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Insect antifeedant sesquiterpene acetals from the liverwort *Lepidolaena clavigera*

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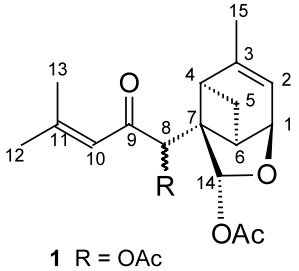
Abstract—Two new oxygenated bergamotane derivatives, clavigerins B and C, were responsible for the insect antifeedant activity of an extract from the New Zealand liverwort *Lepidolaena clavigera*.

The temperate rain forests of New Zealand are a rich source of liverworts, which can form thick carpets on the forest floor and the trunks of older trees. These simple, soft plants lack the physical defences of the woody vascular plants, but generally show little sign of attack from insects and other invertebrates. A range of new compounds with insect antifeedant activity have already been reported from liverworts,[1] so we have included these bryophytes in our search for bioactive natural products as lead compounds for new agrochemicals.[2]

An extract of *Lepidolaena clavigera* (Hook.) Dum. ex Trev. (family Lepidolaenaceae)[3] gave us antifeedant activity against larvae of two insect pests of wool, the carpet beetle *Anthrenocerus australis* (Coleoptera) and the clothes moth *Tineola bisselliella* (Lepidoptera).[4] The first report on the chemistry of *L. clavigera*, which is a New Zealand endemic, was by Asakawa et al. who found clavigerin **1**, plus some common sesquiterpenes.[5] No information was given on the isolation, spectroscopic data or bioactivity of **1**. Our collections of *L. clavigera* have yielded two new poly-oxygenated diterpenes, the first atisanes reported from any liverwort.[6] One of these diterpenes showed cytotoxic and insecticidal activity. We now report the identification of two new sesquiterpene acetoxy acetals, clavigerins B **2** and C **3**, as the main insect antifeedants in our samples of *L. clavigera*.



1 R = OAc
2 R = H
3 R = H; 10,11-dihydro

This liverwort was collected from coastal rain forest on the South Island of New Zealand (voucher specimen OTA 6821). A $CH_3CN/CHCl_3$ extract was subjected to reverse phase flash chromatography using a H_2O:CH_3CN:$CHCl_3$ gradient. The fractions with the highest antifeedant activity against *A. australis* were eluted with 1:3 H_2O:CH_3CN. Silica TLC and ^1H NMR spectra suggested the presence of two major compounds in these fractions. Silica chromatography (flash column, 1:1 cyclohexane:EtOAc; then HPLC, 1:9 isopropyl alcohol:hexane) separated compound **2** (6 mg, approx. 0.02% w/w dry liverwort) and the slightly less polar compound **3** (12 mg, 0.04%).

The various spectra of **2**[7] and **3**[8] showed that these compounds were very similar, but **2** contained one more C=C bond than **3**. ^1H–^1H (COSY) and ^1H–^{13}C (HMQC and HMBC, on **3** only) correlation experiments (Table 1) suggested the two different C-7 to C-13 side chains shown for **2** and **3**. These were confirmed by close matches with ^1H and ^{13}C NMR data for corresponding substructures in other liverwort metabolites.[9]

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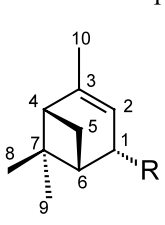
Table 1. NMR data for clavigerins B **2** and C **3**[a]

#	Clavigerin B **2**			Clavigerin C **3**			
	^{1}H	COSY	^{13}C	^{1}H	COSY	^{13}C[f]	HMBC
1	4.81 (dd, 5, 4)	5.49, 2.59	78.6	4.80 (dd, 5, 4)	5.48, 2.56	78.5	2.3, 1.39
2	5.49 (br m)	4.81, 2.43, 1.82	116.1	5.48 (br m)	4.80, 1.80	116.2	4.80, 2.56, 2.3, 1.80
3	–	–	148.4	–	–	148.1	4.80, 2.3, 1.80, 1.39
4	2.43 (td, 6, 2)	5.49, 2.59, 2.38	47.2	2.3 (m)[h]	2.56, 2.12, 1.39[d]	47.1	5.90, 3.06, 2.89, 2.56, 2.3, 1.80, 1.39
5*R*	2.38 (dt, 9, 6)	2.59, 2.43, 1.39	34.6	2.35 (m)	2.56, 2.12, 1.39[d]	34.5	2.3
5*S*	1.39 (d, 9)	2.38	–	1.39 (d,8)	2.35	–	–
6	2.59 (br q, 5)	4.81, 2.43, 2.38	42.7	2.56 (br q,6)	2.3, 4.80	42.5	5.90, 5.48, 3.06, 2.89, 2.3, 1.39
7	–	–	58.2	–	–	57.6	5.90, 4.80, 3.06, 2.89, 2.56, 1.39
8*S*	3.07 (d, 18)	2.91	45.2	3.06 (d,18)	2.89	44.6	2.56
8*R*	2.91 (d, 18)	3.07	–	2.89 (d,18)	3.06	–	–
9	–	–	198.4	–	–	208.2	3.06, 2.89, 2.3
10	6.07 (br m)	2.09, 1.88	123.6	2.3 (m)	2.56, 2.12, 1.39[d]	51.9	2.12, 0.9
11	–	–	155.0	2.12 (m)	2.3, 0.9	24.5	2.3, 0.9
12	1.88 (d, 1)	6.07	20.8[b]	0.90 (d,7)[b]	2.12[e]	22.59[b]	2.3, 2.12, 0.9[d]
13	2.09 (d, 1)	6.07	27.6	0.91 (d,7)[b]	2.12[e]	22.56[b]	2.3, 2.12, 0.9[d]
14	5.93 (s)	None	97.3	5.90 (s)	None	97.2	4.80, 3.06, 2.89, 1.39[g]
15	1.82 (d, 2)	5.49	20.6[b]	1.80 (d,1)	5.48	20.97[c]	2.3[e]
OAc	–	–	169.0	–	–	168.9	5.90, 1.95
OAc	1.91 (s)	None	21.0	1.95 (s)	None	20.95[e]	2.3[e]

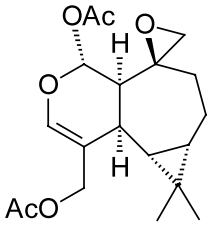
[a]In $CDCl_3$, ^{1}H at 300 MHz, ^{13}C at 75 MHz. [b,c]Asssignments with same superscript interchangeable within columns. [d,e]Correlations from non-resolved signals. [f]One bond ^{1}H–^{13}C correlations shown by HMQC. [g]Four bond correlation. [h]Resolved in C_6D_6: 2.30 (td, 7, 2).

EIMS of **2** and **3** showed only weak molecular ions, which readily lost CH_3CO_2H. The location of this acetate in an acetoxy acetal group was shown by the chemical shifts of C-14 (97.2 ppm in **3**) and H-14 (5.90 ppm), and by an HMBC correlation between H-14 and the acetate carbonyl (Table 1). Similar ^{1}H and ^{13}C NMR data have been reported for the corresponding atoms in synthetic acetoxy acetals.[10] The linkage of C-14 through an oxygen to C-1 was shown by an HMBC correlation between H-1 and C-14 (Table 1).

The completion of the structural assignments for **2** and **3** was complicated by some unusual ^{1}H–^{1}H couplings, particularly a geminal coupling of 8–9 Hz (protons on C-5, Table 1) and a four bond coupling of 5–6 Hz (H-4 to H-6). These were explained by the presence of a bridged cyclobutane substructure, C-1 to C-7, in **2** and **3**. The ^{1}H NMR spectrum of *cis*-verbenol **4** shows the same unusual couplings, due to the presence of the strained cyclobutane.[11] This substructure also explained an HMBC correlation assigned to coupling over four bonds, between H-5 and C-14 (Table 1). An analogous ^4J (C, H) coupling of 3.5 Hz between one H-5 and C-9 reported in α-pinene **5** was due to interaction across the cyclobutane.[12] Therefore, the full structures are assigned as the new bergamotane derivatives **2** and **3**, with oxygenation at C-1, C-9 and C-14. The ^{1}H and ^{13}C NMR spectra of **2** and **3** (Table 1) showed many similarities to the spectra of clavigerin **1**,[13] so we propose the names clavigerin B and C for **2** and **3**, respectively.



4 R = OH
5 R = H



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The relative stereochemistry at C1, C4, C6 and C7 of the clavigerins is fixed by the requirements of the tricyclic ring system, so they are *trans* bergamotane derivatives. The relative stereochemistry at the acetal centre was based on an NOE interaction between H-14 and 15-CH_3 in **2**. Molecular modelling suggested that this interaction was only compatible with the stereochemistry shown.

Apart from clavigerin **1**, the only other reports of other liverwort bergamotanes have been of the hydrocarbons α- and β-bergamotene from a few species.[1] Saritas et al. have shown that these compounds from the liverwort *Dumortiera hirsuta* were enantiomeric to the compounds found in vascular plants.[14] Therefore, the absolute stereochemistry shown for **2** and **3** is also shown to match this.

Clavigerins B **2** and C **3** had significant antifeedant activity against carpet beetle larvae at application rates as low as 0.026% (w/w on wool) for **3** and 0.052% for **2**.[4] Compounds **2** and **3** showed significant insecticidal, as well as antifeedant, activity against clothes moth larvae at application rates of 0.1%. For comparison, the well-

known insect antifeedant azadirachtin showed similar effects against both of these pests at 0.07%.[15] Another insect antifeedant from liverworts, plagiochiline A **6**, is also a sesquiterpene acetoxy acetal, but with a secoaromadendrane skeleton.[1]

The unprecedented carbon–oxygen skeleton of the clavigerins presents a challenge to synthetic chemists. Further work on the chemistry, molecular modelling and bioactivity of this new class of insect antifeedants will be reported elsewhere.

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7. Data for **2**: colourless oil; silica TLC R_F 0.2 (4:1 hexane:EtOAc); $[\alpha]^{577}$ +14 ($c=0.15$, $CHCl_3$); EIMS (70 eV) 230.1311 (14%, M^+–AcOH, $C_{15}H_{18}O_2$ req. 230.1307); IR ν_{max} ($CDCl_3$) 3019, 2965, 1747, 1709, 1681, 1616, 1442, 1380, 1218 cm^{-1}; UV (MeOH) λ_{max} (log ε) 235 nm (3.78); NMR spectra in Table 1.
8. Data for **3**: colourless oil; silica TLC R_F 0.3 (4:1 hexane:EtOAc); $[\alpha]^D$ +15, $[\alpha]^{577}$ +26 (c 0.1, $CHCl_3$); EIMS (70 eV) 292.1666 (1%, M^+, $C_{17}H_{24}O_4$ req. 292.1674), 232.1466 (24%, M^+–AcOH, $C_{15}H_{20}O_2$ req. 232.1463); IR ν_{max} ($CDCl_3$) 2954, 1747, 1709, 1600, 1240 cm^{-1}; NMR spectra in Table 1.
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