Prenylated Anthraquinones and Other Constituents from the Seeds of *Vismia laurentii*

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Two new prenylated anthraquinones, laurenquinone A (1) and B (2) were isolated from the seeds of *Vismia laurentii* together with four known compounds; xanthone V_1 (3), physcion (4), 3-geranyloxyemodin anthrone (5) and friedelin (6). The structures of the new metabolites were determined with the help of spectroscopic data including extensive 2D-NMR spectroscopy. The known compounds were identified by comparison of their physical and spectroscopic data with those reported in the literature. Compounds 1, 4 and 5 exhibited moderate algicidal activity against *Chlorella fusca* and 3 showed moderate activity against the gram-positive bacterium *Bacillus megaterium*.

Key words *Vismia laurentii*; Guttiferae; prenylated anthraquinone; laurenquinone A; laurenquinone B; antimicrobial agent

Vismia laurentii DE WILD. from the family Guttiferae is a large shrub or tree of the secondary forest growth of tropical regions.[1)] Its stem bark and roots have been used in decoctions as tonic and febrifugal. Different parts of this plant are also used in tropical African medicine in the treatment of skin diseases such as dermatitis, leprosy, scabies, eczemas, and wounds.[2)] Previous chemical investigations of the stem bark and roots of this plant have resulted in the isolation of several secondary metabolites including triterpenoids, xanthones, flavonoids and anthraquinones.[3,4)] As part of our ongoing effort to discover molecule natural products with novel structures and/or biological activities from Cameroonian medicinal plants, we have carried out the chemical investigation of the title plant and now report on the constituents of the seeds.

The seeds of *V. laurentii* were air-dried for several days and then ground. The powder (200 g, dry weight) was exhaustively extracted with CH_2Cl_2/MeOH (1/1; v/v) and the extract was separated by chromatography on silica gel, affording the new compounds laurenquinone A (**1**, 38.0 mg) and B (**2**, 20.0 mg) together with the known compounds xanthone V_1 (**3**),[5)] physcion (**4**),[6)] 3-geranyloxyemodin anthrone (**5**),[5)] and friedelin (**6**).[7)] Their structures were elucidated by 1D and 2D NMR spectroscopy and by comparison with spectroscopic data reported in the literature.

The HR-EI-MS of laurenquinone A (**1**) showed a molecular ion peak (M^+) at *m/z* 396.1207, corresponding to the molecular formula $C_{22}H_{20}O_7$. Its IR spectrum exhibited strong vibration bands due to free hydroxy (3427 cm^{-1}), conjugated carbonyl (1666 cm^{-1}) and ester carbonyl (1730 cm^{-1}) groups. These data, together with those obtained from UV (absorptions at 256, 292, 331 nm) and ^{1}H-NMR (two singlets, 1H each at δ_H 12.60 and 12.38 due to two OH *peri* to a carbonyl moiety), suggested the presence of a 9,10-dioxygenated anthraquinone.[8—10)] The ^{1}H-NMR spectrum also displayed resonances for a methoxy (δ_H 3.94) and an aromatic methyl (δ_H 2.54) group, and only two aromatic protons (δ_H 7.54, 7.15). The ^{13}C-NMR spectrum showed signals for 22 carbons including resonances for a methoxy (δ_C 52.6), an aromatic methyl (δ_C 20.1), two carbonyl (δ_C 190.0, 182.3) and one ester carbonyl (δ_C 166.8) group. The aromatic methyl and the hydroxyl groups reside at C-6 and C-3 respectively, based on biogenetic grounds. In the HMBC spectrum (Table 1), the two aromatic proton signals at δ_H 7.54, and 7.15 showed correlations to a carbonyl group at δ_C 182.3, demonstrating their location at *peri* positions (C-4, C-5) to this carbonyl group. The methoxy resonance showed a correlation to the ester carbonyl group at δ_C 166.8 suggesting the presence of a methoxycarbonyl (H_3COCO) group. The EI-MS spectrum of laurenquinone A showed a base peak at *m/z* 336 [M^+-H_3COCO] which was important for the confirmation of the presence of methoxycarbonyl in the structure. Additionally, the ^{1}H-NMR spectrum displayed a set of signals suggesting a prenyl moiety [δ_H 5.20 (t, J=7.1 Hz, vinylic protons), 3.38 (d, J=7.1 Hz, methylene protons), 1.76 and 1.65 (s, each methyl protons)]. This was further confirmed in the ^{13}C-NMR spectrum by resonances at δ_C 22.1 (methylene group), 25.6 and 17.7 (two methyl groups), 120.4 (vinylic carbon), and 132.0 (quaternary sp^2 carbon). The methylene signal at δ_H 3.38 showed cross-peaks with the two

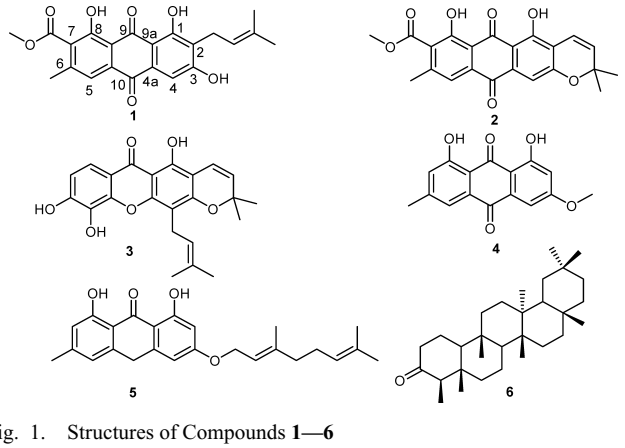

Fig. 1. Structures of Compounds **1—6**

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Table 1. ^{1}H- and ^{13}C-NMR Data for Laurenquinone A (**1**) and B (**2**) (δ (ppm), $CDCl_3/CD_3OD$, 500 and 125 MHz respectively) and ^2J, ^3J Gradient HMBC Correlations for **1** and **2**

C	**1**			**2**		
	δ_H	δ_C	HMBC (H→C)	δ_H	δ_C	HMBC (H→C)
1-OH	12.60 s	162.8	C-1, C-2, C-9a	12.42 s	159.6	C-1, C-2, C-9a
2	—	114.2		—	114.5	
3	—	162.9		—	160.3	
4	7.15 s	108.9		7.20 s	110.3	C-2, C-9a, C-10
4a	—	133.4		—	133.7	
5	7.54 s	120.9	C-7, C-8a, C-10	7.63 s	121.2	
6	—	144.8		—	145.3	
7	—	128.8		—	128.8	
8-OH	12.38 s	159.0	C-7, C-8, C-8a	12.49 s	159.4	C-7, C-8, C-8a
8a	—	113.1		—	114.1	
9	—	190.2		—	190.5	
9a	—	109.2		—	110.0	
10	—	182.3		—	180.0	
10a	—	133.1		—	133.4	
11	3.38 d (7.1)	22.1	C-1, C-3, C-13	6.76 d (10.1)	115.1	C-1, C-3, C-13
12	5.20 t (7.1)	120.4	C-2, C-14, C-15	5.75 d (10.1)	131.1	C-2, C-14, C-15
13	—	132.0		—	78.6	
14	1.76 s	17.7	C-12, C-15	1.50 s	28.4	C-12, C-13, C-15
15	1.65 s	25.6	C-12, C-14	1.50 s	28.4	C-12, C-13, C-14
CH_3-6	2.54 s	20.1	C-5, C-6, C-7	2.43 s	20.3	C-5, C-6, C-7
CH_3OCO	—	166.8		—	166.6	
CH_3O	3.94 s	52.6	C-7	3.98 s	52.6	C-7

oxygenated sp^2 carbons at δ_C 162.9 and 162.8 in the HMBC spectrum. Thus, the prenyl group should be located at C-2. The methoxycarbonyl (CO_2CH_3) group was finally located at C-7, since this was the remaining unoccupied position. Further analysis of the HMBC spectrum showed ^4J correlations from the aromatic proton signal at δ_H 7.54 (H-5) and the aromatic methyl signal at δ 2.54 to the ester carbonyl group (δ_C 166.8). From the above evidence and by comparison of its spectral data with the published values for 2-isoprenylemodin[11,12] and 1,3,8-trihydroxy-6-methylanthraquinone-7-carboxylic acid methyl ester,[13] the structure of compound **1** was established as 1,3,8-trihydroxy-2-(3,3-dimethylallyl)-6-methylanthraquinone-7-carboxylic acid methyl ester (laurenquinone A).

The molecular formula of laurenquinone B (**2**) was assigned $C_{22}H_{18}O_7$ on the basis of HR-EI-MS at *m/z* 394.1051 in conjunction with ^{1}H and ^{13}C spectroscopy data. Its IR spectrum exhibited strong vibration bands at 1650 and 1731 cm^{-1}, suggesting the presence of conjugated carbonyl and ester carbonyl groups. The UV absorption bands (258, 295 sh, 319, 342 nm) were typical for an anthraquinone chromophore. ^{1}H-NMR spectral data of **2** indicated that it was structurally related to laurenquinone A (**1**). Comparison of their NMR data showed that signals assignable to a prenyl group at C-2 of laurenquinone A were missing. However, the ^{1}H-NMR spectrum of **2** did show a spin system that could be assignable to a 2,2-dimethylpyrano group with signals at δ_H 6.76 and 5.75 (d, J=10.1 Hz, olefinic protons), 1.50 (6H, s, CH_3-13). The presence of this group was confirmed in the ^{13}C-NMR spectrum with resonances at δ_C 115.1, 131.1, 78.6, and 28.4. Apart from signals due to prenyl and dimethylpyrano groups, both the ^{1}H- and ^{13}C- (Table 1) NMR spectral data of compound **2** were closely related to those of **1**, with the chemical shifts of the protons and carbons of these two compounds being almost identical. The similarities of the two anthraquinones included the presence of the two hydrogen-bonded hydroxyl group, two aromatic protons, an aromatic methyl group, and a methyl ester group. In the HMBC experiment (Table 1), the proton signal at δ_H 5.75 (olefinic proton) showed ^3J correlations with the carbon signals at δ_C 159.6, 160.3, and 78.6, indicating the attachment of the 2,2-dimethylpyrano group at C-2–C-3. The assignment of carbons and protons (Table 1) could be made through BB, DEPT, HMQC, HMBC spectra and by comparison of the published data for related compounds.[11—13] Thus, the structure of laurenquinone B (**2**) was established to be 1,8-dihydroxy-2-3-(2,2-dimethylpyrano)-6-methylanthraquinone-7-carboxylic acid methyl ester.

Table 2. Biological Activity of Compounds **1**—**5**[a]

Microbial activities	Isolated compounds					
	Control	**1**	**2**	**3**	**4**	**5**
Antibacterial (Bm)	0	—	—	8	—	—
Algicidal (Chl)	0	6	—	—	6	6

a) Compounds **1**—**5** (50 μl at a concentration of 1 μg/μl acetone) were tested in an agar diffusion assay on filter discs (Schleicher and Schuell, 9 mm) for inhibitions of *Bacillus megaterium* (Bm) and *Chlorella fusca* (Chl). The radius of zone of inhibition was measured in mm starting at the outer boundary of the filter disc. (—): inactive. Control=acetone.

In addition to the above two new anthraquinones, four known compounds (**3**—**6**) were isolated. Their structures were established by comparison of NMR data with the corresponding literature or by comparison with the authentic standard compounds.

The isolated compounds were tested for their antibacterial and algicidal properties (Table 2). Laurenquinone A (**1**), physcion (**4**), and 3-geranyloxyemodin anthrone (**5**) were active against the green alga *Chlorella fusca* and xanthone V_1 (**3**) was active against the gram-positive bacterium *Bacillus*

megaterium. Laurenquinone B (**2**) was inactive in these tests. Compounds **1**—**5** were also inactive against *Microbotryum violaceum*. *In situ* the antibacterial activity of the metabolite **3** may contribute to protecting the seeds from bacterial pathogens.

Experimental

General Experimental Procedures Melting points were determined on a Büchi SMP-20 melting point apparatus and are uncorrected. UV spectra were measured with a UV-210 PC, UV. VIS Scanning spectrophotometer (Analytikjena). IR spectra were recorded on a SHIMADZU FTIR-8400S spectrophotometer in KBr disks. EI-MS (ionization voltage 70 eV) and HR-EI-MS mass spectra were recorded on a *Finnigan MAT double focusing spectrometer Model 8230*. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$/CD_3OD using a Bruker-Avance-500 MHz NMR spectrometer and TMS as an internal standard. Column chromatography (CC) was carried out on silica gel 60 F_{254} (Merck) and silica gel 100, respectively. Precoated plates of silica gel 60 GF_{254} were used for analytical purposes and the spots were detected with an UV lamp at 254 and 366 nm and by spraying with 50% H_2SO_4 or ceric sulphate followed by heating.

Plant Material The seeds of *V. laurentii* were collected at Mbalmayo, Center Province of Cameroon in July 2005. Authentication was achieved by Mr. Nana who compared with a voucher specimen (No 1882/SRFK) in the Cameroon National Herbarium, Yaounde.

Extraction and Isolation Air-dried and finely powdered seeds (200 g) of *V. laurentii* were macerated at room temperature for 72 h with a mixture of CH_2Cl_2/MeOH (1/1). Removal of the solvent under reduced pressure yielded 7.0 g of a crude extract which was subjected to column chromatography over silica gel (70—230 mesh). Elution was started with pure hexane followed by step gradients of hexane–EtOAc, and then EtOAc–MeOH. Ninety-five fractions of *ca.* 100 ml each were collected and combined on the basis of TLC analysis to afford six main fractions (F1—6). F1 (1.3 g, hexane–EtOAc 10 : 0, 9 : 1), F2 (0.6 g, hexane–EtOAc 9 : 1, 8 : 2), F3 (0.7 g, hexane–EtOAc 8 : 2), F4 (0.5 g, hexane–EtOAc 7 : 3), F5 (0.9 g, hexane–EtOAc 7 : 3, 6 : 4), F6 (1.0 g, hexane–EtOAc 0 : 10; EtOAc–MeOH 9 : 1, 8 : 2, 0 : 10). Fraction F1 (1.3 g) obtained with pure hexane and hexane/EtOAc (9/1) contained mostly fatty material and was not further investigated. Fraction F2 (0.6 g) eluted with hexane/EtOAc (9/1, 8/2), was also purified by column chromatography over silica gel using hexane–EtOAc (9/1) as eluant to afford compounds **4** (27.3 mg), **5** (25.0 mg) and **6** (16.0 mg). Fraction F3 (0.7 g) obtained with hexane–EtOAc (8/2), was subjected to repeated column chromatography over silica gel with a gradient of hexane–EtOAc to give compound **2** (20.0 mg). Fraction F5 (0.9 g) eluted with hexane–EtOAc (7/3, 6/4), was rechromatographed over silica gel using CH_2Cl_2/EtOAc with an increasing amount of EtOAc to obtain fractions which were regrouped on the basis of TLC analysis. Fractions eluted with CH_2Cl_2–EtOAc (5 : 95) were further purified through Sephadex LH-20 column using CH_2Cl_2/MeOH (1/1) to yield compound **1** (38.0 mg) and **3** (16.0 mg).

Laurenquinone A (**1**): Orange crystals from MeOH; mp: 276 °C; UV (MeOH) λ_{max} (log ε): 247 (3.92), 256 (3.92), 292 (3.87), 331 (4.10) nm; IR (KBr) ν_{max}: 3427, 3083, 2997, 2856, 1730, 1666, 1614, 1593, 1560, 1467, 1390, 1299, 1126, 1072, 850, 763 cm^{-1}; ^{1}H- and ^{13}C-NMR data: see Table 1; HR-EI-MS *m*/*z* 396.12070 (Calcd for $C_{22}H_{20}O_7$, 396.12089); EI-MS *m*/*z* (rel. int.): 396 (70), 379 (25), 364 (80), 347 (27), 336 (97), 321 (80), 309 (50), 293 (10), 284 (60), 240 (100), 183 (17), 155 (30), 139 (10), 98 (30), 69 (70), 44 (45).

Laurenquinone B (**2**): Orange crystals from MeOH; mp: 208—210 °C; UV (MeOH) λ_{max} (log ε): 258 (3.03), 271 (3.00), 295 (3.00), 319 (3.96), 342 (4.32) nm; IR (KBr) ν_{max}: 3056, 2975, 1731, 1650, 1606, 1556, 1460, 1386, 1261, 1139, 1112, 1076, 804, 756 cm^{-1}; ^{1}H- and ^{13}C-NMR data: see Table 1; HR-EI-MS *m*/*z* 394.10518 (Calcd for $C_{22}H_{18}O_7$, 394.10524); EI-MS *m*/*z* (rel. int.): 394 (30), 379 (70), 363 (10), 347 (72), 334 (15), 284 (100), 255 (55), 244 (99), 228 (40), 215 (25), 202 (10), 173 (15), 165 (99), 152 (40), 139 (20), 128 (58), 115 (30), 89 (5), 69 (30), 44 (80).

Bioactivity: Agar Diffusion Assay The compounds to be tested were dissolved in acetone at a concentration of 1 μg/ml. Fifty microliter of the solution were pipetted onto a sterile filter disc (Schleicher and Schuell, 9 mm), which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism.[14] The test organisms were *Bacillus megaterium*, *Microbotryum violaceum* and *Chlorella fusca*. These microorgansims were chosen because a) they are non-pathogenic and b) had in the past proved to be accurate initial test organisms for antibacterial, antifungal and antialgal/herbicidal activities. Commencing at the outer edge of the filter disc, the radius of zone of inhibition was measured in mm.

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References

1) Hutchinson J., Dalziel J., "Flora of West Tropical Africa (Revised by Keavy, R.W.)", Vol. 1, Whitefriars, London, 1954, p. 288.
2) Kuete V., Nguemeving J. R., Beng V. P., Azebaze A. G. B., Etoa F.-X., Meyer M., Bodo B., Nkengfack A. E., *J. Ethnopharmacol.*, **109**, 372—379 (2007).
3) Nguemeving J. R., Azebaze A. G. B., Kuete V., Carly N. N. E., Beng V. P., Meyer M., Blond A., Bodo B., Nkengfack A. E., *Phytochemistry*, **67**, 1341—1346 (2006).
4) Tala M. F., Krohn K., Hussain H., Kouam S. F., Wabo H. K., Tane P., Schulz B., Hu Q., *Z. Naturforsch.*, **62b**, 565—568 (2007).
5) Botta B., Monache G. D., Monache F. D., Bettolo G. B. M., Menichini F., *Phytochemistry*, **25**, 1217—1219 (1986).
6) Kouam S. F., Khan S. N., Krohn K., Ngadjui B. T., Kapche D. G. W. F., Yapna D. B., Zareem S., Amal M. Y. M., Choudhary M. I., *J. Nat. Prod.*, **69**, 229—233 (2006).
7) Kouam S. F., Ngadjui B. T., Krohn K., Wafo P., Ajaz A., Choudhary M. I., *Phytochemistry*, **66**, 1174—1179 (2005).
8) Bilia A. R., Yusuf A. W., Braca A., Keita A., Morelli I., *J. Nat. Prod.*, **63**, 16—21 (2000).
9) Miraglia M. C. M., Mesquita A. A. L., Varejaro De Jesus M. C., Gottlieb O. R., Gottlieb H. E., *Phytochemistry*, **20**, 2041—2042 (1981).
10) Tessier A. M., Delaveau P., Champion B., *Planta Med.*, **41**, 337—341 (1981).
11) Camele G., Delle Monache F., Delle Monache G., Marini B. G. B., Alves De L. R., *Phytochemistry*, **21**, 417—419 (1982).
12) Botta B., Delle Monache F., Delle Monache G., Marini B. G. B., Oguakwa J. U., *Phytochemistry*, **22**, 539—542 (1983).
13) Shibata S., Harada M., Budidarmo W., *Yakugaku Zasshi*, **80**, 620—624 (1960).
14) Höller U., Wright U. A. D., Matthée G. M., König G. F., Draeger S., Aust H.-J., Schulz B., *Mycological Res.*, **104**, 1354—1365 (2000).