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A new labdane diterpene from *Vitex cauliflora* Moldenke from the Madagascar rainforest¹

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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-7,13E-diene on the basis of spectroscopic data (1D and 2D NMR, MS).

Keywords

Vitex cauliflora; Verbenaceae; Diterpene; Triterpene

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 IC₅₀ 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

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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 ($\delta_H = 5.3$, t, $J = 6.4$ and 5.8 , br s) and three acetoxy groups ($\delta_H = 1.97$, 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 ($\delta_C = 170.68$, 170.81 and 171.08), and the two trisubstituted double bonds characterized by the presence of the four olefinic carbons ($\delta_C = 119.19$, 128.08, 134.38 and 141.67) accounted for 6 of the 8 degrees of unsaturation implied by the molecular formula. Additionally, resonances at $\delta_C = 61.18$, 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 $\delta_H = 3.90$ -4.40, and a doublet at $\delta_H = 3.98$ (d, $J = 11.2$), in the 1H -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 1H - and ^{13}C -NMR data suggested that compound **1** was a bicyclic diterpene.

Inspection of the 2D NMR data (1H - 1H COSY, 1H - 1H 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 (Figure 1a) showed $^3J_{CH}$ and/or $^2J_{CH}$ correlations between the following protons and carbons: H-2 ($\delta_H = 2.75$, td, $J = 5.2$, 14.4) to C-3 ($\delta_C = 212.83$), H-19 ($\delta_H = 1.11$) to C-3, C-4 ($\delta_C = 51.15$), C-5 ($\delta_C = 52.27$) and C-18 ($\delta_C = 66.63$), 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** (Figure 1a). The COSY and/or TOCSY correlations observed between H-5 ($\delta_H = 1.76$)/H-6 (2H, $\delta_H = 2.1$), H-6/H-7 ($\delta_H = 5.8$), H-7/H-17a ($\delta_H = 4.48$) and H-7/H-9 ($\delta_H = 1.86$) led to the placement of a trisubstituted double bond at C-7 ($\delta_C = 128.08$)/C-8 ($\delta_C = 134.38$) and an acetoxymethylene group at C-8. Connectivities in the HMBC spectrum from H-17 to C-7, C-8 and C-9 ($\delta_C = 50.66$) corroborated these observations and also required the location of the side chain to be at C-9, whose methine proton H-9 ($\delta_H = 1.86$) formed an isolated proton spin system with H-11 ($\delta_H = 1.38$ and $\delta_H = 1.58$) and H-12 ($\delta_H = 1.94$ and 2.16) as deduced from the COSY and TOCSY spectra.

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

The relative stereochemistry of **1** was determined by ROESY (Figure 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* 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. 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]; 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.

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 CDCl₃ on either Varian INOVA 400 or JEOL Eclipse 500 spectrometers. The chemical shifts are given in δ (ppm) and coupling constants (*J*) are 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 C₁₈ Dynamax column (250 $\times$ 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 (IC₅₀ = 30.7 μ g 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, IC₅₀ = 25.61 μ g mL⁻¹), the chloroform extract (15 g, IC₅₀ = 5.14 μ g mL⁻¹) and the butanol extract (3.4 g, IC₅₀ = 22.36 μ g mL⁻¹). The most active chloroform extract was fractionated by column chromatography over Si (700 g) eluted with CHCl₃-MeOH (100% CHCl₃ to 50:50 CHCl₃:MeOH) into 7 fractions (I-VII). C₁₈ 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, IC₅₀ = 15.15 μ g mL⁻¹) furnished uvaol (6.0 mg). Fraction IV (0.5 g, IC₅₀ = 0.85 μ g mL⁻¹) was further chromatographed over C₁₈ reversed-phase (10 g) using H₂O-MeOH (stepwise, 50 to 100% MeOH) as eluents. The fraction eluted with 80% MeOH (41.9 mg, IC₅₀ = 1.7 μ g mL⁻¹) was purified by reversed-phase HPLC (ODS, H₂O-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 C₂₆H₃₉O₇: 463.2696); ¹H-NMR (400 MHz, CDCl₃): 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, CH₃COO), 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); ¹³C-NMR (125 MHz, CDCl₃): $\delta = 212.83$ (C-3), 170.68, 170.81 and 171.08 (3 CH₃COO), 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 CH₃COO), 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 [18]. Bioassays were performed in triplicate using infected red blood cells as negative control and chloroquine as positive control. Results are expressed as IC₅₀ values, which denote the concentration of sample that reduced the multiplication of parasitemia by 50%.

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

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

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