

Xylodiol, a New Atisane Diterpenoid from *Xylopia Langsdorffiana* St.-Hil. & Tul. (Annonaceae)

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Two diterpenes were isolated from the leaves of *Xylopia langsdorffiana*, the new *ent*-atisane-7 α ,16 α -diol named xylodiol (**1**) and the known 8 (17),12*E*,14-labdatrien-18-oic acid (**2**). The structures of these isolates were deduced by spectroscopic data interpretation. Compound **2** was less toxic than xylodiol on V79 cells and hepatocytes.

Key words: *Xylopia langsdorffiana*, Annonaceae, Atisane, Labdane, Cytotoxicity

Introduction

The genus *Xylopia* (Annonaceae) comprises about 160 species [1]. Various species of this genus are used in folk medicine for rheumatism [2] and as antimicrobial agents [3]. Kaurane, labdane and trachylobane-type diterpenes are encountered in this genus [4, 5, 6]. Atisane diterpenes are rare in the Annonaceae family, having been recorded only in *Xylopia aromatica* [6]. In addition, this group of compounds has not been studied biologically to a large extent as yet. However, other atisane-type diterpenes isolated from different species show anti-HIV [7] and insecticidal activities and are cytotoxic to mouse P388 leukemia cells [8].

Xylopia langsdorffiana St.-Hil. & Tul. is a tree, 5–7 m high, and popularly known in Northeast Brazil as “pimenteira da terra” [3]. As part of our continuing investigations of bioactive molecules from plants of Brazil, we now report on the isolation from the *X. langsdorffiana* leaves and the characterisation of a new atisane named xylodiol (**1**) and the known labdane (**2**) diterpene as well as their cytotoxicity assays against V79 fibroblasts and hepatocytes. Vasorelaxant and hypotensive activities were reported recently for **2** [9].

In vitro systems provide important tools to enhance our understanding of the hazardous effects of novel

pharmacological agents. Most of the attention has been focused on cytochrome P450s (CYPs), the primary enzyme superfamily involved in the metabolism of a broad range of drugs in different species, including man [10]. The cytotoxicity of metabolites produced by hepatic biotransformation is frequently assessed in cultured fibroblasts and hepatocytes [11].

Experimental Section

General procedures

Melting points were measured in a Geahaka model PF1500 version 1.0 apparatus and are uncorrected. Optical rotations were determined on a ADP 220 polarimeter, Bellingham-Stanley Ltd. IR spectra were obtained in KBr disks in a Bomem model MB 100M series spectrophotometer. ¹H and ¹³C NMR spectra were obtained with a Bruker DRX-500 spectrometer, using CDCl₃ as solvent. HREIMS were recorded in a VG-AutoSpec spectrometer, using a direct insertion probe and electron impact at 70 eV. The mass spectra were obtained on a Hewlett-Packard 5971 mass spectrometer by electron impact ionisation at 70 eV.

Plant material

The leaves of *Xylopia langsdorffiana* were collected in the municipality of Cruz do Espírito Santo, State of Paraíba, in July 2002. The plant material was identified by Prof. Maria de Fátima Agra, Head of the Botany Section of the Labo-

ratório de Tecnologia Farmacêutica Prof. Delby Fernandes de Medeiros (LTF). A voucher specimen (AGRA 5541) is deposited at the Herbário Prof. Lauro Pires Xavier (JPB) of the Universidade Federal da Paraíba.

Extraction and isolation

Dried leaves of *X. langsdorffiana* (2 kg) were exhaustively extracted with 95 % EtOH at r. t. Removal of the solvent under reduced pressure in a rotary evaporator yielded a dark green extract (140 g), which was partitioned successively with hexane, CHCl₃ and EtOAc to yield 20, 16 and 12 g of crude residues, respectively. The hexane fraction was subjected to column chromatographic separation with silica gel and eluted with hexane/ethyl acetate in an increasing polarity gradient. Fractions with similar TLC were combined. Fractions 13–28 were purified by preparative TLC over silica gel using hexane/EtOAc (8 : 2) to afford the diterpenoid **1** (72 mg) and fractions 29–40 were purified in the same way using hexane/EtOAc (7 : 3) to afford the diterpenoid **2** (81 mg).

Biological assay

Xylodiol (**1**) and compound **2** were tested for *in vitro* cytotoxic activity against V79 cells and rat hepatocytes using the nucleic acid content (NAC), tetrazolium reduction (MTT) and neutral red uptake (NRU) assays, as described previously [17]. Hepatocytes were isolated from 2 months old Wistar male rats (200–250 g) by a two-step collagenase perfusion method [18].

Ent-atisan-7 α ,16 α -diol or xylodiol (**1**)

Obtained as a crystalline solid, m.p. 108–111 °C. – $[\alpha]_D^{25} = +46$ ($c = 0.19$, CHCl₃). – IR (KBr): $\nu = 3372$, 3325, 2985, 1130 cm⁻¹. – HREIMS: $m/z = 306.2017$ [M⁺], compatible with the molecular formula C₂₀H₃₄O₂.

8 (17),12E,14-labdatrien-18-oic acid (**2**)

Obtained as a crystalline solid, m.p. 139–141 °C. – $[\alpha]_D^{27} = -43$ ($c = 0.19$, CHCl₃). – IR (KBr): $\nu = 3083$, 2934, 2870, 1692, 1643, 1276, 901 cm⁻¹. – EIMS (EI): m/z (%) = 302.6 [M⁺], 119 (100), 122 (48), 147 (78), 159 (45), 161 (29), 176 (55), 242 (10), compatible with the molecular formula C₂₀H₂₈O₂.

Results and Discussion

Compound **1** was obtained as a crystalline solid, m.p. 108–111 °C and $[\alpha]_D^{25} = +46$ in chloroform. The HREIMS of **1** showed a molecular ion peak at $m/z = 306.2017$, compatible with the molecular formula C₂₀H₃₄O₂, having 4 degrees of unsaturation in the molecule. The IR spectrum showed absorptions at 3372 and 3325 cm⁻¹, characteristic of hydroxyl

Table 1. NMR data for xylodiol (**1**).

| C atom | HMQC | | HMBC | ¹ H × ¹ H | |
|--------|-------------------------|----------------------|------------------|---------------------------------|-------|
| | $\delta^{13}\text{C}^a$ | $\delta^1\text{H}^a$ | | COSY | NOESY |
| 1 | 42.0 | 1.45 (m) | | | |
| | | 1.25 (m) | | | |
| 2 | 18.1 | 1.68 (m) | | | |
| | | 1.45 (m) | | | |
| 3 | 39.1 | 1.66 (m) | | | H-3 |
| | | 0.94 (m) | | | |
| 4 | 37.7 | – | | | |
| 5 | 47.3 | 1.42 (m) | H-1 | | |
| 6 | 26.8 | 1.71 (m) | H-7 | | H-6 |
| | | 1.67 (m) | | | |
| 7 | 73.8 | 3.43 (broad) | H-5 / H-9 / H-15 | | H-14 |
| 8 | 32.5 | – | | | |
| 9 | 46.0 | 1.59 (m) | H-13 / H-11 | | |
| 10 | 37.7 | – | | | |
| 11 | 22.4 | 1.77 (m) | | | |
| | | 1.53 (m) | | | |
| 12 | 38.1 | 1.63 (m) | H-13 / H-11 | | |
| 13 | 23.5 | 2.02 (m) | | | H-13 |
| | | 1.19 (m) | | | |
| 14 | 26.7 | 1.70 (m) | | | H-14 |
| | | 0.99 (m) | | | |
| 15 | 51.3 | 1.78 (m) | H-14 | | |
| | | 1.30 (m) | | | |
| 16 | 72.2 | – | H-11 / H-15 | | |
| 17 | 30.5 | 1.35 (s) | | | H-7 |
| 18 | 33.1 | 0.91 (s) | | | |
| 19 | 21.6 | 0.86 (s) | | | |
| 20 | 13.4 | 0.99 (s) | H-5 | | |

^a In CDCl₃, at 500 MHz for ¹H NMR and 125 MHz for ¹³C NMR.

Table 2. Cytotoxicity of xylodiol (**1**) and compound **2** on V79 cells and rat hepatocytes.

| Compound | IC ₅₀ (μmol) | | | Hepatocytes | | |
|----------|-------------------------|---------|-----|-------------|-----|-----|
| | MTT | V79 NRU | NAC | MTT | NRU | NAC |
| Xylodiol | 112 | 56 | 70 | 139 | – | – |
| 2 | 250 | 138 | 220 | – | – | – |

(–): Viability similar to control cells until 300 μmol.

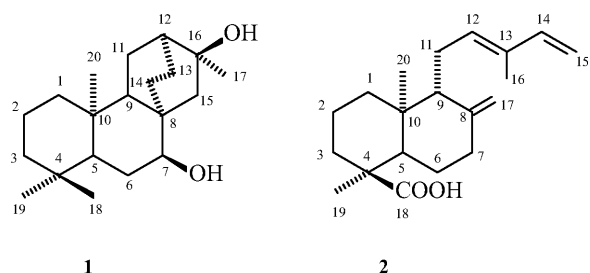


Fig. 1. Diterpenoids **1** and **2** from *Xylopia langsdorffiana*.

groups. The presence of four methyl, eight methylene and four methine groups and four quaternary carbons was supported by the DEPT and HMQC (Table 1). Furthermore, by comparison of the NMR data of **1** with

those of similar structures [12, 13] we concluded that it belongs to the *ent*-atisane diterpene compound class. The location of the hydroxyl groups at C-7 and C-16 was deduced from the HMBC. Correlations between the protons at $\delta_{\text{H}} = 1.42$ (H-5), 1.59 (H-9), and 1.78 (H-15), and the oxygenated carbon at $\delta_{\text{C}} = 73.8$ allowed the location of one hydroxyl at C-7. The other hydroxyl group was located at C-16 due to the correlations between $\delta_{\text{H}} = 1.77$ (H-11), $\delta_{\text{H}} = 1.30$ (H-15), and the carbon at $\delta_{\text{C}} = 72.2$. The α orientation of the hydroxyl group at C-7 was deduced by the presence of a broad signal for H-7 and comparison with literature data [14]. The NOESY spectrum was also used to establish the relative stereochemistry at this center. The one- and two-dimensional $^1\text{H}/^{13}\text{C}$ NMR data are shown in Table 1. Based on the above evidence, the relative stereochemistry of the carbons at C-7 and C-16 is established. Although we have not determined the absolute stereochemistry, the molecule is represented with α orientation for the hydroxyl groups at C-7 and C-16. Therefore, the structure of compound **1**, a new diterpene, was proposed as *ent*-atisan-7 α ,16 α -diol, named xylodiol (Fig. 1).

Compound **2** was obtained as a crystalline solid (m. p. 139–141 °C). The EIMS of **2** showed a molecular ion peak at $m/z = 302.26$, compatible with the

molecular formula $\text{C}_{20}\text{H}_{28}\text{O}_2$, with 7 degrees of unsaturation. The molecular structure of **2** was confirmed by the $^1\text{H}/^{13}\text{C}$ NMR 1D and 2D spectra, and agreed with that previously reported for 8 (17),12*E*,14-labdatrien-18-oic acid [15].

The biological activities of **1** and **2** are summarised in (Table 2). Compound **2** induced no significant suppression on the viability of hepatocytes at concentrations up to 300 μmol . No cytotoxicity was detected in hepatocytes after treatment with up to 300 μmol of xylodiol evaluated by NRU and NAC assays, however it was determined an IC_{50} value of 139 μmol evaluated by MTT reduction assay. These results indicate that this compound is more toxic to mitochondria than the others endpoints measured (NRU and NAC). The different effects observed on V79 cells and rat hepatocytes, especially for xylodiol in the NRU and NAC tests, suggest that the P450-mediated biotransformation of such diterpenes may lead to the formation of less toxic metabolites. In a previous investigation, xylodiol exhibited an IC_{50} value of 40 μmol against the K562 human leukemia cell line [16].

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