

16 α ,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, 16 α ,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 16 α ,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 aglycones. 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 CH₂Cl₂-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 CDCl₃ or CD₃OD solutions. Multiplicities were assigned through DEPT experiments.

16 α ,19-Diacetoxy-*ent*-kaurane (**1**): White powder, mp. 112–114°C. [α]_D = -52.5 (c=0.55, CHCl₃). *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). ¹H NMR (CDCl₃) 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 ¹³C-NMR data see Table I.

16 α -Hydroxy-*ent*-kaurane (**2**): Colourless oil; *R*_f=0.46 (CH₂Cl₂/MeOH 30:1 v/v). ¹³C-NMR as in Hanson *et al.* (1975).

19-Acetoxy-16 α -hydroxy-*ent*-kaurane (**3**): Colourless oil; *R*_f=0.34 (CH₂Cl₂/MeOH 30:1 v/v). For ¹³C-NMR data see Table I.

16 α -Hydroxy-*ent*-kauran-19-al (**4**): Colourless oil; *R*_f=0.35 (CH₂Cl₂/MeOH 30:1 v/v). ¹H-NMR as in Serebryakov *et al.* (1970).

Ent-kaur-16-en-19-oic acid (**5**): Colourless oil; *R*_f=0.52 (CH₂Cl₂/MeOH 30:1 v/v). ¹³C-NMR as in Wu *et al.* (1996).

17-Hydroxy-16 α -*ent*-kauran-19-oic acid (**6**): Colourless oil; *R*_f=0.17 (CH₂Cl₂/MeOH 30:1 v/v). ¹³C-NMR as in Wu *et al.* (1996).

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

(CH₂Cl₂/MeOH 20:1 v/v). ¹H NMR (CD₃OD) 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).



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 $^1\text{H-NMR}$ 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 $^{13}\text{C-NMR}$ spectrum (see Table I) indicated 24 carbons. Carbon types (multiplicities) were established through DEPT experiments and identities were assigned by comparison of carbon resonances with those of the related 19-acetoxy-16 α -

hydroxy-*ent*-kaurane (**3**), also isolated in the present study, and the parent *ent*-Kauran-16 α ,19-diol (**8**), previously isolated from *O. ledifolius* (Arriaga *et al.*, 1998). Acetylation of **8** ($\text{Ac}_2\text{O/Py}$, 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 16 α ,19-diacetoxy-*ent*-kaurane. To our knowledge this compound is reported here for the first time as a natural product.

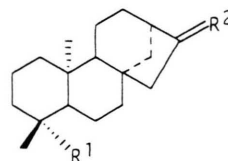


Table I. Comparative CNMR Data for Compounds **1**, **3**, and **8**.

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
OCOCH ₃	q		21.04	20.97
				19.77
OCOCH ₃	s		171.38	171.35
				170.76

	R ¹	R ²
1	CH ₂ OAc	β CH ₃ , α OAc
2	CH ₃	β CH ₃ , α OH
3	CH ₂ OAc	β CH ₃ , α OH
4	CHO	β CH ₃ , α OH
5	COOH	CH ₂
6	COOH	β CH ₂ OH, α H
7	COOH	β COOH, α H
8	CH ₂ OH	β CH ₃ , α OH

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

16 α -*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 ana-

lyzed for their flavonoid aglycones, are now being investigated.

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