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NOTE

# A pterocarpan from the seeds of *Bituminaria morisiana*

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**Abstract** A new prenylated pterocarpan, named morisianine, was isolated together with the known secondary metabolites erybraedin C, psoralen and angelicin from the seeds of *Bituminaria morisiana*. The structures of the compounds were elucidated mainly by 1D and 2D NMR experiments as well as mass spectrometry. The new compound was subjected to cytotoxicity screening against a panel of human cancer cells.

**Keywords** *Bituminaria morisiana* · *Psoralea* · Fabaceae · Pterocarpans · Cytotoxicity

## Introduction

*Bituminaria morisiana* (Pignatti & Metlesics) Greuter (=*P. morisiana* Pignatti & Metlesics) is an endemic shrub of Sardinia (Italy) growing on limestone from 300 to 1000 m above sea level [1, 2]. The genus *Bituminaria* (previously *Psoralea*) is represented only by two species, *B. morisiana* and *B. bituminosa* (L.) C.H. Stirt (Fabaceae). Previously [3], we reported on a series of cytotoxic pterocarpans obtained from the leaves of *B. morisiana*, the most active of which was erybraedin C. We demonstrated that erybraedin C acted as a relatively potent inducer of necrosis in Jurkat T cells [3]. Pterocarpans from *B. morisiana* also exert powerful antioxidant activities [4]. Because of the promising biological profile shown by pterocarpans, covering antifungal, antibacterial, antifeedant, anti-HIV and cytotoxic effects [5], we decided to study the phytochemical composition of the seeds of *B. morisiana*. The phytochemical investigation led to the isolation of a new prenylated pterocarpan **1**, as well as one known pterocarpan and two known furocoumarins (**2**–**4**, Fig. 1). This paper describes the isolation of the four secondary metabolites, as well as the structure elucidation and the cytotoxicity profile of the new pterocarpan **1**.

## Results and discussion

The ethyl acetate extract of the dried and pulverized seeds of *B. morisiana* was subjected to fractionation by vacuum liquid chromatography (VLC), open column chromatography, and semi-preparative normal phase HPLC, yielding one new prenylated pterocarpan **1** and three known compounds **2**–**4** (Fig. 1). The known compounds were identified as erybraedin C (**2**) [6], psoralen (**4**) [7] and angelicin (**3**) [8], by comparing their spectroscopic data with those reported in the literature.

Compound **1** was isolated as a white amorphous powder and its molecular formula was determined to be $C_{22}H_{20}O_4$ by high-resolution electrospray ionisation mass spectrometry (HRESI-MS) (*m/z* 349.1447 $[M + H]^+$), which was also consistent with the $^1H$ and $^{13}C$ NMR data. In the $^1H$ NMR spectrum of compound **1** a set of four protons at $\delta$ 3.64 (1H, t-like, $J = 10.8$ Hz), 4.40 (1H, dd, $J = 10.8$, 4.4 Hz), 3.70 (1H, m) and 5.62 (1H, d, $J = 6.4$ Hz)

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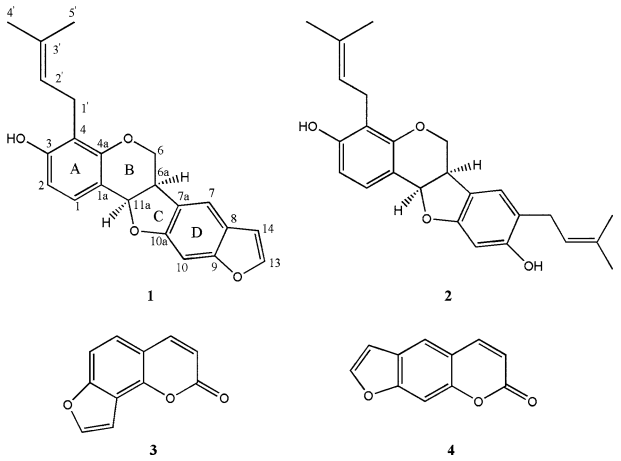


**Fig. 1** Structures of compounds **1**–**4**

suggested the presence of the O–$CH_2$–CH–CH–O unit forming the B and C rings of a pterocarpan nucleus [9]. Other signals in the $^1$H NMR spectrum were consistent with six aromatic protons at $\delta$ 6.58 (1H, d, $J = 8.4$ Hz), 6.81 (1H, d, $J = 2.4$ Hz), 6.96 (1H, s), 7.23 (1H, d, $J = 8.4$ Hz), 7.56 (1H, s), 7.67 (1H, d, $J = 2.4$ Hz), and with a $\gamma$,$\gamma$-dimethylallyl group ($\delta$ 1.70, 1.81, 3.35, 5.25). The $^{13}$C NMR spectrum revealed three signals at $\delta$ 41.1, 67.7 and 81.3 consistent with the C-6a, C-6 and C-11a of the aliphatic ring of a pterocarpan [9]. An HSQC experiment confirmed the attachment of the above-mentioned carbons to the protons at $\delta$ 3.64 (H-6$\beta$), 4.40 (H-6$\alpha$), 3.70 (H-6a) and 5.62 (H-11a), supporting the presence of a pterocarpan skeleton. The proton and carbon chemical shifts of a pair of *ortho*-coupled one-proton doublets at $\delta_H$ 6.58 and $\delta_H$ 7.23, and those of the prenyl chain were almost identical to the shifts found in erybraedin C [6], suggesting the same substitution pattern of the aromatic ring A. The position of the prenyl chain was confirmed by HMBC correlation of the methylene protons at $\delta$ 3.35 (2H, d, $J = 8.0$ Hz) with C-3 ($\delta$ 157.6), C-4a ($\delta$ 156.0) and C-4 ($\delta$ 117.5) (Fig. 2). The main differences between the $^1$H and $^{13}$C NMR data of compound **1** and those of erybraedin C (**2**) are the replacement of the signals of the prenyl chain linked to ring D by two one-proton doublets at $\delta$ 6.81 ($J = 2.4$ Hz) and 7.67 ($J = 2.4$ Hz). For compound **1** these protons were correlated in the HSQC experiment with the carbon signals at $\delta_C = 107.5$ (C-13) and 145.5 (C-14). These data strongly suggested the presence of a unsubstituted furan unit fused to the ring D. This was substantiated by HMBC connectivities between H-13 at $\delta$ 6.81 and C-9 (157.2), C-14 (145.5) and C-8 (122.9) and between H-14 at $\delta$ 7.67 and C-9 (157.2), C-8 (122.9) and C-13 (107.5) (Fig. 2). The absolute stereochemistry at C-6a and C-11a was established on the basis of the coupling constant $J_{6a/11a}$ and of the optical rotation value [10]. The

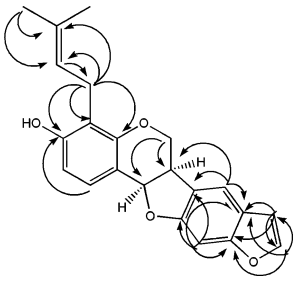

**Fig. 2** Key HMBC correlations of compound **1**

coupling constant of 6.4 Hz between H-6a and H-11a suggested a *cis* arrangement of the two protons. Although pterocarpans contain two chiral centres, only the 6a*R*,11a*R* and 6a*S*,11a*S* configurations are sterically possible [10]. It has been suggested that the absolute configuration of a pterocarpan might be presumed from the sign of its optical rotation [10]: levorotatory pterocarpans have 6a*R* and 11a*R* configurations, whereas the dextrorotatory ones have 6a*S* and 11a*S* configurations. Therefore, based on the negative optical rotation value, the absolute stereochemistry of compound **1** was assigned to be *R*. Thus compound **1** was characterized as 3-hydroxy-4-(3′-methyl-2′-butenyl)-furo[2′,3′:8,9] [6a*R*,11a*R*]pterocarpan and trivially named morisianine.

The putative cytotoxicity of compound **1** was tested against four human cell lines (Jurkat T, HL-60, CaCo-2, MCF-7). Compound **1** did not exhibit any activity up to 60 μM. Considering the structure–activity relationship of the 3-hydroxy-4-(3,3-dimethylallyl)-pterocarpans isolated from the leaves and seeds of *B. morisiana* [3], we conclude that both a free hydroxyl group at C-9 and a prenyl chain at C-8 in the pterocarpan nucleus are essential for the antiproliferative activity.

## Experimental

### General

Optical rotations were measured in MeOH at 25°C by using a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a GBC Cintra 5 spectrophotometer. NMR spectra were recorded at 25°C on a Varian Unity INOVA 400 MHz spectrometer, operating at 400 MHz for $^1$H and 100 MHz for $^{13}$C, respectively. Compounds were measured in tetradeuteromethanol ($CD_3OD$) and $CDCl_3$, and the spectra were referenced against residual non-deuterated solvents. EI-MS were measured on a QMD 1000 instrument at 70 eV by using a direct inlet system. HRESI-MS (positive mode) were measured on an Agilent 6520 Time-of-Flight (TOF) MS instrument. Column chromatography

was carried out by using silica gel (40–63 μm, Merck) and Sephadex LH-20 (25–100 μm, Pharmacia), with monitoring by TLC. For vacuum liquid chromatography (VLC), silica gel (40–63 μm, Merck) was used. TLC was performed on silica gel 60 $F_{254}$ or RP-18 $F_{254}$ (Merck). HPLC was conducted by means of a Hewlett-Packard 1050 instrument. The column was a 250 × 10 mm Spherisorb silica, particle size 5 μm (Waters), and the UV detection wavelength was 254 nm.

### Plant material

The seeds of *B. morisiana* were collected around Burcei (Cagliari, Sardinia, Italy) in September 2003. The plant material was identified by Dr. Marco Leonti and Prof. Bruno De Martis (University of Cagliari, Dipartimento di Scienze Botaniche), and a voucher specimen is deposited in the Herbarium of the Dipartimento Farmaco Chimico Tecnologico, University of Cagliari, Italy.

### Extraction and isolation

Air-dried and powdered seeds of *B. morisiana* (100.9 g) were ground and extracted with EtOAc (2 L) by percolation at room temperature to give 7.5 g dried extract. The EtOAc extract was subjected to VLC (silica gel, 40 g, 40–63 μm) by using a step gradient of hexane–$CH_2Cl_2$/EtOAc to yield 13 fractions (F1–F13). Fraction F6 (60 mg) was separated by normal phase HPLC by using hexane/EtOAc (8:2, flow 3.5 mL/min) to give compound **4** ($t_R$ = 16.4 min, 5.6 mg). Fraction F7 (102.8 mg) was subjected to column chromatography over silica gel [2.0 × 40 cm] (15 g, 40–63 μm), using hexane/EtOAc (9.5:0.5) as eluent, resulting in 2 subfractions (F7.1–F7.2). F7.2 (21.4 mg) was further purified by normal phase HPLC by using hexane/EtOAc (8.25:1.75, flow 3.5 mL/min) to afford compound **1** ($t_R$ = 17.9 min, 2.7 mg). Fraction F9 (100 mg) was chromatographed by column chromatography over silica gel [2.0 × 40 cm] (12 g, 40–63 μm) by using hexane–EtOAc (8.5:1.5) as eluent to yield compound **3** (13.8 mg). Fraction F11 (580 mg) was purified on Sephadex LH-20 [2.5 × 40 cm] with MeOH as eluent to give compound **2** (18.5 mg).

### Morisianine (**1**)

White amorphous powder. $[\alpha]_D^{25}$ −20° (*c* 0.1, MeOH). UV (MeOH): $\lambda_{max}$ (log ε) = 244 (3), 252 (2.9), 296 (2). HRESI-MS *m/z*: 349.1447 $[M + H]^+$, ($C_{22}H_{21}O_4$ calcd. 349.1440). EI-MS *m/z* (rel. int.): 348 (94) $[M]^+$, 331 (9), 292 (100), 221 (17), 171 (36), 158 (82), 147 (26), 135 (30), 69 (25), 43 (55). $^1$H NMR ($CD_3OD$) δ: 1.70 (3H, s, H-5′), 1.81 (3H, s, H-4′), 3.35 (2H, d, *J* = 8 Hz, H-1′), 3.64 (1H, t-like, *J* = 10.8, H-6β), 3.70 (1H, m, H-6a), 4.40 (1H, dd, *J* = 10.8, 4.8 Hz, H-6α), 5.25 (1H, t, *J* = 8 Hz, H-2′), 5.62 (1H, d, *J* = 6.4 Hz, H-11a), 6.58 (1H, d, *J* = 8.4 Hz, H-2), 6.81 (1H, d, *J* = 2.4 Hz, H-13), 6.96 (1H, s, H-10), 7.23 (1H, d, *J* = 8.4 Hz, H-1), 7.56 (1H, s, H-7), 7.67 (1H, d, *J* = 2.4 Hz, H-14). $^{13}$C NMR ($CD_3OD$) δ: 17.9 (C-4′), 23.1 (C-1′), 25.9 (C-5′), 41.1 (C-6a), 67.7 (C-6), 81.3 (C-11a), 94.3 (C-10), 107.5 (C-13), 109.7 (C-2), 113.3 (C-1a), 117.5 (C-4), 117.8 (C-7), 122.9 (C-8), 123.9 (C-2′), 125.5 (C-7a), 129.5 (C-1), 131.7 (C-3′), 145.5 (C-14), 156.0 (C-4a), 157.2 (C-9), 157.6 (C-3), 159.3 (C-10a).

### Cytotoxic activity

The cytotoxicity of compound **1** against Jurkat human leukaemia T cells (ATCC TIB-152), HL-60 cells (ATCC CCL-240 cells), CaCo-2 cells (ATCC HTB-37 cells) and MCF-7 (ATCC HTB-22) was determined after 2.5, 20 and 72 h in a WST-1-based cell viability assay, as described previously [11]. All compounds were tested in a concentration range between 0.5 and 80 μM. Maximal standard deviation was 10% (absolute).

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