Bioactive Constituents from Three *Vismia* Species

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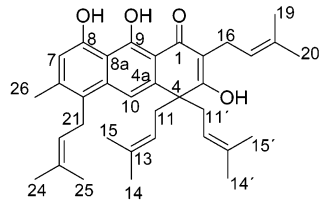
Bioassay-guided fractionation of the methanolic extracts of *Vismia baccifera*, *V. jefensis*, and *V. macrophylla* against human breast, CNS, and lung cancer cell lines resulted in the isolation of a new compound, ferruginin C (**1**), and seven known compounds, ferruginins A (**2**) and B (**3**), vismin (**4**), harunganin (**5**), vismione B (**6**), deacetylvismione H (**7**), and deacetylvismione A (**8**), as active constituents. In addition, bivismiaquinone (**9**) and vismiaquinone (**10**) were obtained as inactive constituents. The structure of ferruginin C was elucidated by spectroscopic means. Compounds **6**−**8** were the most active, and the cytotoxic activity of compounds **2**−**5** and **7** is reported for the first time.

Within the framework of an International Cooperative Biodiversity Groups (ICBG) project, three *Vismia* species were studied for their potential anticancer activity. The tribe *Vismieae* belongs to the family Clusiaceae and comprises three genera, *Vismia*, *Harungana*, and *Psorospermum*. It is distributed in tropical and subtropical regions.[1] Several triterpenoids, prenylated anthrones, anthraquinones, bianthraquinones, benzophenones, and lignans have been characterized from the genus *Vismia*.[2−12] Cytotoxic[13,14] and antifeedant[15] activities have been reported for various *Vismia* constituents. Vismiones A and B, ferruginins A and B, and ferruanthrone have been reported previously from the fruits of *Vismia baccifera* (L.) Triana & Planch.[10,16] However, the leaves of *V. baccifera* and the plants *Vismia macrophylla* Kunth and *Vismia jefensis*, N. Robson have not been studied phytochemically before.

The methanolic extracts of the young leaves of *V. baccifera*, *V. jefensis*, and *V. macrophylla* each showed cytotoxic activity against three different cancer cell lines (see Table 1). The fractionation process was carried out using both liquid−liquid partitioning and column chromatography (see Experimental Section). Five active compounds were isolated from the young leaves of *V. macrophylla*, namely, ferruginin C (**1**), ferruginins A (**2**)[17] and B (**3**),[17] vismin (**4**),[18] and harunganin (**5**).[19] Three active compounds, vismione B (**6**),[11] deacetylvismione H (**7**),[16] and deacetylvismione A (**8**),[18] were isolated from *V. baccifera*. A bianthrone which has been named bivismiaquinone (**9**) was isolated with vismiaquinone (**10**)[20] as inactive constituents from the same source. The bivismiaquinone (**9**)[11] is reported for the first time as a natural product. Two compounds were isolated from *V. jefensis*, which are identical to compounds **7** and **8** isolated from *V. baccifera*. Compound **1** was obtained as orange crystals from the hexane partition of a methanolic extract of young leaves of *V. macrophylla*. The molecular formula of **1** was established as $C_{35}H_{44}O_4$ by HRCIMS, which showed a molecular ion peak at *m*/*z* 528. The ^{1}H and ^{13}C NMR data of **1** suggested a tetraprenylated dihydroanthracene nucleus with a nonaromatic C ring.[6] The NMR data of **1** were similar to those reported for vismin (**4**)[18,21] isolated from the same plant. The spectral data indicated the existence of two additional prenyl groups in **1**, and the absence of two aromatic proton signals H-2 and H-5 (δ_H 5.80, 7.09, respectively) in vismin (**4**) suggested the structure **1**. HMBC cross-peak connectivities of H-17 (δ_H 5.09) to C-2 (δ_C 116.3), and H-22 (δ_H 5.04) to C-5 (δ_C 125.4), confirmed the two prenyl groups at positions 2 and 5 (Figure 1). Additional spectroscopic data of HSQC, COSY 45, HMBC,

Table 1. Cytotoxic Activities of Plant Extracts and Compounds **1**−**8**

compound/extract	GI_{50} (μg/mL) MCF-7	H-460	SF-268
V. baccifera total extract	2.4	4.4	3.7
V. jefensis total extract	<1.0	1.3	2.3
V. macrophylla total extract	0.5	0.5	0.4
ferruginin C (**1**)	4.8	5.4	4.0
ferruginin A (**2**)	5.0	3.3	4.9
ferruginin B (**3**)	5.0	5.0	4.0
vismin (**4**)	5.2	7.3	5.7
harunganin (**5**)	6.0	6.4	6.0
vismione B (**6**)	1.6	2.8	2.5
deacetylvismione H (**7**)	0.4	0.6	0.6
deacetylvismione A (**8**)	1.8	1.9	2.2
adriamycin	6.2×10^{-7}	3.6×10^{-7}	5.3×10^{-7}



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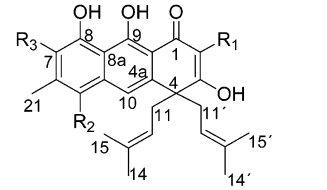

2 R_1 (H) R_2 (H) R_3 (Prenyl)
3 R_1 (Prenyl) R_2 (H) R_3 (H)
4 R_1 (H) R_2 (H) R_3 (H)
5 R_1 (H) R_2 (Prenyl) R_3 (H)

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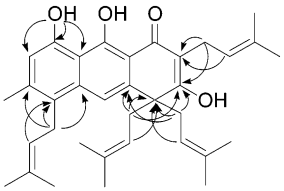
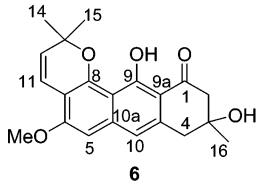


Figure 1. Selected HMBC correlations for **1**.



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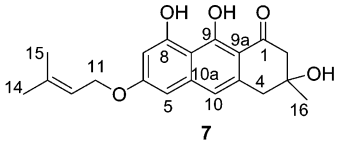

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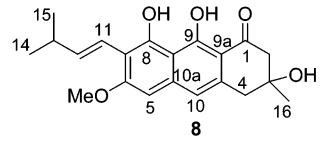

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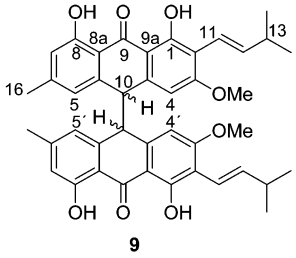

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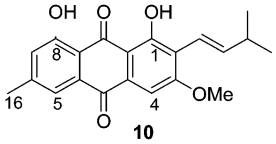

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and NOESY were used to confirm the structure of **1**, which we have named ferruginin C. To the best of our knowledge, compound **1** [2,5-di(3-methyl-2-butenyl)vismin] is the first tetraprenylated ferruginin isolated from a natural source.

Compound **9** was obtained as yellow crystals from the ethyl acetate partition of *V. baccifera*. It has a molecular formula $C_{42}H_{42}O_8$ as deduced from HRFABMS, which showed a $[M + 1]^+$ peak at m/z 675. ^1H and ^{13}C NMR (Table S1) with 2D NMR data supported the structure of **9**, named bivismiaquinone, which was described previously as a transformation product of vismione A.[6,10,11] However, the presence of compound **9** is reported here for the first time as a naturally occurring constituent of *V. baccifera* young leaves, since it was detected in the total extract by TLC and neither vismione A, a possible precursor of **9**, nor its transformation intermediates could be isolated from the extract.

Table 1 shows the GI_{50} values of compounds **1**−**8** against the three cancer cell lines. Compounds **6**−**8** showed more potent activity than **1**−**5**, with **7** showing the highest activity among the tested compounds. GI_{50} values of **1**−**5** showed no major differences from each other. The cytotoxic activities of compounds **1**−**5** and **7** are reported for the first time, while vismione B (**6**) and deacetylvismione A (**8**) have been reported to be active against KB and P388 cell lines.[13,24]

Experimental Section

General Experimental Procedures. Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. NMR spectra were recorded using a Brüker Avance 300 spectrometer in $CDCl_3$ or acetone-d_6 at 300 MHz for ^1H and 75.0 MHz for ^{13}C NMR. Mass spectra were obtained on a Kratos MS50TC mass spectrometer. Silica gel [Merck, Kieselgel 60 (0.063−0.200 mm) and (0.015−0.040 mm)], LiChroprep RP-18 (Merck, 9303), and Sephadex LH-20 (Sigma, 904-37-6) were used for column chromatography. Silica gel plates (Merck, Kieselgel 60 F_{254s}) were used for TLC.

Cytotoxicity Bioassays. The cytotoxicity bioassay was carried out against breast (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines according to the method given by Monks et al.[25] During the isolation process, the activity of all fractions was monitored using the three cell lines.

Plant Material. Young leaves of *V. macrophylla* were collected from Soberanía National Park (N 9°4′57.62″, W 79°39′56.92″; April 2002, voucher number SCZ 51411). Young leaves of *V. baccifera* were collected from Altos de Campana National Park (N 9°9′57.52″, W 79°50′10.36″; April 2002, voucher number SCZ 51365). Young leaves of *V. jefensis* were collected from Chagres Cerro Jefe National Park (N 9°13′45.26″, W 79°22′56.48″; April 2002, voucher number SCZ 51366). Voucher specimens are deposited in the Herbarium of the Smithsonian Tropical Research Institute, Panama.

Extraction and Isolation. Plant materials were subjected to the following procedures. The fresh leaves were homogenized with cold methanol for 30 s in a Waring blender followed by treatment with a Polytron homogenizer (Brinkmann Instruments, Inc. Westbury, NY) for at least 2 min or until the suspension of leaf material was homogeneous. The mixture was filtered under a vacuum through Whatman #4 filter paper, and the marc was then washed with EtOAc. The MeOH and EtOAc extracts were combined and filtered through Whatman #1 filter paper. The combined extract was concentrated in vacuo at <40 °C in a rotary evaporator and stored at −80 °C until further use. The solvent partition protocol involved suspension of the crude extract in water followed by successive extraction with CH_2Cl_2 and EtOAc. The CH_2Cl_2 partition was concentrated to dryness, redissolved in 80% MeOH, and extracted with hexane.

Isolation of Active Constituents of *V. macrophylla*. Fresh, young leaves (574 g) were extracted and subjected to solvent partitioning as described above. The activity was retained in the hexane partition (9 g). Flash chromatography of the hexane partition (9 g) on a Si gel column, using as eluent gradient mixtures of hexane and EtOAc (0 to 100% EtOAc), yielded 11 fractions, MH1−11. Fraction MH1 (2.0 g), upon standing overnight, gave crystals that were separated and washed with hexane, to afford **1** (150 mg, 0.00026%). Crystallization of the second fraction, MH2 (2.1 g), using an EtOAc−hexane mixture gave **3** (130 mg, 0.00022%). Fraction MH5 (0.5 g) was chromatographed on a Si gel column eluted with 20% EtOAc in hexane, followed by passage over a RP-18 Lobar column using 80% aqueous MeOH, giving **2** (45 mg, 0.00008%). Fraction MH6 (45 mg) was chromatographed on a RP-18 Lobar column and eluted with MeOH, affording **4** (40 mg, 0.00007%). Fraction MH8 (0.3 g) was chromatographed on a Si gel column, eluted with a gradient of EtOAc in hexane (5−20%), and then chromatographed over a RP-18 Lobar column using MeOH as eluent, yielding **5** (25 mg, 0.00004%).

Isolation of Active Constituents of *V. baccifera*. Fresh, young leaves (448 g) were extracted and partitioned as described before, and the EtOAc (15.7 g) and hexane (5.0 g) partitions were subjected to additional purification. Chromatography of the extract derived from the of EtOAc partition (6

g) on Sephadex LH-20 using MeOH as eluent yielded four fractions, BE1−4. Fraction BE3 (640 mg) was subjected to Si gel flash chromatography using 5% MeOH in $CHCl_3$ followed by chromatography on a RP-18 Lobar column with 15% aqueous MeOH, yielding **6** (22.4 mg, 0.00005%), **7** (8.6 mg, 0.000019%), and **8** (128 mg, 0.00028%). The hexane partition (5 g) was purified on a Si gel column using a gradient of $CHCl_3$−MeOH (1 to 10% MeOH) to yield 11 fractions, BH1−11. Purification of BH2 (55.6 mg) by preparative TLC on Si gel using EtOAc−hexane (2:8) yielded **9** (22 mg, 0.000049%) and **10** (8 mg, 0.000017).

Isolation of Active Constituents of *V. jefensis*. Fresh, young leaves (370 g) were extracted and partitioned as described above. The chromatographic processing of the MeOH partition (7.5 g) yielded **7** (80 mg, 0.00021%) and **8** (40 mg, 0.00010%).

Ferruginin C (3,8,9-Trihydroxy-6-methyl-2,4,4,5-tetrakis(3-methyl-2-butenyl)-1(4*H*)-anthracenone, 1): orange crystals, mp 110−112 °C; IR (KBr) ν_{max} 3450−3100, 2990, 2960, 1290, 1630, 1600, 1455, 1380 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.25 (s, OH-8), 7.52 (s, H-10), 6.64 (s, H-7), 5.09 (tq, J = 6.8, 1.1 Hz, H-17), 5.04 (tq, J = 6.2, 1.3 Hz, H-22), 4.64 (tq, J = 7.0, 1.3 Hz, H-12, 12′), 3.65 (d, J = 6.2 Hz, H-21), 3.31, 3.29 (d each, J = 7.0 Hz, H-11a, 11′a), 3.28 (d, J = 6.8 Hz, H-16), 2.72, 2.71 (d each, J = 7.0 Hz, H-11b, 11′b), 2.40 (s, Me-26), 1.93 (s, Me-25), 1.75 (s, Me-20), 1.65 (s, Me-19, 24), 1.48 (s, Me-15,15′), 1.45 (s, Me-14,14′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.9 (s, 1), 174.9 (s, 3), 164.1 (s, 9), 156.1 (s, 8), 140.5 (s, 4a), 139.7 (s, 6), 137.0 (s, 10a), 134.2 (s, 13, 13′), 132.9 (s, 18), 131.1 (s, 23), 125.4 (s, 5), 124.2 (d, 22), 122.6 (d, 17), 119.1 (d, 12,12′), 116.3 (s, 2), 112.9 (d, 7), 112.3 (s, 8a), 112.3 (d, 10), 108.5 (s, 9a), 50.1 (s, 4), 41.1 (t, 11,11′), 27.6 (t, 21), 25.5 (q, 24), 25.5 (q, 20), 25.4 (q, 14,14′), 20.8 (q, 26), 20.8 (t, 16), 17.9 (q, 25), 17.8 (q, 15,15′), 17.7 (q, 19); CIMS m/z 528 M^+ (30), 459 (100), 403 (80), 361 (14), 334.9 (25), 293 (15); HRCIMS m/z 528.32285 (calcd for $C_{35}H_{44}O_4$, 528.32396).

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Supporting Information Available: ^1H and ^{13}C NMR data of compounds **2**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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