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The First Atisane Diterpenoids from a Liverwort: Polyols from *Lepidolaena clavigera*

Nigel B. Perry,*,† Elaine J. Burgess,† Seung-Hwa Baek,‡ and Rex T. Weavers†

Plant Extracts Research Unit, New Zealand Institute for Crop & Food Research Ltd., Department of Chemistry, University of Otago, Box 56, Dunedin, New Zealand, and Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan 570-749, Korea

perryn@crop.cri.nz

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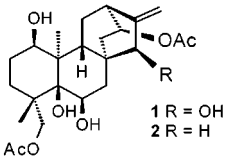
ABSTRACT



Two new diterpenoids were isolated from the New Zealand liverwort *Lepidolaena clavigera*. Spectroscopic studies identified the structures as atisanes 1 and 2, with an unprecedented level of oxygenation. This is the first report of the atisane skeleton from a liverwort. Compound 2 showed cytotoxic and insecticidal activity.

The simple nonvascular plants known as liverworts have proved a rich source of secondary metabolites, including a wide range of diterpenoids.[1] Many of the diterpenoid carbon skeletons found in vascular plants have also been found in liverworts, including tetracyclic kauranes. For example, we identified six oxygenated *ent*-kauranes from the New Zealand species *Lepidolaena taylorii*.[2] However, the tetracyclic beyerane and atisane skeletons, biosynthesized by carbocationic rearrangements alternate to those giving kauranes,[3] have not yet been found in liverworts.[1]

Continuing our search for bioactive natural products from plants[4] we examined an extract of the New Zealand endemic liverwort *Lepidolaena clavigera* (Hook.) Dum. ex Trev. (family Lepidolaenaceae).[5] The only previous report on the chemistry of this species described a new oxygenated sesquiterpenoid.[6] In the same genus, *L. hodgsoniae* gave an insecticidal epoxy ether sesquiterpene with a new skeleton,[7] and *L. taylorii* and *L. palpebrifolia* yielded oxygenated *ent*-kauranes with cytotoxic activity.[2] We now report two new poly-oxygenated atisanes **1** and **2** from *L. clavigera* and describe the cytotoxic and insecticidal activity of compound **2**.

Lepidolaena clavigera was collected from tree trunks in temperate rain forest.[8] An extract was subjected to two stages of RP chromatography[9] to give pure compounds **1**[10] and **2**.[11] Both compounds gave similar NMR spectra (Table 1), which showed the presence of two acetates, two quaternary methyls, and an exocyclic methylene.

Electron impact (EI) MS on **1** gave a highest *m*/*z* ion at 392.2173 Da, appropriate for $C_{22}H_{32}O_6$. However, the NMR spectra (Table 1) showed 24 carbon signals. Electrospray

† University of Otago.
‡ Wonkwang University.

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for Atisanes **1** and **2** in $CDCl_3$

	tetraol **1**		triol **2**	
no.	^{13}C	^{1}H	^{13}C	^{1}H
1	71.8	3.37 (br s)	71.6	3.44(br t, 3)
2α	24.9	2.0 (t)[b]	25.1	2.07(td,11,3)
2*β*		1.53 (br d, 12)		1.56 (d)[b]
3α	27.8[a]	1.21 (br d, 12)	28.0	1.23 (d)[b]
3*β*		1.85 (td, 14, 4)		1.90 (td,15,5)
4	42.7		42.7	
5	79.8		79.7	
6	69.1	4.21 (dd,11,5)	69.3	4.18(dd,11,5)
7α	30.2	1.45 (dd, 16, 6)	36.7	1.61 (dd, 14,5)
7*β*		2.17 (dd, 14, 12)		1.52 (t)[b]
8	41.8		36.72	
9	28.4	2.96 (br dd,11,9)	37.3	2.71(dd,11,8)
10	44.3		44.6	
11α	27.5[a]	1.26 (br d, 12)	27.5	1.30(ddd,13,8,2)
11*β*		1.71 (br t, 11)		1.73 (ddt,13,11,3)
12	36.0	2.43 (br s)	36.1	2.36 (br m)
13*R*	35.1	1.42 (dd, 14, 2)	36.07	1.48 (d)[b]
13*S*		2.09 (td, 12, 2)		2.1 (t)[b]
14	69.8	5.34 (dd, 10,2)	69.9	5.26(dt,10,2)
15*S*	69.4	4.21 (br s)	40.1	2.67(dt,17,2)
15*R*				1.85 (dd,16,2)
16	154.2		149.6	
17exo	109.9	5.04 (br s)	105.7	4.78(ddd,2,2,2)
17endo		5.12 (br s)		4.62(ddd,2,2,2)
18	25.2	1.21 (s)	25.6	1.23(s)
19	68.1	4.33 (d, 11)	68.6	4.31(d,12)
19		4.24 (d, 11)		4.28(d,12)
20	17.6	1.06 (s)	17.6	1.06(s)
14-OAc	170.8		171.1	
14-OAc	21.2	2.03 (s)	21.3	2.04(s)
19-OAc	171.2		171.0	
19-OAc	21.0	2.09 (s)	21.0	2.11(s)

[a] Assignments interchangeable. [b] Position and coupling (>8 Hz) from HSQC spectrum.

(ES) MS gave strong ions at 475 and 491 Da, which were assigned as MNa^+ and MK^+ of a compound with molecular formula $C_{24}H_{36}O_8$, in accord with the NMR data. The EI MS therefore showed a facile loss of acetic acid, and ions corresponding to two losses of water were also present. The NMR spectra (Table 1) showed signals for 32 protons directly attached to carbons and only one carbon−carbon double bond. These observations, in combination with the molecular formula required four OH groups and four rings.

Gradient-enhanced HMQC and HMBC correlation experiments gave enough data to define the molecular connectivity. One of the acetate carbonyl groups correlated to a CH_2 with protons showing only geminal ^{1}H−^{1}H coupling (H19s, Table 1). Two and three bond correlations to these protons from a CH_3 (H19/C18), a CH_2 (H19/C3), a quaternary carbon (H19/C4), and an oxygenated quaternary carbon (H19/C5) established sub-structure *A* (Figure 1). This carbon (C5) also

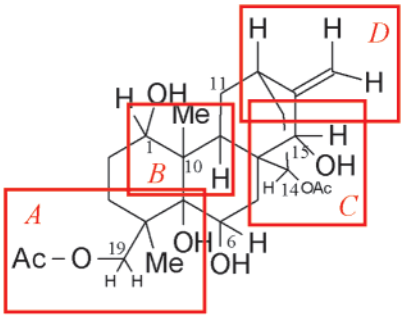


Figure 1. Stages in establishing the connectivity of atisane **1**.

showed a correlation to another CH_3 (C5/H20), as did another quaternary carbon (C10/H20), a CH (C9/H20) and an oxygenated CH (C1/H20). This extended the connectivity to sub-structure *B* (Figure 1).

A second entry point was provided by the CH (H14, Table 1) correlated to the other acetate carbonyl, which showed vicinal ^{1}H−^{1}H couplings to a CH_2 (H14/H13). ^{1}H−^{13}C correlation between the 14CH and a quaternary C (H14/C8), an oxygenated CH (H14/C15) and a CH_2 (H14/C7) established sub-structure *C* (Figure 1). The CH at 69.4 ppm (C15) correlated with the exocyclic methylene protons (C15/H17), as did another CH (C12/H17), extending the connectivity to sub-structure *D*.

COSY and HMBC correlations involving the CH at 2.44 ppm (H12) linked sub-structures *B* and *D* via a CH_2 (no. 11, Table 1). Further correlations allowed placement of the remaining CH_2 (no. 2) and oxygenated CH (no. 6) to give the full proposed molecular connectivity for **1** shown in Figure 1. This comprises a new compound with the known atisane carbon skeleton.[3]

The NOESY spectrum was used to establish the relative stereochemistry. Although we have not yet determined the absolute stereochemistry we have pictured the molecules with the *ent*-stereochemistry as was found in the kauranes from *L. taylorii*.[2] NOE interactions between 20CH_3 and H1, both H19s, H6, H14 and one H11 (Figure 2) showed that all of these groups were on the same face of the molecule, which led to the stereochemistry at C1, C4, C5, C6, C8, C9, and C14 proposed in Figure 2. The other question to be settled was the configuration at C15. The only NOE interaction noted involving H15 was with one H17, whereas a strong

(9) **Experimental Procedure.** Dried, ground liverwort (37 g) was extracted with MeCN and $CHCl_3$, and the extract (2.47 g) was subjected to RP flash chromatography (Aldrich C18). Fractions eluted with 3:1 and 1:1 H_2O/MeCN were combined (111 mg) and subjected to preparative RP HPLC (Merck Li Chrospher 100 RP-18, 250 mm × 10 mm, 5 mL/min 40:60 H_2O/MeOH, 206 nm detection) to give pure **1** (retention time 3.3 min, 47 mg). RP flash fractions eluted with 1:1 and 1:2 H_2O/MeCN were combined (162 mg) and subjected to preparative RP HPLC on the same column (5 mL/min, 55:45 H_2O/MeCN) to give more of **1** (4.5 min, 7 mg) and pure **2** (11.4 min, 32 mg).

(10) Data for **1**: colorless gum; $[\alpha]^{24}_D$ − 54° (*c* 1.7 mg/mL, MeOH); IR ν_{max} (film) 3418, 1734, 1715, 1651, 1248 cm^{-1}; NMR in Table 1; +EIMS *m*/*z* 392.2173 (M^+ − AcOH, 2%, calcd for $C_{22}H_{32}O_6$ 392.2199), 374(2), 356(3), 314(3), 294(12), 276(40), 234(17), 216(100), 199(52); + ESMS *m*/*z* 491 (MK^+, 60%), 475 (MNa^+, 100).

(11) Data for **2**: colorless gum; $[\alpha]^{27}_D$ − 47°(*c* 20 mg/mL, $CHCl_3$); IR ν_{max} (film) 3412, 2927, 1734, 1715, 1247 cm^{-1}; NMR in Table 1; +EIMS *m*/*z* 376.2235 (M^+ − AcOH, 2%, calcd for $C_{22}H_{32}O_5$ 376.2250), 358(7), 340 (3), 298 (5), 278 (32), 218 (100), 201 (62).

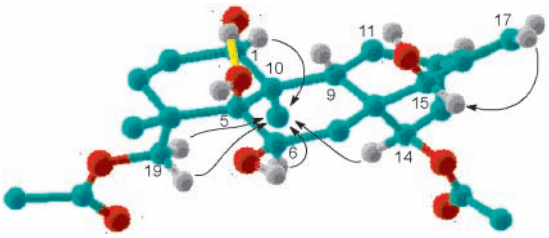
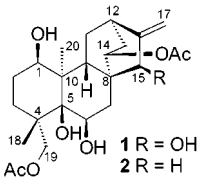


Figure 2. Predicted most stable conformation of atisane **1** from molecular modeling, showing selected NOE interactions (most protons omitted, hydrogen bond from 1-OH to 5-O).

H15−H9 interaction would be expected for the C15 configuration opposite to that shown in Figure 2.

The proposed structure for compound **1** was supported by molecular modeling.[12] Conformational searching found one preferred skeletal conformation (Figure 2) with several energetically accessible orientations of the hydroxyl groups. The chair conformations of the C1−C10 and C5−C10 rings accounted for all the experimental NOESY and $^1H-^1H$ coupling data for the protons in these rings (Table 1). The boat conformations of the rings linking C8 and C12 accounted for the other conformational data, e.g., the strong NOE interaction and the 10−12 Hz coupling between the eclipsed protons H14 and H13S (Table 1).



The less polar compound **2** gave an EI MS spectrum with a highest *m*/*z* ion at 378.2235 Da, indicating one less oxygen atom than compound **1**. The ^{13}C NMR spectrum of **2** showed one fewer oxygenated CH than **1** and one more CH_2 (Table 1). Full analysis of the NMR correlation spectra of **2** showed the same molecular connectivity as atisane **1**, but with no hydroxyl at C15. The chemical shifts and $^1H-^1H$ coupling data (Table 1), plus NOE interactions, showed that the stereochemistry of **2** was the same as that of **1** at the other chiral centers.

Compounds **1** and **2** are the first atisanes reported from liverworts[1] and the most oxygenated naturally occurring compounds with this skeleton. The most oxygenated atisane that we could find previously reported was a triol derivative.[13] Hexaol and pentaol derivatives **1** and **2** continue the pattern of highly oxygenated bioactive terpenoids in the genus *Lepidolaena*.[2,6,7]

The less polar atisane **2** showed slight cytotoxic activity against mouse leukemia cells (P388 IC_{50} 16 μg/mL)[2] and moderate insecticidal activity against blow fly larvae.[7] Compound **1** was less active in both assays. We note that the grayanotoxins, which are also highly oxygenated tetracyclic diterpenes, show insecticidal activity and mammalian toxicity.[14]

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Supporting Information Available: Tables of full NMR data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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