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Nitrogen-Containing Dimeric *nor*-Multiflorane Triterpene from a *Turraea* sp.

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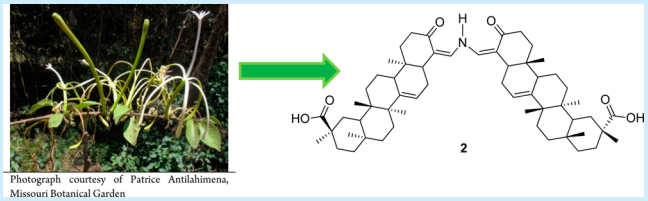
Supporting Information

ABSTRACT: The new triterpene turranoic acid (**1**) and the new N-containing *nor*-triterpene turraenine (**2**), along with triptocallic acid B (**3**) and esculentoic acid (**4**) were isolated from leaves of a *Turraea* sp. Compounds **1**−**3** showed weak to moderate in vitro antiplasmodial activity against the chloroquine-resistant *Plasmodium falciparum* strain FCM29. Compound **1** also displayed weak cytotoxic activity against the nonsmall lung cancer cell line H522-T1 with an IC_{50} value of 16.4 μM.



Plants from the genus *Turraea* (family Meliaceae, order Rutales) have been extensively investigated due to their high content of bioactive limonoids[1−7] and triterpenoids such as turrapubesols[8−10] and pregnanes.[8,9,11,12] These compounds have been reported to have a wide range of biological activities including cytotoxic, insect antifeedant, and mosquito larvicidal activity. As part of a joint International Cooperative Biodiversity Group (ICBG) research program to search for new antimalarial and anticancer secondary metabolites from the natural resources of Madagascar, we selected a plant species from the genus *Turraea* for investigation. The genus *Turraea* contains approximately 85 species, about 60 of them found in tropical and southern Africa, one species in Australia, and 24 species in Madagascar.[13] In African ethnobotany, species of this genus have been used as an aphrodisiac and to treat wounds, parasites (bilharzias), abscesses, and impotence, and the ripe fruit and the bitter bark of *Turraea* species are used in Madagascar to treat throat problems.

A crude EtOH extract made from leaves of a Madagascar species of *Turraea* was selected for investigation since it demonstrated in vitro antiplasmodial activity at 3.9 μg/mL against the chloroquine-resistant strain FCM29 of *Plasmodium falciparum* during our preliminary screening. Bioassay-guided fractionation of this extract resulted in the isolation of turranoic acid (**1**), a new triterpenoid with a multiflorane skeleton, and turraenine (**2**), a new nitrogen-containing *nor*-multiflorane-type triterpene. Triptocallic acid B (**3**) and esculentoic acid (**4**) were also isolated (Figure 1). In this paper, we report the isolation and the structural elucidation of the new compounds **1** and **2** and the biological activity of all four compounds.

The EtOH extract was subjected to liquid−liquid partitioning followed by column chromatography over silica gel, RP-C18

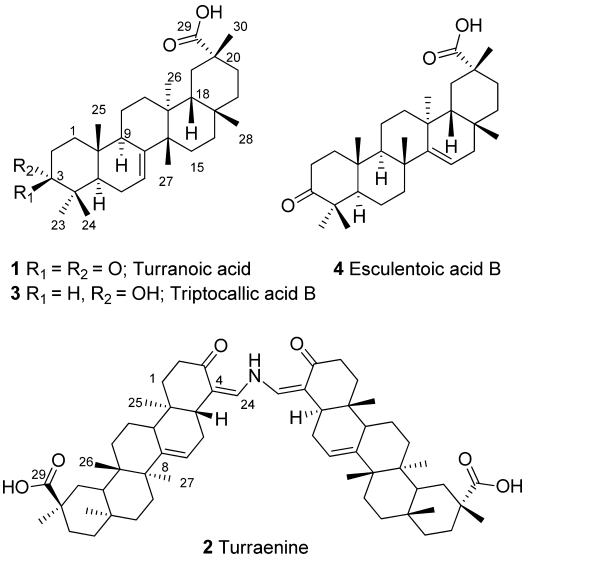


Figure 1. Structures of compounds**1**−**4**.

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silica gel, and Sephadex LH-20. Final crystallization afforded the four compounds **1**−**4**. Compounds **3** and **4** were identified as triptocallic acid B and esculentoic acid, respectively, based on the comparison of their spectroscopic data with those reported in the literature.[14,15]

The positive-ion high-resolution electrospray ionization (HRESI) mass spectrum of compound **1** displayed a protonated molecular ion peak at m/z 455.3517 corresponding to the molecular formula $C_{30}H_{47}O_3$ (required for $[M + H]^+$: m/z 455.3520). The IR spectrum showed a typical absorption band at 3443 cm^{-1} and strong absorptions at 1709 and 1732 cm^{-1} which were suggestive of hydroxyl, ketone carbonyl, and carboxyl functions. The ^{1}HNMR spectroscopic data of **1** (Table 1) exhibited seven methyl singlets (δ 0.93, 3H; 0.98, 6H;1.01, 3H; 1.03, 3H; 1.10, 3H; 1.23, 3H) and one olefin methine at δ 5.47 (dd, 6.4, 3.2 Hz) which was coupled to a methylene in a cyclohexene ring. The ^{13}C NMR data (Table 1) displayed 30 carbon resonances which were assigned to one ketone carbonyl (δ 217.2), one carboxylic acid (δ184.5), one olefin methine (δ 117.3), one sp^2-hybridized quaternary carbon (δ 145.6), 10 methylenes, three methines, six sp^3-hybridized quaternary carbons, and seven methyls by DEPT 135 experiments. The above NMR data were very similar to those of triptocallic acid B (**3**, Table 1), a multiflorane-type triterpene acid which was first isolated from a callus cell culture of *Trypterigium wilfordii* (Celastraceae).[14]

Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of **3** indicated that the chemical shifts arising from the methyl, the methylene, and the methine groups were essentially the same except for the signals due to the A-ring. The ^{13}C NMR spectrum of **1** showed the presence of a signal at δ 217.2 (C-3) instead of the oxygen-bearing methine (δ 79.3) in **3**. The carbon chemical shifts of C-23 and C-24 shifted from 27.8 and 15.0 in **3** to 24.0 and 21.8, respectively, while the other signals remained almost the same.

In addition, the methylene signals at δ 2.25 dt (J = 14.5, 3.7 Hz, H-2a) and δ 2.73 dt (J = 14.5, 5.5 Hz, H-2b) in the proton spectrum of **1** corroborated the presence of a ketone carbonyl at C-3. Two-dimensional NMR data of **1** confirmed the assignment of the carbonyl to C-3, the olefin methine at C-7, the methyl groups at C-4, -10, -13, -14, -17, and -20, and the carboxyl group at C-29. The HSQC spectrum was used to assign the proton-bearing carbons (Table 1). The ^2J and ^3J couplings observed in the HMBC spectrum were then interpreted. The carbonyl carbon at C-3, the methyl group at C-10, and the location of the olefin methine (C-7) of the decalin were assigned by observing the long-range correlations from H-1 to C-3; CH_3-23 and −24 to C-3, C-4, and C-5; CH_3-25 to C-1, C-9, and C-5; and CH-7 to C-5, C-9, and C-14. The HMBC cross peaks between CH_3-26 and C-12, C-18, and C-27 on one hand and between CH_3-30 and C-29, C-19, and C-21 on the other hand indicated the presence of a carboxylic acid at C-29. In the same manner, the methyl groups at C-13 and C-14 were allocated. The relative and absolute configurations at C-20 and at other chiral centers were assigned by interpretation of the NOESY spectroscopic data and X-ray diffraction analysis of a single crystal of **1**. The X-ray structure of **1** showing anisotropic displacement ellipsoids at the 50% probability level is shown in Figure 2.[16] These findings confirmed the structure of **1** as multiflor-3-on-7-en-29-oic acid, named turranoic acid.

Table 1. ^{1}H NMR Data of Compound 1 and ^{13}C NMR Data of Compounds 1 and 3 in $CDCl_3$ (δ in ppm)[a]

	1		3
position	δ_H (multiplicity (m), J in Hz)	δ_C	δ_C
1	1.44, m	38.5	37.6
	2.00, m		
2	2.25, dt (14.5, 3.7)	35.0	27.6
	2.73, td (14.5, 5.5)		
3		217.2	79.3
4		47.8	39.2
5	1.64, dd (7.8, 6.5)	52.1	50.7
6	2.08, m	24.7	24.5
7	5.47, dd (6.4, 3.2)	117.3	117.6
8		145.6	145.7
9	2.12, m	48.1	48.3
10		35.3	35.5
11	1.52, m, 1.63, m	17.4	17.5
12	0.86 br d (13.8)	32.8	33.3
	2.00, td (13.8, 4.3)		
13		36.9	37.3
14		42.5	42.5
15	1.47−1.41[b]	29.4	30.9
16	2.00, m[b]	36.9	37.3
17		31.2	31.6
18	1.48, m	47.2	45.8
19	1.67, m	30.4	30.9
	2.37, br d (16.0)		
20		40.4	40.6
21	1.37−1.41[b]	29.4	29.5
22	1.37, m, 1.76, m	35.6	36.0
23	0.98, s	24.0	27.8
24	1.10, s	21.8	15.0
25	0.98, s	13.0	13.4
26	1.03, s	24.7	25.2
27	0.93, s	25.5	27.8
28	1.01, s	31.4	31.5
29		184.5	182.8
30	1.23, s	33.1	33.5

[a] ^{1}H NMR recorded at 600 MHz; ^{13}C NMR recorded at 150 MHz. [b] Signals overlapped.

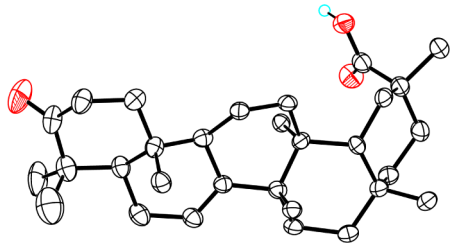

Figure 2. Displacement ellipsoid drawing (50% probability) of the single-crystal X-ray structure of **1**. One of two crystallographically independent molecules is shown. Aliphatic H atoms are omitted for clarity.

Compound **2** had the molecular formula $C_{58}H_{83}NO_6$ as determined by positive high-resolution ESIMS (observed m/z 890.6311, required for $C_{58}H_{84}NO_6$ $[M + H]^+$, 890.6293). Its ^{1}HNMR spectrum displayed a deshielded triplet resonance due to a hydrogen-bonded amine proton (δ14.0, t, J = 11.4 Hz, 1H), two 2H olefin methine signals (δ 6.46, d, J = 11.4, 2H, H-24 and -24′ and δ 5.40, brs, 2H, H-7 and -7′), and two sets of signals superposable on those of **1**. Excitation of the proton at δ

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14.0 by 1D TOCSY collapsed the 2H olefin methine signal at δ 6.46, indicating the presence of the partial structure =CHNHCH= in **2**.

The ^{13}C NMR spectrum of **2** showed signals for conjugated ketone carbonyl (δ 200.0, C-3) and carboxyl (δ 185.9) groups, signals due to olefin methine carbons (δ 114.0, C; 115.8, CH; 141.5, CH and 146.5, C), one of which was assignable to a methine attached via a heteroatom, and signals ascribable to a multiflorene-type triterpene dimer.

Inspection of the ^{1}H and ^{13}C NMR data (Table 2) of **2** revealed the presence of two sets of signals which were very

Table 2. ^{1}H and ^{13}C NMR Data for 2 in $CDCl_3$ (δ in ppm)[a]

position	δ_H (multiplicity (m), J in Hz)	δ_C
1,1′	2.49 m, 1.41−1.44[b]	34.9
2, 2′	1.40 m, 1.98 m	33.7
3,3′		200.0
4,4′		114.0
5,5′	2.39 dd (10.1, 5.6)	42.4
6,6′	1.30−1.38[b]	29.1
7,7′	5.40 br	115.8
8,8′		146.5
9,9′	2.11 brd (13.6)	44.8
10,10′		34.4
11,11′	1.48 m, 1.78 m	17.9
12,12′	0.90 m, 2.04 m	32.8
13,13′		36.7
14,14′		43.0
15,15′	1.68 m, 2.44 m	30.3
16,16′	1.36 m, 1.70 m	36.8
17,17		31.2
18,18′	1.49 brd (7.7)	47.2
19,19′	1.35−1.39[b]	29.2
20,20′		40.4
21,21′	1.35−1.39[b]	29.3
22,22′	1.38, m, 1.82, m	35.5
24,24′	6.46, d (11.4)	141.5
25,25′	0.67, s	11.6
26,26′	0.96, s	24.4
27,27′	0.89, s	25.1
28,28′	1.01, s	31.4
29,29′		185.9
30,30′	1.25, s	33.1
NH	14.0, t (11.4)	

[a] ^{1}H NMR recorded at 600 MHz; ^{13}C NMR recorded at 150 MHz. [b] Signals overlapped.

similar to those of **1**. In addition, the IR spectroscopic data of **2** showed absorption bands superposable to those of **1**, suggesting that compound **2** also had ketone carbonyl, carboxyl, and olefin methine functions in its skeleton. A comparison of the ^{1}H NMR and ^{13}C NMR spectroscopic data of **2** with those of **1** disclosed the absence of the two methyl signals at δ_H 0.98 (CH_3-23) and 1.10 (CH_3-24) of **1** in compound **2** and the presence instead of the olefin methine signal at δ 6.46. These data suggested that the CH_3-23 and -24 methyls were replaced by an N-bearing olefin methine which could be the dimerization site of two multiflorane-type triterpenes.

In order to assign the allocations of all functionalities present in **2**, to confirm the site of dimerization, and to elucidate its planar structure, an HMBC experiment was carried out. ^{2}J and ^{3}J long-range correlations between the hydrogen-bonded secondary amine proton at δ_H 14.0 and two olefin methine and quaternary carbons at δ_C 141.5 (C-24, C-24′) and 114.0 (C-4, C-4′) were observed. The ^{3}J long-range correlation between the two olefin protons at δ 6.46 (H-24, 24′) and their bearing carbons confirmed that the C-24 (24′) of the two units present in **2** were connected with the tertiary amine at δ_H14.0. Moreover, the presence of a ketone carbonyl at C-3 was evidenced by the cross-peaks observed from H-24 (24′) to the carbonyl at δ 200.0 and to the methine at C-5 (C-5′, δ 42.4). The double bond at C-7 (C-8), the carboxyl group at C-20, and the locations of the methyl groups were evidenced by interpretation of the HMBC spectroscopic data. The key correlations observed to support the structure of **2** are shown in Figure S3 (Supporting Information). The molecular formula of **2** required 18 degrees of unsaturation, 9 of which could be assigned to 23-*nor*-multiflora-7(8),4(24)-dien-3-on-29-oic acid. The remaining 9 were thus due to the second occurrence of the same monomer.

The relative configuration of **2** was determined by a NOESY experiment and by X-ray diffraction analysis of a single crystal obtained from a chloroform/methanol solution of **2**. The absolute configuration of **2** was assumed to be the same as the absolute configuration of **1**. The X-ray structure of **2** showing anisotropic displacement ellipsoids at the 50% probability is depicted in Figure 3. The structure of turraenine (**2**) was thus determined to be as shown in Figure 1.

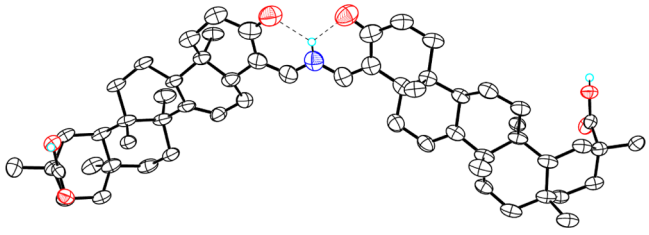

Figure 3. Displacement ellipsoid drawing (50% probability) of the single-crystal X-ray structure of **2**. Aliphatic H atoms and a methanol solvate are omitted for clarity.

The dimeric structure of turraenine immediately raised the question of whether it might be a simple artifact of the reaction of ammonia with a suitable aldehyde precursor, as observed in a study of acid hydrolysis of some 16-methylamino steroids.[17] This is considered to be highly unlikely for three reasons. In the first place, the required aldehyde precursor, 13-methyl-3,23-dioxo-24,26-bisnorolean-7-en-29-oic acid, is not a known natural product and would be relatively unstable. Second, no ammonia was used in the initial extraction process or in any subsequent purification step. Finally, compound **2** was shown to be present in the crude extract by direct ^{1}H NMR analysis, which clearly showed the presence of the triplet due to the hydrogen-bonded secondary amine (Figure S4, Supporting Information). When this signal was excited, the doublet signal due to the olefin methine (δ_H 6.46, d, J = 11.4) was collapsed.

Compound **2** is the first example known of a naturally occurring N-linked triterpene dimer. The biogenesis of **2** can be plausibly traced from **1**. Successive oxidation and decarboxylation of **1** would lead to a C-23 demethylated compound which could be oxidized to afford C-23 aldehyde **S3**. Schiff base formation with ammonia, followed by tautomerism and reaction with another molecule of aldehyde **S3** and loss of

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water, would give the dimerized compound **2** (Figure S5, Supporting Information).

All of the isolated compounds (**1**–**4**) were evaluated for antimalarial activity against the chloroquine-resistant strain FCM29 of *Plasmodium falciparum*. Turranoic acid (**1**) was the most active (IC_{50} 5.2 μM) among all compounds tested. The activities of triptocallic acid B (**3**) and turraenine (**2**) are comparable (16.4 and 16.6 μM, respectively) while esculentoic acid (**4**) was not active. Among the four compounds isolated, only compound **1** exhibited antiproliferative activity, albeit weak, against the A2780 human ovarian cancer cell line (IC_{50} 20 μM). Compound **1** was inactive against the H522-T1 nonsmall cell lung cancer cell line (IC_{50} > 20 μM) and showed weak activity against the human A2058 melanoma cancer cell line (IC_{50} = 16.4 μM).

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the isolation of compounds **1**–**4**; characterization data for compounds **1** and **2**; key HMBC correlations of **1** and **2** and NOESY correlations of **1**; single-crystal X-ray data for compounds **1** and **2**; ^{1}H NMR and 1D-TOCSY spectra of the crude ethanol extract of *Turraea* sp.; plausible biosynthetic pathway for **2**; ^{1}H, ^{13}C, DEPT 135, HSQC, and HMBC spectra of compounds **1** and **2**; 1D-TOCSY spectrum of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(16) Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 980410 (**1**) and no. CCDC 980561 (**2**). These data can be obtained free of charge on application to Cambridge Crystallographic Data Centre, 2 Union Road, Cambridge CB2 1EZ, UK [fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk].

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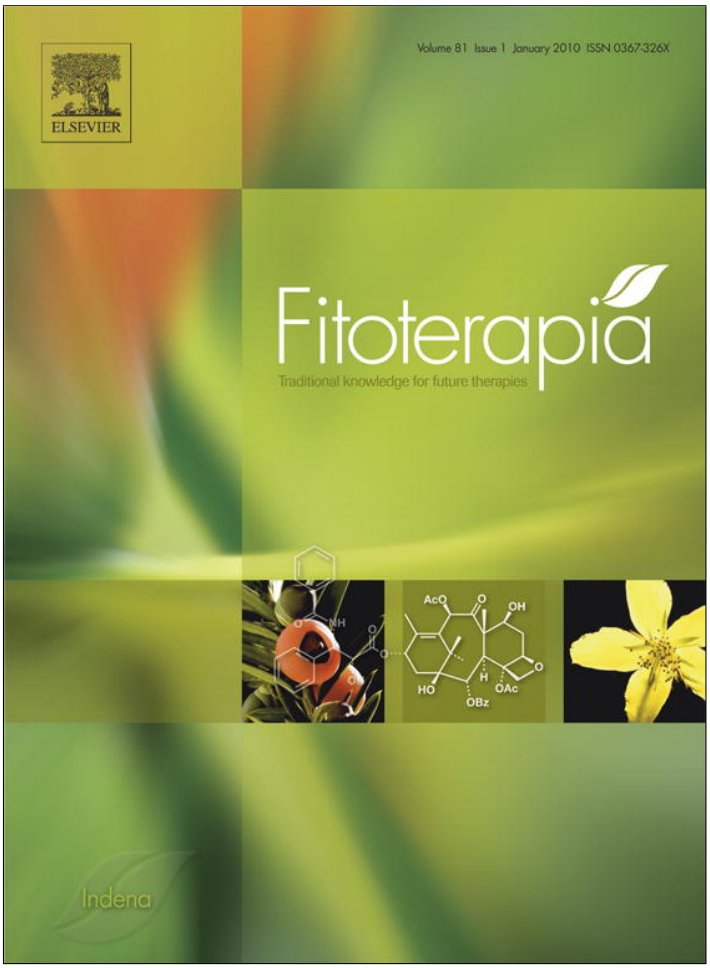
■ NOTE ADDED AFTER ASAP PUBLICATION

In the Supporting Information, Figure S5 was submitted in its uncorrected form. Figure S5 was replaced on April 29, 2014.



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A new labdane diterpene from *Vitex cauliflora* Moldenke from the Madagascar rainforest ☆

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ABSTRACT

Fractionation of an antiplasmodial ethanolic extract from the aerial parts of *Vitex cauliflora* led to the isolation of the new labdane diterpene **1** together with the known triterpene uvaol. The structure of the new compound **1** was established as 3-oxo,15,17,18-triacetoxy-labda-7,13*E*-diene on the basis of spectroscopic data (1D and 2D NMR, MS).

1. Introduction

The genus *Vitex* (Verbenaceae) consists of hermaphrodite shrubs to medium trees, and comprises about 250 recognized species distributed in tropical to temperate areas. Forty two *Vitex* species are found only in Madagascar [1]. A literature survey showed that *Vitex* species are a source of sesquiterpenes, iridoids, flavonoids, lignans, steroids and diterpenes, some of which have shown antimalarial, antioxidant, cytotoxic, antibacterial and tyrosinase inhibitory effects [2–8]. *V. cauliflora* Moldenke is an endemic shrub encountered in the Madagascar rainforest from 0 to 1600 m altitude. No information is available on its traditional uses, but its poisonous property has been reported [9]. There are no previous reports on biological or chemical studies of this species. During an ongoing screening for antiplasmodial activity of plants from Madagascar, the ethanolic extract of *V. cauliflora* showed antiplasmodial activity against the African multidrug-resistant strain FCM 29 of *P. falciparum* with an IC_{50} value of 30.7 μg mL^{-1}. Investigation of this extract led to the isolation of a new but inactive labdane-type diterpene **1** along with the known triterpene uvaol. In this paper, we describe the isolation and structure elucidation of the new compound **1**; studies to isolate the active antimalarial constituent to the extract have not yet been completed.

2. Results and discussion

Compound **1** had a molecular formula of $C_{26}H_{38}O_7$ determined by HRFABMS (m/z = 463.2697 $[M+H]^+$, calcd for $C_{26}H_{39}O_7$ 463.2696). Its ^1H NMR spectrum showed signals for three tertiary methyl groups (δ_H 1.01, s; 1.11, s and 1.67, s), two trisubstituted double bonds (δ_H = 5.3, t, J = 6.4 and 5.8, br s) and three acetoxyl groups (δ_H = 1.97, s; 2.02, s and 2.04, s). In the ^{13}C NMR spectrum, a ketone carbon at δ_C 212.83, the three acetoxy carbonyl carbons (δ_C = 170.68, 170.81 and 171.08), and the two trisubstituted double bonds characterized by the presence of the four

☆ Biodiversity Conservation and Drug Discovery in Madagascar, Part 37. For Part 36, see B. T. Murphy, S. Cao, P. Brodie, J. Maharavo, H. Andriamanantoanina, P. Ravelonandro, D. G. I. Kingston, J. Nat. Prod. 2009;72:1338.

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olefinic carbons (δ_C = 119.19, 128.08, 134.38 and 141.67) accounted for 6 of the 8 degrees of unsaturation implied by the molecular formula. Additionally, resonances at δ_C = 61.18, 66.63 and 66.95 in the ^{13}C NMR spectrum were attributable to three oxymethylene groups, consistent with the appearance of deshielded overlapping signals at δ_H = 3.90–4.40, and a doublet at δ_H = 3.98 (d, J = 11.2), in the ^{1}H NMR spectrum. Since the molecular formula contains seven oxygen atoms, and one ketone and three acetoxy substituents are required from the data indicated above, these oxymethylenes must all be acetoxymethylenes. The above ^{1}H and ^{13}C NMR data suggested that compound **1** was a bicyclic diterpene.

Inspection of the 2D NMR data (^{1}H–^{1}H COSY, ^{1}H–^{1}H TOCSY, HSQC and HMBC) allowed the assignments of all the signals observed in the 1D NMR spectra and revealed the labdane skeleton of the diterpene **1**. The HMBC spectrum (Fig. 1a) showed $^3J_{CH}$ and/or $^2J_{CH}$ correlations between the following protons and carbons: H-2 (δ_H = 2.75, td, J = 5.2, 14.4) to C-3 (δ_C = 212.83), H-19 (δ_H = 1.11) to C-3, C-4 (δ_C = 51.15), C-5 (δ_C = 52.27) and C-18 (δ_C = 66.63), indicating the presence of a carbonyl function at C-3 and an acetoxymethylene group at C-4.

The COSY and TOCSY spectra delineated some structural fragments corresponding to the bicyclic moiety and the side chain of **1** (Fig. 1a). The COSY and/or TOCSY correlations observed between H-5 (δ_H = 1.76)/H-6 (2H, δ_H = 2.1), H-6/H-7 (δ_H = 5.8), H-7/H-17a (δ_H = 4.48) and H-7/H-9 (δ_H = 1.86) led to the placement of a trisubstituted double bond at C-7 (δ_C = 128.08)/ C-8 (δ_C = 134.38) and an acetoxymethylene group at C-8. Connectivities in the HMBC spectrum from H-17 to C-7, C-8 and C-9 (δ_C = 50.66) corroborated these observations and also required the location of the side chain to be at C-9, whose methine proton H-9 (δ_H = 1.86) formed an isolated proton spin system with H-11 (δ_H = 1.38 and δ_H = 1.58) and H-12 (δ_H = 1.94 and 2.16) as deduced from the COSY and TOCSY spectra.

A second trisubstituted double bond was located at C-13 (δ_C = 141.67) and C-14 (δ_C = 119.19) on the basis of $^2J_{C-H}$ and $^3J_{C-H}$ HMBC correlations from Me-16 (δ_H = 1.67) to C-12 (δ_C = 40.57), C-13 and C-14, and from the olefinic proton H-14 (δ_H = 5.3) to C-12 (Fig. 1).

The relative stereochemistry of **1** was determined by ROESY (Fig. 1b). ROESY correlations between H-18 and Me-20 and between H-5 and Me-19 indicated the β-axial orientation of the acetoxymethylene group at C-4 and the A/B *trans* ring junction. The double bond at C-13 was assigned as the *E*

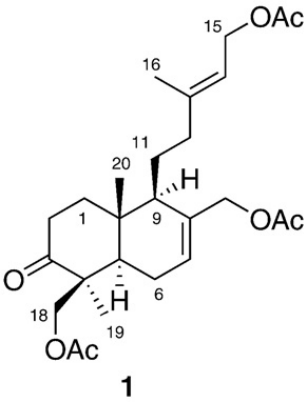

Fig. 2. Structure of labdane diterpenoid **1**.

configuration because of the ROESY correlation observed between H-15 and Me-16, and by comparison of NMR data with those of related compounds [10,11].

Hence, the structure of **1** was established as the new compound 3-oxo,15,17,18-triacetoxy-labda-7,13*E*-diene (Fig. 2). The closest reported analogs in the literature are 3-oxo-7,13*E*-labdadien-15-ol (**2**) [11] and (−)-labda-7,13*E*-diene-3,15-diol (**3**) [12,13] (Fig. 3). The absolute configuration of **1** shown was assigned by analogy with compounds **2** and **3**; regrettably the small amount of compound isolated decomposed before it was possible to obtain its optical rotation, so this assignment is not assured (Fig. 3).

The known triterpenoid uvaol (**4**) [14,15] was also isolated. Neither compound **1** nor uvaol showed antiplasmodial activity against *P. falciparum*. The fact that *V. cauliflora* has been reported to be toxic [9] raises the question of whether compound **1** could be the toxic principle of this plant. The small amount of isolated compound prevented a direct answer to this question, and no biological activities have been reported for the closely similar compounds **2** and **3**. However, the somewhat similar compounds **5** [16] and **6** [17] have been reported to be non-cytotoxic [16,17], and it is thus unlikely that compound **1** is toxic to any significant extent.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded in $CDCl_3$ on either Varian INOVA 400 or JEOL Eclipse 500 spectrometers. The chemical shifts are given in δ (ppm) and coupling constants (J) are

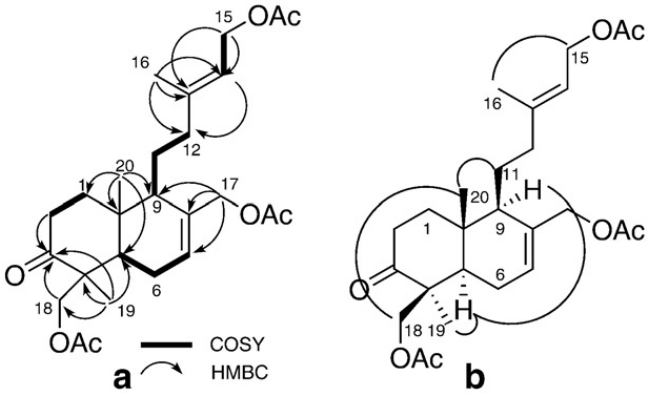

Fig. 1. a. Key COSY and HMBC correlations of **1**. b. Key ROESY correlations of **1**.

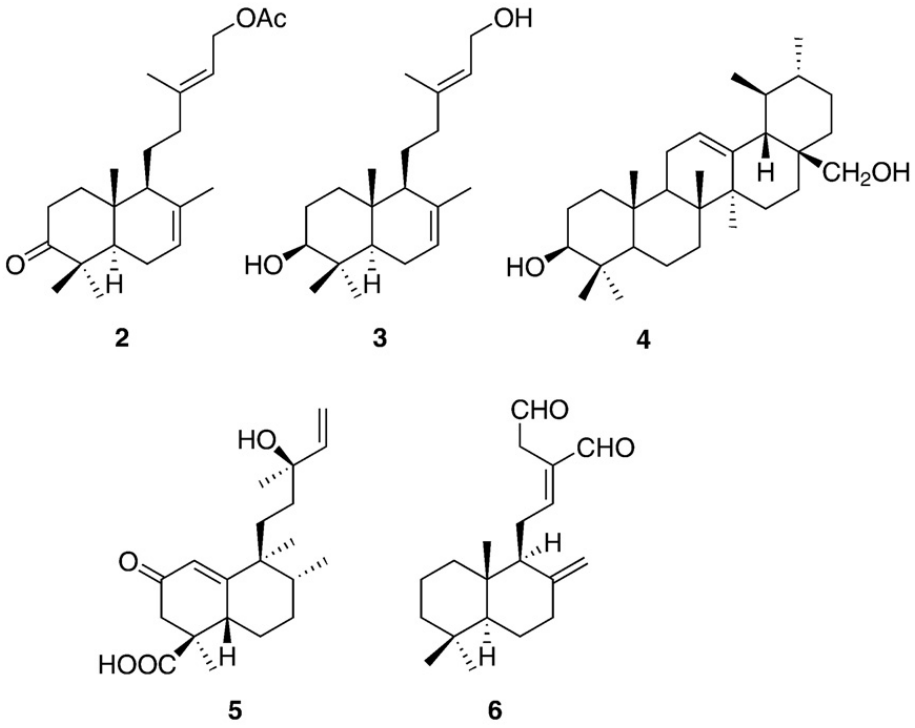

Fig. 3. Structures of compounds **2**–**6**.

reported in Hz. The HR mass spectra were obtained on a JEOL HX-110 instrument. Reversed-phase HPLC was performed on a Shimadzu LC-10AT instrument with an ODS C_{18} Dynamax column (250 × 10 mm).

3.2. Plant material

V. cauliflora Moldenke (Verbenaceae) was initially collected in 2000 (collector number F. Ratovoson 252) near the Parc National de Zahamena at coordinates 17°28′45″S 048°44′10″E at an elevation of 996–1250 m. The bushy shrub was 5 m in height, with clear green sepals and orange petals. A recollection of the aerial parts of the plant was made in the same region in July 2005. Voucher specimens have been deposited at the Parc Botanique and Zoologique de Tsimbazaza (TAN) and at the Centre National d'Application des Recherches Pharmaceutiques (CNARP) in Antananarivo, Madagascar; the Missouri Botanical Garden in St. Louis, Missouri (MO); and the Muséum National d'Histoire Naturelle in Paris, France (P).

3.3. Extraction and isolation

Dried and powdered aerial parts of *V. cauliflora* (2.2 kg) were extracted by maceration in EtOH for a week at room temperature. After filtration by suction, the ethanolic solution was evaporated to dryness under reduced pressure. The residue 74 g (IC_{50} = 30.7 μg mL^{-1}) was submitted to liquid–liquid partition with hexane/water, chloroform/water and n-butanol/water to furnish the hexane extract (20.2 g, IC_{50} = 25.61 μg mL^{-1}), the chloroform extract (15 g, IC_{50} = 5.14 μg mL^{-1}) and the butanol extract (3.4 g, IC_{50} = 22.36 μg mL^{-1}). The most active chloroform extract was fractionated by column chromatography over Si (700 g) eluted with $CHCl_3$–MeOH (100% $CHCl_3$ to 50:50 $CHCl_3$:MeOH) into 7 fractions (I–VII). C_{18} reversed-phase column chromatography followed by Si prep-TLC (Hexane:EtOAc; 8.5:1.5; five developments; R_f = 0.33) of fraction III (37.8 mg, IC_{50} = 15.15 μg mL^{-1}) furnished uvaol (6.0 mg). Fraction IV (0.5 g, IC_{50} = 0.85 μg mL^{-1}) was further chromatographed over C_{18} reversed-phase (10 g) using H_2O–MeOH (stepwise, 50 to 100% MeOH) as eluents. The fraction eluted with 80% MeOH (41.9 mg, IC_{50} = 1.7 μg mL^{-1}) was purified by reversed-phase HPLC (ODS, H_2O–MeOH 68 %) to yield compound **1** (5.3 mg, t_R: 21.1 min) as the major component. The known triterpene uvaol was identified by comparison of its NMR data with literature values [12,13].

3.3.1. 3-oxo,15,17,18-triacetoxy-labda-7,13E-diene (**1**)

Amorphous solid; HRFABMS: m/z = 463.2697 $[M+H]^+$ (Calcd for $C_{26}H_{39}O_7$: 463.2696); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.8 (1H, bs, H-7), 5.3 (1H, t, J = 6.4 Hz, H-14), 4.48–4.58 (5H, m, H-15, H-17 and H-18b), 3.98 (1H, d, J = 11.2 Hz, H-18a), 2.75 (1H, dt, J = 5.2, 14.4 Hz, H-2), 2.28 (1H, dt, J = 3.6, 14.4 Hz, H-2), 2.10–2.16 (4H, m, H-1b, H-6 and H-12b), 1.97, 2.02, 2.04 (each 3H, s, CH_3COO), 1.76–1.94 (3H, m, H-5, H-9 and H-12a), 1.67 (3H, s, H-16), 1.38–1.58 (3H, m, H-1a and H-11), 1.11 (3H, s, Me-19), 1.01 (3H, s, Me-20); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 212.83 (C-3), 170.68, 170.81 and 171.08 (3 $CH_3\underline{C}OO$), 141.67 (C-13), 134.38 (C-8), 128.08 (C-7), 119.19 (C-14), 66.95 (C-17), 66.63 (C-18), 61.18 (C-15), 52.27 (C-5), 51.15 (C-4), 50.66 (C-9), 40.57 (C-12), 37.97 (C-1), 36.41 (C-10), 35.06 (C-2), 25.02 (C-11), 24.25 (C-6), 20.77, 21.01 and 21.09 (3 $\underline{C}H_3COO$), 20.47 (C-19), 16.52 (C-16), 14.19 (C-20).

3.4. Antiplasmodial assay

The African multidrug-resistant strain FCM29 of *P. falciparum* was obtained from the "Institut Pasteur de Madagascar", Antananarivo, and the antiplasmodial assay was carried out

according to the *in vitro* semi-microtest method developed by Le Bras and Deloron [18]. Bioassays were performed in triplicate using infected red blood cells as negative control and chloroquine as positive control. Results are expressed as IC_{50} values, which denote the concentration of sample that reduced the multiplication of parasitemia by 50%.

Acknowledgements

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Phytochemical communication

Additional pregnane glycoside from *Baseonema acuminatum*

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Abstract

The isolation of baseonemoside C (**1**), a new pregnane glycoside from the aerial parts of *Baseonema acuminatum* is reported.

Keywords: *Baseonema acuminatum*; Baseonemoside C; Pregnane glycoside

Plant. *Baseonema acuminatum* P. Choux (Asclepiadaceae), aerial parts were collected in June 1991 at Ambatondrazaka, Madagascar and identified at the Botany and Ethnobotany of the Centre National d'Application des Recherches Pharmaceutiques (CNARP), Madagascar, where a voucher specimen N° 1041 is deposited.

Uses in traditional medicine and other reported activities. A decoction of the aerial parts is used for the treatment of coughs [1]. An aqueous extract has been shown to have anti-coughing, anti-anaphylactic and anti-microbial properties [2–5].

Previously isolated classes of constituents. Pregnane glycosides, triterpenoids, steroids and sugars [6].

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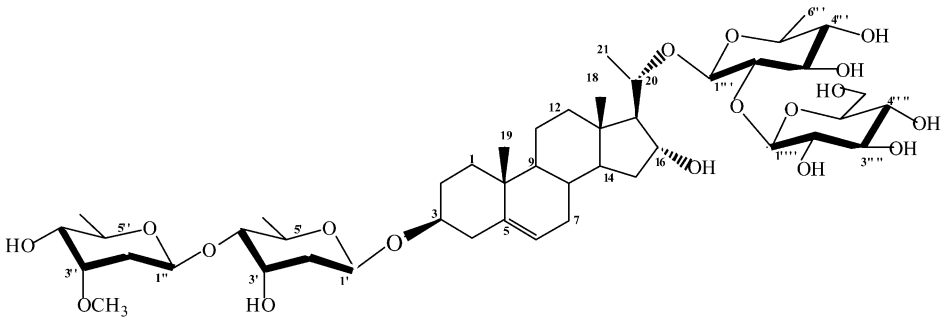
PII: S0367-326X(02)00129-6

New-isolated constituents. Pregn-5-en-3β,16α,20(*S*)-triol 3-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside 20-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-6-deoxyglucopyranoside (baseonemoside C) (**1**) (yield: 0.0007% on dried wt.)



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Baseonemoside C (**1**). Mp 232–234 °C; ^{1}H-NMR (500 MHz, CD_3OD): δ 0.75 (3H, *s*, H-18), 1.02 (3H, *s*, H-19), 1.23 (3H, *d*, *J* 6.6 Hz, H-6′), 1.24 (3H, *d*, *J* 6.5 Hz, H-6″), 1.28 (3H, *d*, *J* 7.7 Hz, H-6‴), 1.38 (3H, *d*, *J* 6 Hz, H-21), 1.42 (1H, *m*, H-17), 1.60–2.20 (2H, *m*, H-2″), 1.65–1.99 (2H, *m*, H-2′), 3.46 (3H, *s*, 3″-OMe), 3.50 (1H, *m*, H-3), 3.64–3.83 (2H, *m*, H-6⁗), 3.78 (1H, *m*, H-20), 4.32 (1H, *m*, H-16), 4.50 (1H, *d*, *J* 7.4 Hz, H-1‴), 4.80 (1H, *d*, *J* 7.7 Hz, H-1⁗), 4.85 (1H, *d*, *J* 11 Hz, H-1″), 4.96 (1H, *d*, *J* 9 Hz, H-1′), 5.40 (1H, *br s*, H-6); ^{13}C-NMR (125 MHz, CD_3OD): 39.4 (C-1), 31.7 (C-2), 77.5 (C-3), 40.4 (C-4), 136.9 (C-5), 118.7 (C-6), 33.8 (C-7), 33.3 (C-8), 51.4 (C-9), 38.5 (C-10), 23.0 (C-11), 40.7 (C-12), 44.2 (C-13), 54.9 (C-14), 35.9 (C-15), 75.5 (C-16), 67.9 (C-17), 16.0 (C-18), 21.3 (C-19), 80.4 (C-20), 24.5 (C-20), 94.5 (C-1′), 38.9 (C-2′), 67.3 (C-3′), 81.8 (C-4′), 68.4 (C-5′), 20.1 (C-6′), 97.9 (C-1″), 36.2 (C-2″), 77.5 (C-3″), 71.9 (C-4″), 70.2 (C-5″), 20.3 (C-6″), 57.6 (3″-OMe), 101.3 (C-1‴), 78.6 (C-2‴), 73.0 (C-3‴), 74.5 (C-4‴), 70.8 (C-5‴), 18.5 (C-6‴), 101.7 (C-1⁗), 74.2 (C-2⁗), 76.5 (C-3⁗), 69.7 (C-4⁗), 76.8 (C-5⁗), 61.8 (C-6⁗); ESI-MS/MS (positive mode) *m/z*: 939 $[M + Na]^+$, 795 $[M + Na\text{-}Cym]^+$, 777 $[M + Na\text{-}Glu]^+$, 665 $[M + Na\text{-}Cym\text{-}Dig]^+$, 631 $[M + Na\text{-}Glu\text{-}DeoxyGlu]^+$, 335 $[Aglycon + H]^+$, 317 $[Aglycon + H\text{-}H_2O]^+$, 299 $[Aglycon + H\text{-}2H_2O]^+$, 281 $[Aglycon + H\text{-}3H_2O]^+$. The ^{1}H-NMR and ^{13}C-NMR assignments were confirmed through ^{1}H–^{1}H COSY, HMQC, HMBC experiments and data reported for related compounds [6].

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IRIDOID GLUCOSIDE FROM PHYLLARTHRON BERNIERIANUM

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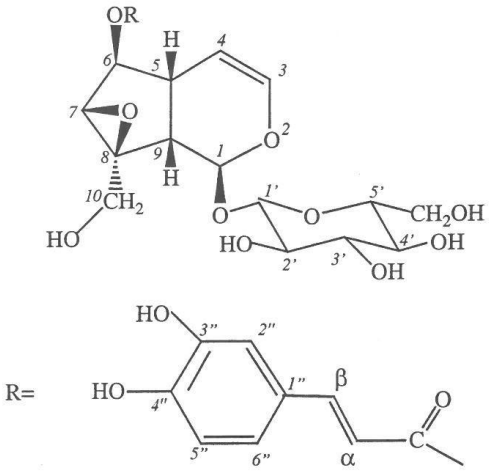
ABSTRACT

Chromatographic resolution of the hydroethanolic extract of the aerial parts of *Phyllarthron bernierianum* afforded an iridoid glucoside, the structure of which was defined as 6-*O*-*trans*-caffeoyl-catalpol (1) on the basis of spectral data including 1D and 2D-homonuclear and heteronuclear correlation NMR techniques. The triterpene ursolic acid was also obtained as a major constituent.

INTRODUCTION

Phyllarthron bernierianum Seem. (Bignoniaceae) is a tree 4 m tall growing in the southern region of Madagascar.[1] It is locally used as an antifebrile and a sedative.[2]

Following our research on endemic medicinal plants from Madagascar, we have investigated this plant on which no pharmacological and chemical studies are recorded in the literature. Previous work on two other *Phyllarthron* species concerned the identification of a quinone and a flavone.[3,4] The present communication deals with the isolation and the structure elucidation of the iridoid glucoside 6-*O*-*trans*-caffeoyl-catalpol (1) from *P. bernierianum*.



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RESULTS AND DISCUSSION

The dried aerial parts of *P. bernierianum* were successively extracted with ethanol and the mixture ethanol :water (80:20). The combined extracts were evaporated to dryness and fractionated over silica gel column to give 6 fractions: F_1-F_6. Repeated crystallization of fraction F_2 from methanol gave ursolic acid whose spectral and physical properties (Mp, MS, ^{1}H- and ^{13}C-NMR) were identical to those of an authentic sample.

Separation of fraction F_5 by column chromatography and flash chromatography over silica gel yielded compound **1**. Its ^{1}H-NMR spectrum showed two *trans* coupled olefinic protons at δ 6.22 (d, J=16 Hz, H-α) and 7.50 (d, J=16 Hz, H-β), and three aromatic protons coupled in an AMX system at δ 6.96 (d, J=2 Hz, H-2"), 6.87 (dd, J=8 and 2 Hz, H-6") and 6.68 (d, J=8 Hz, H-5"). In the HMBC spectrum, the following ^{1}H-^{13}C long-range couplings were observed: H-α and H-β to the carbonyl carbon (δ 168.93), H-β to C-2" (δ 115.18) and C-6'' (δ 123.13), H-2" (δ 6.96) to C-3" (δ 146.82) and C-4" (δ 149.75), H-5" (δ 6.68) to C-3" and C-4" (figure 1). These data suggested the presence of a *trans*-caffeoyl unit.

The ^{13}C-APT spectrum of **1** displayed 24 signals corresponding to 17 methyls and methines, and 7 methylenes and quaternary carbons. An anomeric carbon resonated at δ 99.68. After subtracting carbon signals ascribable to the caffeoyl unit, the characteristics (positions and CH multiplicities) of the remaining 15 carbon signals were comparable to those of catalpol, except for C-5, C-6 and C-7 which were shifted by ± 2 ppm.[5] Correlations in the HMBC spectrum confirmed this skeleton (figure 1).

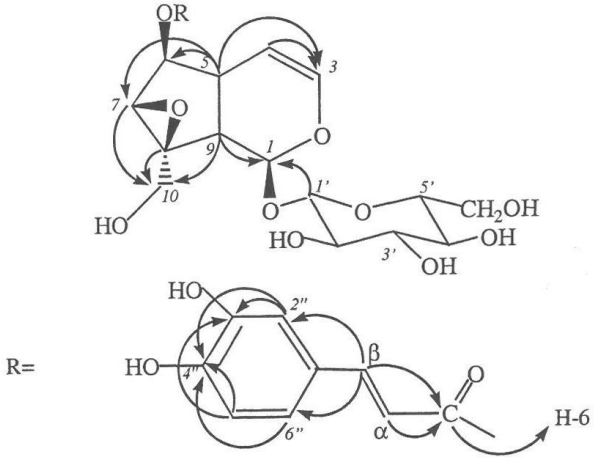

Figure 1: Important correlations observed in the HMBC spectrum of **1**

The ^{1}H-^{13}C long-range connectivity between the oxymethine proton H-6 (δ 4.92) and the carbonyl carbon (δ 168.93) indicated the attachment of the caffeoyl unit to C-6 through an ester linkage.

Therefore, the structure of **1** was determined to be 6-*O-trans*-caffeoyl-catalpol. Literature search revealed that **1** is a known iridoid glucoside named verminoside. [6,7] The ^{1}H- and ^{13}C-NMR spectra of **1** and verminoside matched in all respects.

To the best of our knowledge, this is the first report on the occurrence of iridoids from species of the genus *Phyllarthron* and thus reinforces the view that these compounds are widespread in the Bignoniaceae family.[8,9]

EXPERIMENTAL

General experimental procedures: ^{1}H- and ^{13}C-NMR spectra were recorded in CD_3OD with a VARIAN UNITY 300. Column fractions were monitored by Si-TLC using CH_2Cl_2/MeOH (90:10) acidified by adding three drops of acetic acid as eluent.

Plant material. The sample was collected from Tuléar, Madagascar in 1995. It was identified as *Phyllarthron bernierianum* at the Botany and Ethnobotany Department of the "Centre National d'Application des Recherches Pharmaceutiques" (CNARP), Antananarivo, where a voucher specimen is also preserved.

Extraction and isolation. Dried and powdered aerial parts (275 g) of *P. bernierianum* were extracted first with ethanol and then with ethanol:water (80:20) for 48 hours at room temperature. The combined extracts were evaporated to dryness under vacuum to give 29.2 g of crude extract. A part (10 g) of this crude extract was subjected to column chromatography over silica gel (60-200 mesh, 100 g) using increasing amounts of MeOH in CH_2Cl_2 as eluents to afford six fractions: F_1-F_6 . Ursolic acid (140 mg) was obtained from the fraction F_2 eluted with 3% MeOH and recrystallized from MeOH. The fraction F_5 (3 g) eluted with 12% MeOH was rechromatographed on silica gel under gradient conditions with the same mixtures of solvents. The effluent was collected in 15 ml fractions. Fractions 32 to 37 eluted with 10% MeOH were further purified by flash chromatography eluted with 8% MeOH to furnish verminoside **1** (14 mg).

Verminoside **1** : Amorphous powder. R_f-value in the above TLC system = 0.4. ^{1}H-NMR (CD_3OD, 300 MHz): δ 2.51 (2H, m, H-5 and H-9); 3.56 (1H, d, J=6.3 Hz, H-7); 3.73 and 4.07 (2H, d each, J=12.9 Hz, C$\underline{H}_2$-10); 4.69 (1H, d, J=8.1 Hz, H-1'); 4.87 (1H, m, H-4); 4.92 (1H, m, H-6); 5.06 (1H, d, J=9 Hz, H-1); 6.22 (1H, d, J=15.9 Hz, H-α); 6.27 (1H, d, J=6 Hz, H-3); 6.68 (1H, d, J=8 Hz, H-5"); 6.87 (1H, dd, J=2 and 8 Hz, H-6"); 6,96 (1H, d, J=2 Hz, H-2"); 7.50 (1H, d, J=15.9 Hz, H-β). ^{13}C-NMR (CD_3OD, 75 MHz): δ 36.74 (C-5), 43.17 (C-9), 60.26 (C-7), 61.28 (C-10), 62.92 (C-6'), 66.81 (C-8), 71.76 (C-4'), 74.84 (C-2'), 77.69 (C-5'), 78.62 (C-3'), 81.29 (C-6), 95.06 (C-1), 99.68 (C-1'), 102.92 (C-4), 114.48 (C-α), 115.18 (C-2"), 116.50 (C-5"), 123.13 (C-6"), 127.61 (C-1"), 142.36 (C-3), 146.82 (C-3"), 147.56 (C-β), 149.75 (C-4"), 168.93 (O$\underline{C}$O).

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