Anthraquinones from *Vismia mexicana*

Ricardo Reyes-Chilpa[a,*], Rocío Gómez-Cansino[a,b], Silvia Laura Guzmán-Gutiérrez[a,c], Simón Hernández-Ortega[a], María Campos-Lara[d], Elisa Vega-Avila[c], and Antonio Nieto-Camacho[a]

[a] Instituto de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, 04510, México, D. F. Fax: (52+55) 56162203. E-mail: chilpa@unam.mx
[b] Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, 04510, México, D. F.
[c] Universidad Autónoma Metropolitana, Unidad Iztapalapa, Departamento de Ciencias de la Salud, San Rafael Atlixco 186, Del. Iztapalapa, 09340, México, D. F.
[d] Unidad de Investigación en Farmacología, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Av. Cuauhtémoc 330, Del. Cuauhtémoc, 06720, México, D. F.

* Author for correspondence and reprint requests

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Vismia mexicana (Clusiaceae) is a small tropical tree found from Mexico to Honduras. The CH_2Cl_2/MeOH extract from the leaves has been reported to have inhibitory properties against reverse transcriptase of human immunodeficiency virus type 1 (HIV-1 RT). In order to characterize some of its chemical constituents, the EtOAc-soluble fraction of this extract was subjected to column chromatography. A new natural product was isolated and designated vismiaquinone D [1-hydroxy-6-methoxy-7,8-(3′,3′-dimethyl-pyrano) anthraquinone]. In addition, vismiaquinone was obtained. The structures of vismiaquinone and vismiaquinone D were determined by ^{1}H and ^{13}C NMR spectroscopy, unambiguous assignments were achieved with DEPT, HSQC, and HMBC experiments, and corroborated by X-ray diffraction studies. The isolated anthraquinones were tested against HIV-1 RT. However, none showed relevant activity, suggesting that other compounds in this extract may be responsible for its HIV-1 RT inhibitory properties.

Key words: Vismia mexicana, Anthraquinones, HIV Reverse Transcriptase

Introduction

Vismia mexicana Schltdl. is a small tree found from Mexico to Honduras, growing in the Tropical Montane Cloud and Rain Forests. The leaves are brown-golden coloured on the abaxial side, and the trunk exudes a yellow latex after injury. The people of Ejido Benito Juarez, State of Oaxaca, Mexico, use the cortex of the trunk to prepare an infusion used as a mouthwash and as a women's douche, and this species is also used for tanning (Martínez Alfaro, 1970). The chloroform extract of *V. mexicana* leaves contains the anthranoids vismione A, vismione B, ferruginin A, and *cis*-γ-hydroxyferruginin A, the anthraquinones physcion, γ,γ'-dihydroxyferruginin A, and 7-(*trans*-3-methyl-1-butenyl)-physcion (vismiaquinone), as well as isocaryophyllene and *cis*-α-farnese, while the acetone extract contains quercetin, (−)-epicatechin, and procyanidin B_2 (Moura Pinheiro *et al*., 1984).

The CH_2Cl_2/MeOH extract of *V. mexicana* leaves inhibited human immunodeficiency virus type 1 (HIV-1) replication in a human MT2 cell assay *in vitro* at 50 μg/mL; it was non-toxic to human lymphocytes in culture at this concentration. It acts by inhibiting HIV-1 reverse transcriptase (RT) (Huerta-Reyes *et al*., 2004). The antiviral active compounds have not been characterized to date. In this contribution, we report on the isolation of the anthraquinones vismiaquinone D (**1**), vismiaquinone (**2**), as well as physcion from this extract. Structures of compounds **1** and **2** were determined by ^{1}H and ^{13}C NMR spectroscopy, and corroborated by X-ray diffraction. The isolated anthraquinones were tested against HIV-1 RT, but none inhibited this viral enzyme to any extent, suggesting that other com-

pounds in this extract must be responsible for its antiviral properties.

Experimental

General

^{1}H, ^{13}C NMR, and HMBC data were acquired with a Varian Unity Inova 500 MHz instrument (Palo Alto, CA, USA) using $CDCl_3$. UV spectra were obtained using a Shimadzu U160 spectrophotometer (Columbia, MD, USA). EI mass spectra were acquired on a JMS AX-505 HA spectrometer (Tokyo, Japan) at 70 eV. X-ray crystallographic data were collected using a Bruker SMART APEX CCD-based X-ray diffractometer system (Madison, WI, USA) equipped with a Mo-target X-ray tube ($\lambda = 0.71073$ Å).

Plant material

Vismia mexicana leaves were collected at "Cascada de la Monja", Municipio de Xico, Estado de Veracruz, México. A voucher specimen has been deposited (FECME 91674) in the herbarium of Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM), Coyoacán, México.

Extraction and isolation of phytoconstituents

The dried leaves were extracted with CH_2Cl_2/MeOH (1:1, v/v). The solvent was evaporated with a rotary evaporator giving a brown gum (14.65 g) which was further macerated with EtOAc, providing an insoluble brown solid (10.75 g) and a soluble fraction (3.90 g). The latter was impregnated into celite and subjected to column chromatography (silica gel 70 – 230, 78 g; Merck, Darmstadt, Germany) eluting with *n*-hexane, EtOAc, and mixtures of these solvents in order of increasing polarity.

Fractions 10 – 13 eluted with *n*-hexane/EtOAc (98:2, v/v) afforded a mixture (157.5 mg) which was subjected to column chromatography (silica gel C-18, 15 g; Merck) eluting with acetone/H_2O (9:1). Subfractions 3 – 6 afforded a yellow gum which was identified as physcion (13 mg) by its ^{1}H NMR spectral data which were in agreement with those previously reported (Takahashi *et al*., 1977). Subfractions 7 – 13 yielded vismiaquinone (**2**) (18 mg) as a red gum, which was recrystallized by slow evaporation of a mixture of *n*-hexane and CH_2Cl_2 as solvent system, obtaining red crystals (6 mg).

Fractions 14 – 34 eluted with *n*-hexane/EtOAc (98:2) yielded an orange gum which was analysed by thin-layer chromatography (TLC) (silica gel 60; Merck) eluted with *n*-hexane/EtOAc (7:3) and shown to contain vismiaquinone (**2**) (R_f 0.66) and vismiaquinone D (**1**) (R_f 0.74). This mixture (137 mg) was subjected to preparative TLC using five plates (silica gel, plate thickness, 1 mm; Merck) with *n*-hexane/EtOAc (98:2) as eluent which allowed to obtain the two quinones.

Vismiaquinone D (**1**): Amber crystals (CH_2Cl_2/MeOH). – M.p. 186 – 188 °C [188 – 189 °C according to Delle Monache *et al*. (1979)]. – EIMS: m/z (%) = 350 (10) [M^+] ($C_{21}H_{18}O_5$), 335 (100) [M^+ – CH_3], 336 (22), 320 (12), 167 (14). – UV (MeOH): $\lambda_{max}(\log\varepsilon) = 425$ (4.12), 288 (4.48), 265 (4.45), 225 (4.72), 206 nm (4.65). – ^{1}H and ^{13}C NMR: see Table I.

Vismiaquinone (**2**): Red crystals. – M.p. 197 – 198 °C [197.3 – 198.7 °C according to Nagem and de Oliveira (1997)]. – EIMS: m/z (%) = 352 (74) [M^+] ($C_{21}H_{20}O_5$), 309 (100) [M^+ – C_3H_7], 337 (40) [M^+ – CH_3], 323 (12), 297 (53), 267 (12), 161 (10), 125 (7). – UV (MeOH): $\lambda_{max}(\log\varepsilon) = 441$ (4.09), 292 (4.44), 261 (4.28), 216 nm (4.76). – ^{1}H and ^{13}C NMR: see Table II.

X-ray diffraction studies

Crystalline amber and red, respectively, prisms were grown for vismiaquinone D (**1**) and vismiaquinone (**2**) independently by slow evaporation of acetone/EtOAc and *n*-hexane/CH_2Cl_2 as solvent systems, respectively, and mounted on glass fibers. In all cases, the X-ray intensity data were measured at 298 K with a Mo-target X-ray tube ($\lambda = 0.71073$ Å). The detector was placed at a distance of 4.837 cm from the crystals in all cases. A total of 1,800 frames were collected with a scan width of 0.3 in x and an exposure time of 10 s/frame. The frames were integrated with the Bruker SAINT software package using a narrow-frame integration algorithm. The data were integrated using a monoclinic unit cell for vismiaquinone D (**1**) and triclinic for vismiaquinone (**2**) to yield a total of 24,026 and 12,374 reflections, respectively, to a maximum 2θ angle of 50.00°, of which 3,012 [R(int) = 0.0741] for vismiaquinone D (**1**) and 3,071 [R(int) = 0.0443] for vismiaquinone (**2**) were independent. Analysis of the data showed in all cases negligible decay during data collections. The structures

Table I. ^{1}H and ^{13}C NMR spectral data of vismiaquinone D (**1**).

Position	^{1}H[a] (*J* in Hz)	^{13}C[b]	HMBC (H → C)
1		162.6 s	
1a		114.9 s	
2	7.06 d (1.5)	124.5 d	C-1, C-4, Me-3, C-1a
3		146.6 s	
4	7.56 d (1.5)	119.7 d	C-10, C-2, Me-3, C-1a
4a		135.4 s	
5	7.43 s	102.8 d	C-10, C-6, C-4a
5a		132.6 s	
6		158.7 s	
7		116.3 s	
8		156.3 s	
8a		115.4 s	
9		187.2 s	
10		182.7 s	
1′	6.73 d (10)	116.1 d	C-6, C-8, C-3′
2′	5.83 d (10)	132.1 d	C-3′, Me-4′, Me-5′, C-7
3′		77.7 s	
Me-4′,Me-5′	1.57 s	27.9 q	C-2′, C-1′
OMe-6	4.01 s	56.2 q	C-6
Me-3	2.42 s	21.9 q	C-3, C-2, C-4, C-1a
OH	13.19		C-1, C-3, C-2, C-1a

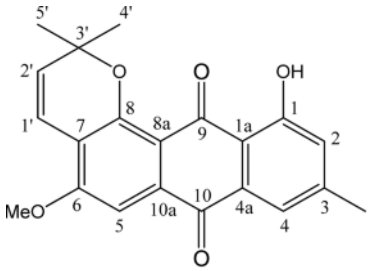
[a] 500 MHz/$CDCl_3$. [b] 125 MHz.

Table II. ^{1}H and ^{13}C NMR spectral data of vismiaquinone (**2**).

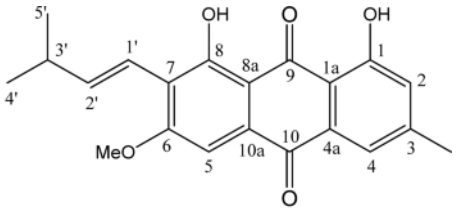
Position	^{1}H[a] (*J* in Hz)	^{13}C[b]	HMBC (H → C)
1		162.4 s	
1a		113.7 s	
2	7.06 d (1.6)	124.4 d	C-4, C-1a
3		148.4 s	
4	7.61 d (1.6)	121.1 d	C-10, C-2, C-1a
4a		133.2 s	
5	7.40 s	103.3 d	C-10, C-7, C-8a
5a		132.0 s	
6		163.0 s	
7		120.0 s	
8		162.0 s	
8a		110.5 s	
9		191.4 s	
10		181.9 s	
1′	6.65 dd (1.2, 16)	115.7 d	C-6, C-3′
2′	6.91 dd (7, 16)	146.7 d	C-7
3′	2.52 qdd (7.2, 7, 1.2)	33.3 d	C-2′, C-1′, Me-4′, Me-5′
Me-4′,Me-5′	1.13 d (7.2)	22.1 q	C-2′, C-3′
OMe-6	4.04 s	56.3 q	C-6
Me-3	2.44 s	22.4 q	C-3, C-2, C-4
OH-1	12.09 s		C-1, C-2, C-1a
OH-8	12.94 s		C-8, C-7, C-8a

[a] 500 MHz/$CDCl_3$. [b] 125 MHz.

were solved by the direct method using the SHELXS-97 program. The remaining atoms were located via a few cycles of least-squares refinements and difference Fourier maps, using the space group $P2_1/n$ with $Z = 4$ for vismiaquinone D (**1**) and $P\bar{1}$ with $Z = 2$ for vismiaquinone (**2**). Hydrogen atoms were put at calculated positions on the atoms to which they are attached. Thermal parameters were refined for hydrogen



Vismiaquinone D (**1**)



Vismiaquinone (**2**)

Fig. 1. Compounds isolated from *Vismia mexicana* leaves.

atoms on the phenyl groups using a $U_{eq} = 1.2$ times to precedent atom in all cases. For all complexes, the final cycle of refinement was carried out on all non-zero data using SHELXTL and anisotropic thermal parameters for all non-hydrogen atoms. Supplementary data for compounds **1** and **2** have been deposited at the Cambridge Crystallographic Data Centre. Copies of this information are available free of charge on request from: The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336 033, E-mail: deposit@ccdc.ac.uk, or internet address: http://www.ccdc.cam.ac.uk) quoting the deposition numbers 012345 and 678901, respectively.

Biological assays

Compounds **1** and **2** and physcion were evaluated by a commercial non-radioactive immunocolorimetric assay – Lenti RT Activity Assay (Cavidi Tech, Uppsala, Sweden) – in a microtiter plate reader (ELx 808; BIOTEK Instruments, Winoski, VT, USA) at 405 nm, as previously described (Huerta-Reyes *et al.*, 2004).

Results and Discussion

Isolation of compounds

The EtOAc fraction of the CH_2Cl_2/MeOH extract of *V. mexicana* leaves afforded vismiaquinone D (**1**), vismiaquinone (**2**), and physcion (Fig. 1). To the best of our knowledge, this is the first time vismiaquinone D (**1**) has been obtained as a natural product, even though it has been reported as the main alkaline decomposition product (pyridine or NaOH in the presence of O_2) of the anthranoid vismione B isolated from the fruits of *V. baccifera* (Delle Monache *et al.*, 1979).

The EI mass spectrum of vismiaquinone D (**1**) exhibited the molecular ion at m/z 350 congruent with the molecular formula $C_{21}H_{18}O_5$ (10%), with the base peak at m/z 335 [$M^+ - CH_3$] (100%). The ^{13}C NMR spectrum showed signals for 21 carbon atoms, two of them were carbonyl carbon atoms (187.2 ppm, s, and 182.7 ppm, s) and nine aromatic quaternary carbon atoms (Table I). The DEPT experiment revealed signals for five C-H carbon atoms, three of them were aromatic (124.5 ppm, d, 119.7 ppm, d, and 102.8 ppm, d) and two vinylic (116.1 ppm, d, and 132.1 ppm, d). Signals for CH_3 groups were observed, one at 21.9 ppm (q) and two overlapped at 27.9 ppm (q). A methoxy group was revealed at 56.2 ppm (q). Unambiguous ^{13}C NMR assignments were achieved with HSQC and HMBC experiments (Table I). The ^1H NMR spectrum showed one phenolic hydroxy group at 13.19 ppm (s, 1H); its chemical shift at very low magnetic field suggested that it is chelated with a C=O group, while the HMBC experiment showed coupling of this hydrogen atom with C-1, therefore it was placed at this position. Signals for three aromatic protons were also observed. A singlet at 7.43 ppm (1H) was assigned to H-5. Two doublets at 7.06 (1H) and 7.56 ppm (1H), both with $J = 1.5$ Hz, were assigned to *meta*-H-2 and H-4, respectively. A methyl group on an aromatic ring was observed at 2.42 ppm (s, 3H), which on basis of the HMBC experiment could be placed on C-3. Signals for a dimethyl pyran ring were observed as a singlet at 1.57 ppm (s, 6H) assigned to the hydrogen atoms of the methyl groups Me-4′ and Me-5′, and two doublets at 6.73, and 5.83 ppm (both $J = 10$ Hz, 1H) were assigned to H-1′ and H-2′. The structure of vismiaquinone D (**1**) was corroborated by X-ray-diffraction (Fig. 2).

Our ^1H NMR data of vismiaquinone (**2**) were in agreement with those previously reported (do Carmo *et al.*, 1981); however, differences were noticed regarding the chemical shifts of some aromatic protons and carbon atoms reported in the literature. Therefore, unam-

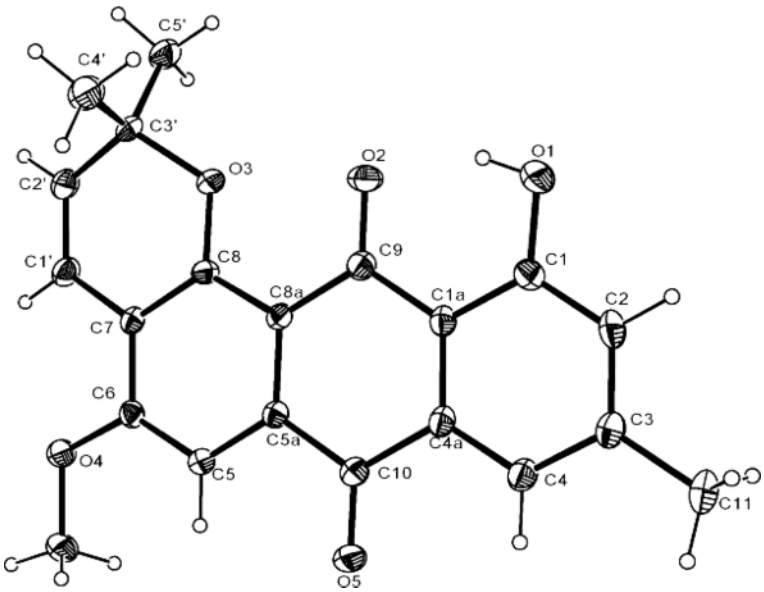


Fig. 2. X-ray structure of vismiaquinone D (**1**).

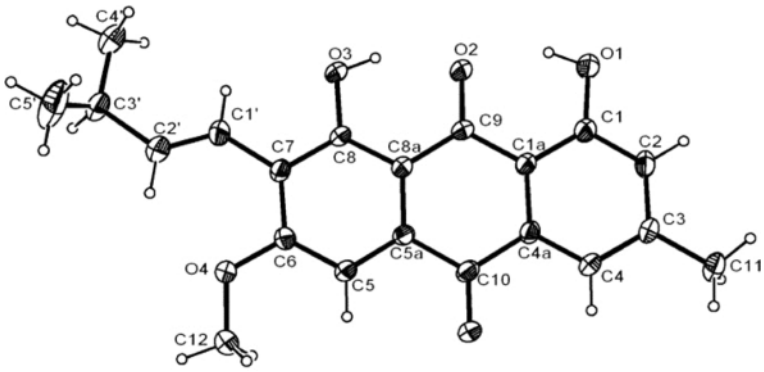


Fig. 3. X-ray structure of vismiaquinone (**2**).

biguous ^{13}C and ^{1}H NMR assignments (Table II) were achieved with the aid of HSQC and HMBC experiments. The structure of vismiaquinone (**2**) was corroborated by X-ray diffraction (Fig. 3). Vismiaquinone (**2**) had first been isolated from the wood of *V. japurensis* (do Carmo *et al*., 1981), thereafter from the leaves of *V. reichardtiana* (Goncalves and Mors, 1981), *V. parviflora* (Nagem and de Oliveira, 1997), and *V. laurentii* (Noungoue *et al*., 2009). The crude extract from the stem bark of the latter species, growing in Africa, as well as **2** exhibited high activity against *Plasmodium falciparum* (Noungoue *et al*., 2009).

Physcion has previously been obtained as one of the alkaline decomposition products of the anthranoid vismione A from the berries of *V. baccifera* (Delle Monache *et al*., 1979). It was further obtained as a natural product from the wood of *V. japurensis* (do Carmo *et al*., 1981), and the berries of *V. mexicana* and *V. cayennensis* (Moura Pinheiro *et al*., 1984).

Table III. HIV-1 RT inhibition (%) by the anthraquinones (50 μg/mL) from *Vismia mexicana*.

Compound	Mean	Standard deviation
Vismiaquinone D (**1**)	22.7	$\pm$ 1.18
Vismiaquinone (**2**)	0.0	$\pm$ 1.26
Physcion	0.64	$\pm$ 4.34

$n = 5$. Positive control, nevirapine, $IC_{50} = 0.01$ μM.

HIV-1 RT inhibition

Vismiaquinone D (**1**), vismiaquinone (**2**), and physcion were tested against reverse transcriptase of human immunodeficiency virus type 1 (HIV-1 RT) at 50 μg/mL; however none of these compounds showed relevant inhibitory activity (Table III).

Conclusion

The EtOAc-soluble fraction of the CH_2Cl_2/MeOH extract of *Vismia mexicana* leaves contains the anthraquinones vismiaquinone D (**1**), vismiaquinone (**2**), and physcion. These were inactive against HIV-1 RT, suggesting that other compounds must be responsible for the HIV-1 RT inhibitory properties reported for the whole extract.

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