



DITERPENES FROM THE LEAF EXUDATE OF *PSIADIA PUNCTULATA*

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Abstract—Two known diterpenes, (*ent*)-2-oxokaur-16-en-18,19-diol (psiadin) and (*ent*)-kaur-16-en-2 α ,18,19-triol, and three novel diterpenes, (*ent*)-16 β ,17-dihydroxykauran-20-oic acid, 2-oxotrachyloban-18,19-diol and trachyloban-2 β ,6 β ,19-triol have been isolated from the leaf exudate of *Psiadia punctulata*. The structures of the diterpenes was established on the basis of spectroscopic analysis and previously published data on the known compounds. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Psiadia Jacq. (Compositae) is an Old World genus of some 60 species [1]. *Psiadia punctulata* (DC) Vatke is an East African species, the leaf decoction of which is used locally for the treatment of colds and fevers [2], and for the removal of ectoparasites from cattle [3]. The shrub is known to be avoided by browsing herbivores like giraffe and goats, even during severe drought. The leaves, especially when young, are covered by a gummy exudate which may be responsible for the deterrent effects.

The only reference to the chemistry of *P. punctulata* (in which it was regarded as synonymous with *P. arabica* Jaub and Spach.) recorded the presence of fifteen flavonoids [4]. *P. arabica* on the other hand has been reported to contain polymethylated flavonoids and kaurane diterpenes [5–8]. This combination of non-glycosidated methylated flavonoids and diterpenes is common in resinous surface exudates [9]. Here we describe the isolation and characterization of five kaurene and trachylobane diterpenes from the leaf resin exudate of *P. punctulata*.

RESULTS AND DISCUSSION

Dipping fresh leaves in acetone for short periods (*ca* 15 sec) gave a mixture of diterpenes, which were separated by column chromatography to give three pure compounds and a (1 : 1) mixture of two others.

The second diterpene to elute analysed for C₂₀H₃₂O₃

by HREIMS. The ¹H NMR spectrum showed AB quartet resonances for two isolated hydroxymethyl groups (no further coupling) and an oxymethine with a *W*₁₂ of 16 Hz, indicating an axial orientation for the proton. Assignment of the three hydroxyl groups to C-18, C-19 and C-2 followed from an analysis of the long-range heteronuclear coupling spectrum (HMBC [10]) which, together with the direct heteronuclear coupling information gained from the HC-COBI experiment [11], allowed assignment of the ¹³C NMR spectrum (Table 1). On the basis of these data the diterpene was identified as (*ent*)-kaur-16-en-2 α , 18, 19-triol (**1**) which has previously been reported from *Psiadia arabica* [5, 6]. Some of the ¹H and ¹³C NMR chemical shift assignments were found to be at variance with those reported previously and have been revised (Experimental and Table 1). The specific rotation, run in methanol, was considerably higher than previously recorded in chloroform [5].

The third diterpene fraction to be obtained proved to be an intractable mixture of two compounds. The HREIMS of the mixture indicated that both components were C₂₀H₃₀O₃ while the ¹H NMR spectrum revealed the occurrence of two exocyclic methylene and several oxymethylene substituents. An analysis of the ¹³C NMR spectrum of the mixture run in pyridine-*d*₅ revealed resonances that correlated closely with those previously recorded for psiadin (**2**) which has also been isolated from *P. arabica* [5]. The resonance values previously proposed for C-1 and C-3 should be reversed (confirmed by ³*J* correlation of Me-20 to C-1 and of 18- and 19-hydroxymethyl to C-3 in HMBC experiment). The second component of this mixture will be discussed below.

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Table 1. ^{13}C NMR shift values for isolated compounds

	1[5]	1	2[5]	2	3
1	36.9	49.6	45.2	56.6	36.8*
2	66.2	66.7	212.3	215.6	21.9**
3	49.1	37.4	56.1	45.7	43.4
4	40.3	43.2	48.0	48.5	34.7
5	47.3	47.9	47.9	48.4	57.5
6	21.4	21.9	20.9	21.4	21.7**
7	41.0	41.5	40.9	41.4	40.6*
8	44.3*	44.8	44.3*	44.8	45.9
9	56.3	56.9	54.8	55.3	56.0
10	42.7*	40.8	43.2*	44.0	49.4
11	19.0	19.5	18.7	19.1	19.7
12	33.3	33.8	33.0	33.5	25.9
13	44.2	44.7	44.1	44.6	46.3
14	39.5	40.2	39.3	39.8	43.0
15	49.1	49.6	48.9	49.5	54.9
16	155.8	156.3	155.4	155.9	82.2
17	103.5	104.0	103.8	104.2	67.0
18	66.2	66.8	66.9	67.4	23.6
19	69.4	70.0	63.7	64.2	33.9
20	23.1	23.5	19.5	20.0	179.6

*Chemical shift values interchangeable.

The first compound to be obtained from the column revealed a weak $[\text{M} + \text{H}]^+$ ion (FABMS) at 337 ($\text{C}_{20}\text{H}_{32}\text{O}_4 + \text{H}$). The ^1H NMR spectrum showed the presence of only two methyls and one oxymethylene and was otherwise rather uninformative. The ^{13}C NMR spectrum (Table 1) identified five quaternary carbons, including one oxygenated sp^3 (δ 82.2) and a carboxylic acid (δ 179.6), three methines, 10 methylenes, including the oxymethylene (δ 67.0), and two methyls. Given the presence of a carboxylic acid, the remaining two oxygens must be associated with

the sp^3 and oxymethylene carbons as tertiary and primary alcohols, respectively.

The HMBC spectrum revealed correlation of the two methyls with each other and with three other carbons, indicative of a 4,4-dimethyl diterpene and so precluding either C-18 or C-19 being oxidised. The oxymethylene protons showed coupling to the carbon at δ 82.2 as well as to a methine (C-13) and a deshielded methylene (C-15), which is typical for a 16,17-oxygenated kaurane. Identification of the H-13 resonance from an HC-COBI allowed, through the HMBC experiment, identification of C-12, C-15 and C-8. Assuming that the diterpene was a kaurane then the third methine must be assigned to C-9 (δ 56.0) and from this the HCCOBI allowed identification of H-9 centred at δ 1.41. This proton showed a total of seven 2J and 3J correlations in the HMBC spectrum which identified but did not distinguish C-1 and C-7 and allowed unambiguous assignment of C-8, C-10, C-11, C-12, C-14 and, most importantly, the carboxylic acid at C-20 (Fig. 1). The ^{13}C NMR of **3**, when compared with that of other 16,17 dihydroxy kaurane diterpenes, showed chemical shift values for C-13, C-16 and C-17 which were typical of a 16α orientation [12]. Thus the diterpene must be (*ent*)-16 β ,17-dihydroxy-kauran-20-oic acid (**3**), which appears to be novel.

The most polar of the diterpenes eluted gave a molecular ion by HREIMS which resolved for $\text{C}_{20}\text{H}_{30}\text{O}_5$. The IR spectrum revealed a strong hydroxyl band but no carbonyls. The ^1H NMR spectrum revealed two axial oxymethine resonances as a *ddd* at δ 4.41 (2 axial-axial and 1 axial-equatorial couplings) and as a *dddd* at δ 4.21 (2 axial-axial and 2 axial-equatorial) and an oxymethylene as an ABq at δ 4.44 and 3.85. There were three methyl singlets, one strongly deshielded at δ 1.75, and two methine resonances at δ 0.86 and 0.60 suggesting a cyclopropane ring.

The HMBC spectrum showed one of the methyls (δ 1.16) correlation with a quaternary carbon (δ 23.2), the two carbons associated with the cyclopropane protons (δ 21.0 and 25.1) and a methylene (51.5 ppm). The resonance positions for the methyl (δ 21.1) and for C-12, C-13 and C-16 were in close agreement with those previously reported for ciliaric acid (**6**) run in the same solvent (pyridine- d_5) [13]. Furthermore, a ^1H doublet at δ 1.50, which could be assigned to the

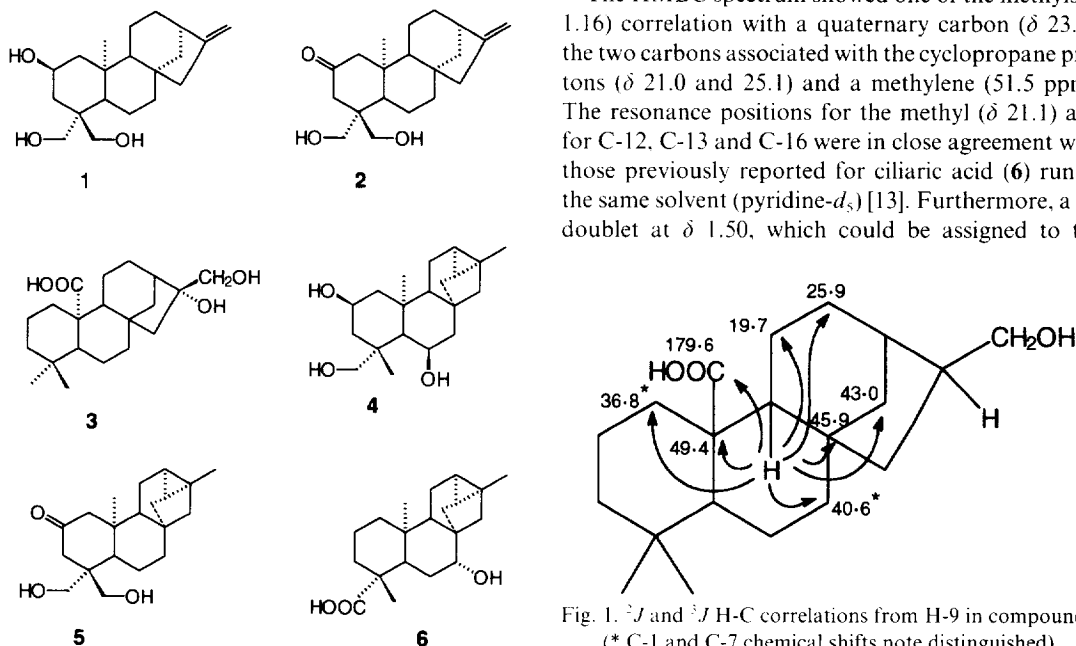


Fig. 1. 2J and 3J H-C correlations from H-9 in compound **3** (* C-1 and C-7 chemical shifts note distinguished).

Table 2. ^{13}C NMR chemical shift for the trachylobane diterpenes **4** and **5** (spectra run in pyridine- d_5)

	4	5
1	50.3	55.5
2	63.4	215.8
3	50.6	46.0
4	42.2	49.1
5	61.4	48.6
6	69.2	21.5
7	49.9	39.4
8	41.7	41.5
9	53.8	52.9
10	42.5	43.5
11	20.8	20.5
12	25.1	25.0
13	21.0	21.2
14	35.0	33.5
15	51.5	50.6
16	23.2	23.1
17	21.1	21.1
18	33.0	64.2
19	67.2	67.3
20	17.3	16.9

isolated methylene at C-14 showed a 3J correlation to C-16 as well as other 3J couplings to C-7 (δ 49.9), C-9 (δ 53.8) and C-11 (δ 20.8). From these data this diterpene was assigned a trachylobane structure.

The hydroxymethyl protons and those of the deshielded methyl correlated with each other, indicating their placement as geminal substituents on C-4. Identical correlations from both identified C-3 (methylene), C-4 and C-5 (methine). The strongly deshielded resonance for the methyl (δ 33.0) is indicative of it being equatorial (C-18), so placing the hydroxymethyl at C-19. The two hydroxyl substituents can now be assigned to C-2 (*dddd*) and C-6 (*ddd*). Unfortunately, neither H-2 nor H-6 revealed any long-range correlations in the HMBC spectrum. However, the COSY-45 spectrum allowed identification of H-3 (axial) at δ 1.53 (HMBC correlations to C-18, C-19, C-1, C-2, C-4 and C-5) and H-7 (axial) at δ 1.83 (HMBC correlations to C-5, C-6, C-8, C-14 and C-15). On the basis of these data the diterpene was characterised as trachyloban-2 β ,6 β ,19-triol (**4**), which again appears to be novel. Full ^{13}C NMR data are given in Table 2.

The final compound to be identified was the second component of the mixture containing **2**. That this was a trachylobane diterpene was established by the cyclopropane resonances which were identical to those observed in **4**. Comparison of ^{13}C NMR data for **4** with signals identified as belonging to the trachylobane component of the mixture showed rings C–E to be identical (Table 2). The molecular formula of this compound could be assumed to be $\text{C}_{20}\text{H}_{32}\text{O}_3$ and the HMBC correlations indicated 18, 19 hydroxymethyls. The remaining oxygen is a ketone and this must be placed at C-2 in view of the deshielded

resonances for C-1 and C-3 (seen through long range correlations from 18/19 hydroxymethyl and 20-methyl, respectively). This compound must therefore be 2-oxo-trachyloban-18,19-diol (**5**), which again appears to be novel.

The presence of trachylobane as well as kaurane diterpenes in *P. punctulata* at present differentiates it from *P. arabica* which has to date yielded only kauranes. It would be interesting to re-examine material that can unequivocally be assigned to *P. arabica* to confirm this distinction.

EXPERIMENTAL

Plant material. Aerial parts of *P. punctulata* were collected from open road side by the Nairobi National Park in September 1994. A voucher specimen is deposited at University Herbarium, Department of Botany, University of Nairobi.

Extraction of diterpenes. The whole fresh plants (within 24 hr of collection) were extracted by dipping into Me_2CO for less than 15 sec. The solvent was removed to leave a pale yellow gum (22 g from 394 g aerial dry parts).

Isolation of diterpenes. The crude extract (10 g) was subjected to CC over silica gel (150 g). Elution with mixtures of hexane and CH_2Cl_2 and then CH_2Cl_2 and MeOH so that there was a constantly increasing polarity gave four bands as follows. **3** (85 mg), **1** (150 mg), **2** + **5** (950 mg) and **4** (88 mg).

(ent)-Kaur-16-en-2 α , 18, 19-triol (**1**). Amorphous solid from MeOH, mp 230–232 (lit. [5] 237–238°). $[\alpha]_{\text{D}}^{25} = 111$ (*c* 2.94, MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3410, 1642. ^1H NMR pyridine- d_5 : δ 4.92, 4.67 (2 $\times$ 1H, 2 $\times$ *s*, H-17), 4.56 (1H, *m*, H-2), 4.38, 4.20 (2H, ABq, *J* = 10.7 Hz, H-18), 4.20, 4.02 (2H, ABq, *J* = 10.4 Hz, H-19), 2.63 (1H, *br s*, H-13), 2.47 (1H, *dd*, *J* = 13.7, 3.9 Hz, H-3_{eq}), 2.36 (1H, *dd*, *J* = 13.7, 8.7 Hz, H-3_{ax}), 1.47 (3H, *s*, Me-20), 1.17 (1H, *dd*, *J* = 9.5, 5.5 Hz, H-9), 1.04 (1H, *dd*, *J* = 11.3, 4.9 Hz, H-14). ^{13}C NMR—see Table 1. HRFABMS *m/z* (rel. int.): 343.22 [$\text{M} + \text{Na}$] $^+$, 321.252 [$\text{M} + \text{H}$] $^+$ ($\text{C}_{20}\text{H}_{33}\text{O}_3$) (6.2), 320.239 ($\text{C}_{20}\text{H}_{32}\text{O}_3$) (1.4).

Psiadin (**2**) and 2-oxotrachyloban-18,19-diol (**5**). Amorphous solid from MeOH. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3410, 1698, 1645. For ^{13}C NMR see Table 1 for **2** and Table 2 for **5**. FABMS *m/z* 319 [$\text{M} + \text{H}$] $^+$.

(ent)-16 β ,17-Dihydroxykaur-20-oic acid (**3**). Amorphous solid from MeOH, mp 226–229°. $[\alpha]_{\text{D}}^{25} = 35^\circ$ (*c* 2.86, MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400–2500. ^1H NMR (pyridine- d_5): δ 4.19, 4.11 (2H, ABq, *J* = 10.8 Hz, H-17), 2.97 (1H, *d*, *J* = 12.5 Hz, H-14), 2.41 (1H, *br s*, H-13), 1.24 (3H, *s*, Me-19), 0.98 (3H, *s*, Me-18). ^{13}C NMR—see Table 1. FABMS *m/z*: 337.542 [$\text{M} + \text{H}$] $^+$ ($\text{C}_{20}\text{H}_{33}\text{O}_4$).

Trachyloban-2 β ,6 β , 19-triol (**4**). Needles from MeOH, mp 216–217°. $[\alpha]_{\text{D}}^{25} = 60$ (*c* 4.20, MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3350. ^1H NMR pyridine- d_5 : δ 4.44, 3.85 (2H, ABq, *J* = 10.4 Hz, H-19), 4.41 (1H, *td*, *J* = 11.0, 3.8 Hz, H-6_{ax}), 4.21 (1H, *tt*, *J* = 11.5, 4.0 Hz, H-2_{ax}),

1.83 (1H, *t*, *J* = 11.9 Hz, H-7_{ax}), 1.76 (3H, *s*, Me-18), 1.53 (1H, *t*, *J* = 12.2 Hz, H-3_{ax}), 1.50, 1.37 (2H, AB_q, *J* = 11.1 Hz, H-14), 1.21 (3H, *s*, Me-20), 1.16 (3H, *s*, Me-17), 0.86 (1H, *dd*, *J* = 7.8, 3.2 Hz, H-13), 0.62 (1H, *br d*, *J* = 7.8 Hz, H-12). ¹³C NMR—see Table 2. EIMS *m/z* (rel. int.): 320.2346 (calc for C₂₀H₃₂O₃ 320.2351) (6.2), 284 (65), 271 (15), 253 (30), 218 (34).

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