16α,19-Diacetoxy-*ent*-kaurane, a New Natural Diterpene from the Exudate of *Ozothamnus scutellifolius* (Asteraceae)

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Ozothamnus scutellifolius, Asteraceae, Leaf and Stem Exudate, Diterpenoids, Novel Kaurane Derivative

Leaf and stem exudate of *Ozothamnus scutellifolius*, previously reported to contain 21 flavonoid aglycones, was now investigated for its terpenoid composition. Seven structurally related diterpenoids were identified. One of them, 16α,19-diacetoxy-*ent*-kaurane, is a novel natural product.

In a previous paper, dealing with rare flavonoids occurring in the leaf and stem exudates of 2 *Odixia* and 11 *Ozothamnus* species from Australia, *O. scutellifolius* was reported to contain fourteen flavonols and seven flavones (Wollenweber *et al.*, 1997). The terpenoid portion of the exudate of this species has now been analyzed. In the present paper, we report the isolation of seven diterpenes belonging to the *ent*-kaurane group. One of them, identified by spectroscopic methods as 16α,19-diacetoxy-*ent*-kaurane (**1**), is a new natural product.

Material and Methods

Branches of *Ozothamnus ledifolius* were field collected by C. Puttock between The Springs and Chalet, Tasmania on 28. Dec. 1995. A voucher (*Puttock 1305*) is kept at the Herbarium Australiense, CSIRO, in Canberra, Australia (CANB). Air-dried plant material was briefly rinsed with acetone to dissolve the lipophilic exudates. The concentrated solution was defatted and passed over Sephadex LH-20 to separate terpenoids from flavonoid aglcyones. The terpenoid portion (3.6 g) was subjected to repeated column chromatography on "flash" Si-gel using binary mixtures of increasing polarity (from Hex-AcOEt 10:1 v/v to CH_2Cl_2-MeOH 20:1 v/v) furnishing **1** (19 mg), **2** (72 mg), 3 (12 mg), **4** (25 mg), **5** (12 mg), **6** (23 mg) and **7** (9 mg).

Mass spectra were measured on a HP 5890 at 70 eV *via* GC-MS. NMR spectra were recorded on a Bruker AC-300 (300/75.4 MHz) in $CDCl_3$ or CD_3OD solutions. Multiplicities were assigned through DEPT experiments.

16α,19-Diacetoxy-*ent*-kaurane (**1**): White powder, mp. 112–114°C. $[\alpha]_D$ = -52.5 (c=0.55, $CHCl_3$). R_f =0.44 (Hex/AcOEt 10:1). MS *m/z* (% rel. int.): 390 (M^+, -), 330 (M^+ -AcOH, 13%), 315 (M^+ -AcOH-Me, 2), 270 (M^+ -2AcOH, 4), 257 (13), 242 (7), 221 (14), 161 (16), 119 (23), 106 (47), 94 (100), 81 (24), 67 (16), 55 (22) and 43 (73). ^{1}H NMR ($CDCl_3$) δ 4.20 d (10, H-19), 3.87 brd (10, H-19), 2.32 m (H-13), 2.03 s (3H), 1.99 dd (1H, 15, 2), 1.95 s (3H), 1.58 s (3H), 1.02 s (3H), 0.93 s (3H) and 0.77 br dt (1H, 12.5, 5). For ^{13}C-NMR data see Table I.

16α-Hydroxy-*ent*-kaurane (**2**): Colourless oil; R_f =0.46 (CH_2Cl_2/MeOH 30:1 v/v). ^{13}C-NMR as in Hanson *et al.*(1975).

19-Acetoxy-16α-hydroxy-*ent*-kaurane (**3**): Colourless oil; R_f =0.34 (CH_2Cl_2/MeOH 30:1 v/v). For ^{13}C-NMR data see Table I.

16α-Hydroxy-*ent*-kauran-19-al (**4**): Colourless oil; R_f =0.35 (CH_2Cl_2/MeOH 30:1 v/v). ^{1}H-NMR as in Serebryakov *et al.* (1970).

Ent-kaur-16-en-19-oic acid (**5**): Colourless oil; R_f =0.52 (CH_2Cl_2/MeOH 30:1 v/v). ^{13}C-NMR as in Wu *et al.* (1996).

17-Hydroxy-16α-*ent*-kauran-19-oic acid (**6**): Colourless oil; R_f =0.17 (CH_2Cl_2/MeOH 30:1 v/v). ^{13}C-NMR as in Wu *et al.* (1996).

ent-kauran-17,19-dioic acid (**7**): Colourless oil; R_f =0.38

(CH_2Cl_2/MeOH 20:1 v/v). ^{1}H NMR (CD_3OD) δ 2.66 m (H-16), 2.48 bs (1H), 1.22 s (3H), 1.00 s (3H) and 0.88 br dt (1H, 12.5, 5).

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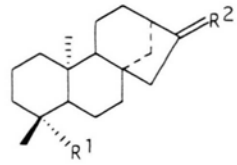
Results and Discussion

The terpenoid portion of the resinous exudate of *Ozothamnus scutellifolius*, previously obtained by CC on Sephadex (Wollenweber *et al.*, 1997), was flash-chromatographed on Si gel to yield 7 products.

Compound **1** was obtained as an amorphous white powder. The molecular ion at *m/z* 390 could not be observed, but peaks at *m/z* 330 and 270 indicated two consecutive losses of acetic acid. The ^{1}H-NMR spectrum in $CDCl_3$ exhibited signals for three tertiary methyl groups at δ 0.93, 1.02 and 1.58 ppm. The other major features were two one-proton doublets at 4.20 and 3.87 ppm with a geminal coupling constant (10Hz) and two methyl singlets at 2.03 and 1.95 ppm. These signals suggested the structure of an *ent*-kaurane diterpene with two acetoxy groups, one of them on a quaternary carbon and the other attached to a methylene group. The ^{13}C-NMR spectrum (see Table I) indicated 24 carbons. Carbon types (multiplicities) were established through DEPT experiments and identitites were assigned by comparison of carbon resonances with those of the related 19-acetoxy-16α-hydroxy-*ent*-kaurane (**3**), also isolated in the present study, and the parent *ent*-Kauran-16α,19-diol (**8**), previously isolated from *O. ledifolius* (Arriaga *et al.*, 1998). Acetylation of **8** (Ac_2O/Py, overnight) yielded **3** but not **1**, thus confirming the existence of an acetoxy group on a quaternary carbon in the latter. The structure of **1** is thus determined as 16α,19-diacetoxy-*ent*-kaurane. To our knowledge this compound is reported here for the first time as a natural product.

Table I. Comparative CNMR Data for Compounds **1**, **3**, and **8**.
a,b: Signals are interchangeable within the same column.

Carbon		**1**	**3**	**8** [15]
1	t	42.19	42.36	42.29
2	t	18.18[a]	18.05	18.16[a]
3	t	36.28	36.38	35.50
4	s	36.98	37.06	38.81
5	d	56.59[b]	56.75[a]	56.92
6	t	20.53	20.59	20.81
7	t	40.22	40.28	40.28
8	s	44.59	45.30	45.47
9	d	56.63[b]	56.97[a]	57.14
10	s	39.13	39.20	39.38
11	t	18.13[a]	18.05	17.93[a]
12	t	26.21	26.79	26.96
13	d	45.47	49.02	49.13
14	t	37.30	37.51	37.39
15	t	55.44	57.88	57.99
16	s	90.69	79.25	79.54
17	q	22.71	24.48	24.65
18	q	27.48	27.53	27.25
19	t	67.14	67.13	65.60
20	q	18.16	18.22	18.44
OCO**CH$_3$**	q		21.04	20.97
				19.77
O**CO**CH$_3$	s		171.38	171.35
				170.76



	R^1	R^2
1	CH_2OAc	βCH$_3$, αOAc
2	CH_3	βCH$_3$, αOH
3	CH_2OAc	βCH$_3$, αOH
4	CHO	βCH$_3$, αOH
5	COOH	CH_2
6	COOH	βCH_2OH, αH
7	COOH	βCOOH, αH
8	CH_2OH	βCH$_3$, αOH

Fig. 1. Structures of the kaurane derivatives **1–8.**

16α-*ent*-kauranol **2** is a widespread natural diterpene, that has been reported from fungi as well as from various higher plants (c.f. Serebryakov *et al.*,1970; Patra *et al.*, 1980; Jakupovic *et al.*, 1991), and the kaurenoic acid **5** has been reported from at least five sources (c.f. Wu *et al.*, 1996). The monoacetyl ester **3** was isolated from roots of *Ichthyothere terminalis* (Bohlmann *et al.*, 1982) and from *Helichrysum davii* Jakupovic *et al.*, 1987). The aldehyde **4** was found in *Fusarium moniliforme* (Serebryakov *et al.*,1970) and later in *Ruilopezia margarita* (Usubillaga and Nakano, 1979) and *Espeletia semiglobulata* (Usubillaga and Capra, 1988). The hydroxyacid **6** was isolated from *Annona reticulata* (Este *et al.*, 1987) and *A. squamosa*

(Wu *et al.*, 19996), while the diacid **7** was found in *Ricinocarpus stylosus* (Henrick *et al.*, 1964).

We recently identified the kauranol **2** in the exudate of *Ozothamnus hookeri* among other terpenoids (Wollenweber *et al.*, 1997), while the diol **8** was found in the exudate of *O. ledifolius* (Arriaga *et al.*, 1998). A study on *O. obcordatus* revealed only prenylated aromatics, no terpenoids (Zdero *et al*, 1991). The terpenoid portions of the exudates of further species of this genus, previously analyzed for their flavonoid aglycones, are now being investigated.

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