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Phytochemistry 69 (2008) 1024–1028
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Anthraquinones from the fruits of *Vismia laurentii*

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Received 12 July 2007; received in revised form 19 October 2007
Available online 11 December 2007

Abstract

Phytochemical study of the fruits of *Vismia laurentii* resulted in the isolation of five structurally related compounds. Three of them are constituents, namely, laurentiquinone A (**1**) (methyl 1,6,8-trihydroxy-3-methyl-7-(3-methylbut-2-enyl)-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate), laurentiquinone B (**2**) (methyl 5,7-dihydroxy-2,2,9-trimethyl-6,11-dioxo-6,11-dihydro-2*H*-anthra[2,3-*b*]pyran-8-carboxylate) and laurentiquinone C (**3**) (methyl 9-(ethanoyloxymethyl)-5,7-dihydroxy-2,2-dimethyl-6,11-dioxo-6,11-dihydro-2*H*-anthra[2,3-*b*]pyran-8-carboxylate) and two are known compounds, emodin (**4**) and isoxanthorin (**5**). Their structures were elucidated by spectroscopic means. Crude extracts of hexane and EtOAc showed anti-plasmodial activity against the W2 strain of *Plasmodium falciparum*.

Keywords: *Vismia laurentii*; Guttiferae; Anthraquinones; Fruits; Laurentiquinones A, B and C

1. Introduction

As a part of our ongoing search for new bioactive constituents of plant origin (Lenta et al., 2007a; Ngouela et al., 2006), the fruits of *Vismia laurentii* were subjected to phytochemical investigation. *V. laurentii* De wild (Guttiferae) is a small tree that grows in many parts of the tropical region of Cameroon. According to Hutchinson and Dalziel (1954), this species belongs to the *Vismieae* tribe, Hyperiocoideae subfamily, Guttiferae family. The fifty main species of the *Vismia* genus consist of shrubs, small trees or large trees growing in the tropical regions of the world (Delle Monache, 1997). They are used in folk medicine as purgatives, tonics or febrifugal agents and also for the treatment of skin diseases (Kerharo, 1974; Macfoy and Sama, 1983). Previous phytochemical investigations of some *Vismia* species led to the isolation of benzophenones, xanthones, anthranoids and triterpenoids (Seo et al., 2000; Nagem and de Oliveira, 1997; Botta et al., 1983). Cytotoxic and antifeedant activities have also been reported for some *Vismia* constituents (Hussein et al., 2003; Seo et al., 2000; Simmonds et al., 1985). However, to the best of our knowledge, the fruits of *V. laurentii* have not been studied phytochemically before. The present paper deals with the isolation from the fruits of *V. laurentii* of five compounds, three of which were new constituents.

2. Results and discussion

Air-dried and ground fruits of *V. laurentii* were extracted successively with hexane, EtOAc and MeOH. The extracts were concentrated to dryness under vacuum. Repeated column chromatographic separations of the EtOAc extract on silica gel resulted in the isolation of laurentiquinone A (**1**), laurentiquinone B (**2**) and laurentiquinone C (**3**)

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0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.10.026

together with two known anthraquinones, emodin and isoxanthorin.

Compound **1** was isolated as yellow needles, with melting point 269–271 °C, which gave a positive Bornträger's test characteristic of anthraquinone derivatives. It reacted positively with $FeCl_3$, indicating its phenolic nature. Its molecular formula $C_{22}H_{20}O_7$, could be deduced from its high resolution electrospray ionisation-time of flight (HR-ESI-TOF) mass spectrum showing a pseudomolecular ion peak $[M+H]^+$ at *m/z* 397.1282 (calcd. for $C_{22}H_{21}O_7$ 397.1293) and corresponding to 13° of unsaturation. The UV spectrum showed specific absorptions with maxima at 230 (3.31), 252 (3.40), 287 (3.35) and 442 (3.59) nm and its IR spectrum revealed absorption bands at 1614 and 1563 cm^{-1} due to the free and chelated carbonyl groups, respectively. Both UV and IR data indicated that compound **1** was a 9,10-anthraquinone derivative (Wijnsma and Verpoorte, 1986; Tessier et al., 1981). The IR spectrum showed also at 3418 and 1727 cm^{-1} important bands due to hydroxyl and ester groups. The ^{13}C NMR spectrum (Table 1) displayed 22 carbon signals which were sorted by DEPT and J-mod techniques into three methyl, one methoxyl and one methylene groups, three sp^2 methine and fourteen quaternary carbons including three carbonyls at δ_C 189.6, 180.6 and 165.7. In the ^1H NMR spectrum of **1**, two chelated hydroxyl groups were observed at δ_H 12.30 and 11.25. In addition, it showed the characteristic resonances of a 3,3-dimethylallyl moiety [δ_H 1.74 (3H, s); 1.63 (3H, s); 3.25 (2H, nd) and 5.17 (1H, t, $J = 6.0$ Hz)] together with signals for one methoxyl group [δ_H 3.89 (3H, s)], one aromatic methyl group [δ_H 2.35 (3H, s)] and two aromatic protons [δ_H 7.53 (1H, s) and 7.25 (1H, s)]. The presence of the 3,3-dimethylallyl group was further confirmed by the set of signals at δ_C 17.5, 21.5, 25.3, 120.6 and 131.6 in the ^{13}C NMR spectrum. In the HMBC spectrum of **1** (Fig. 2) the correlation observed between the methoxyl protons and the carbonyl carbon at δ_C 165.7 suggested that the ester group was a methoxycarbonyl linked to the anthraquinone skeleton. Furthermore, the two aromatic protons at δ_H 7.53 (H-4) and 7.25 (H-5) gave cross-peaks with the carbonyl carbon at δ_C 180.6 (C-10) and with 3-Me (δ_C 19.4), C-8b (δ_C 113.9), C-2 (δ_C 128.4) and C-8a (δ_C 108.6), C-7 (δ_C 121.2), respectively, consistent with their location on carbons C-4 and C-5, *peri* to the carbonyl group C-10 (δ_C 180.6). In the same experiment, the methylene protons of the 3,3-dimethylallyl moiety at δ_H 3.25 (2H, nd) showed correlations with three quaternary carbons, C-7 (δ_C 121.2) and the oxygenated C-8 (δ_C 161.9) and C-6 (δ_C 162.3). This result indicated that the 3,3-dimethylallyl group was attached to C-7 and that the free hydroxyl was on C-6 in ring A. The remaining methyl with methoxycarbonyl substituents were placed on ring C. The aromatic methyl at δ_H 2.35 had cross-peaks with C-2, C-3 and C-4. In addition, it showed NOE associations with

Table 1
^1H and ^{13}C NMR spectroscopic data (200 MHz) for compounds **1**–**3**

Position	**1** (DMSO-d_6)		Position	**2** ($CDCl_3$)		**3** ($CDCl_3$)	
	δ_C (ppm)	δ_H (ppm) (*J* in Hz)		δ_C (ppm)	δ_H (ppm) (*J* in Hz)	δ_C (ppm)	δ_H (ppm) (*J* in Hz)
1	157.8	–	2	78.6	–	78.8	–
2	128.4	–	3	131.2	1H, 5.73, d (10.0)	131.3	1H, 5.74, d (10.0)
3	144.1	–	4	115.1	1H, 6.73, d (10.0)	115.0	1H, 6.73, d (10.0)
4	120.2	1H, 7.53, s	4a	114.5	–	114.5	–
4a	131.9*	–	5	159.6	–	159.7	–
4b	133.0*	–	5a	110.0	–	109.9	–
5	108.3	1H, 7.25, s	6	190.5	–	190.4	–
6	162.9	–	6a	114.1	–	115.8	–
7	121.2	–	7	159.4	–	159.6	–
8	161.9	–	8	128.8	–	127.2	–
8a	108.6	–	9	145.4		142.9	
8b	113.9	–	10	121.3	1H, 7.59, s	118.5	1H, 7.78, s
9	189.6	–	10a	133.6*	–	134.1*	–
10	180.6	–	11	181.0	–	180.5	–
1'	21.5	2H, 3.31, nd[a]	11a	133.5*	–	133.5*	–
2'	120.6	1H, 5.17, t (6.0)	12	110.3	1H, 7.21, s	110.6	1H, 7.23, s
3'	131.6	–	12a	160.3	–	160.6	–
4'	25.3	3H, 1.63, s					
5'	17.5	3H, 1.74, s	(2-Me)2	28.5	6H, 1.49 s	28.5	6H, 1.49, s
2- CO_2Me	52.3 s	3H, 3.89, s	8-CO_2Me	52.4	3H, 3.97, s	52.9	3H, 3.97, s
2- CO_2Me	165.7	–	8-CO_2Me	166.6	–	165.6	–
3-Me	19.4	3H, 2.35, s	9-Me/9-$CH_2OC(O)Me$	20.3	3H, 2.40, s	20.7	3H, 2.12, s
1-OH	–	12.30, s	9-CH_2O_2CMe	–	–	63.3	2H, 5.18, s
8-OH	–	11.25	9-CH_2O_2CMe	–	–	170.2	–
	–		7-OH	–	12.46, s	–	12.57, s
			5-OH	–	12.38, s	–	12.32, s

* Interchangeable signals.
[a] Signal partially obscured by water.

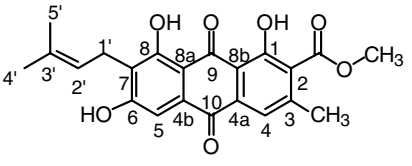
the aromatic proton H-4. These observations clearly demonstrated that the methyl group was located at C-3 and hence the methoxycarbonyl was at C-2. On the basis of the above results, the structure of laurentiquinone A (**1**) was determined to be methyl 1,6,8-trihydroxy-3-methyl-7-(3-methylbut-2-enyl)-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (Fig. 1).

Compound **2** was obtained as red needles in MeOH, mp 234–236 °C, and gave also a positive Bornträger's test for anthraquinones. Its molecular formula was established as $C_{22}H_{18}O_7$ by HR-ESI-TOF mass spectroscopy which showed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 395.1139 (calcd. for $C_{22}H_{19}O_7$, 395.1131) implying 14° of unsaturation. Like compound **1**, **2** appeared to be a 9,10-anthraquinone derivative based on its UV spectrum with maxima at 231 (3.43), 264 (3.44), 300 (sh) (3.60), 445 (3.26) nm and IR spectrum which exhibited absorption bands at 1602 and 1553 cm^{-1} due to the free and chelated carbonyl, respectively. The IR spectrum also revealed an important absorption band at 1727 cm^{-1} due to an ester group. The ^1H NMR data (Table 1) of compound **2** were similar to those of laurentiquinone A (**1**). The differences were proton resonances in ring A. The prenyl resonances in **1** were replaced by characteristic signals of the dimethylpyran ring [δ_H 6.73 (1H, d, $J = 10.0$ Hz), 5.73 (1H, d, $J = 10.0$ Hz) and 1.49 (6H, s)] in compound **2**. The HMBC correlations from the *cis* olefinic proton at δ_H 6.73 (H-4) to C-2 (δ_C 78.6), C-5 (δ_C 159.6) and C-12a (δ_C 160.3), and from the other olefinic proton at δ_H 5.73 (H-3) to C-2 (δ_C 78.6) and C-4a (δ_C 114.5) established the fusion of the dimethylpyran ring at C-4a and C-12a with an ether linkage at C-12a. Consequently compound **2**, named laurentiquinone B, was assigned structurally as methyl 5,7-dihydroxy-2,2,9-trimethyl-6,11-dioxo-6,11-dihydro-2*H*-anthra[2,3-*b*]pyran-8-carboxylate (Fig. 1).

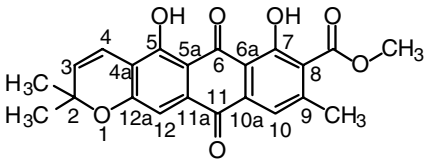
Compound **3** was obtained as an orange powder, with mp 191–193 °C, and gave a positive Bornträger's test for anthraquinones. Its molecular formula was established as $C_{24}H_{20}O_9$ by HR-ESI-TOF mass spectrometry which showed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 453.1174 (calcd. for $C_{24}H_{21}O_9$, 453.1186) implying 15° of unsaturation. Its UV and IR spectra were similar to those of compound **2** with maxima at 231 (3.47), 264 (3.41), 305 (sh) (3.50), 446 (3.20) nm for UV and important bands at 1616, 1603, 1746 cm^{-1} for IR. These data suggested that, like compounds **1** and **2**, compound **3** was also a 9,10-anthraquinone derivative. The ^{13}C NMR and J-mod spectra of **3** showed 24 carbon atoms shared between three methyls, one methoxyl, one methylene bearing an oxygen atom (δ_C 63.3), four methines and 15 quaternary carbon atoms including four carbonyl groups (δ_C 190.4, 180.5, 170.2 and 166.6). On comparing ^{13}C NMR assignments of compound **2** with those of **3** (Table 1), some discrepancies were observed. Unlike **2** which showed the aromatic methyl at δ_H 2.40/δ_C 20.3 (9-Me), compound **3** exhibited signals of an oxymethylene at δ_H 5.18/δ_C 63.3 and acetyl group (δ_H 2.12/δ_C 20.7, 170.2), suggesting that this methyl had been replaced by a methylene carbon linked to an acetate group in **3**. This was confirmed by the correlations observed in the HMBC spectrum of **3** (Fig. 2) which showed cross-peaks between the oxymethylene protons (δ_H 5.18) and C-8 (δ_C 127.2), C-10 (δ_C 118.5) and the carbonyl at δ_C 170.2 (acetyloxy) which further correlated with the methyl at δ_H 2.12. These results indicated clearly that the methylene was attached to C-9 and to an acetate group. All the remaining carbon signals in the two compounds **3** and **2** were very similar (Table 1). Thus, the structure of laurentiquinone C (**3**) was determined as methyl 9-(ethanoyloxymethyl)-5,7-dihydroxy-2,2-dimethyl-6,11-dioxo-6,11-dihydro-2*H*-anthra[2,3-*b*]pyran-8-carboxylate (Fig. 1).

In addition to compounds **1**, **2** and **3**, two known compounds, emodin (Buchalter, 1969) and isoxanthorin (Yagi et al., 1977) were also isolated.

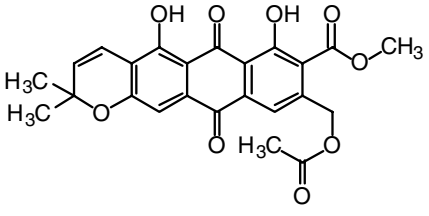
Hexane and EtOAc crude extracts of *V. laurentii* were evaluated for their anti-plasmodial activity in vitro according to the method previously described (Lenta et al.,



laurentiquinone A (**1**)



laurentiquinone B (**2**)



laurentiquinone C (**3**)

Fig. 1. Structures of compounds **1–3**.

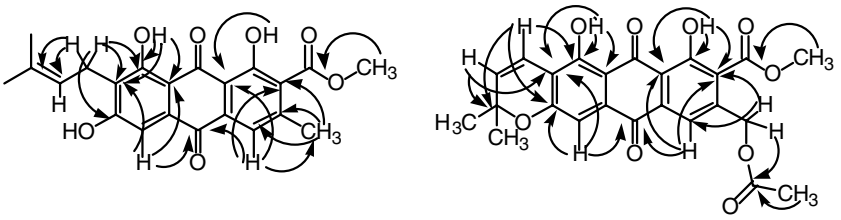


Fig. 2. Selected HMBC correlations for compounds **1** and **3**.

2007b). The two extracts exhibited anti-plasmodial activity with an IC_{50} of 10 μg/ml.

3. Experimental

3.1. General methods

Melting points were measured on a Stuart Scientific melting point apparatus SMP3 (UK) and are uncorrected. UV spectra were recorded on Shimadzu-2401 PC spectrophotometer. IR spectra were recorded on a Nicolet 380 spectrophotometer, using the ATR technique. NMR experiments were recorded using a Bruker Avance 200 spectrometer (200 MHz for ^{1}H and 50 MHz for ^{13}C). Five millimeter, Dual or BBi probe heads were used depending on the NMR results. For NMR analysis, all the compounds were dissolved in 0.5 ml of $CDCl_3$ or DMSO-d_6. Spectra were calibrated on the residual non deuterated solvent. So, $CHCl_3$ or DMSO were used as internal standard and the temperature was stabilised at 25 °C for all experiments. One and two dimensional experiments were performed using the Bruker software. For the mass spectra, electrospray ionization MS was performed on an HP1100MSD mass spectrometer, for high resolution measurements, Micro-TOF, Bruker instrument was used in ESI mode. Silica gel [Merck, Kieselgel 60 (0.063–0.200 mm)] and Sephadex LH-20 were used for column chromatography. Silica gel plates (Merck, Kieselgel 60 F_{254}) were used for TLC.

3.2. Plant material

The fruits of *V. laurentii* were collected from the bank of the Nyong river near Nkolmaka Lake (Endome) in Center Province, Cameroon on 17th October 2004 by Mr. Nana Victor. A voucher specimen (No. 1882/SRFK) has been deposited in the National Herbarium, Yaoundé, Cameroon.

3.3. Extraction and isolation

Plant material was subjected to the following procedures: Dried fruits (0.988 kg) were ground and exhaustively extracted by maceration successively with hexane, ethyl acetate and methanol. In each extraction 3 × 5 l of solvent were used for a period of 3 × 24 h and the extracts obtained were concentrated to dryness to give green (62.3 g), brown (43.6 g) and brown (22.1 g) crude viscous residues from hexane, EtOAc and MeOH extracts, respectively. The EtOAc extract (40 g) was subjected to flash column chromatography on silica gel 60 (0.063–0.200 mm, Merck, 500 g) as a stationary phase eluting with cyclohexane–EtOAc–MeOH mixtures of increasing polarity. Twenty-four fractions of 200 ml each were collected and grouped on the basis of TLC analysis to afford two main fractions A (11.7 g) and B (17.3 g). Fraction A was chromatographed on a silica gel column, using as eluent gradient mixtures of cyclohexane and EtOAc and yielded three main fractions (A1–A3). Fraction A1 (3.2 g) afforded, after filtration, emodin (35 mg) as crystals. Fraction A2 (2.2 g) was subjected to further column chromatography to afford, after filtration, laurentiquinone A (**1**) (10 mg) as crystals. Meanwhile fraction A3 was eluted with a gradient of mixtures of cyclohexane, EtOAc and MeOH to afford isoxanthorin (12 mg) and laurentiquinone B (**2**) (16 mg). Elution of fraction B on Sephadex LH-20 with a water–MeOH system gave laurentiquinone C (**3**) (5 mg) from sub-fractions 10–12.

3.4. Laurentiquinone A (*1*)

Yellow needles (MeOH); mp 269–271 °C; UV ($CHCl_3$) λ_{max} nm ($\log\varepsilon$) 230 (3.31), 252 (3.40), 287 (3.35), 442 (3.59); IR (KBr) ν_{max} cm^{-1} 3418, 1727, 1666, 1614, 1563, 1386, 1337, 1247; ^{1}H and ^{13}C-NMR, see Table 1; ESI-TOF-MS *m/z* 397 $[M+H]^+$; HRESI-MS *m/z* 397.1282 (calcd. for $C_{22}H_{20}O_7 + H$, 397.1293).

3.5. Laurentiquinone B (*2*)

Red needles (MeOH); mp 234–236 °C; UV ($CHCl_3$) λ_{max} nm ($\log\varepsilon$) 231 (3.43), 264 (3.44), 300 (sh) (3.60), 445 (3.26); IR (KBr) ν_{max} cm^{-1} 1727, 1670, 1644, 1602, 1553, 1374, 1248; ^{1}H and ^{13}C-NMR, see Table 1; ESI-TOF-MS *m/z* 395 $[M+H]^+$; HRESI-MS *m/z* 395.1139 (calcd. for $C_{22}H_{18}O_7 + H$, 395.1131).

3.6. Laurentiquinone C (*3*)

Orange powder (MeOH); mp 191–193 °C; UV ($CHCl_3$) λ_{max} nm ($\log\varepsilon$) 231 (3.47), 264 (3.41), 305 (sh) (3.50), 446 (3.20); IR (KBr) ν_{max} cm^{-1} 1746, 1675, 1616, 1603, 1557, 1386, 1374, 1242; ^{1}H and ^{13}C-NMR, see Table 1; ESI-

TOF-MS *m/z* 453 $[M+H]^+$; HRESI-MS *m/z* 453.1174 (calcd. for $C_{24}H_{20}O_9 + H$, 453.1186).

Acknowledgements

The authors thank the "Service de Coopération et d'Action Culturelle de l'Ambassade de France au Cameroun" for fellowship awarded to D.T. Noungoue at ULP Strasbourg, Pr Philip Jon ROSENTHAL for the anti-plasmodial tests and M. Patrick Wehrung (PCBis, IFR85, CNRS, Strasbourg) for the mass spectra.

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