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Cytotoxic 8,9-Secokaurane Diterpenes from a New Zealand Liverwort, *Lepidolaena taylorii*

Nigel B. Perry*, Elaine J. Burgess and Raymond S. Tangney†

Plant Extracts Research Unit, New Zealand Institute for Crop & Food Research Limited, Department of Chemistry, University of Otago, Box 56, Dunedin, New Zealand

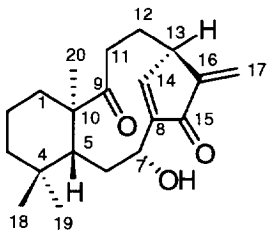
†Department of Botany, University of Otago, Box 56, Dunedin, New Zealand

Abstract: The main cytotoxic compound from the liverwort *Lepidolaena taylorii* is the known *ent*-8,9-seco-7-hydroxy-kaura-8(14),16-dien-9,15-dione **1**. The relative stereochemistry of a new cytotoxic compound, 16,17-dihydro derivative **2**, was supported by molecular modelling. Another new cytotoxic compound was identified as 8,14-epoxide **3** by preparation from **1**. These compounds showed differential cytotoxic activity against human tumor cell lines. Copyright © 1996 Elsevier Science Ltd

We are searching for bioactive natural products from New Zealand's native plants,[1] focussing on liverworts because of their rich array of secondary metabolites.[2] Crude extracts of the liverwort *Lepidolaena taylorii* (Gott.) Trev. (family Lepidolaenaceae) showed strong cytotoxic activity in P388 leukemia assays. There are no published reports of *Lepidolaena* chemistry. Bioactivity-directed fractionation led to three cytotoxic 8,9-secokauranes **1** - **3**. This is the first report of 8,9-secokauranes from any source other than the higher plant genus *Rabdosia* (family Lamiaceae).[3-5] The two most active compounds, rabdoumbrosanin **1** and epoxide **3**, showed differential cytotoxicity in the human disease oriented *in vitro* assays of the US National Cancer Institute (NCI).[6]

An extract of *L. taylorii* (4.05 g, P388 IC_{50} 1.3 μg/ml)[7] subjected to reverse phase (C18) flash chromatography gave cytotoxic activity (IC_{50}'s < 0.5 μg/ml) spread across fractions eluted with 1:1, 1:3 and 1:9 H_2O:MeCN. Silica TLC showed that these fractions were mostly complex mixtures. One of the most cytotoxic fractions (96 mg, 1:3 H_2O:MeCN, IC_{50} 0.06 μg/ml), which contained one major compound by ^1H NMR, was fractionated by silica column chromatography and C18 HPLC.[8] The major compound **1** was finally separated from a minor (10%) compound **2** by changing the C18 HPLC mobile phase from H_2O:MeCN to H_2O:MeOH.[9]

The high resolution MS of compound **1** was consistent with the molecular formula $C_{20}H_{28}O_3$.[10] The IR, ^1H and ^{13}C NMR spectra suggested the presence of -OH, two ketone C=O, a trisubstituted C=C bond, a 1,1-disubstituted $C=CH_2$, and three methyl singlets.[10] A search of the Chemical Abstracts Registry file retrieved only one compound which matched all these structural requirements. The spectral data, especially ^{13}C NMR shifts,



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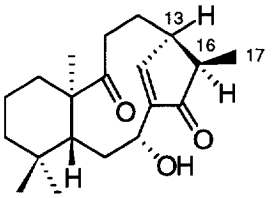

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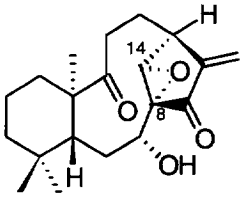

3

reported for rabdoumbrosanin **1** matched our cytotoxic compound from *L. taylorii*,[5,10] and the optical rotations showed the same absolute stereochemistry. Rabdoumbrosanin **1** is an *ent*-8,9-secokaurane diterpenoid which has only been reported once before, with no mention of biological activity, from the higher plant *Rabdosia umbrosa* (Maxim.) Hara.[5]

The MS of minor compound **2** was similar to the MS of **1**, but with the higher *m/z* ions all +2 Daltons and a molecular ion consistent with the formula $C_{20}H_{30}O_3$.[11] The ^{1}H NMR spectrum of **2** showed a methyl doublet, and lacked the signals for a 1,1-disubstituted C=CH_2 group,[11] suggesting that this compound was a 16,17-dihydro derivative **2** of rabdoumbrosanin **1**. No 16,17-dihydro-8,9-secokauranes have been reported previously, although 16,17-dihydrokaurane derivatives have been found in *Rabdosia* species.[4] The ^{13}C NMR spectrum of **2**[11] showed signals within 1 ppm of the signals assigned to C-1 to C-11 and C-18 to C-20 of **1**.[5] Therefore **2** was assigned the same relative stereochemistry as **1** at C-5, C-7, C-10 and C-13. The stereochemistry at C-16 of **2** was assigned by comparing proton NOE interactions and coupling data with the results of molecular modelling. Conformational searching and molecular mechanics minimisations[12] were used to predict the most stable conformations of the C-16 *R* and *S* configurations of **2**. The NMR data, particularly the H-16 to H-13 coupling of 7 Hz, best matched the C-16 *R* configuration of **2** (Fig. 1).

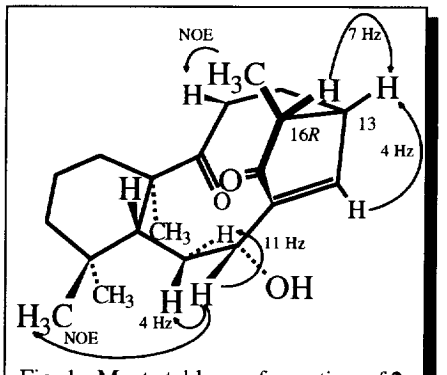

Fig. 1. Most stable conformation of **2** predicted by molecular modelling, plus selected NMR interactions.

In order to obtain more of compound **1** for biological testing (see below), a second cytotoxic fraction from C18 flash chromatography (220 mg, 1:3 H_2O:MeCN, IC_{50} 0.04 µg/ml) was subjected to preparative C18 HPLC.[13] In addition to **1** and **2**, compound **3** was obtained pure. High resolution MS[14] was consistent with the molecular formula $C_{20}H_{28}O_4$, with one more oxygen atom than **1**. The ^{1}H NMR spectrum of **3** was similar to that of **1**, but lacked the low-field H-14 signal and showed an additional singlet at 3.71 ppm. This suggested that **3** was the previously unreported 8,14-epoxide of **1**. This was proved by epoxidation of **1**, which occurred selectively at the trisubstituted 8,14 bond,[12] to give **3**.[15] Three 8,14-epoxy-8,9-secokauranes have been reported, and showed ^{1}H and ^{13}C NMR signals similar to **3**.[3,4] Molecular modelling of 8,9-secokauranes (Fig. 1) suggested

epoxidation was only possible on the "outside" face of the cyclopentenone to give the 14*R* stereochemistry shown for **3**.

All three 8,9-secokauranes from *L. taylorii* showed strong cytotoxic activity against P388 leukemia cells: **1** had an IC_{50} of 0.06 μg/ml (SD ± 0.02, n = 2), equivalent to 0.2 μM; **2** had an IC_{50} of 0.8 μg/ml (SD ± 0.2, n = 2); and **3** had an IC_{50} of 0.27 μg/ml (SD ± 0.06, n = 4). The *in vitro* cytotoxicity and *in vivo* antitumor activity reported for other 8,9-secokauranes has been ascribed to Michael addition of bio-nucleophiles to the α,β-unsaturated ketone group.[3] If this is the mode of action, it seems that either the exocyclic or the endocyclic double bond can undergo Michael addition, since both 16,17-dihydro derivative **2** and 8,14-epoxide **3** are cytotoxic. Compound **1** (containing 10% of **2**) and compound **3** showed differential cytotoxicity in *in vitro* human tumor assays.[6] Growth of five leukemia cell lines was inhibited (mean IC_{50} of 0.4 μM for **1**) but the cells were not killed (mean LC_{50} > 50 μM). By contrast, other tumor cell lines were killed. For example, the mean LC_{50} of **1** against seven colon cancer cell lines was 6 μM. Compounds **1** and **3** are now being evaluated for *in vivo* antitumor activity. We are continuing investigations of the other cytotoxic compounds in *L. taylorii*.

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7. Collected from Bruce Bay, West Coast, South Island, New Zealand in June 1995 (University of Otago Herbarium number OTA 6803). Dried liverwort (100 g) was extracted with EtOH then $CHCl_3$. For the P388 assay method, see Lorimer *et al.*.[1]
8. Flash chromatography on silica with 3:1 cyclohexane:EtOAc; followed by C18 HPLC (Merck Lichrospher 100 RP-18 10 μm, 250 x 10 mm, mobile phase 5 ml/min 1:3 H_2O:MeCN, detection at 206 nm): peak retention time (RT) 4.7 min (**1** plus **2**, 12 mg).
9. C18 HPLC as above except with 3:7 H_2O:MeOH: peak RTs 10.8 min (**1**) and 12.4 min (**2**).

10. *Rabdoumbrosanin (Registry No. 123941-77-5)* **1**.- colourless oil; silica TLC R_F 0.3 (2:1 cyclohexane:EtOAc, UV plus yellow with anisaldehyde dip); $[\alpha]^{21}_D$ -66° (*c* 0.5, MeOH); UV (MeOH) λ max (log ε) 245 (3.71) nm; IR (film) ν max 3442 (br, OH), 2948 (CH), 1694 (both C=O), 1646 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.24 (1 H, br d, 2 Hz, H-14), 6.12 (1 H, br s, H-17), 5.43 (1 H, s, H-17), 4.68 (1 H, dd, 12, 5 Hz, H-7), 3.59 (1 H, br m, H-13), 2.58 (1 H, td, 12, 4 Hz, H-12), 2.3 (1 H, m, H-11), 1.9-1.1 (m), 1.00 (3 H, s), 0.94 (3 H, s), 0.92 (3 H, s), 0.88 (1 H, dd, 6, 2 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 215.3 (s, C-9), 195.2 (s, C-15), 159.7 (d, C-14), 148.5 (s, C-16), 146.0 (s, C-8), 116.9 (t, C-17), 64.4 (d, C-7), 53.9 (s, C-10, 43.5 (d, C-5), 42.4 (d, C-13), 41.4 (t, C-3), 36.7 (t, C-6), 34.7 (s, C-4), 34.1 (t, C-1), 33.7 (q, C-18), 30.8 (t, C-11), 25.9 (t, C-12), 22.4 (q, C-19), 18.1 (t, C-2), 16.6 (q, C-20); EIMS (70 eV) *m/z* 316.2042 (M^+, 3%, $C_{20}H_{28}O_3$ req 316.2038), 301 (2), 298 (9), 283 (4), 242 (6), 192 (48), 179 (100), 151 (18), 138 (31), 123 (70).

11. *16,17-Dihydro-rabdoumbrosanin* **2**.- colourless oil; silica TLC R_F 0.2 (2:1 cyclohexane:EtOAc, purple spot with anisaldehyde dip); $[\alpha]^{21}_D$ -11° (*c* 0.15, $CHCl_3$); UV (MeOH) λ max (log ε) 213 (4.58) nm; IR (film) ν max 3436 (br, OH), 2942 (CH), 1697 (both C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34 (1 H, br d, 3 Hz, H-14), 4.58 (1 H, dd, 12, 5 Hz, H-7), 3.20 (1 H, br m, H-13), 2.50 (1 H, quintet, 7 Hz, H-16), 2.42 (1 H, m, H-12), 2.02 (1 H, br dd, 18, 13 Hz, H-11), 1.9-1.2 (m), 1.14 (3 H, d, 7 Hz, H-17), 1.04 (3 H, s, H-18), 0.95 (3 H, s, H-20), 0.92 (3 H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 214.4 (s, C-9), 210.0 (s, C-15), 162.7 (d, C-14), 145.3 (s, C-8), 64.3 (d, C-7), 53.8 (s, C-10), 44.7 (d, C-16), 42.7 (d, C-5), 42.3 (d, C-13), 41.3 (t, C-3), 36.9 (t, C-6), 34.5 (s, C-4), 34.3 (t, C-1), 33.4 (q, C-18), 31.4 (t, C-11), 22.3 (q, C-19), 21.2 (t, C-12), 18.1 (t, C-2), 16.4 (q, C-20), 10.0 (q, C-17); EIMS (70 eV) *m/z* 318.2192 $[M]^+$ (3%,$C_{20}H_{30}O_3$ req 318.2195), 300 (6), 244 (12), 194 (24), 181 (100), 138 (55), 123 (99).

12. For modelling methods, see Hinkley, S. F.; Perry, N. B.; Weavers, R. T. *Phytochemistry* **1994**, 35, 1489-1494.

13. C18 HPLC as above except with 2:3 H_2O:MeCN: peak RTs 7.4 min (**1** plus **2**, 18 mg) and 9.7 min (**3**, 26 mg).

14. *8,14-Epoxy-rabdoumbrosanin* **3**.- colourless oil; silica TLC R_F 0.5 (2:1 cyclohexane:EtOAc, UV spot); $[\alpha]^{25}_D$ -53° (*c* 0.46, MeOH); UV (MeOH) λ max (log ε) 235 (3.57) nm; IR (film) ν max 3490 (br, OH), 2951 (CH), 1726 (C-15=O), 1694 (C-9=O), 1643 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.29 (1 H, br s, H-17), 5.53 (1 H, d, 1 Hz, H-17), 4.61 (1 H, dd, 12, 5 Hz, H-7), 3.71 (1 H, s, H-14), 3.22 (1 H, br m, H-13), 2.65 (2 H, m), 2.0-1.1 (m), 1.06 (3 H, s), 0.99 (3 H, s), 0.94 (3 H, s); ^{13}C NMR δ 214.5 (s, C-9), 196.9 (s, C-15), 145.0 (s, C-16), 123.0 (t, C-17), 64.6 (s, C-8), 61.9 (d, C-14), 60.6 (d, C-7), 53.7 (s, C-10), 42.0 (d, C-5), 41.5 (t, C-3), 40.0 (d, C-13), 34.9 (s, C-4), 33.9 (t, C-6), 33.4 (q, C-18), 33.2 (t, C-1), 31.4 (t, C-11), 25.2 (t, C-12), 22.0 (q, C-19), 18.1 (t, C-2), 16.4 (q, C-20); EIMS (70 eV) *m/z* 332.1987 (M^+, 1%, $C_{20}H_{28}O_4$ req 332.1988), 314 (5), 299 (3), 258 (5), 209 (17), 195 (8), 192 (8), 179 (20), 178 (14), 165 (10), 150 (18), 149 (11), 138 (11), 137 (20), 123 (100).

15. *m*-Chloroperoxybenzoic acid (2.7 mg) was added to **1** (5 mg) dissolved in CH_2Cl_2 (1 ml), and stirred with aq $NaHCO_3$ (0.5 M, 0.3 ml). After 2 days, silica TLC and ^1H NMR spectroscopy showed conversion to **3**.

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