



11-Oxygenated cytotoxic 8,9-secokauranes from a New Zealand liverwort, *Lepidolaena taylorii*

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Abstract

Four new cytotoxic 8,9-secokauranes have been identified from the liverwort *Lepidolaena taylorii*. The 11-oxygenation found in three of these has not been encountered in the 8,9-secokauranes known from higher plants. NMR studies were combined with molecular modelling to determine the preferred conformations. Six structurally related kauren-15-ones were also found, including three new compounds. Some of these compounds showed differential cytotoxic activity against human tumor cell lines. The probable mode of cytotoxic action was supported by Michael addition of a thiol. Two 8,9-secokauranes were the main cytotoxins in another New Zealand liverwort, *L. palpebrifolia*. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Lepidolaena taylorii*; *L. palpebrifolia*; Liverworts; Bioactive plant products; Cytotoxicity; Diterpenes; 8,9-Secokauranes; Kauren-15-ones

1. Introduction

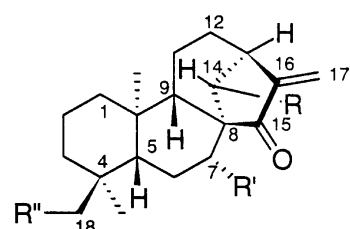
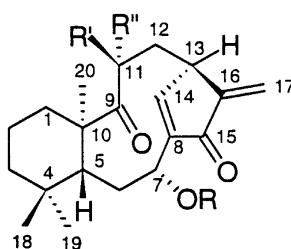
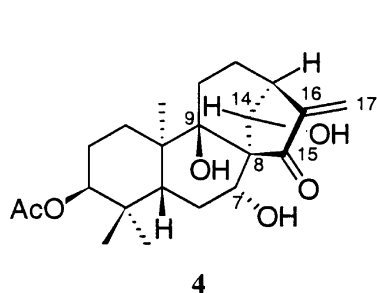
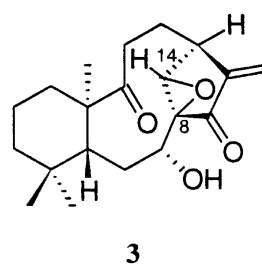
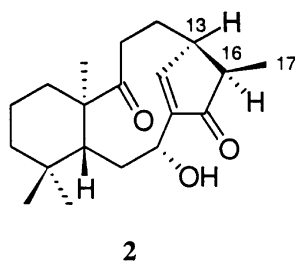
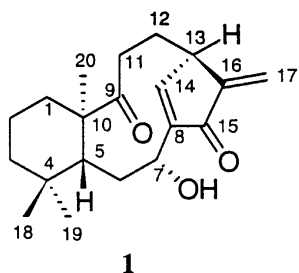
Liverworts are the structurally simplest of the terrestrial plants, but they contain a complex array of secondary metabolites (Asakawa, 1995). Diterpenes are often found, including a range of oxygenated kauranes (Buchanan, Connolly, Kadir, & Rycroft, 1996; Nagashima, Tanaka, Takaoka, & Asakawa, 1996; Lorimer, Perry, Burgess, & Foster, 1997). We recently reported the bioactivity-directed isolation of three cytotoxic diterpenes from the leafy liverwort *Lepidolaena taylorii* (Gott.) Trev. 8,9-Secokaurane (**1**) was the main cytotoxin, with the less toxic dihydro and epoxy derivatives **2** and **3** present at lower concentrations (Perry, Burgess, & Tangney, 1996).

The only other reported source of 8,9-secokauranes is the higher plant genus *Rabdosia* (or *Isodon*, Labiatae) (Fujita & Node, 1984; Takeda & Otsuka, 1995). Rabdoubrosanin **1** was first isolated from *Rabdosia umbrosa* (Maxim.) Hara, along with other 8,9-secokauranes and the 7,9,14-trihydroxykauren-15-one shikoccidin **4** (Takeda, Ichihara, Fujita, & Ueno, 1989). The 8,9-seco-

kauranes are thought to be derived biosynthetically from 7,9,14-trihydroxykauren-15-ones by a retrograde aldol reaction cleaving the 8,9 bonds and loss of H₂O to give the 8,14-double bond (Fujita & Node, 1984). The reverse 8,9 cyclization was achieved by Backhaus and Paquette (1997), as an adjunct to their synthesis of 8,9-secokauranes (Paquette, Backhaus, & Braun, 1996).

Eight liverwort species have been assigned to the genus *Lepidolaena* (family Lepidolaenaceae), with *L. taylorii* being the most common of these in New Zealand (Grolle, 1967). This endemic species is widely distributed throughout the country, especially in wet forest (Allison & Child, 1975). The only other report on *Lepidolaena* chemistry is of several sesquiterpenes, including a new bergamotane diacetate, from another New Zealand species *L. clavigera* (Hook.) Dum. ex Trev. (Asakawa, Toyota, Nakaishi, & Tada, 1996). We now report the isolation of four new 8,9-secokauranes from *L. taylorii*, plus a series of kauren-15-ones. The cytotoxic activities of all these compounds are described, and related to their structures. Results are presented showing differential cytotoxicity in the human disease oriented in vitro assays of the US National Cancer Institute (NCI) (Boyd & Paull, 1995). The presumed mode of cytotoxic action, Michael addition of biological nucleophiles (Fujita, Nagao, Kaneko, Nakazawa, &

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5 R=Ac, R'=R''=H

6 R=Ac, R'=OH, R''=H

7 R=Ac, R'=H, R''=OH

8 R=H, R'=OAc, R''=H

9 R=H, R'=OH, R''=H

10 R=R'=OH, R''=H

11 R=R'=R''=OH

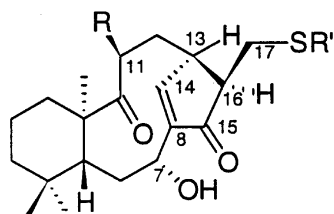
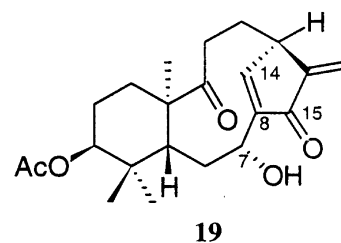
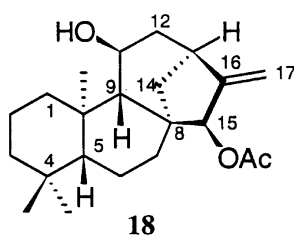
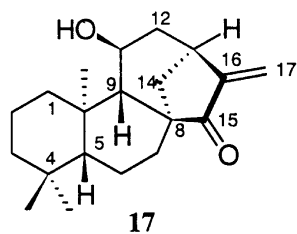
12 R=R'=R''=H

13 R=OH, R'=OAc, R''=H

14 R=OAc, R'=OH, R''=H

15 R=R''=H, R'=OH

16 R=OH, R'=R''=H



20 R=H, R'=Ph

21 R=OH, R'=Me

Kuroda, 1976), is discussed in relation to the addition of thiophenol to **1**. 8,9-Secokauranes **1** and **3** were found to be the main cytotoxins in another New Zealand endemic liverwort, *L. palpebrifolia* (Hook.) Dum. ex Trev.

2. Results and discussion

2.1. NMR assignments and conformation of **1**

The ^1H and ^{13}C NMR spectra of rabdoumbrosanin **1** were fully assigned in order to assist with the structure determination of new 8,9-secokauranes (Takeda et al. assigned the ^{13}C NMR spectrum and the absolute configuration of **1** by comparison with other 8,9-secokauranes (Takeda et al., 1989)). ^1H – ^1H (COSY) and one bond (HMQC) and two/three bond (HMBC) ^1H – ^{13}C correlation spectra were combined to give the assignments in Tables 1 and 2. Assignments of the diastereotopic proton signals at C-6, C-11 and C-12 were assisted by molecular modelling. Molecular mechanic calculations (MM2 force field (Allinger, 1977)) and conformational searching (MacroModel Monte Carlo method (Mohamadi et al., 1990)) were used to predict the solution conformation of **1**. The two most stable conformers, predicted to be >98% populated in the gas

phase, differed only in orientation of the C-7 hydroxyl group. They had the same skeletal conformation as found in the crystal structure of another 8,9-secokaurane, shikoccin monoacetate (Fujita et al., 1979), with the six-membered ring in a chair conformation and the C-9 carbonyl oxygen *syn* with the C-10 methyl (Fig. 1). H-7 had a dihedral angle of about 180° to the pro-*R* H-6 (Fig. 1), so the H-6 signal with two 12 Hz couplings was assigned as pro-*R* (Table 1). Likewise, the pro-*S* H-11 was predicted to have a dihedral angle of about 180° to one proton on C-12 (H* in Fig. 1), so the H-11 signal at 2.30 ppm and the H-12 signal at 2.58 ppm with 12 Hz vicinal coupling were assigned to these protons (Table 1). The modelling also suggested that these same protons would be in the plane of the C-9 carbonyl group (Fig. 1), which explained their deshielding relative to their geminal partners. Our ^{13}C NMR assignments (Table 2) matched those of Takeda et al. (1989), except for a reversal of the C-8 and C-16 assignments based on HMBC correlations.

2.2. Other 8,9-secokauranes

Four new 8,9-secokauranes were discovered during the isolation of larger quantities of **1** and **3** for in vivo anti-tumour testing at the NCI (see below). We found that a

Table 1
 ^1H NMR data for 8,9-secokauranes^a

Proton	1	5	6	8	9^d
1 (ax)	1.66 (tm) ^b	1.65 (NR) ^b	2.55 (td, 12, 4)	NR	2.90 (td, 13, 5)
1 (eq)	1.24 (d) ^b	1.28 (NR) ^b	1.2 (d) ^b	NR	1.30 (NR)
2	1.46 (NR) ^c	1.46 (NR) ^b	1.3–1.5 (NR)	NR	1.5–1.9 (NR)
3 (eq)	1.42 (dm) ^b	1.44 (dm) ^b	1.2–1.4 (NR) ^b	NR	NR
3 (ax)	1.2 (t) ^b	1.2 (NR) ^b	1.2–1.4 (NR) ^b	NR	NR
5	0.88 (dd, 6, 2)	0.93 (NR) ^b	1.9 (NR) ^b	1.73 (dd, 6, 2)	2.22 (NR)
6S	1.86 (dt, 14, 6)	1.94 (ddd, 13, 6, 5)	1.9 (ddd, 12, 6, 4)	1.90 (ddd, 13, 6, 5)	1.96 (ddd, 14, 7, 5)
6R	1.27 (td, 12, 2)	1.3 (NR) ^b	1.4 (br t, 12)	1.45 (NR)	1.55 (NR)
7	4.68 (dd, 12, 5)	5.51 (dd, 12, 5)	5.47 (dd, 12, 4)	4.71 (dd, 12, 4)	4.69 (dd, 12, 5)
11R	1.75 (d) ^b	1.8 (NR) ^b	4.40 (ddd, 5, 3, 1)	5.23 (dd, 5, 1)	4.52 (dd, 5, 1)
11S	2.30 (ddm, 17, 12)	2.34 (NR) ^b	–	–	–
12R	2.58 (td, 12, 4)	2.61 (td, 12, 4)	2.99 (ddd, 15, 5, 1)	2.91 (ddd, 15, 5, 2)	2.98 (ddd, 14, 5, 2)
12S	1.74 (NR) ^b	1.79 (dm, 15)	2.07 (ddd, 15, 5, 3)	2.32 (ddd, 15, 6, 3)	2.30 (ddd, 14, 5, 3)
13	3.59 (br m)	3.62 (br m)	3.60 (br m)	3.57 (br m)	3.70 (br m)
14	7.24 (br d, 2)	7.19 (br d, 3)	7.13 (br d, 2)	7.25 (br d, 3)	7.20 (br d, 3)
17 (E)	5.43 (br s)	5.45 (br s)	5.35 (br s)	5.24 (br s)	5.41 (t, 1)
17 (Z)	6.12 (br s)	6.16 (br s)	5.95 (br s)	5.88 (br s)	5.83 (q, 1)
18 (eq)	1.00 (s)	1.10 (s)	1.05 (s)	1.03 (s)	1.13 (s) ^e
19 (ax)	0.92 (s)	0.93 (s)	0.91 (s)	0.95 (s)	1.090 (s) ^e
20	0.94(s)	0.96 (s)	0.99 (s)	1.01 (s)	1.094 (s) ^e
7-OAc	–	2.00 (s)	1.97 (s)	–	–
11-OAc	–	–	–	2.00 (s)	–
11-OH	–	–	1.39 (d, 3)	–	–

^aIn CDCl_3 unless otherwise stated; shift in ppm (multiplicity, coupling in Hz).

^bShift and multiplicity from HMQC spectrum.

^cNot resolved.

^dIn $(\text{CD}_3)_2\text{CO}$.

^eAssignments interchangeable within column.

Table 2
¹³C NMR data for 8,9-secokauranes^a

Carbon	1 ^b	5 ^b	6 ^b	8	9 ^c
1	34.1	34.1	32.1	31.8	33.3
2	18.1	18.1	18.1	17.9	19.3
3	41.4	41.3	40.7	41.5	42.0
4	34.7	34.7	34.2	34.3	35.2
5	43.5	43.2	39.6	40.6	42.5
6	36.7	33.1	33.5	32.4	36.4
7	64.4	67.0	66.6	63.8	64.3
8	148.5	144.9	145.1	148.5	150.1
9	215.3	215.4	215.2	212.2	216.7
10	53.9	53.9	54.9	54.7	55.6
11	30.8	30.9	78.1	77.7	78.4
12	25.9	25.9	35.6	37.1	38.6
13	42.4	42.5	41.2	41.0	40.8
14	159.7	159.8	158.4	159.1	158.2
15	195.2	194.2	193.6	194.7	195.2
16	146.0	145.6	149.1	148.2	151.2
17	116.9	117.2	112.9	113.0	111.4
18 (eq)	33.7	33.5	33.6	34.1	34.5
19 (ax)	22.4	22.4	22.2	22.2	23.0
20	16.6	16.6	18.1	18.3	19.0
OCOCH ₃	–	169.9	169.9	169.1	–
OCOCH ₃	–	21.1	21.1	20.8	–

^aIn CDCl₃ unless otherwise stated; shift in ppm.

^bAssigned by HMQC and HMBC experiments.

^cIn (CD₃)₂CO.

Soxhlet extraction with CHCl₃ gave improved recovery of **1** and **3** from plant material, compared to our previous extraction with cold EtOH (Perry et al., 1996). Analytical reversed-phase (RP) HPLC revealed the presence of a range of other UV-active compounds. These were purified by further chromatography, especially preparative

HPLC. Four compounds were recognised as 8,9-secokauranes by the characteristic ¹H NMR signal of H-14, a 2–3 Hz doublet at 7.13–7.25 ppm (Table 1).

One of these compounds was readily identified as acetate **5**, since its ¹H NMR spectrum was very similar to that of **1**, apart from an acetate signal and deshielding of the signal of H-7 to δ 5.51 (δ 4.68 in **1**; see Table 1). Acetylation of **1** confirmed the assignment of structure **5**, previously unreported.

A second new compound **6** gave a ¹H NMR spectrum which also contained an acetate signal and a signal appropriate for H-7 at δ 5.47. However, the ¹H NMR and ¹³C NMR spectra showed the presence of an additional oxygenated methyne and one less sp³ methylene, compared to **5** (Tables 1–2). The mass spectrum of **6** supported the molecular formula C₂₂H₃₀O₅ (**5** is C₂₂H₃₀O₄). Several of the known 8,9-secokauranes have acetate or hydroxyl groups at C-1 or C-3 (Fujita & Node, 1984; Takeda & Otsuka, 1995), but mass spectral and ¹³C NMR evidence suggested that this was not the case in **6**. The mass spectra of **1**, **2** and **3** showed strong ions at *m/z* 138 and 123 (Perry et al., 1996), due to cleavage of the 9,10-bond and either the 6,7-bond to give C₁₀H₁₈⁺, or the 5,6-bond to give C₉H₁₅⁺. These same ions were present in the mass spectrum of **6**, suggesting a non-oxygenated 6-membered ring. In addition, all the signals assigned to this ring in **1** and **5** were closely matched in the ¹³C NMR spectrum of **6** (Table 2). The structure was fully determined by NMR spectroscopy, using COSY, HMQC and HMBC experiments. Two particularly important HMBC correlations were from one oxygenated methyne proton signal (δ 4.40, assigned to H-11) to carbon signals at δ 41.2 (C-13) and δ 215.2 (C-9). Analysis of the full set of correlations showed **6** to be an 8,9-secokaurane with a C-7 acetate and a C-11 hydroxyl. None of the previously reported 8,9-secokauranes are oxygenated at C-11 (Fujita & Node, 1984; Takeda & Otsuka, 1995).

The relative stereochemistry of **6** was determined by a combination of NMR spectroscopy and molecular modelling (the absolute stereochemistry at C-1 was assumed to be the same as for rabdoubrosanin **1**). The similarities in the NMR spectra of **1**, **5** and **6** (Tables 1–2) suggested that these compounds had the same configurations at C-5, C-7 and C-13. Both *R*- and *S*-configurations have been reported for *ent*-kauranes oxygenated at C-11 (Fujita & Node, 1984; Takeda & Otsuka, 1995), so two structures were modelled: 11*S*-**6** and 11*R*-**7**. The most stable conformations predicted for both **6** and **7** had the same skeletal conformation as found for **1** (Fig. 1). The predicted proton–proton couplings and distances for **6** and **7** were compared with the experimental couplings and NOE interaction data. These were consistent with the proposed configurations at C-5, C-7 and C-13. The *R*-configuration at C-11, i.e. **7**, would place H-11 *anti* to one of the C-12 protons, leading to one predicted proton–proton coupling of 12 Hz for H-

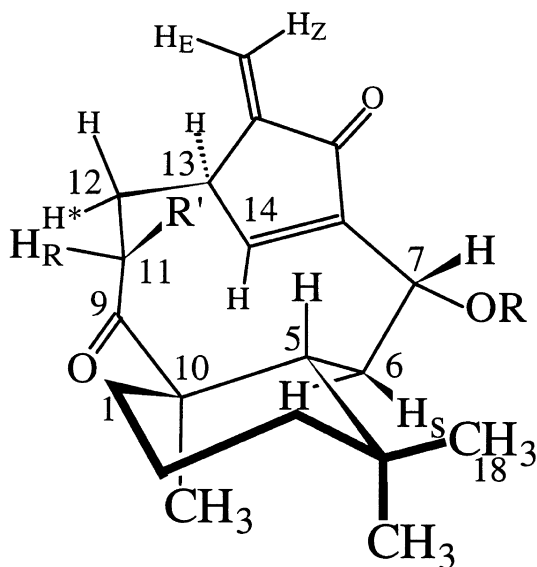


Fig. 1. Conformations of **1** (R = R' = H) and **6** (R = Ac, R' = OH) from molecular modelling.

11. In fact, H-11 of the natural product showed couplings of 1 and 5 Hz to the C-12 protons (Table 1), consistent with the predicted conformation of **6**, with H-11 almost at a 90° dihedral angle to one C-12 proton (Fig. 1). Structure **6** was further supported by the observed NOE interactions between H-11 and both C-12 protons, whereas structure **7** would only lead to an NOE interaction between H-11 and the one C-12 proton in a *gauche* relationship.

A third new compound **8** had a similar mass spectrum to **6**, with the same $C_{22}H_{30}O_5$ molecular ion and prominent losses of H_2O and $AcOH$. The ^{13}C NMR spectra of **6** and **8** were also similar (Table 2), so **8** was an isomeric 11-oxygenated 8,9-secokaurane. The 1H NMR spectrum of **8** showed a signal appropriate for H-7 at δ 4.71 very similar to the signal of H-7 in **1** (Table 1). The H-11 signal of **8** (δ 5.23) was deshielded relative to H-7 in **6** (δ 4.40). Therefore **8** had a C-7 hydroxyl and a C-11 acetate. The H-11 coupling constants showed the *S*-configuration at C-11.

The most polar compound **9** purified from *L. taylorii* had NMR and mass spectra consistent with the molecular formula $C_{20}H_{28}O_4$. The NMR spectra, of a $(CD_3)_2CO$ solution, were very similar to those of the other 8,9-secokauranes (Tables 1–2). A signal at δ 4.69 closely matched the H-7 signals of **1** and **8** and a signal at δ 4.52 closely matched the H-11 signal of **6**. Therefore **9** had hydroxyls at both C-11 and C-7, again with the *S*-configuration at C-11.

2.3. Kauren-15-ones

In view of the co-occurrence of 8,9-secokauranes with kauren-15-ones in *Rabdosia* species (Fujita & Node, 1984; Takeda et al., 1989; Takeda & Otsuka, 1995), we searched for these possible biosynthetic intermediates in *L. taylorii*. Several of our liverwort metabolites showed 1H NMR signals typical of a $C=CH_2$ group conjugated to a carbonyl, but lacked the characteristic low field H-14 signal of the 8,9-secokauranes (Table 3). The most polar of these compounds, **10**, had NMR and mass spectra consistent with the molecular formula $C_{20}H_{30}O_3$. The ^{13}C NMR spectrum (Table 3) showed only one carbonyl signal, plus the expected $C=CH_2$ group, so four rings were indicated. The DEPT spectra showed 28 protons attached to carbons, so **10** was a diol. Therefore, it seemed likely that **10** was a dihydroxykauren-15-one, with the same carbon skeleton as shikoccidin **4**. A combination of COSY, HMQC and HMBC spectra confirmed this skeleton, with $CHOH$ groups at C-7 and C-14. A search of the Chemical Abstracts registry file retrieved some 7,14-dihydroxykauren-15-ones with further oxygenation, which are often found in *Rabdosia* species (Fujita & Node, 1984; Takeda & Otsuka, 1995). The close similarity of our 1H NMR and ^{13}C NMR data on **10** (Table 3) with published data on compounds such as excisanin C (**11**) (Takeda & Otsuka, 1995) showed that the relative stereochemistry was the same.

The absolute stereochemistry of this new compound **10** was assumed to be *ent*, on the basis of the co-occurrence with *ent*-kauren-15-one (**12**).

We first detected kauren-15-one in *L. taylorii* by GC–MS analysis of a crude extract. A range of common plant volatiles, especially sesquiterpenes, were also detected, along with kaurene. The main GC–MS peak had a mass spectrum appropriate for kauren-15-one (**12**), which has been found in several other liverwort species (Asakawa, 1995). Compound **12** was purified from a bulk extract of *L. taylorii* and its identity confirmed by direct comparison of its 1H NMR spectrum with that of a sample isolated from the liverwort *Jungermannia exsertifolia* ssp. *cordifolia* (Nagashima et al., 1996). The ^{13}C NMR spectrum of **12** matched that recently published (Fraga, Gonzalez, Guillermo, Hernandez, & Perales, 1995) and the optical rotation confirmed the *ent* absolute stereochemistry.

Two further kauren-15-ones were not well separated on RP HPLC, but silica gel HPLC gave pure samples. Both compounds showed single acetate signals in their 1H NMR spectra, and mass spectra supported the same molecular formula, $C_{22}H_{32}O_4$. These mass spectra showed quite strong ions due to the loss of $CH_2=C=O$. Compound **13** was readily assigned as the 7-acetate of **10** (previously unreported) because of the similarity of their 1H and ^{13}C NMR spectra (Table 3), apart from the diagnostic downfield shift for H-7 in **13**. The isomeric compound was tentatively assigned as the 14-acetate **14**, but it could not be fully characterised because it gradually isomerised to **13** in $CDCl_3$ solution. Molecular modelling was used to predict the most stable conformations of structures **13** and **14**. The lowest energy conformation of **13** included an intramolecular hydrogen bond from 14-OH to 7-O, which explained the observation of a sharp singlet for 14-OH in the 1H NMR spectrum of **13** (Table 3). The lowest energy conformation of **14** had 7-O only 2.9 Å from the carbonyl carbon of the C-14 acetate, which explained the facile trans-esterification of **14** to **13**. The lowest energy conformation of **13** was calculated (MM2* force field (Allinger, 1977; Mohamadi et al., 1990) to be about 2 kJ/mol more stable than the lowest energy conformation of **14**.

Two monohydroxykauren-15-ones, $C_{20}H_{30}O_2$, were obtained pure. One of these compounds showed a secondary carbinol signal at δ 4.06 (dd, 12 and 4 Hz) appropriate for 7-hydroxykauren-15-one **15** (compare **10**, Table 3). This compound has been reported once before, from the liverwort *Jungermannia truncata*, with spectroscopic data and optical rotation matching ours (Buchanan et al., 1996). The other monohydroxy compound showed a secondary carbinol signal at δ 4.55 (br s) appropriate for 14-hydroxykauren-15-one (**16**) (compare **10** and **13**, Table 3). This structure, previously unreported, was confirmed by comparison of its ^{13}C NMR spectrum with the spectra of **10** and **13** (Table 3) and of **12** (Fraga et al., 1995).

Table 3
¹H and ¹³C NMR data for kauren-15-ones^a

Position	10 (¹ H)	10 (¹³ C)	13 (¹ H)	13 (¹³ C)	16 (¹ H)	16 (¹³ C)
1 (ax)	0.69 (td, 14, 4)	39.6	0.74 (td, 13, 4)	39.5	0.76 (td, 13, 4)	39.7
1 (eq)	1.7 (NR) ^b	—	1.74 (NR)	—	1.7 (NR)	—
2	0.79, 1.43 (NR) ^b	18.6 ^c	NR	18.5 ^c	NR	18.6 ^c
3	1.13, 1.40 (NR) ^b	41.6	NR	41.5	NR	41.7
4	—	33.3	—	33.3	—	33.3
5	0.92 (dd, 12, 2)	53.7	NR	53.3	0.95 (dd, 12, 2)	55.1 ^f
6	1.92, 1.98 (NR) ^b	28.0	1.6, 2.04 (NR)	25.3	NR	25.3
7	4.35 (dd, 12, 4)	75.3	5.42 (dd, 12, 4)	76.8	NR	18.3
8	—	62.1	—	61.6	—	59.0
9	1.18 (dd, 15, 8)	54.4	NR	55.0	NR	55.4 ^f
10	—	40.1	—	40.1	—	40.2
11	0.93 (NR) ^b	17.5 ^c	NR	17.3 ^c	NR	17.8 ^c
12	1.77, 1.97 (NR) ^b	31.2	NR	31.2	1.8, 2.05 (NR)	32.4
13	3.05 (br m)	46.1	3.06 (br m)	45.9	3.04 (br m)	46.5
14	4.88 (d, 0.5)	75.0	4.88 (br s)	74.5	4.55 (br s)	73.7
15	—	208.1	—	205.2	—	209.2
16	—	147.7	—	147.1	—	146.9
17 (E)	5.39 (br s)	118.0	5.39 (br s)	118.2	5.34 (br t, 1)	117.2
17 (Z)	6.16 (br s)	—	6.14 (br s)	—	6.10 (br s)	—
18 (eq)	0.90 (s)	33.6	0.91 (s)	33.4	0.88 (s)	33.6
19 (ax)	0.84 (s)	21.7	0.82 (s)	21.7	0.81 (s)	21.6
20	1.05 (s)	18.2	1.07 (s)	18.2	1.02 (s)	17.9
7-OAc	—	—	2.00 (s)	168.2, 21.4	—	—
14-OH	NO ^d	—	4.03 (s)	—	NO	—

^aIn CDCl₃; shift in ppm (multiplicity, coupling in Hz).

^bShift and multiplicity from HMQC spectrum.

^cNot resolved.

^dNot observed.

^eAssignments interchangeable within column.

^fAssignments interchangeable within column.

2.4. Cytotoxic activity

We have tested all the 8,9-secokauranes and kaurenes obtained pure from *L. taylorii* against P388 leukemia cells (Table 4). The results on two related kaurene derivatives **17** and **18**, purified by Nagashima et al. (1996) from the liverwort *Jungermannia exsertifolia* ssp. *cordifolia*, are also included.

Our in vitro P388 results (Table 4) compare well with the results of Fuji et al. (1985) on a series of 8,9-secokauranes and kauren-15-ones from a *Rabdosia* species, tested against HeLa cells. Their most active compound was shikoccin (**19**), with a GI₅₀ of 0.08 µg/ml. 7-Methoxy and 8,14-epoxy derivatives of **19** were less cytotoxic (GI₅₀s of 0.30 and 0.25 µg/ml), as were our 7-acetoxy **5** and 8,14-epoxy **3** derivatives of **1** (Table 4). Fuji et al. (1985) also found that kauren-15-ones hydroxylated at C-7 and C-14, such as shikoccidin (**4**) (GI₅₀ 0.10 µg/ml), were only a little less cytotoxic than 8,9-secokauranes.

The results of testing five of the *L. taylorii* 8,9-secokauranes against the NCI's panel of cell lines are also summarised in Table 4. This panel contains 60 human tumor cell lines, including six leukemia cell lines and a range of organ-specific cancers (Boyd & Paull, 1995).

Table 4
 Cytotoxicity of 8,9-secokauranes and kauren-15-ones^a

Compound	Mouse P388 leukemia		Human tumors
	µg/ml ^b	µM ^b	µM ^c
1	0.10 (0.05)	0.3	1.2 (0.11 to 7.44)
20	0.156 (0.002)	0.4	NT ^d
9	0.165 (0.002)	0.5	1.5 (0.04 to 16.2)
13	0.22 (0.03)	0.6	NT
3	0.27 (0.06)	0.8	2.5 (0.29 to 20.2)
10	0.3 (0.1)	0.9	NT
8	0.345 (0.006)	0.9	NT
15	0.37 (0.07)	1.2	NT
17	0.48 (0.06)	1.6	NT
5	0.7 (0.5)	1.9	NT
16	0.8 (0.2)	2.6	NT
12	1.1 (0.5)	3.7	NT
6	1.2 (0.6)	3.2	11.0 (4 to >25)
2	1.9 (1.3)	5.9	21.4 (0.99 to >100)
18	>25	>72	NT

^aValues are GI₅₀s, i.e. concentration that inhibited growth to 50% of control.

^bMean from at least two separate assays (standard deviation).

^cMean from 60 different human tumor cell lines (range).

^dNot tested.

There was a good correlation between the activity against mouse P388 leukemia cells and the mean activity against the NCI cell lines ($r=0.99$ for log-transformed data). However, the 8,9-secokauranes were not indiscriminant cell poisons, since they showed quite wide ranges of cytotoxic activity against the different human tumor cell lines (Table 4). For example, compound **9** was most active against the leukemia cell lines (mean GI_{50} 0.3 μ M) and least active against the central nervous system cancer cell lines (mean GI_{50} 6 μ M). The activity profiles (GI_{50} data) of compounds **1** and **3** against the 60 human tumor cell lines were searched against the NCI database of active compounds, using the 'COMPARE' algorithm. The aim was to look for compounds that might have similar mechanisms of cytotoxic action (for a detailed discussion, see Boyd and Paull (1995)). The usefulness of this approach was supported when the profile of **1** showed high COMPARE correlations with both **3** and with another 8,9-secokaurane submitted independently, shikoccin (**19**) from *Rabdosia* species (Takeda et al., 1989). The other classes of natural products that showed high COMPARE correlations with **1** and **3** were α -methylene lactones (both sesquiterpenes and diterpenes) and quinones, plus some α,β -unsaturated or α,β -epoxy ketones. These compounds are known Michael acceptors, which probably act by alkylating cellular thiols (Kupchan, Fessler, Eakin, & Giacobbe, 1970; Ahn & Sok, 1996). Therefore this is likely to be the mode of cytotoxic action for the 8,9-secokauranes.

Fujita et al. (1976) found that a cytotoxic kauren-15-one from *Rabdosia* reacted with butane thiol to give a 17-S-Bu derivative that was not cytotoxic. However, no such model reactions have been done with 8,9-secokauranes, which have two possible sites for thiol addition: the 16,17-exocyclic double bond; and the 8,14 endocyclic double bond. Thiophenol reacted immediately with **1**, at room temperature in the presence of a base, to give a single product **20** in good yield. The NMR spectra of **20** showed that the thiol addition had occurred only at the 16,17-exocyclic double bond, since there were no $C=CH_2$ signals in the 1H NMR spectrum and the ^{13}C NMR spectrum showed that the characteristic low field signal of C-14 (δ 163.1) was still present. In order to determine the stereochemistry at C-16, the 1H NMR spectrum had to be run in $(CD_3)_2CO$ solution (the signals of H-16 and one H-17 overlapped in $CDCl_3$). The H-16 to H-13 coupling was 6 Hz, very similar to the corresponding coupling in the 16,17-dihydro derivative **2** (Perry et al., 1996) and these two protons also showed a strong NOE interaction. Therefore the stereochemistry at C-16 of **20** was *R*, corresponding to thiol addition to the less hindered 'outer' face of the macrocycle (see Fig. 1). Treatment of the 16,17-dihydro derivative **2** with thiophenol under the conditions used to form **20** gave no reaction, and heating had no effect.

These results show that the 16,17 exocyclic double

bond of the 8,9-secokauranes is the most reactive site for thiol addition, and could explain the relative cytotoxicities of 8,14-epoxy derivative **3** and 16,17-dihydro derivative **2** (Table 4). Oxygenation at C-11 leads to reduced cytotoxicity (compare **8** and **9** with **1**, Table 4). This may be because of some steric crowding of 11-O and 17- CH_2 destabilising the proposed thiol adducts. Molecular modelling of a hypothetical thiol adduct **21** suggested an 11-O to 17-C distance of 3.2 Å. Acetylation at C-7 also leads to reduced cytotoxicity (compare **5** with **1**, and **6** with **9**, Table 4). This is not thought to be due to an intramolecular 7-OH to 15=O hydrogen bond in **1** promoting Michael addition, since molecular modelling showed no such hydrogen bond (see above and Fig. 1). Instead, we assume that 7-OH assists attack at biologically important nucleophiles in tumor cells, as suggested by Fujita and Nagao (1977). This would also explain why kauren-15-ones with C-7 and/or C-14 hydroxyl groups were more cytotoxic than the simple kauren-15-one (**12**). Compound **18** was inactive because of the absence of a carbonyl group to allow Michael addition. One surprising result in Table 4 was the cytotoxic activity of thiophenol adduct **20**, only slightly less than that of the parent compound **1**. We postulate that this was due to elimination of thiophenol under cellular conditions.

Compounds **1** and **3** were tested in an in vivo model system for antitumor activity. This involves implanting human tumor cells, encapsulated in hollow fibres, in mice, which are then treated with compounds intra-peritoneally or sub-cutaneously (Hollingshead et al., 1995). Unfortunately neither compound was active at the doses tested (150 and 100 mg/kg for **1** and **18** and 12 mg/kg for **3**).

2.5. *Lepidolaena palpebrifolia*

Crude extracts of another *Lepidolaena* species, *L. palpebrifolia*, also showed strong cytotoxic activity in P388 leukemia assays. This species is closely related to *L. taylorii*, from which it differs only in having ciliate toothed branches and partly ciliated leaves (Hodgson, 1959). Bioactivity-directed fractionation led to rabdoubrosanin **1** and epoxide **3** as the main cytotoxins.

3. Conclusions

8,9-Secokaurane (**1**) is the main cytotoxic compound in *L. taylorii*, with a purified yield of about 0.5 mg of **1** per g of dried liverwort. Six new 8,9-secokauranes are present at lower levels, representing a significant jump in the number of compounds known in this class from the nine (including **1**) reported from *Rabdosia* species (Fujita & Node, 1984; Takeda & Otsuka, 1995). Also present in *L. taylorii* are kaurene, *ent*-kauren-15-one (**12**) and a series of C-7 and/or C-14 oxygenated derivatives of **12**.

No C-9 oxygenated kauren-15-ones, possible precursors of the 8,9-secokauranes, were detected. However, only one such compound, shikoccidin **4**, has been reported in the many papers on *Rabdosia* diterpenes (Fujita & Node, 1984; Takeda & Otsuka, 1995). 8,9-Secokauranes **1** and **3** showed some selective toxicity amongst human tumor cell lines, in a pattern similar to other natural products known to act as Michael acceptors for biological nucleophiles. This mode of action was supported by the facile addition of a thiol to the 16,17 double bond of **1**, but the 8,14 double bond of **2** was relatively unreactive. The occurrence of 8,9-secokauranes in both *L. taylorii* and *L. palpebrifolia* reflects their morphological similarity (Hodgson, 1959) and their placement together in the same section of *Lepidolaena* by Grolle (1967). We have found completely different bioactive compounds in two other New Zealand liverworts from different sections of *Lepidolaena* (unpublished results).

4. Experimental

4.1. General

All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 35°C. Octadecyl functionalised silica gel (C_{18}) was used for reversed-phase (RP) flash chromatography and Davisil, 35–70 μ m, 150 Å was used for silica gel flash chromatography. Analytical HPLC was done on a RP column (Merck LiChroCART 100 RP-18, 250 \times 4 mm, 5 μ m) with a guard column (Merck LiChroCART 100 RP-18, 4 \times 4 mm, 5 μ m), a mobile phase of 3:2 $CH_3CN:H_2O$ at 1 ml/min and detection at 235 nm. Preparative (prep.) HPLC was done on either a RP column (Merck LiChroCART 100 RP-18, 250 \times 10 mm, 10 μ m) with a guard column (Merck LiChroCART 100 RP-18, 25 \times 4 mm, 5 μ m) or a silica gel column (Merck LiChroCART Si 60, 250 \times 10 mm, 10 μ m) with a guard column (Merck LiChroCART Si 60, 25 \times 4 mm, 5 μ m), both with 5 ml/min of an appropriate mobile phase (see below) and detection at 206 nm. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mass, UV and IR spectra were recorded on Kratos MS-80, Shimadzu UV 240 and Perkin-Elmer 1600 FTIR instruments, respectively. NMR spectra, at 25°C, were recorded at 300 MHz for 1H and 75 MHz for ^{13}C on a Varian VXR-300 spectrometer. Chemical shifts are given in ppm on the δ scale referenced to the solvent peak $CHCl_3$ at 7.25 and $CDCl_3$ at 77.0 or $(CHD_2)_2CO$ at 2.20 and $(CD_3)_2CO$ at 30.2. Parameters in HMQC experiments were optimised for one bond $^1H-^{13}C$ couplings of 145 Hz and in HMBC experiments for two/three bond $^1H-^{13}C$ couplings of 8 Hz. Conformational searching and molecular modelling methods are described by Hinkley, Perry, and Weavers (1994).

4.2. Plant material

L. taylorii was collected from tree trunks in the Cascade Valley, on the West Coast of the South Island of New Zealand, in March 1997. A voucher specimen, collection code 970321-07, has been deposited in the University of Otago Herbarium (OTA). A collection from the same area in June 1995 (OTA046804) was the source of compound **16** (see below) and a collection from November 1995 (OTA046824) was used for GC–MS analysis. *L. palpebrifolia* was collected from near Lake Brunner, on the West Coast of the South Island, in June 1995 (950630-01). Collections were air dried (30°C) then stored at room temperature.

4.3. GC–MS analyses

These were carried out as described by Asakawa et al. (1996). In addition to kauren-15-one (**12**), the following compounds were identified by matching with a mass spectral library: α -pinene; 3-acetoxyoct-1-ene; two isomers of bicyclogermacrene; caryophyllene; caryophyllene oxide and kaurene.

4.4. Extraction and purification

Ground material (80 g) was extracted with $CHCl_3$ (1 l) in a Soxhlet apparatus for 24 h, then the extract was dried to give a green gum (4 g). Two further 24 h extractions yielded a total crude extract of 13 g. This was coated onto C_{18} (39 g), packed onto a C_{18} column (200 g) and subjected to RP flash chromatography using an $H_2O \rightarrow CH_3CN \rightarrow CHCl_3$ gradient. Twelve fractions were collected and analysed by RP HPLC. Compounds **1** and **2** predominated in the fractions eluted with H_2O –MeCN (1:1) and **1** was purified from these fractions by the method previously reported (Perry et al., 1996) to provide material for in vivo antitumor testing.

The next fraction from RP flash chromatography, eluted with H_2O –MeCN (1:3), was further fractionated by prep. RP HPLC with H_2O –MeCN (2:3) (total 1000 mg injected) to give a series of fractions with peaks at 4.5, 6.8, 7.5, 9.8 and 11.2 min. The 4.5 min fraction (19 mg) was further purified by prep. RP HPLC with H_2O –MeOH (3:7) to give compound **9** (6 mg). The 6.8 min fraction (17 mg) was further purified by prep. RP HPLC with 3:7 H_2O –MeOH to give compound **8** (4 mg). The 7.5 min fraction (106 mg) contained further amounts of compounds **1** and **2**. The 9.8 min fraction was compound **3** (150 mg), previously reported (Perry et al., 1996). The 11.2 min fraction (42 mg) was further purified by prep. RP HPLC with H_2O –MeOH (3:7) to give compound **6** (16 mg).

A further fraction from RP flash chromatography, also eluted with H_2O –MeCN (1:3), was further fractionated by prep. RP HPLC with H_2O –MeCN (2:3) (total 80 mg

injected) to give a series of fractions with peaks at 16.1, 20.2, 21.6, 22.3 and 31.0 min. The 16.1 min fraction was compound **10** (7 mg). The 20.2 min fraction was compound **5** (5 mg). The 21.6 min (9 mg) and 22.3 min (27 mg) fractions were mostly compounds **13** and **14**. The 22.3 min fraction was further purified by prep. silica gel HPLC with hexane–isopropyl alcohol (4:1) to give compounds **14** (4 mg, 4.1 min) and **13** (8 mg, 5.4 min). The 31.0 min fraction (13 mg) was further purified by prep. silica gel HPLC with hexane–isopropyl alcohol (4:1) to give compound **15** (5 mg, 3.5 min).

The later fractions from RP flash chromatography, eluted with MeCN→CHCl₃, were combined and a sub-sample (1 g) was further fractionated by silica gel CC (sample coated on 2 g silica gel, on a 10 g column) using an cyclohexane→cyclohexane–EtOAc (9:1) gradient. Fractions eluted with cyclohexane–EtOAc (19:1) were combined (370 mg) and subjected to RP (sample coated on 1.2 g C₁₈, on a 8 g column) using an H₂O–MeOH (1:4)→H₂O–MeOH (1:19) gradient. Fractions eluted with 1:9 and 1:19 H₂O–MeOH were combined to give compound **12** (36 mg).

Compound **16** was obtained from a cold EtOH extract of an earlier collection of *L. taylorii* (see above) by a similar combination of RP flash chromatography and HPLC. Final purification was by prep. silica gel HPLC with hexane–isopropyl alcohol (4:1) to give more of compound **15** (3 mg, 3.5 min) plus compound **16** (3 mg, 4.0 min).

4.5. *ent*-8,9-*Seco*-7 α -*acetoxykaura*-8(14),16-dien-9,15-dione (**5**)

Colourless oil: $[\alpha]_{589}^{20} -72^\circ$, $[\alpha]_{577} -82^\circ$, $[\alpha]_{546} -98^\circ$, $[\alpha]_{435} -173^\circ$, $[\alpha]_{405} -111^\circ$ (*c* 0.2, CHCl₃); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 244 (3.8). IR ν_{\max}^{film} cm⁻¹: 2930 (CH), 1736 (C=O), 1697 (C=O), 1236. ¹H NMR and ¹³C NMR in Tabs. 1–2. EIMS (70 eV) *m/z* (rel. int.): 358.2138 (M^+ , <1%, C₂₂H₃₀O₄ req. 358.2144), 298 (35), 221 (15), 179 (100), 138 (13), 123 (35).

4.6. *ent*-8,9-*Seco*-7 α -*acetoxy*-11 β -*hydroxykaura*-8(14),16-dien-9,15-dione (**6**)

Colourless oil: $[\alpha]_{589}^{25} +2^\circ$, $[\alpha]_{578} +3^\circ$, $[\alpha]_{546} +6^\circ$, $[\alpha]_{435} +50^\circ$ (*c* 0.46, MeOH); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 244 (3.62). IR ν_{\max}^{film} cm⁻¹: 3470 (br, OH), 2925 (CH), 1735 (C=O), 1700 (C=O), 1368, 1240, 1023, 919, 725. ¹H NMR and ¹³C NMR in Tabs. 1–2. EIMS (70 eV) *m/z* (rel. int.): 374 (M^+ (<1), 332.1978 [$\text{M}^+ - \text{C}_2\text{H}_2\text{O}$, C₂₀H₂₈O₄ req. 332.1988] (5), 314.1887 [$\text{M}^+ - \text{C}_2\text{H}_4\text{O}_2$, C₂₀H₂₆O₃ req. 314.1882] (55), 299 [$\text{M}^+ - \text{C}_2\text{H}_4\text{O}_2 - \text{CH}_3$] (34), 286 [C₁₉H₂₆O₂] (18), 271 (4), 242 [C₁₇H₂₂O] (68), 237 [C₁₂H₁₃O₅] (29), 195 [C₁₀H₁₁O₄] (100), 138 [C₁₀H₁₈] (34), 123 [C₉H₁₅] (73), 109 (43), 91 (26), 81 (29), 69 (30).

4.7. *ent*-8,9-*Seco*-7 α -*hydroxy*-11-*acetoxykaura*-8(14),16-dien-9,15-dione (**8**)

Colourless oil: $[\alpha]_{589}^{20} -31^\circ$, $[\alpha]_{577} -42^\circ$, $[\alpha]_{546} -54^\circ$, $[\alpha]_{435} -131^\circ$, $[\alpha]_{405} -126^\circ$ (*c* 0.2, CHCl₃); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 245 (3.84). IR ν_{\max}^{film} cm⁻¹: 3490 (br, OH), 2950 (CH), 1746 (C=O), 1700 (C=O), 1220, 1027. ¹H NMR and ¹³C NMR in Tabs. 1–2. EIMS (70 eV) *m/z* (rel. int.): 374.2099 [M^+ , C₂₂H₃₀O₅ req. 374.2093] (4), 356 (4), 296 (11), 237 (100) 195 (54), 138 (44), 123 (86).

4.8. *ent*-8,9-*Seco*-7 α ,11 β -*dihydroxykaura*-8(14),16-dien-9,15-dione (**9**)

Colourless oil: $[\alpha]_{589}^{19} -3^\circ$, $[\alpha]_{577} -2^\circ$, $[\alpha]_{546} +1^\circ$, $[\alpha]_{435} +53^\circ$, $[\alpha]_{405} +165^\circ$ (*c* 0.2, CHCl₃); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 246 (3.87). IR ν_{\max}^{film} cm⁻¹: 3400 (br, OH), 2900 (CH), 1690 (C=O). ¹H NMR and ¹³C NMR in Tables 1–2. EIMS (70 eV) *m/z* (rel. int.): 332.1991 [M^+ , C₂₀H₂₈O₄ req. 332.1987] (1), 314 (4), 299 (10), 286 (15), 242 (55), 195 (51), 177 (20), 123 (100).

4.9. *ent*-7 α ,14 β -*Dihydroxykaur*-16-en-15-one (**10**)

Colourless oil: $[\alpha]_{589}^{20} -75^\circ$, $[\alpha]_{577} -83^\circ$, $[\alpha]_{546} -100^\circ$, $[\alpha]_{435} -206^\circ$, $[\alpha]_{405} -216^\circ$ (*c* 0.2, CHCl₃); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 232 (3.6). IR ν_{\max}^{film} cm⁻¹: 3312 (OH), 2929 (CH), 1729 (C=O), 1649, 1456, 1251, 1091. ¹H NMR and ¹³C NMR in Table 3. EIMS (70 eV) *m/z* (rel. int.): 318.2196 [M^+ , C₂₀H₃₀O₃ req. 318.2195] (33), 300 (57), 221 (16), 194 (51), 179 (72), 149 (32), 123 (100), 91 (41).

4.10. *ent*-*Kaur*-16-en-15-one (registry No. 14140-75-1) (**12**)

Colourless oil: $[\alpha]_{589}^{20} -125^\circ$, $[\alpha]_{577} -141^\circ$, $[\alpha]_{546} -173^\circ$, $[\alpha]_{435} -402^\circ$, $[\alpha]_{405} -545^\circ$ (*c* 0.2, CHCl₃); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 232 (3.82). IR ν_{\max}^{film} cm⁻¹: 2916, 2864 (CH), 1721 (C=O), 1644, 1442, 930. ¹H NMR and ¹³C NMR match literature (Fraga et al., 1995; Nagashima et al., 1996); EIMS (70 eV) *m/z* (rel. int.): 286.2294 [M^+ , C₂₀H₃₀O req. 286.2297] (100), 271 (35), 253 (9), 189 (8) 153 (19), 123 (24), 77 (38).

4.11. *ent*-7 α -*Acetoxy*-14 β -*hydroxykaur*-16-en-15-one (**13**)

Colourless oil: $[\alpha]_{589}^{20} -79^\circ$, $[\alpha]_{577} -93^\circ$, $[\alpha]_{546} -112^\circ$, $[\alpha]_{435} -239^\circ$, $[\alpha]_{405} -267^\circ$ (*c* 0.2, CHCl₃); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 225 (4.2). IR ν_{\max}^{film} cm⁻¹: 3550 (OH), 2928 (CH), 1730 (C=O), 1237. ¹H NMR and ¹³C NMR in Table 3. EIMS (70 eV) *m/z* (rel. int.): 360.2306 [M^+ , C₂₂H₃₂O₄ req. 360.2301] (2), 318 (23), 300 (100), 285 (22), 244 (7), 217 (22), 143 (33), 109 (36).

4.12. *ent-7 α -Hydroxykaur-16-en-15-one* (registry No. 180050-84-4) (**15**)

Colourless oil: $[\alpha]_{589}^{20} - 107^\circ$, $[\alpha]_{577} - 124^\circ$, $[\alpha]_{546} - 154^\circ$, $[\alpha]_{435} - 355^\circ$, $[\alpha]_{405} - 462^\circ$ (c 0.2, CHCl_3); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm ($\log \epsilon$): 227 (3.92). IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3450 (br, OH), 2927 (CH), 1730 (C=O). ^1H NMR and ^{13}C NMR match literature (Buchanan et al., 1996). EIMS (70 eV) m/z (rel. int.): 302.2244 [M^+ , $\text{C}_{20}\text{H}_{30}\text{O}_2$ req. 302.2246] (100), 274 (54), 245 (14), 199 (5), 165 (20), 123 (30).

4.13. *ent-14 β -Hydroxykaur-16-en-15-one* (**16**)

Colourless oil: $[\alpha]_{589}^{19} - 87^\circ$, $[\alpha]_{577} - 100^\circ$, $[\alpha]_{546} - 117^\circ$, $[\alpha]_{435} - 269^\circ$, $[\alpha]_{405} - 330^\circ$ (c 0.2, CHCl_3); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm ($\log \epsilon$): 230 (3.85). IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3490 (br, OH), 2930 (CH), 1730 (C=O). ^1H NMR and ^{13}C NMR in Table 3. EIMS (70 eV) m/z (rel. int.): 302.2245 [M^+ , $\text{C}_{20}\text{H}_{30}\text{O}_2$ req. 302.2246] (53), 287 (72), 248 (9), 217 (4), 164 (39), 137 (100).

4.14. Acetylation of **1**

Treatment of **1** with acetic anhydride and pyridine gave **5** with ^1H NMR spectrum identical to the natural product (above).

4.15. Reaction of **1** with thiophenol

A sample of **1** (10 mg) was dissolved in CDCl_3 (1 ml) with thiophenol (3 μl) and Et_3N (4 μl). The ^1H NMR spectrum showed that complete reaction of **1** had occurred immediately. The reaction mixture was dried down, redissolved in CH_2Cl_2 , rinsed with NaOH_{aq} (2 M), dried over MgSO_4 and the solvent removed to give pure **20** (7 mg).

4.16. *ent-8,9-Seco-7 α -hydroxy-17-thiophenylkaur-8(14)-en-9,15-dione* (**20**)

Colourless oil: UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm ($\log \epsilon$): 247 (3.57). IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3412 (OH), 2915 (CH), 1696 (C=O). ^1H NMR (CDCl_3): δ 7.39 (dm, $J = 7$ Hz, 3H, S-Ph + H-14), 7.30 (tm, $J = 7$ Hz, 2H, S-Ph), 7.22 (tt, $J = 7$, 1 Hz, 1H, S-Ph), 4.56 (dd, $J = 12$, 5 Hz, 1H, H-7), 3.71 (q, $J = 10$ Hz, 1H, H-17), 3.35 (br m, 1H, H-13), 2.7–2.4 (m, 3H), 2.1–1.9 (m, 3H), 1.82 (dt, $J = 14$, 5 Hz, 1H), 1.7–0.9 (various m), 1.04 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H). ^1H NMR ($\{\text{CD}_3\}_2\text{CO}$): δ 7.43 (dm, $J = 7$ Hz, 2H, S-Ph), 7.35 (tm, $J = 7$ Hz, 2H, S-Ph), 7.28 (br d, $J = 3$ Hz, 1H, H-14), 7.24 (tt, $J = 7$, 1.5 Hz, 1H, S-Ph), 4.45 (dd, $J = 12$, 5 Hz, 1H, H-7), 3.61 (dd, $J = 13$, 3 Hz, 1H, H-17), 3.33 (br m, 1H, H-13), 2.81 (dd, $J = 13$, 12 Hz, 1H, H-17), 2.60 (dddd, $J = 12$, 6, 4, 1 Hz, 1H, H-16), 2.5–1.1 (various m), 1.02 (s, 3H), 0.98 (dd, $J = 6$, 2 Hz, 1H, H-5), 0.94 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (CDCl_3): δ 214.1 (C-9), 207.2 (C-15),

163.1 (C-14), 145.2 (C-8), 135.2 (S-Ph), 129.7 (2C, S-Ph), 129.2 (2C, S-Ph), 126.6 (S-Ph), 64.3 (C-7), 53.8 (C-10), 49.3 (C-16), 42.8 (C-5), 41.3 (C-3), 40.9 (C-13), 36.8 (C-6), 34.6 (C-4), 34.3 (C-1), 33.4 (C-18), 31.6 (C-17), 30.0 (C-11), 22.3 (C-19), 20.8 (C-12), 18.2 (C-2), 16.5 (C-20). EIMS (70 eV) m/z (rel. int.): 426.2239 [M^+ , $\text{C}_{26}\text{H}_{34}\text{O}_3\text{S}$ req. 426.2229] (11), 289 (13), 193 (10), 192 (27), 180 (13), 179 (100), 123 (56).

4.17. Cytotoxicity assays

For the P388 assay a two-fold dilution series of the sample was incubated for 72 h with murine leukemia cells (ATCC CCL 46 P388D1). The concentration of the sample required to inhibit cell growth to 50% of the growth of a solvent control was determined using the absorbance obtained upon staining with MTT tetrazolium. As a positive control for this assay, mitomycin C at a concentration of 0.06 $\mu\text{g}/\text{ml}$ inhibited the growth of P388 cells by 43–75%. References to the NCI's in vitro 60 human cell line assays are given by Boyd and Paull (1995). The NCI's in vivo hollow fiber assay is described by Hollingshead et al. (1995).

4.18. *L. palpebrifolia* extraction and purification

Dried material (6.5 g) was extracted by blending with EtOH (200 ml, then 2×100 ml) and CHCl_3 (2×100 ml) to give after solvent removal a green gum (0.39 g). The purification of **1** (3 mg) and **2** (8 mg) followed the methods described above. These compounds were identified by comparison of their ^1H NMR and ^{13}C NMR spectra with the spectra of the compounds isolated from *L. taylorii* (Perry et al., 1996).

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