New Constituents of the Leaf and Stem Exudate of *Ozothamnus hookeri* (Asteraceae)

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The exudate of *Ozothamnus hookeri* has been investigated for its non-flavonoid constituents. A new natural C_6-C_3 ester of a long chain fatty acid and seven structurally related kaurane-diterpenoids were isolated. Three of the latter are new natural products, too. A rare 8-methoxy flavonol was also identified.

Introduction

The lipophilic leaf and stem exudates of several species of *Ozothamnus*, an Asteraceae genus occurring in Australia, New Zealand and New Caledonia (Bremer, 1994), were previously reported to contain a number of more or less rare flavonoid aglycones (Wollenweber *et al.*, 1997; Rumbero *et al.*, 2000) and an oxyprenyl coumarin (Rumbero *et al.*, 2000). The major portion of these *Ozothamnus* exudates consists of terpenoids. A series of kaurane type diterpenoids were found in *O. scutellifolius* (Arriaga-Giner *et al.*, 1999), while in *O. ledifolius* four sesquiterpenes, a diterpene diol and two pentacyclic triterpene acids have been identified, along with three phenylethyl esters (Arriaga-Giner *et al.*, 1998). From the non-flavonoid portion of the exudate of *O. hookeri* we now identified seven diterpenoids and a novel natural phenolic ester of a long-chain fatty acid. From a flavonoid fraction, an additional rare flavonol was identified.

Material and Methods

The lipophilic exudate material from several twigs of *Ozothamnus hookeri* Sond., collected in Tasmania, was recovered and treated as previously reported (Wollenweber *et al.*, 1997). Several Sephadex fractions were combined to yield 8.5 g of resinous material. This was subjected to repeated column chromatography on "flash" Si-gel using binary mixtures of increasing polarity (Hex-EtOAc 4:1, 1:1 and 1:3 v/v) furnishing docosanoic acid (17 mg), 3-(4-hydroxyphenyl)-propan-1-ol (15 mg), **1** (12 mg), **2** (560 mg), **3** (120 mg), **5** (15 mg), **7** (60 mg), **8** (21 mg), **9** (10 mg) and **10** (30 mg).

Mass spectra were measured on a HP 5890 at 70 eV *via* solid probe. 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.

3-(4-Hydroxyphenyl)-propyl docosanoate (**1**). Colourless oil. R_f =0.49 (Hex/EtOAc 4:1 v/v). MS *m/z* (% rel. int.): 474 (M^+, 0.7), 134 (100) 107 (17). ^{1}H NMR ($CDCl_3$) δ ppm: 7.03 d (2H, 8.5), 6.77 d (2H, 8.5), 4.09 t (2H, 7), 2.61 t (2H, 7), 2.31 t (2H, 7), 1.91 m (2H), 0.89 t (3H, 7). ^{13}C NMR ($CDCl_3$): δ174.16 (s), 153.91 (s), 133.22 (s), 129.43 (d, 2xC), 115.26 (d, 2xC), 63.61 (t), 34.39 (t), 31.91 (t), 30.43 (t), 29.67 (t, 14xC), 29.60 (t), 29.45 (t), 29.34 (t), 29.26 (t), 29.17 (t), 25.02 (t) 22.67 (t) and 14.09 (q).

16α-hydroxy-*ent*-kaurane (**2**). Colourless oil. R_f =0.55 (Hex/EtOAc 1:1 v/v). ^{13}C NMR as Hanson *et al.* (1975).

19-hydroxy-16α-H-*ent*-kauran-17-al dimethyl acetal (**3**). Colourless oil. R_f =0.50 (Hex/EtOAc 1:1 v/v). MS *m/z* (% rel. int.): 350 (M^+, -), 318 (M^+ -MeOH, 3), 287 (M^+ -MeOH -CH_2OH, 5), 255 (M^+ –2MeOH -CH_2OH, 4), 123 (8), 105 (7), 95 (7), 91 (10), 81 (10) and 75 (100). ^{1}H NMR ($CDCl_3$) δ ppm: 4.03 d (9, H-17), 3.71 d (10, H-

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19), 3.40 d (10, H-19) 3.28 s (2xOCH$_3$), 0.95 s (3H) and 0.92 s (3H). ^{13}C NMR ($CDCl_3$): see Table I.

19-acetoxy-16α-H-*ent*-kauran-17-al dimethyl acetal (**5**). Colourless oil. R_f = 0.42 (Hex/EtOAc 4:1 v/v). MS *m/z* (% rel. int.): 392 (M^+, -), 360 (M^+ -MeOH, 6), 329 (M^+ -MeOH -CH_2OH, 4), 269 (M^+ -MeOH -CH_2OH-AcOH, 9), 135 (10), 123 (16), 105 (11), 95 (12), 91 (16), 81 (18) and 75 (100). ^{1}H NMR ($CDCl_3$) δ ppm: 4.21 d (10, H-19), 4.03 d (9, H-17), 3.85 d (10, H-19) 3.28 s (2xOCH$_3$), 2.03 s (OAc), 0.98 s (3H) and 0.91 s (3H).^{13}C NMR ($CDCl_3$): see Table I.

19-acetoxy-16β, 17-dihydroxy-*ent*-kaurane (**7**). Colourless oil. R_f = 0.23 (Hex/EtOAc 1:3 v/v). MS *m/z* (% rel. int.): 364 (M^+, -), 346 (M^+ -H_2O, 5), 333 (M^+ -CH_2OH, 98), 273 (M^+ -CH_2OH-AcOH, 100), 255 (33), 135 (44), 123 (95), 109 (63) and 81 (84). ^{1}H NMR ($CDCl_3$) δ ppm: 4.19 d (10, H-19), 3.85 d (10, H-19) 3.74 brd (10, H-17), 3.62 brd (10, H-17), 2.01 s (OAc), 0.98 s (3H) and 0.90 s (3H). ^{13}C NMR ($CDCl_3$ and MeOD) : see Table I.

16α,19-dihydroxy-*ent*-kaurane (**8**). Colourless oil. R_f = 0.40 (Hex/EtOAc 1:3 v/v). ^{13}C NMR as in Satake *et al.* (1983).

16α,17,19-trihydroxy-*ent*-kaurane (**9**). Colourless oil. R_f = 0.18 (Hex/EtOAc 1:3 v/v). ^{13}C NMR as in Kuraishi *et al.* (1983).

16β,17,19-trihydroxy-*ent*-kaurane (**10**). Colourless oil. R_f = 0.13 (Hex/EtOAc 1:3 v/v). ^{13}C NMR as in Wu *et al.* (1996).

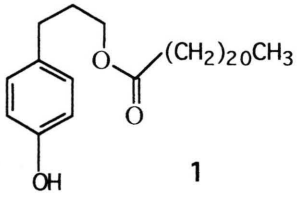
Results and Discussion

Flash-chromatography on Si-gel of the non-flavonoid portion of the resinous exudate of *Ozothamnus hookeri* yielded ten compounds. The less polar first eluates were found to contain docosanoic (behenic) acid, 3-(4-hydroxy-phenyl)-propanol and the ester **1**.

Compound **1** shows the molecular ion at *m/z* 454, corresponding to $C_{31}H_{54}O_3$. Its ^{1}H NMR spectrum exhibits signals for a p-disubstituted aromatic ring, two one-proton doublets at δ 6.77 and 7.03 ppm, as well as three two-proton triplets, easily attributable to CH_2-**CH_2**-OCO- (4.09 ppm), -CH_2-**CH_2**-COO- (2.61 ppm) and CH_2-**CH_2**-Ph (2.31 ppm). ^{13}C NMR (see Experimental) and the above data lead us to identify **1** as 3-(4-hydroxyphenyl)-propyl docosanoate.

Esters of long chain fatty acids and phenols are very unusual. From the stembark of *Buddleja globosa*, 4-hydroxy-cinnamyl and 4-hydroxyphenylethyl esters of C_{20} and C_{22} acids have been reported (Houghton, 1989), but to our knowledge this is the first time that such a C_6-C_3 ester of docosanoic acid is reported as a natural plant component.

Compound **2,** the main constituent, was identified as 16α-hydroxy-*ent*-kaurane, a widespread member of this family of diterpenes, which was recently isolated from *O. scutellifolius* (Arriaga-Giner *et al.*, 1999).



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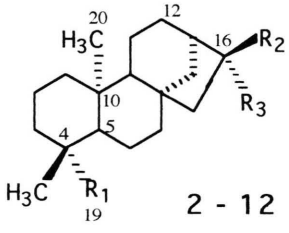

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	R_1	R_2	R_3
2	CH_3	CH_3	OH
3	CH_2OH	$CH(OCH_3)_2$	H
4	CH_2OH	CHO	H
5	CH_2OAc	$CH(OCH_3)_2$	H
6	CH_2OAc	CHO	H
7	CH_2OAc	OH	CH_2OH
8	CH_2OH	CH_3	OH
9	CH_2OH	CH_2OH	OH
10	CH_2OH	OH	CH_2OH
11	COOH	H	CH_2OH
12	COOH	CH_2OH	H

Compound **3** did not show the molecular ion at *m/z* 350 ($C_{22}H_{38}O_3$), but loss of methanol was observed (*m/z* 318). The ^{1}H NMR spectrum displayed three deshielded one-proton doublets at 4.03, 3.71 and 3.40 ppm, a six-proton singlet at 3.28 ppm, but only two three-proton singlets at 0.95 and 0.92 ppm. ^{13}C NMR data (see Table I) suggested the structure of an *ent*-kaurane diterpenoid, in accordance to other similar compounds isolated from species of this genus (Arriaga-Giner *et al.*, 1999). A hydroxymethyl group at C-19 (65.37 ppm) and an unusually deshielded methine at 107.44 ppm was attributed to a **C**H-$(OCH_3)_2$ group supported by two methoxyls at 52.54 and 52.49 ppm. DEPT experiments and comparison of data with related terpenoids indicated an *ent*-kaurane with a hydroxy group at C-19 and a dimethyl acetal at C-17. The stereochemistry of C-16 was assigned by comparison of the ^{13}C NMR data with those of the pair of epimers **11/12** (**#4/5** in Wu *et al.*, 1996) which showed a similar substitution pattern at the corresponding carbon atom. The α or β position of the substituent at C-16 strongly affects the shift of C-12 and C-13, too. A β-methyloxy function at C-16 deshielded C-12 (about 31 ppm), while the α-epimer shifted that carbon to 21 ppm (see Table I). These observations lead us to describe **3** as 19-hydroxy-16α-H-*ent*-kauran-17-al dimethyl acetal. It is assumed that such a derivative was formed during the elution of the acetone extract through the Sephadex column, where MeOH was used as eluent (Wollenweber *et al.*, 1997). 19-Hydroxy-16α-H-*ent*-kauran-17-al (**4**) must be the natural compound existing in the plant. The ability of an aldehyde function at C-17 to form the dimethyl acetal was earlier observed during purification of the dialdehyde at C-17 and C-19 (Henrick and Jefferies, 1964). When MeOH was used as solvent for chemical reactions, the mono-dimethyl acetal derivative was the only recovered product. Later, aldehydes at C-17 have been obtained during acid hydrolysis of kaurane-16,17-diol glycosides due to the loss of water (Kuraishi *et al.*, 1983). This reaction is not observed when enzymatic hydrolysis is carried out (Satake *et al.*, 1983). A natural aldehyde, epimer at C-17, was isolated from *Baccharis minutiflora* (Bohlmann *et al.*, 1982).

Table I. ^{13}C NMR data of compounds **3, 5, 7** and **9–12.** Indices a, b, c indicate interchangeable assignments in the same column.

Compound	**3**	**5**	**7**		**9**		**10**		**11/12**
Carbon/solv	$CDCl_3$	$CDCl_3$	$CDCl_3$	MeOD	Py-d_5[7]	MeOD	$CDCl_3$[5]	MeOD	$CDCl_3$[5]
1	40.46	40.33	40.15	42.37	40.8	42.73	40.7	42.57	41.6/42.0
2	18.27[a]	18.21[a]	18.26[a]	20.31[a]	19.3	20.67[a]	18.8[a]	20.31[a]	19.0/19.1
3	37.71	37.70	36.96	39.02	36.2	37.66	36.9	37.60	37.2
4	39.13[z]	37.01	36.23	38.36	39.3	40.70	39.2	40.66	44.7
5	56.72[b]	56.70[b]	56.26[b]	58.88[b]	57.8	59.91[b]	57.0[b]	59.05[b]	56.9
6	20.92	20.87	20.55	21.74	20.7	22.15	21.1	22.51	22.1/22.4
7	41.83	41.72	42.16	44.43	42.8	44.52	43.1	44.60	40.4/40.7
8	44.90	44.90	44.54	46.61	43.9	45.69	44.9	46.63	43.6/43.7
9	56.28[b]	56.34[b]	56.70[b]	59.30[b]	57.0	59.16[b]	57.3[b]	59.41[b]	55.3/56.4
10	38.60[z]	39.11	39.10	41.31	39.6	41.42	39.6	41.34	39.6
11	18.65[a]	18.67[a]	18.08[a]	19.69[a]	18.8	20.24[a]	18.8[a]	20.17[a]	18.9
12	31.38	31.38	26.06	28.12	27.5	28.73	26.7	28.12	20.0/31.4
13	37.87	37.90	45.26	47.25	41.8	43.23	46.0	47.23	36.9/38.1
14	35.56	36.28	37.01	39.10	38.5	40.11	37.7	39.04	37.8
15	44.01	44.01	52.93	54.75	53.5	54.16	53.9	54.80	44.2/45.0
16	42.73	42.78	81.87	83.67	79.6	81.55	81.5	83.67	43.3/43.1
17	107.44	107.49	66.12	67.75	70.4	71.49	66.4	67.73	64.2/67.4
18	27.05	25.52	27.44	28.90	28.1	28.73	28.0	28.74	28.9
19	65.37	67.13	67.09	69.12	64.1	66.07	64.1	65.97	183.7
20	17.99	17.96	18.13	20.12	18.4	19.64	18.6	19.87	15.5
$HC(O\mathbf{CH}_3)_2$	52.54	52.57							
	52.49	52.45							
$O\mathbf{CO}CH_3$		171.35	171.46	174.03					
$OCO\mathbf{CH}_3$		21.00	20.95	22.66					

Compound **5** also lacked a molecular ion (*m/z* 392, $C_{22}H_{40}O_4$), but showed the peak corresponding to a loss of methanol (*m/z* 360). The ^{1}H NMR spectrum showed a pattern similar to that of diterpenoid **3**. Three deshielded one-proton doublets at 4.21, 4.03 and 3.85 ppm, a six-proton singlet at 3.28 ppm, a three-proton singlet at 2.03 ppm, attributed to an acetyl, and two three-proton singlets at 0.98 and 0.91 ppm corresponding to the diterpenoid skeleton were observed. Comparison with deshielded protons of compound **3** revealed that two one-proton doublets appeared shifted to low field (4.21 and 3.85 ppm). This fact lead us to assign the location of the acetoxy group at C-19. The remaining one-proton doublet at 4.03 ppm is the one corresponding to H-17, which supported two methoxyls (as in **3**). The ^{13}C-NMR data (see Table I) are also similar to those of compound **3**, with the same dimethyl acetal group (107.49, 52.57 and 52. 45 ppm). The methylene group at C-19 appeared at 67.13 ppm, and C-4 was shifted to 37.01, indicating that the acetyl group (171.35 and 21.00 ppm) was at C-19. The structure proposed for **5** is thus 19-acetoxy-16α-H-ent-kauran-17-al dimethyl acetal. As discussed above for compound **3**, formation of the acetal **5** is again assumed to be produced during the column elution with acetone. Hence the 19-acetoxy-16α-H-ent-kauran-17-al **6** should be the natural compound in the plant. Natural diterpene aldehydes are relatively uncommon, but we recently also isolated 16α-hydroxy-*ent*-kauran-19-al from *O. scutellifolius* (Arriaga-Giner *et al.*, 1999).

The EI mass spectrum of ompound **7** also lacked the molecular ion *m/z* 364 ($C_{22}H_{36}O_4$), but the ion corresponding to loss of water was observed at *m/z* 346. Its ^{1}H NMR spectrum showed two pairs of deshielded one-proton doublets corresponding to methylenes having an oxygenated function. The lowest doublet, at 4.19 and 3.85 ppm, is supporting the acetyl group (2.01 ppm). The ^{13}C NMR data (see Table I) pointed to the presence of three hydroxyl substituents, because three carbons appeared at low field (81.87s, 66.12t and 67.09t). Assignment of the relative location of oxygenated functions was achieved by comparison of the MeOD spectrum with those of the triols **9** and **10**, epimers at C-16. Using that solvent, the signals appeared generally shifted about 2 ppm downfield relative to the corresponding signals recorded in $CDCl_3$. Comparison of the signals of carbons C-13, C-16 and C-17 revealed that those of **7** and **10** agree for the substituents at C-16. Differences observed for C-3, C-4 and C-19 confirmed that the acetoxy group is at C-19 in **7**. Thus, its structure is confirmed as 19-acetoxy-16β,17-dihydroxy-*ent*-kaurane. To our knowledege this is a new natural product, because only both epimers at C-16 of the parent triol have been previously described.

The diterpene-diol **8** was identified as 16α,19-dihydroxy-*ent*-kaurane. It was recently reported from *O. ledifolius* (Arriaga-Giner *et al.*, 1998), while the first report was from *Xylopia aethiopica* (Ekong *et al.*, 1969).

The epimeric triols **9** and **10** were identificated based on their ^{13}C NMR data. They have been isolated previously from *Ricinocarpus stylosus* and *Bahia glandulosa* (**9**; Henrick and Jefferies, 1964; Pérez-Castorena *et al.,* 1997) and from *Annona squamosa* (**10**; Wu *et al.*, 1996), respectively.

In phenolic fractions remaining from the earlier studies (Wollenweber *et al.*, 1997), we identified a previously unreported flavonol, gossypetin-3,8-dimethyl ether, by direct comparisons with an authentic sample, isolated from *Gutierrezia microcephala* (Roitman and James, 1985), as well as with a synthetic sample (Horie *et al.*, 1987). This rather rare flavonol was found in both collections of *O. hookeri*. It should be added to the list of *Ozothamnus* flavonoids presented in Wollenweber *et al.* (1997).

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