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Three *ent*-trachylobane diterpenes from the leaf exudates of *Psiadia punctulata*

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Abstract

Three *ent*-trachylobane diterpenes have been isolated from the leaf exudates of *Psiadia punctulata* and characterised as 6α,17,19-*ent*-trachylobantriol; 2α,18,19-*ent*-trachylobantriol; and 2β,6α,18,19-*ent*-trachylobantetraol. The structures were determined on the basis of spectroscopic evidence.

Keywords: *Psiadia punctulata*; Asteraceae (Compositae); Leaf exudates; Diterpenes; *ent*-Trachylobane; 6α,17,19-*ent*-Trachylobantriol; 2α,18,19-*ent*-Trachylobantriol; 2β,6α,18,19-*ent*-Trachlobantetraol

1. Introduction

Psiadia punctulata (DC) Vatke (Compositae) is an erect, round-topped, glabrous shrub, which is covered with a gum-like secretion, mainly on young leaves (Agnew, 1974). It is used for relief of fever and abdominal pain in East Africa (Kokwaro, 1983). This East African taxon is considered by some taxonomists to be synonymous with *P. arabica* found in Saudi Arabia and the Sudan (Beentje, 1994). We have earlier reported the presence of kaurene and trachylobane diterpenes (Midiwo et al., 1997), flavones and phenylpropanoids (Juma et al., 2001) from the leaf exudates of *P. punctulata*. We have also noted that the compounds isolated from *P. punctulata* are distinct from those reported from *P. arabica* (Abou-Zaid et al., 1991, El-Feraly et al., 1990), and disputed the suggested synonymy between these two taxa (Juma et al., 2001). Here we report the isolation and characterization of three new trachylobane diterpenes from the leaf exudates of *P. punctulata*, which further demonstrates that this plant is a rich source of trachylobane diterpenes.

2. Results and discussion

Compound **1** was isolated as colourless crystals from CH_2Cl_2/MeOH. TOF-EIMS analysis showed a molecular ion peak at *m/z* 320.2334 corresponding to $C_{20}H_{32}O_3$. The ^1H and ^{13}C NMR (Table 1) spectra are consistent with this compound being a diterpene (20 carbons) and showed signals for two methyls, two oxymethylenes and one secondary alcohol, thus accounting for the three oxygens. The HMBC spectrum revealed cross correlations between one methyl and one oxymethylene with the protons of both showing further correlations to a quaternary carbon (39.5 ppm), a methine (61.5 ppm) and a methylene (40.8 ppm). These observations indicate geminal methyl and oxymethylene substituents placed on C-4 of a decalin nucleus. The strongly deshielded resonance of the methyl

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Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (δ) of diterpens **1–3** in pyridine-d_5

	1	**2**	**3**	**1**	**2**	**3**
1	0.81 dd (12.7, 3.9)	1.21 m	1.75 m	40.2	49.9	48.8
	1.42 dt (12.8, sm)	2.28 dt (11.3, sm)	1.98 m			
2	1.30 m	4.42 brt	4.48 m	18.7	64.2	65.2
	1.50 m					
3	1.25 m	1.90 t (12.5)	1.98 m	40.8	41.3	38.2
	1.70 m	2.92 ddd (12.7, 4.3, 2.0)	2.56 dd (13.4, 3.8)			
4				39.5	45.3	45.0
5	1.18 d (10.9)	1.63 d (11.7)	1.33 m	61.5	50.6	53.7
6	4.38 m	1.55 m	4.33 td (10.5, 3.5)	69.3	21.2	67.6
		1.90 m				
7	1.86 t (12.1)	1.44 m	1.75 m	49.8	40.0	48.6
	2.01 dd (12.1, 3.3)	1.50 m	1.86 m			
8				41.5	41.3	41.3
9	1.30 m	1.38 m	1.33 m	54.0	54.1	53.5
10				40.7	40.0	41.6
11	1.70 m	1.80 ddd (11.5, 6.3, 2.0)	1.82 m	20.8	20.6	20.3
	1.93 d (11.1)	1.90 m	1.98 m			
12	1.00 d (7.7)	0.62 d (7.8)	0.62 d (7.7)	19.2	21.4	21.2
13	1.21 dd (7.7, 3.1)	0.87 d (7.8, 3.0)	0.87 dd (7.7, 2.9)	22.8	25.1	25.0
14a	1.32 m	1.27 m	1.26 brd (11.8)	34.6	34.2	34.0
14b	2.14 d (11.8)	2.11 d (11.8)	2.08 d (11.8)			
15a	1.93 d (11.1)	1.27 d (11.2)	1.33 m	47.5	51.1	51.1
15b	1.68 d (11.1)	1.37 d (11.2)	1.47 d (11.1)			
16				31.0	23.1	22.7
17	3.90 d (11.1)	1.14 s	1.15 s	66.8	21.1	20.9
	3.95 d (11.1)					
18	1.63 s	4.25 d (10.8)	4.09 d (10.8)	32.6	70.1	68.8
		4.02 d (10.8)	4.65 d (10.6)			
19	3.71 dd (10.3, 2.6)	4.22 d (10.8)	4.25 d (10.6)	67.8	64.7	65.8
	4.38 m	4.01 d (10.8)	4.57 d (10.6)			
20	1.13 s	1.14 s	1.43 s	16.4	17.0	21.6

J values in Hz.

(δ 32.6) indicated that it is equatorial (C-18), allowing the placement of the OH at C-19 (Midiwo et al., 1997). HMBC correlations from the second methyl also correlated with the methine at 61.5 ppm as well as with a quaternary carbon (40.7 ppm), a second methine (54.0 ppm) and a methylene (40.2 ppm). As there is a common correlation to the methine at 61.5 ppm the second methyl must be attached to C-10 of the decalin.

The remaining oxymethylene protons revealed four HMBC correlations to a quaternary carbon at 31.0 ppm (C-16), two methines at 19.2 ppm (C-12) and 22.8 ppm (C-13), and a methylene at 47.5 ppm (C-15) allowing its placement at C-16 of a trachyloban skeleton; as in other trachylobans H-13 showed coupling with one of the C-14 protons (Midiwo et al., 1997). However the resonances were deshielded for the cyclopropane protons in comparison with compounds where the C-16 substituent is a methyl rather than an oxymethylene.

The hydroxymethine proton appeared at δ 4.38, overlapping with a signal from the C-4 hydroxymethylene substituent. It showed COSY cross peaks with the protons at δ 2.01 (H-7), 1.86 (H-7) and 1.18 (H-5) and could therefore be assigned to H-6. The large coupling constants ($J = 12.1$ Hz to δ 1.86) and ($J = 10.9$ Hz to H-5) requires that H-6 should also be axial (6-OH then is equatorial).

A series of nOe difference experiments were performed to examine the relative stereochemistry of **1**. The most instructive of these involved irradiation of the C-20 methyl (δ 1.13) which resulted in enhancements of H-14 (δ 2.14), H-11 (δ 1.70), the oxymethylene proton (δ 3.71, 4.38, CH_2-19), H-6ax (δ 4.38) and H-2ax (δ 1.50) (Table 2). By contrast irradiation of the second methyl (Me-18) resonance gave no enhancements.

On the basis of these observations compound **1** must be (*rel*)-6β,17,19-trachylobanetriol. Given the strong negative optical rotation it is presumed that the actual structure will be 6α,17,19-*ent*-trachylobanetriol (Midiwo et al., 1997; Leong and Harrison, 1997; Block et al., 2004).

HR-EIMS analysis of compound **2** also showed a molecular ion peak for $C_{20}H_{32}O_3$. ^{1}H and ^{13}C NMR (Table 1) indicated that this compound is also a trachylobane-type diterpene with three hydroxyl substituents. As in **1**, two of the hydroxyl groups are primary (hydroxymethelenes, each with non-equivalent protons resonating at δ 4.02 and 4.25 for CH_2-18, δ 4.01 and 4.22 for CH_2-19) and the third a secondary hydroxymethine (δ 4.42).

Although the two methyl resonances were almost identical it was possible from the HMBC spectrum to assign one to C-17 and the other to C-20 as all ^{2}J and ^{3}J correlations for both were visible. It was noteworthy that as the C-16

Table 2
Major nOe enhancements seen upon irradiation of Me-17 (in **2** and **3**) and Me-20 (in **1-3**)

	1	**2**	**3**
Irradiated group	nOe enhancements	nOe enhancements	nOe enhancements
Me-17		H-12, H-13, H-15b	H-12, H-13, H-15b
Me-20	H-2ax, H-6ax, H-11eq, H-14b, CH_2-19	H-2ax, H-6ax, H-11eq, H-14b, CH_2-19	H-6ax, H-11eq, H-14b, CH_2-19

substituent reverted to the normal methyl the resonances for the cyclopropane protons reverted to their normal highly shielded positions (H-12, δ 0.62; H-13, δ 0.87). This required that both the C-4 substituents were oxymethylenes and this was supported by HMBC correlations between them and from them to C-3, C-4 and C-5.

The COSY spectrum of **2** revealed that the oxymethine proton coupled with protons assigned to C-1 and C-3 and the hydroxy group must therefore be placed at C-2. This was reflected by the appreciable change in ^{13}C resonance for C-1 of **2** in comparison with **1** (Table 1). The oxymethine proton H-2 occurred as a broad triplet reflecting two large couplings thereby requiring it to be axial.

Although the correspondence of Me-17 and Me-20 made an nOe difference experiment complex it was possible to distinguish which enhancements were caused by each methyl as the two are so far apart within the molecule (Table 2). Enhancements attributable to Me-20 were essentially as would be predicted from those noted for **1** and included H-2, so confirming that proton must be axial (Fig. 1). Consequently **2** must be (*rel*)-2β,18,19-trachylobanetriol and, in view of the levorotatory nature of the isolate it is presumed to be 2α,18,19-*ent*-trachylobanetriol.

The final compound, **3**, showed an $[M]^+$ peak at *m/z* 336.2281 ($C_{20}H_{32}O_4$). The NMR spectra (Table 1) once again indicated a trachylobane-type diterpene with four hydroxyl (two primary and two secondary) substituents. The HMBC spectrum once again revealed that the two methyls could be assigned as Me-17 and Me-20 and consequently the oxymethylenes must be placed as C-18 and C-19. The placement of the hydroxyl groups at C-2 and C-6 was confirmed through HMBC and HMQC experiments. As in **1** the H-6 proton showed two axial-axial couplings and must therefore be axial. In comparison the coupling observed for H-2 was unresolved.

In nOe difference experiments (Table 2), irradiation of the Me-20 showed the anticipated enhancements with H-14, H-11, the oxymethylene resonating at δ 4.57 and 4.25, and with H-6, confirming this proton is axial. However, in contrast with **2**, there was no enhancement of H-2, suggesting that this proton was equatorial. A change in the configuration at C-2 is also supported by the considerable deshielding observed for Me-20 which is now 1,3-diaxial with the C-2 hydroxyl group.

Thus the final new compound is identified as (*rel*)-2α,6β,18,19-trachylobantetraol (**3**). Once again the strong negative optical rotation is consistent with this compound being 2β,6α,18,19-*ent*-trachylobantetraol.

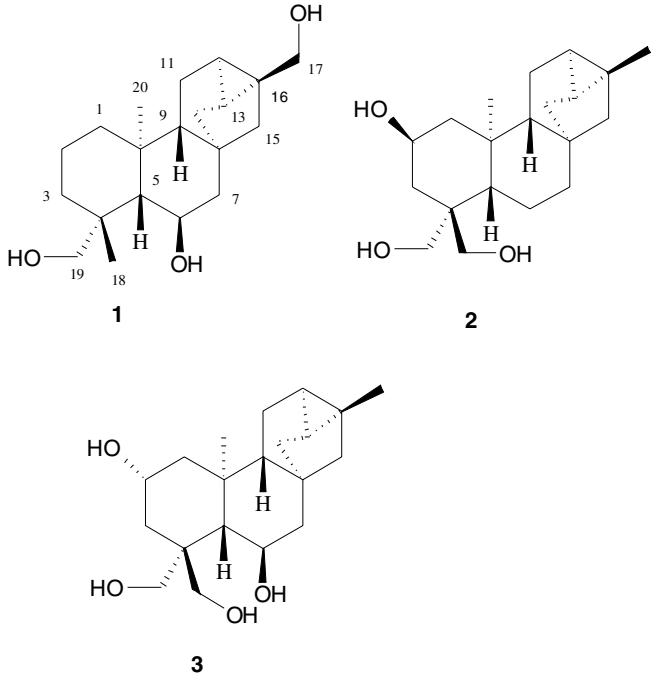

Fig. 1. New *ent*-Trachylobanin the exudate of *P. punctulata*.

3. Experimental

3.1. General

Analytical TLC: Merck pre-coated silica gel 60 F_{254} plates. CC on silica gel 60 (70–230 mesh). EIMS: direct inlet, 70 eV, on a SSQ 710, Finnigan MAT mass spectrometer. ^1H and ^{13}C NMR on Bruker DRX-500 spectrometer with TMS as int. standard. HMQC and HMBC spectra were acquired using the standard Bruker software.

3.2. Plant material

Refer to Juma et al. (2001) for authentication of the plant material.

3.3. Extraction and isolation

The exudates on the leaves of *Psiadia punctulata* was extracted with ethyl acetate as described in Midiwo et al. (1997). Removal of the solvent gave a brown gummy extract (494 g) from a dry weight of 1.7 kg. A portion of the ethyl acetate extract (100 g) was subjected to column chromatography on silical gel (500 g) and eluted with

hexane/CH_2Cl_2 and then CH_2Cl_2/MeOH mixtures with increasing polarities as described in Juma et al. (2001). The fraction eluted with 10% MeOH in CH_2Cl_2 showed two spots on TLC (hexane/EtOAc; 4:1, multiple development) and were separated by further column chromatography on silica gel (eluted with 5% MeOH in CH_2Cl_2) to give **1** (1.3 g) and **2** (500 mg). From the original column, the fraction eluted with 20% MeOH in CH_2Cl_2 gave **3** (800 mg).

3.4. 6α,18,19-ent-Trachylobantriol (*1*)

White crystals from MeOH/CH_2Cl_2, mp 193–194 °C. $[\alpha]_D^{25}$ −75.0° (*c* 0.1, MeOH) ^{1}H NMR (Table 1). ^{13}C NMR (Table 1). EIMS *m/z* (rel. int.): 320 (4, $[M]^+$), 302 (26, $[M\text{-}H_2O]^+$), 284 (100, $[M\text{-}2H_2O]^+$), 271 (31), 255 (22). HRMS $[M]^+$ found 320.2334, $C_{20}H_{32}O_3$, calculated for 320.2343.

3.5. 2α,17,18-ent-Trachylobantriol (*2*)

White crystals from MeOH/CH_2Cl_2, mp 201–202 °C. $[\alpha]_D^{25}$ −35.0° (*c* 0.1, MeOH). ^{1}H NMR (Table 1). ^{13}C NMR (Table 1). EIMS *m/z* (rel. int.): 320 (17, $[M]^+$), 302 (23, $[M\text{-}H_2O]^+$), 284 (100, $[M\text{-}2H_2O]^+$), 271 (37), 254 (76). HRMS $[M]^+$ found 320.2334, $C_{20}H_{32}O_3$, calculated for 320.2343.

3.6. 2β,6α,18,19-ent-Trachylobantetraol (*3*)

White crystals from MeOH/CH_2Cl_2, mp 198–199 °C. $[\alpha]_D^{25}$ −91.1° (*c* 0.7, MeOH). ^{1}H NMR (Table 1). ^{13}C NMR (Table 1). EIMS *m/z* (rel. int.): 336 (17, $[M]^+$), 318 (7, $[M\text{-}H_2O]^+$), 301 (27, $[M\text{-}2H_2O]^+$), 288 (75), 270 (100), 255 (37). HRMS $[M]^+$ found 336.2281, $C_{20}H_{32}O_4$, calculated for 336.2292.

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