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Tambouranolide, a new cytotoxic hydroxybutanolide from a *Tambourissa* sp. (Monimiaceae)^φ

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Abstract

A new cytotoxic hydroxybutanolide, tambouranolide, has been isolated by solid phase extraction from an ethanol extract of the dried roots of a species of *Tambourissa* (Monimiaceae) from the Madagascar rainforest. The structure was elucidated through the interpretation of spectral data and its comparison to data reported in the literature for related molecules. The compound showed moderate *in vitro* cytotoxicity with an IC₅₀ of 8 µg/mL in the A2780 human ovarian cancer cell line assay.

Keywords

Tambourissa; Monimiceae; Hydroxybutanolide

1. Introduction

The genus *Tambourissa* is one of the largest members of the Monimiaceae family of flowering plants, which is typically found in tropical and subtropical areas of the southern hemisphere [1]. Members of the Monimiaceae family are especially common around of the Indian Ocean and in the South Pacific. More than forty species of *Tambourissa* are known to grow exclusively on the islands of the southwest Indian Ocean, including Madagascar and the Mascarene Islands [2]. Only two published phytochemical investigations have been performed on this genus; these revealed the presence of a number of volatile terpenes such as limonene, bergamotene, and curcumene, from *T. leptophylla* [3] and other miscellaneous constituents from *T. quadrifidia* [4]. These plants were obtained from the islands of the Comores, Réunion and Mauritius. The crude petrol ether extract of *T. leptophylla* was shown to be slightly antifungal, although no individual constituents were specifically examined for biological activity.

In continuation of our investigation of bioactive compounds from plant collections in the Madagascar rainforest, as part of an International Cooperative Biodiversity Group (ICBG) [5], we acquired a sample of an ethanol extract from the roots of an unknown species of

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Tambourissa. The dry, crude material yielded a positive test in our mammalian cytotoxicity bioassay with an IC₅₀ of 22 µg/mL. Bioassay-guided fractionation (liquid-liquid partitioning and solid phase extraction) afforded a new hydroxybutanolide, tambouranolide (**1**), whose structure was deduced from ¹H NMR, ¹³C NMR, and MS data. The isolation and characterization of this new compound is reported here.

2. Results and discussion

Tambouranolide (**1**) was obtained as a pale yellow solid. Its positive ion HRFABMS indicated a molecular ion at *m/z* 419.3486, in agreement with the molecular formula of C₂₇H₄₆O₃. A broad doublet at δ_H = 5.25 (H-3), a doublets of doublets at δ_H = 4.71 (H-5a) and δ_H = 4.94 (H-5b), and a triplet of doublets at δ_H = 7.07 (H-6) in the ¹H-NMR spectrum suggested that the compound was a hydroxylated α,β-unsaturated-γ-lactone. Signals at δ_C = 166.8 (C-1), 127.4 (C-2), 66.5 (C-3), 157.8 (C-4), 91.4 (C-5), and 150.3 (C-6) in the ¹³C-NMR spectrum further supported this identification. For the remaining 21 carbons, eight resolved peaks (including two at δ_C = 129.9 and 130.0) and numerous overlapping signals in the region 29.4 – 30.0 ppm were observed. The observations indicated that the compound contained a side chain with several methylenes and one double bond. HMBC data was used to establish the position of the double bond at Δ²², since both H-24 and H-27 showed correlations with C-25 and C-26 and H-24 further correlated with C-22 and C-23, as shown in Figure 1.

The *Z* stereochemistry of the disubstituted double bond was unequivocally confirmed from the ¹³C-NMR data of *Z* and *E* isomers of other long chain compounds [6]. The allylic (δ_C = 27.0 and 27.3) and olefinic (δ_C = 129.9 and 130.0) carbon signals of **1** were coincident with those typical of *Z* isomers (allylic carbons: δ_C = 27.2, olefinic carbons: δ_C = 129.8 and 129.9), but not those of *E* isomers (allylic carbons: δ_C = 32.6, olefinic carbons: δ_C = 130.3). The spectral data of **1** resembled those reported for linderanolides and isolinderanolides from *Lindera benzoin* [7] and *Lindera glauca* [8], and the ¹³C-NMR data of these compounds were examined to assign the stereochemistry of the monosubstituted double bond. The allylic (δ_C = 66.5) and olefinic (δ_C = 150.3) carbon signals of **1** were coincident with those of isolinderanolide and isolinderanolide *E* (allylic carbons: δ_C = 66.3 and 66.5, olefinic carbons: δ_C = 150.2 and 150.3), which are *E* isomers, but not those of linderanolide and linderanolide *E* (allylic carbons: δ_C = 68.9, olefinic carbons: δ_C = 151.4), which are *Z* isomers. Compound **1** ([α]_D +20°) was also dextrorotatory, in analogy with linderanolide *E* and isolinderanolide *E*, which have (3*R*)-hydroxyl groups, rather than levorotatory in analogy with linderanolide and isolinderanolide, which have (3*S*)-hydroxyl groups. Hence, the structure of **1** was determined to be (3*R*,2*E*)-3-hydroxy-4-methylene-2-((17*Z*)-17-docosenylidene)butanolide, as shown.

Compound **1** was tested in the A2780 assay, and it was moderately active with an IC₅₀ value of 8 µg/mL, using actinomycin D as a positive control (IC₅₀ = 1–3 ng/mL).

3. Experimental

3.1.1. General Experimental Procedures

Solid phase extraction was performed with Supelco Discovery DSC-NH₂ and DSC-Si tubes. Optical rotation data was obtained on a JASCO J-720 spectropolarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. NMR spectra were obtained on either a JEOL Eclipse (at 500 MHz for ¹H NMR and 125 MHz for ¹³C NMR) or Varian Inova (at 400 MHz for ¹H NMR and 100 MHz for ¹³C NMR) spectrometer. Chemical shifts are given in δ (ppm) and coupling constants (*J*) are reported in Hz.

3.2. Plant material

The roots of a *Tambourissa* species (Monimiaceae) were collected by Fidisoa Ratovoson and co-workers on July 26, 2003. The specimens were collected in the forest of Ampitsahambe, north-west of the village of Androrangabe, around the Natural Reserve of Zahamena in the province of Toamasina, Madagascar. Duplicate voucher specimens have been deposited at the Centre National d'Application des Recherches Pharmaceutiques (CNARP) and the Direction des Recherches Forestieres et Piscicoles Herbarium (TEF) in Antananarivo, Madagascar; the Missouri Botanical Garden in St. Louis, Missouri (MO); and the Museum National d'Histoire Naturelle in Paris, France (P).

3.3. Extraction and isolation

The roots of the *Tambourissa* sp. were dried, ground and extracted with ethanol to give extract MG2090 (163.5 g). The crude bioactive extract ($IC_{50} = 22 \mu\text{g/mL}$, 1.06 g) was partitioned between hexanes (200 mL) and MeOH-H₂O (4:1, $2 \times 100 \text{ mL}$). Water was added to the MeOH-H₂O fraction to yield a MeOH-H₂O solution (3:2) that was subsequently partitioned with CH₂Cl₂. Evaporation of the organic solvents yielded bioactive ($IC_{50} = 11 \mu\text{g/mL}$) fractions of 903 mg (hexanes) and 106 mg (CH₂Cl₂). The two fractions were combined and subjected to further fractionation through a Discovery DSC-NH₂ solid phase extraction (SPE) cartridge with a mixture of hexanes and isopropanol. The first (hexanes, 710 mg, $IC_{50} = 9 \mu\text{g/mL}$) and second (hexanes-isopropanol, 19:1, 101 mg, $IC_{50} = 8 \mu\text{g/mL}$) fractions displayed the greatest cytotoxicity. These two fractions were combined, and one-fourth of the material was subjected to further fractionation through a Discovery DSC-Si SPE cartridge with a mixture of hexanes and CH₂Cl₂. The fourth (hexanes-CH₂Cl₂, 7:3, 24 mg), fifth (hexanes-CH₂Cl₂, 3:2, 24 mg), and sixth (hexanes-CH₂Cl₂, 1:1, 23 mg) fractions all contained the same pure bioactive compound **1**.

3.3.1. Tambouranolide (1)—Yellow amorphous solid; $[\alpha]_D^{+20} (c 0.11, \text{CHCl}_3)$; UV λ_{max} (log ϵ); IR ν_{max} ; HRFABMS: $m/z = 419.3486 [M + H]^+$ (calcd for C₂₇H₄₇O₃: 419.3525); ¹H-NMR (500 MHz, CDCl₃): $\delta = 7.07$ (1H; td; $J = 7.9, 2.2 \text{ Hz}$; H-6), 5.34 (2H, m, H-22 and H-23), 5.25 (1H, br s, H-3), 4.94 (1H; dd; $J = 2.8, 1.7 \text{ Hz}$; H-5b), 4.71, (1H; dd; $J = 2.8, 1.4 \text{ Hz}$; H-5a), 2.46 (2H, m, H-7), 2.24 (1H, br s, HO-3), 2.00 (4H, m, H-21 and H-24), 1.51 (2H, qui, $J = 7.9 \text{ Hz}$, H-8), 1.30 (4H, m, H-25 and H-26), 1.24 (24H, br s, H-9 to H-20), 0.88 (3H, br t, $J = 7.2 \text{ Hz}$, H-27); ¹³C-NMR (125 MHz, CDCl₃): $\delta = 166.8$ (C-1), 157.8 (C-4), 150.3 (C-6), 130.0 (C-23), 129.9 (C-22), 127.4 (C-2), 91.4 (C-5), 66.5 (C-3), 32.1 (C-25), 29.4–30.0 (C-9 to C-20), 29.7 (C-7), 28.4 (C-8), 27.3 (C-24), 27.0 (C-21), 22.4 (C-26), 14.1 (C-27).

3.4. Cytotoxicity bioassays

The A2780 human ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported [9]. Compound **1** had an IC_{50} of $8 \mu\text{g/mL}$ in this assay.

Acknowledgments

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Figure 1.
Selected HMBC correlations of 1.