; δ_C 21.4 CH_3, 170.5 C) and a newly observed oxygenated methine (δ_H 5.13; δ_C 69.0). Furthermore, the structure of **5** was determined to be a monoacetate by the ^{1}H–^{1}H COSY, HMBC and NOESY spectra. Compound **6** ($C_{15}H_{24}O_2$ Calcd for 236.1776) was found to be a monoalcohol by spectral analysis (Tables 1, 2). Accordingly, the structure of **2** was determined to be a bazzanane-type sesquiterpene with a ketone at C-2 and an ether linkage between C-4 and C-11. Thus the structure of **2**, named bazzanenoxide, was established as shown in Fig. 3. Although the absolute configuration of **2**,

shown in Fig. 3, was assumed by the analysis of its CD spectrum ($\Delta\varepsilon_{295}$ +0.57),[14] further experiment is needed.

The isolation of sesquiterpenoids and aromatic compounds from *F. falciloba*, *e.g.*, *ent*-cyclocolorenone (**7**), β-bazzanene (**8**), 3-hydroxy-4,3′-dimethoxybibenzyl (**9**), and 3-(4′-methoxybenzyl)-5,7-dimethoxyphthalide (**10**), has previously been reported by our group[15,16] and Mali *et al.*[17] Bazzananetype sesquiterpenoids are very rare in the plant kingdom. This type of compound is mainly distributed in the liverwort *Bazzania* genus, making bazzananes important chemical markers of the *Bazzania* species. In the present study, we report the first isolation of two new bazzanenes **1** and **2** from *F. falciloba*. The presence of bibenzyl or phthalide derivatives could not be detected.

Experimental

General Methods Optical rotations were measured on a Jasco DIP-1000 polarimeter. IR spectra were recorded on a Shimadzu FTIR 8400S infrared spectrophotometer. UV spectra were recorded on a Shimadzu UV-1650PC UV-visible spectrophotometer. CD spectra were recorded on a Jasco J-725 spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were measured on Varian Unity-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) and Jeol Eclipse-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) NMR spectrometers. Chemical shift values are expressed in δ (ppm) downfield from tetramethylsilane as an internal standard (^{1}H-NMR), and relative to the solvent $CDCl_3$ (δ: 77.03) as a standard (^{13}C-NMR). Mass spectra were obtained on a JEOL Mstation JMS 700 instrument. TLC was performed on silica gel 60F_{254} plates (Merck). Column chromatography was performed on silica gel 60 (Merck, 230—400, 35—70 mesh) and Sephadex LH-20 (Amersham Pharmacia Biotech, 1 : 1 CH_2Cl_2–MeOH as solvent). TLC spots were visualized under UV (254 nm) light and by spraying with Godin reagent[18] and 30% H_2SO_4, followed by heating.

Plant Material *Frullania falciloba* TAYL. ex LEHM. (NZ-267) was collected in Tree Trunk Gorge, New Zealand, in December 2000 and identified by Dr. J. E. Braggins (University of Auckland, New Zealand), and a voucher specimen was deposited in the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and Isolation The ether extract (205 mg) of *F. falciloba* was divided into seven fractions by column chromatography (CC) on silica gel using an *n*-hexane–EtOAc gradient. Fraction 2 was chromatographed on Sephadex LH-20, silica gel, and preparative HPLC (Chemcosorb 5Si-U, 17 : 3 *n*-hexane–EtOAc) to give 2-oxobazzanene (**1**, 9.0 mg). Bazzanenoxide (**2**, 10.4 mg) was isolated from Fr. 4 by CC on Sephadex LH-20 and preparative HPLC (Chemcosorb 5Si-U, 7 : 3 *n*-hexane–Et_2O).

2-Oxobazzanene (**1**): Oil, $[\alpha]_D^{18}$ +8.4° (c=0.69). FT-IR cm^{-1}: 1685. UV λ_{max} (EtOH) nm (log ε): 238 (3.12) (c=6.12×10^{-4}). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-CI-MS (*iso*-butane) *m/z*: 219.1738 (Calcd for $C_{15}H_{23}O$: 219.1749). CI-MS (*iso*-butane) *m/z*: 219 $[M+H]^+$, EI-MS *m/z* (int.): 218 $[M]^+$ (2), 205 (2), 163 (4), 149 (9), 123 (100), 109 (14), 95 (91), 81 (29), 67 (18), 55 (19), 41 (11).

Bazzanenoxide (**2**): Amorphous, $[\alpha]_D^{18}$ +76.2° (c=0.81). FT-IR cm^{-1}: 1703. CD (EtOH): $\Delta\varepsilon_{295}$ +0.57° (c=5.77×10^{-4}). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-EI-MS *m/z*: 234.1623 (Calcd for $C_{15}H_{22}O_2$: 234.1620). EI-MS *m/z* (int.): 234 $[M]^+$ (13), 207 (3), 175 (2), 163 (100), 145 (18), 135 (10), 119 (9), 107 (11), 94 (36), 79 (21), 63 (14), 55 (15), 43 (58).

Oxidation of 3 Tetrapropylammonium perruthenate (14 mg) was added to a stirred mixture of bazzanenol **3** (42 mg), 4-methylmorpholine-*N*-oxide (64.8 mg) and 4 Å molecular sieves (180.2 mg) in dry CH_2Cl_2 (3 ml) at room temperature for 1.5 h. The reaction mixture was chromatographed on silica gel to yield **1** (12.6 mg). The ^{1}H- and ^{13}C-NMR spectra and optical rotation ($[\alpha]_D^{18}$ +7.4°, c=0.95) of the reaction derivative were identical with those of natural **1**.

Reduction and Acetylation of 2 To a suspension of $LiAlH_4$ (10 mg) in dry Et_2O (3 ml) was added the compound **2** (4.9 mg) in dry Et_2O (2 ml). The solution was stirred for 20 min at room temperature, providing a mixture after the usual work-up. Pyridine (1 ml) and Ac_2O (1 ml) were added to the unpurified mixture and the solution was kept at room temperature overnight. After work-up, the resulting mixture was chromatographed on Sephadex LH-20 to yield the monoacetate **5** (2.9 mg) and the unreacted monoalcohol **6** (1.6 mg).

Monoacetate (**5**): Oil, $[\alpha]_D^{21}$ +267.1° (c=1.74). FT-IR cm^{-1}: 1740, 1240, 1100. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-EI-MS *m/z*: 278.1888 (Calcd for $C_{17}H_{26}O_3$: 278.1882). EI-MS *m/z* (int.): 278 $[M]^+$ (6), 236 (3), 218 (19), 203 (6), 185 (9), 163 (71), 145 (20), 135 (15), 125 (31), 119 (32), 107 (66), 94 (100), 79 (71), 67 (17), 55 (34), 43 (79).

Monoalcohol (**6**): Oil, $[\alpha]_D^{21}$ +263.8° (c=0.94). FT-IR cm^{-1}: 3422. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-EI-MS *m/z*: 236.1765 (Calcd for $C_{15}H_{24}O_2$: 236.1776). EI-MS *m/z* (int.): 236 $[M]^+$ (17), 218 (5), 208 (4), 200 (6), 163 (34), 145 (12), 135 (13), 125 (14), 119 (22), 110 (44), 108 (33), 94 (100), 79 (49), 67 (14), 55 (21), 43 (21).

Esterification of 3 To a suspension of **3** (10 mg) in pyridine (2 ml) and CH_2Cl_2 (1 ml) was added *p*-bromobenzoylchloride (42 mg) and 4-dimethylaminopyridine (10 mg) and the mixture was stirred overnight at room temperature. The reaction mixture was filtered and purified on silica gel CC to yield the benzoate derivative **4** (6.2 mg): UV λ_{max} (EtOH) nm (log ε): 245 (3.73) (c=6.82×10^{-5}). CD (EtOH): $\Delta\varepsilon_{245}$ −6.77° (c=6.82×10^{-5}). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.00 (3H, s, H-13), 1.04 (3H, s, H-12), 1.38 (1H, m, H-11β), 1.42 (1H, m, H-10), 1.58 (1H, t, J=12.1 Hz, H-1α), 1.66 (1H, m, H-10), 1.68 (3H, br s, H-12), 1.71 (1H, br d, H-5β), 1.87 (1H, m, H-11α), 2.11 (1H, ddd, J=12.1, 6.0, 2.2 Hz, H-1β), 2.20 (1H, m, H-9), 2.30 (1H, m, H-9), 2.33 (1H, br d, J=18.4 Hz, H-5α), 4.81 (1H, d, J=2.7 Hz, H-15), 4.98 (1H, s, H-15), 5.57 (1H, m, H-4), 5.60 (1H, br s, H-2), 7.58 (2H, d, J=8.8 Hz, Br$C_6\overline{H}_4$COO–), 7.93 (2H, d, J=8.8 Hz, Br$C_6\overline{H}_4$COO–). FAB-MS (*m*-NBA) *m/z*: 425, 427 $[M+Na]^+$; (*m*-NBA+KCl) *m/z* 441, 443 $[M+K]^+$. EI-MS *m/z* (int.): 307 $[M-C_7H_{12}]^+$ (8), 202 (16), 183 (58), 155 (12), 149 (7), 107 (100), 106 (89), 96 (34), 91 (29), 81 (14), 67 (9), 55 (11), 41 (7).

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