Laurentixanthone C: A New Antifungal and Algicidal Xanthone from Stem Bark of *Vismia laurentii*

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Laurentixanthone C (**1**), a new xanthone, was isolated from the stem bark of *Vismia laurentii* (Guttiferae or Clusiaceae), in addition to the known compounds vismiaquinone (**2**), bisvismiaquinone (**3**), and dammaradienol (**4**). The structures were established based on spectroscopic studies, notably of the 2D NMR spectra. Preliminary results showed that **1** is algicidal and strongly antibacterial against the Gram-positive bacteria *Bacillus megaterium* and *Chlorella fusca*, respectively.

Key words: Vismia laurentii, Xanthone, Anthraquinone, Biological Activity

Introduction

The tribe Vismieae belongs to the family of Guttiferae and comprises three genera, *Vismia*, *Harungana* and *Psorospermum*. It is distributed in the tropical and subtropical regions of the world. *Vismia laurentii* De Wild is a large shrub or tree which is found in the Centre Province of Cameroon where it is locally called "atondo owse". The bark and roots are employed in decoctions as tonic and are febrifugal, and the plant is also used in tropical Africa as a remedy for the treatment of skin diseases (such as dermatitis, leprosy, scabies, eczemas) and wounds, using coconut as vehicle. Previous chemical studies have reported the presence of xanthones, anthraquinones, and prenylated anthrones in the plant [1]. As part of our systematic search for new bioactive lead structures from African medicinal plants, one new xanthone, laurentixanthone C (**1**), together with three known compounds identified as vismiaquinone (**2**), bisvismiaquinone (**3**), and dammaradienol (**4**) were isolated from *V. laurentii* (Fig. 1).

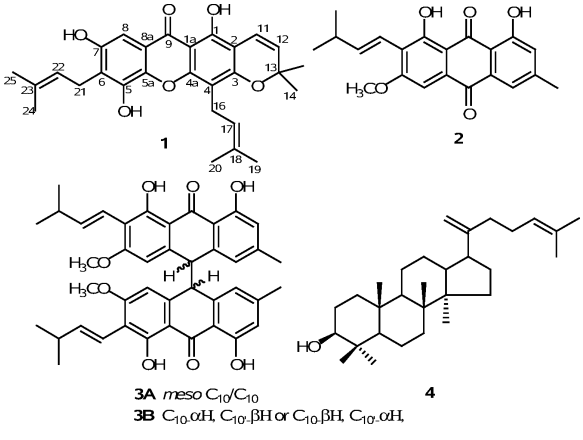

Fig. 1. Structures of compounds **1** – **4**.

Results and Discussion

Dried and powdered stem bark of *Vismia laurentii* was extracted with acetone. The residue obtained after evaporation of the solvent was subjected to conventional purification procedures, and resulted in the isolation of four constituents, including one new xanthone named laurentixanthone C (**1**).

Compound **1** gave a molecular ion peak at m/z = 462.2028 in its HREIMS, corresponding to the elemental formula $C_{28}H_{30}O_6$. The IR spectrum showed an absorption band at 3310 cm^{-1} for one or more hydroxyl groups and at 1610 cm^{-1} for a conjugated car-

bonyl functionality which also appeared at δ = 180.9 (C-9) in the ^{13}C NMR spectrum of **1** (see Experimental Section).

This carbonyl group formed a hydrogen bond with a hydroxyl group, as evidenced by a proton signal at δ = 13.28 (OH-1). One aromatic proton signal was observed at δ = 7.45 (H-8), and six oxygenated aromatic carbon signals appeared at δ = 157.7 (C-1), 155.2 (C-3), 149.0 (C-5), 155.6 (C-4a), 154.0 (C-5a), and 145.3 (C-7). These results indicated that **1** has a xanthone skeleton [2]. The ^{1}H NMR spectrum of **1** (see Experimental Section) showed the characteristic resonances of two prenyl groups [δ = 1.70 (3H, s) and 1.54 (3H, s); 1.63 (3H, s) and 1.60 (3H, s); 3.42 (2H, d, J = 7.2 Hz) and 3.28 (2H, d, J = 7.2 Hz); 5.24 (1H, t, J = 7.2 Hz) and 5.15 (1H, t, J = 7.2 Hz)] and the dimethylpyran ring at δ = 1.35 (6H, s), 5.46 (1H, d, J = 10 Hz) and 6.58 (1H, d, J = 10 Hz). The presence of the dimethylpyran ring was further supported by the set of signals at δ = 28.0, 77.7, 115.5 and 127.1 in the ^{13}C NMR spectrum [3]. Correlations in the HMBC spectrum (Fig. 2) of **1** showed that the two prenyl groups were attached to C-4 and C-6, and the pyran ring to C-2 and C-3 of ring A. Thus the methylene proton of one prenyl group at δ = 3.42 (d, J = 7.2 Hz, H-16) showed cross peaks with C-3, C-4, C-4a, and the second prenyl methylene at δ = 3.28 (d, J = 7.2 Hz, H-21) showed cross peaks to C-5, C-6, C-7. The position of the pyran ring to C-2 and C-3 of ring A was confirmed by HMBC correlations of the AB system of the pyran ring at δ = 6.58 (d, J = 10 Hz, H-11) and 5.46 (d, J = 10 Hz, H-12) to C-1, C-2, and C-3 (Fig. 2). A pyran system at positions C-2 and C-3 is very common among prenylated xanthones [3 – 13]. Finally, the position of the proton at C-8 was confirmed by HMBC correlations to C-9 and C-7. Consequently, the structure was established to be 5,8,10-trihydroxy-2,2-dimethyl-9,12-bis(3-methylbut-2-enyl)pyrano [3,2-b]xanthen-6(2H)-one (**1**, Fig. 1), named laurentixanthone C, after the producing organism, *Vismia laurentii*.

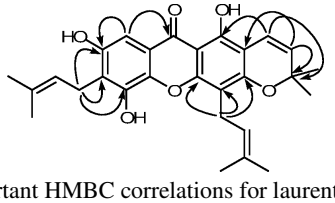

Fig. 2. Important HMBC correlations for laurentixanthone C (**1**).

Table 1. Biological activity of laurentixanthone C[a] (**1**) in an agar diffusion test.[a]

Compound	algicidal		antibacterial			
	Chl		Bm		Ech	
Laurentixanthone C (**1**) [mg/mL]	1	5	1	5	1	5
Zone of inhibition	0	6	10	7	0	0

[a] *Chlorella fusca* (Chl), *Bacillus megaterium* (Bm) and *Escherichia coli* (Ech). 0.05 μL (1 or 5 mg/mL) of dissolved xanthone were applied to a filter disc and sprayed with a suspension of the test organism. Radius of zone of inhibition in mm.

Three known compounds, vismiaquinone (**2**) [14], bisvismiaquinone (**3**) [15] and dammaradienol (**4**) [16] were identified by comparison with published data. The ^{1}H NMR spectrum of bisvismiaquinone showed two sets of signals, one set for the *meso* isomer (**3A**) and one for the racemic form (**3B**) [17].

Laurentixanthone C (**1**), vismiaquinone (**2**), bisvismiaquinone (**3**), and dammaradienol (**4**) were tested for algicidal and antifungal activities. Laurentixanthone C (**1**) showed strong antibacterial activity against the Gram-positive bacterium *Bacillus megaterium* and algicidal activity against *Chlorella fusca* (Table 1). Interestingly, related prenylated xanthones, isolated from *Calophyllum* species, also showed marked antibacterial activity [18, 19]. Vismiaquinone (**2**), bisvismiaquinone (**3**), and dammaradienol (**4**) were inactive in these tests.

Material and Methods

General experimental procedure

^{1}H, 2D ^{1}H-^{1}H COSY, ^{13}C, 2D HMQC and HMBC spectra were recorded with a Bruker Avance 500 MHz spectrometer. Chemical shifts (in ppm) are referenced to internal TMS (δ = 0) and coupling constants J are reported in Hz. Optical spectra were recorded with a NICOLET 510P FT-IR spectrometer, a UV-2101PC spectrometer, and a Perkin-Elmer 241 polarimeter.

Plant material

The stem bark of *V. laurentii* De Wild was collected at Mbalmayo, Center Province of Cameroon in July 2005. Botanical identification was achieved through comparison with a voucher specimen (No 1882/SRFK) in the National Herbarium, Yaounde (Cameroon).

Extraction and isolation

Air-dried and finely powdered stem bark (2.4 kg) was extracted successively at r. t. with acetone and MeOH and the solvent removed under reduced pressure to yield 50 g and 60 g of the respective extracts. The acetone extract

(50 g) was subjected to column chromatography over silica gel as stationary phase eluting with hexane-ethyl acetate of increasing polarity. Eighty-six fractions of 300 mL each were collected and grouped on the basis of TLC analysis to yield seven main fractions labelled A-G. Fraction A contained mostly fats and was not further investigated. Crude crystals from fraction B were purified on a silica gel column eluting with *n*-hexane-EtOAc (95 : 5) to afford laurentixanthone C (**1**) (740 mg). Further column chromatography of fractions C, D and E over silica gel, eluting with a step gradient of *n*-hexane-EtOAc and by gel permeation on Sephadex LH-20 (CH_2Cl_2-MeOH 1 : 1) yielded vismiaquinone C (**2**) (20 mg), bivismiaquinone (**3**) (100 mg), and dammaradienol (**4**) (152 mg).

5,8,10-trihydroxy-2,2-dimethyl-9,12-bis(3-methylbut-2-enyl)pyrano[3,2-b]xanthen-6(2H)-one; *Laurentixanthone C (**1**):* Yellow crystals, m. p. 109 – 110 °C. – UV ($CHCl_3$): λ_{max} = 253, 285, 350. – IR: ($CHCl_3$) ν_{max} = 3310, 1610, 1590, 710 cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$ + CD_3OD, 5 drops): δ = 1.35 (s, 6H, H-14, H-15), 1.54 (s, 3H, H-20), 1.60 (s, 3H, H-25), 1.63 (s, 3H, H-24), 1.70 (s, 3H, H-19), 3.28 (d, *J* = 7.2 Hz, 2H, H-21), 3.42 (d, *J* = 7.2 Hz, 2H, H-16), 5.15 (t, *J* = 7.2 Hz, 1H, H-17), 5.24 (t, *J* = 7.2 Hz, 1H, H-22), 5.46 (d, *J* = 10 Hz, 1H, H-12), 6.58 (d, *J* = 10 Hz, 1H, H-11), 7.45 (s, 1H, H-8), 13.28 (OH-1). – ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 5 drops): δ = 17.4 (C-19), 17.6 (C-24), 21.1 (C-16), 25.4 (C-20), 25.5 (C-25), 27.9 (C-21), 28.0 (C-14), 77.7 (C-13), 102.7 (C-2), 104.1 (C-1a), 107.4 (C-4), 112.9 (C-6), 115.5 (C-11), 116.5 (C-8), 121.2 (C-23), 122.4 (C-17), 125.9 (C-8a), 131.4 (C-18), 133.3 (C-22), 145.3 (C-7), 149.0 (C-5), 154.0 (C-5a), 155.2 (C-3), 155.6 (C-4a), 157.7 (C-1), 180.9 (C-9). – HREIMS: *m/z* = 462.2028 (calcd. 462.2040 for $C_{28}H_{30}O_6$). – EIMS (rel. int.): *m/z* (%) = 462.2 (40) $[M]^+$, 447.2 $[M–CH_3]^+$, 424 (23), 409 (100), 391 (5), 325 (10), 311 (22), 297 (12), 271 (12), 271 (28), 257 (58), 231 (13), 205 (14), 187 (19), 161 (22), 123 (45), 109 (83).

Bioactivity tests: Agar diffusion test

The tested compounds (**1** – **4**) were dissolved in acetone at a concentration of 1 mg/mL and 5 mg/mL. 50 μL each of the solutions were pipetted onto sterile filter discs, which were placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the respective test organism [20]. The test organisms were *Bacillus megaterium* (NB medium), *Microbotryum violaceum* (MPY-medium) and *Chlorella fusca* (MPY-medium); the radius of zone of inhibition was measured in mm.

Acknowledgements

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