Guttiferones K and L, Antiproliferative Compounds of *Rheedia calcicola* from the Madagascar Rain Forest[1]

Shugeng Cao,[†] Peggy J. Brodie,[†] James S. Miller,[‡] Fidy Ratovoson,[§] Chris Birkinshaw,[§] Sennen Randrianasolo,[⊥] Etienne Rakotobe,[⊥] Vincent E. Rasamison,[⊥] and David G. I. Kingston*[,†]

Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, and Centre National d'Application et Recherches Pharmaceutiques, B.P. 702, Antananarivo 101, Madagascar

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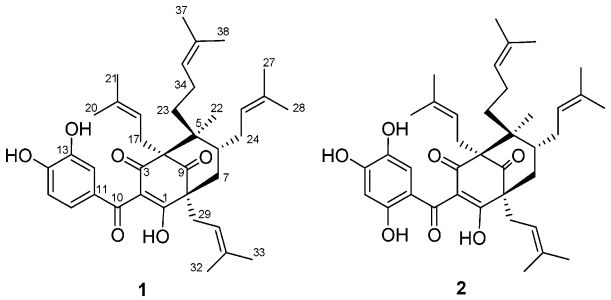
Bioassay-guided fractionation of the ethanol extract obtained from the fruits of *Rheedia calcicola* led to the isolation of two new guttiferone analogues, guttiferones K (**1**) and L (16-hydroxyguttiferone K) (**2**). The structures of **1** and **2** were established on the basis of extensive interpretation of one- and two-dimensional NMR spectroscopic data. Both compounds were tested for their antiproliferative activity against the A2780 human ovarian cancer cell line.

As part of our continuing investigation of Madagascar plants for antiproliferative principles,[1] we found that an ethanol extract (MG 2796) of the fruits of *Rheedia calcicola* Jum. & H. Perrier (Clusiaceae) showed antiproliferative activity in the A2780 assay with an IC_{50} value of 15 μg/mL. This extract was selected for bioassay-guided fractionation on the basis of its antiproliferative activity against the A2780 human ovarian cancer cell line and also on the absence of any previous chemical investigation of the species. Our bioassay-guided fractionation of *R. calcicola* resulted in the isolation of two new antiproliferative guttiferone analogues, guttiferones K (**1**) and L (16-hydroxyguttiferone K) (**2**).



1

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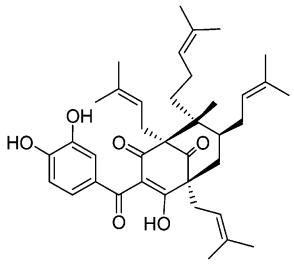

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The genus *Rheedia* has been found to be a rich sources of xanthones,[2−4] biflavonoids,[5−7] polyisoprenylated benzophenones (7- and 15-epiclusianone and xanthochymol),[7−9] and triterpenoids.[10] Their biological properties including brine shrimp lethality,[11] as well as antibacterial[10,11] and analgesic activity,[6] have been reported. 7-Epiclusianone and xanthochymol showed anti-HIV activity,[12] and xanthochymol also displayed antimicrobial activity[13] and cytotoxicity.[14] There is no information on traditional uses of the plant, and only lemurs eat its fruit.[15]

Extract MG 2796 was partitioned between hexane, CH_2Cl_2, and MeOH, and the CH_2Cl_2 extract was found to be the most active, with an IC_{50} value of 10 μg/mL. The CH_2Cl_2 extract was purified by filtration through a C18 cartridge followed by HPLC on a C18 column to yield compound **1** from the second fraction. Further HPLC separation of the first fraction using a C8 column yielded compound **2**.

Compound **1** was obtained as a yellow oil. Its positive ion HRFABMS revealed a pseudomolecular ion $[(M + H)^+]$ consistent with the molecular formula $C_{38}H_{50}O_6$, requiring 14 double-bond equivalents. The IR spectrum for **1** displayed bands for hydroxyl (3348 cm^{-1}) and carbonyl groups (1728, 1670, 1640 cm^{-1}), while the UV absorptions at λ_{max} 241, 255, and 325 revealed aromatic and conjugated carbonyl chromophores. NMR data were collected in CD_3OD/0.1% TFA for comparisons with the literature data, and in pyridine-d_5 to reduce signal overlap for the purpose of structure elucidation. The ^1H NMR spectrum (Table 1) exhibited the presence of a 1,2,4-trisubstituted benzene ring. Four olefinic protons, one tertiary methyl and eight vinyl methyl groups, six methylenes, and one methine were also observed in the ^1H NMR and HSQC spectra of **1**, indicating the presence of four 3-methylbut-2-enyl groups and a fifth C5 unit.

The ^{13}C NMR spectrum of **1** (Table 1, CD_3OD/0.1% TFA) showed resonances for six aromatic carbons, a conjugated carbonyl group at δ_C 196.7, an enolized 1,3-diketone (δ_C 191.0, 119.9, and 196.7), a nonconjugated carbonyl at δ_C 209.2, two quaternary carbons [δ_C 69.4 (C-4) and 64.1 (C-8)], and 25 signals assignable to four isoprene units and a fifth C5 unit. ^2J and ^3J HMBC correlations (Figure 1) indicated the presence of two fragments, I (a 3,4-dihydroxybenzoyl group) and II (a 2,2-dimethylbicyclo[3.3.1]-nonane ring system substituted with four 3-methylbut-2-enyl groups). The ^1H and ^{13}C NMR spectra of **1** were very similar to those of guttiferone A (**3**),[12b] suggesting that **1** was a stereoisomer of guttiferone A. The correlations between CH_3-22 and CH_2-17/H-7α in the ROESY spectrum of **1** indicated that CH_3-22 (δ_H 0.81/δ_C 16.4, in CD_3OD/0.1% TFA) must be in the α-orientation like CH_2-17. The ^{13}C NMR chemical shift of C-6 at δ_C 42.0 in CD_3OD/0.1% TFA suggested that the C-6 substituent was in the equatorial position, since the signal of C-6 with an axial substituent is located at lower field (δ_C 46−48).[12a] The coupling patterns of H-7α (δ_H 1.44, dd, $J_{7\alpha,7\beta} = 13.3$, $J_{7\alpha,6} = 12.9$ Hz, in CD_3OD/0.1% TFA) further revealed axial orientations for CH_3-22, H-6, and H-7α and equatorial orientations for H-7β, the 3-methylbut-2-enyl group

* To whom correspondence should be addressed. Tel: (540) 231-6570. Fax: (540) 231-7702. E-mail: dkingston@vt.edu.
[†] Virginia Polytechnic Institute and State University.
[‡] Missouri Botanical Garden, St. Louis.
[§] Missouri Botanical Garden, Madagascar.
[⊥] Centre National d'Application des Recherches Pharmaceutiques.

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for Compounds **1** and **2**[a]

	1				**2**		
	^{1}H		^{13}C		^{1}H		^{13}C
no.	MeOH-d_4	C_5D_5N	MeOH-d_4	C_5D_5N	MeOH-d_4	C_5D_5N	MeOH-d_4
1			196.7	190.9			190.4
2			119.9	121.9			120.4
3			191.0	187.1			182.5
4			69.4	69.4			70.3
5			51.6	49.4			51.7
6	1.75 m	2.62 dd 13.5, 10.1	42.0	41.0	1.75 m	2.11 m	39.4
7	2.03 dd 13.3, 3.3	2.47 dd 14.0, 3.9	43.2	41.8	2.04 dd 13.1, 3.5	2.43 dd 13.3, 3.2	43.8
	1.44 dd 13.3, 12.9	1.73 dd 14.0, 10.1			1.52 m	1.75 m	
8			64.1	62.9			66.5
9			209.2	210.6			207.5
10			196.7	196.9			196.1
11			130.3	131.3			117.9
12	7.20 d 2.1	7.92 br s	117.4	117.4	7.44 s	8.06 s	109.8
13			146.5	147.2			147.4
14			152.6	152.7			151.2
15	6.69 d 8.4	7.17 d 8.3	115.3	115.7	6.82 s	7.36 s	104.2
16	6.95 dd 8.4, 2.1	7.66 dd 8.3, 2.5	125.1	124.0			155.0
17	2.73 dd 13.0, 7.8	3.10 dd 13.8, 7.1	26.7	26.7	3.00 dd 14.2, 9.0	3.18 m	26.7
	2.65 dd 13.0, 4.3	3.03 dd 13.8, 4.2			2.87 dd 14.2, 4.0	3.18 m	
18	4.88 m	5.52 br t 7.0	121.5	123.4	4.70 m	5.21 br t 7.0	120.8
19			135.2	132.1[b]			135.3
20	1.69 s	1.78 s	18.5	18.7	1.83 s	1.68 s	18.8
21	1.62 s	1.57 s	26.4	26.3	1.50 s	1.47 s	26.2
22	0.81 s	0.99 s	16.4	16.6	0.90 s	0.99 s	17.6
23	1.68 m	1.98 br t 8.1	37.6	37.0	1.95 m; 1.58 m	1.67 m	37.5
24	2.07 m; 1.77 m	2.25 m; 1.91 m	30.2	29.9	2.00 m; 1.28 m	2.09 m; 1.85 m	30.3
25	5.00 br t 7.0	5.22 m	123.7	124.0	4.95 m	5.00 m	123.2
26			134.7	133.2[b]			134.9
27	1.67 s	1.60 s	18.3	18.3	1.59 s	1.58 s	18.3
28	1.57 s	1.56 s	26.0	26.0	1.55 s	1.57 s	25.8
29	2.51 dd 14.5, 8.8	2.91 d 6.4	31.8	31.6	2.52 m	2.94 m; 2.85 m	31.4
	2.44 dd 14.5, 4.8						
30	5.10 br t 7.1	5.73 br t 7.0	121.1	122.5	4.95 m	5.42 br t 7.0	120.9
31			135.7	133.3			135.4
32	1.67 s	1.73 s	18.4	18.5	1.65 s	1.75 s	18.3
33	1.71 s	1.65 s	26.4	26.3	1.59 s	1.52 s	26.2
34	1.97 m	2.55 m; 2.25 m	25.3	25.0	1.78 m	2.07 m	24.3
35	5.04 br t 6.9	5.24 m	125.6	126.1	4.95 m	5.12 m	124.7
36			132.7	132.0			133.3
37	1.66 s	1.63 s	26.1	26.1	1.69 s	1.63 s	26.0
38	1.59 s	1.63 s	18.0	18.1	1.59 s	1.37 s	17.7

[a] δ (ppm), 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR; multiplicities; J values (Hz). [b]Interchangeable.

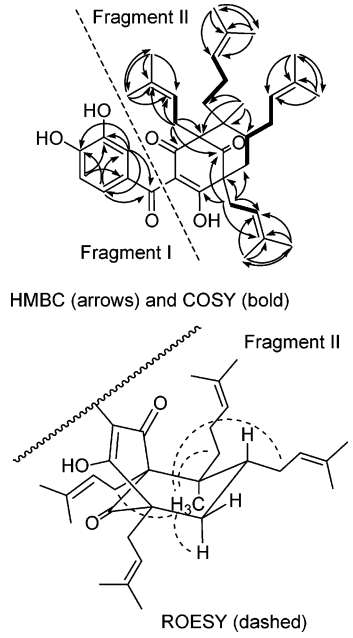

Figure 1. Key HMBC, COSY, and ROESY correlations for **1**.

at the 6-position, and the 4-methylpent-3-enyl group at the 5-position. These data supported a chair conformation of the bicyclo[3.3.1]nonane ring system and enabled the structure of **1** to be assigned as 5-*epi*,6-*epi*-guttiferone A. The orientations of CH_3-22 and H-6 of **1** were the same as those of guttiferone G,[16a] isolated from *Garcinia macrophylla* from the Suriname rainforest, and of its enantiomer (+)-oblongifolin C.[16b] The optical rotation of **1** was small but negative, suggesting but not demanding that it belongs to the guttiferone G series rather than the oblongifolin C series.

Compound **2** was also obtained as a yellow oil, and its molecular formula was determined as $C_{38}H_{50}O_7$ by HRFABMS and ^{13}C NMR spectroscopy. The ^{1}H NMR signals of fragment II were very similar to those of **1**, but fragment I was identified as a 1,2,4,5-tetrasubstituted benzoyl group by the presence of two singlets (δ_H 6.82 and 7.44, s, in CD_3OD/0.1% TFA) in the aromatic region. The ^{13}C NMR chemical shifts of fragment I of **2** were similar to those of orirubenones A and B,[17] indicating that it was a 2,4,5-trihydroxybenzoyl group. The stereochemistries of the 4-, 5-, 6-, and 8-positions were determined to be the same as those of **1** on the basis of observed ROESY correlations between CH_3-22 and CH_2-17/H-7α. The structure of **2** was thus assigned as guttiferone L (16-hydroxyguttiferone K).

It is reported that guttiferones I and J from *Garcinia virgata* were weakly cytotoxic against the KB cancer cell line, with IC_{50} values of 4.7 and 5.0 μg/mL, respectively.[16c] Guttiferone G isolated from *Garcinia macrophylla* also displayed weak antiproliferative activity against the A2780 ovarian cancer cell line, with an IC_{50} value of 8.00 μg/mL.[16a] Compounds **1** and **2** were evaluated for their antiproliferative activity against the A2780 human ovarian cancer cell line and had IC_{50} values of 3.6 and 3 μg/mL, respectively, which is in good agreement with previous observations that guttiferone analogues are weakly active against certain cancer cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on a Spectrum One FT-IR spectrometer (Perkin-Elmer Instruments) and a Shimadzu UV-1201 spectrophotometer, respectively. NMR spectra were obtained at room temperature on a JEOL Eclipse 500 spectrometer (5 mm BB probe for ^{1}H and ^{13}C NMR, and PFG probe for 2D NMR) and a Varian INOVA 400 spectrometer (5 mm AUTOSW PFG probe) in CD_3OD/0.1% TFA (δ_H 3.31 and δ_C 49.5) or C_5D_5N (δ_H 8.71 and δ_C 149.9). The chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectra were obtained on a JEOL JMS-HX-110 instrument, in the positive ion mode. HPLC was performed on a Shimadzu LC-10AT instrument with a semipreparative C8 Varian Dynamax column (5 μm, 250 × 10 mm) and a preparative C18 Varian Dynamax column (8 μm, 250 × 21.4 mm). Finnigan LTQ LC/MS with a C18 Hypersil column (5 μm, 100 × 2.1 mm) was also used for crude sample analysis.

Antiproliferative Bioassays. Antiproliferative activity measurements were performed at Virginia Polytechnic Institute and State University against the human A2780 ovarian cancer cell line as previously described.[1] The A2780 cell line is a drug-sensitive ovarian cancer cell line.[18]

Plant Material. Fruits of *Rheedia calcicola* Jum. & H. Perrier (Clusiaceae) were collected from a tree 8 m high with a trunk diameter at breast height of 10 cm, growing on sand near a stream, on November 12, 2004, in the forest of Sahafary/Saharenana, in the Province of Antsiranana, Madagascar (12.34.36 S/49.27.15 E, elevation 268 m). The herbarium voucher specimen for the sample is Sennen Randrianasolo et al. 503. Duplicate voucher specimens were deposited at herbaria of the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP), and of the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN), and at the Missouri Botanical Garden, St. Louis, Missouri (MO), and the Muséum National d'Histoires Naturelles, Paris, France (P).

Extraction and Isolation. Dried fruit of *R. calcicola* (130 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at rt to give the crude extract MG 2796 (7.0 g), of which 1.3 g was shipped to Virginia Polytechnic Institute and State University (VPISU) for fractionation. MG 2796 (IC_{50}: 15 μg/mL, 106.1 mg) was suspended in aqueous MeOH (MeOH/H_2O, 9:1, 10 mL) and extracted with hexane (3 × 10 mL portions). The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 10 mL portions). Both the hexane and the CH_2Cl_2 extracts were evaporated *in vacuo* to leave 31.3 and 44.8 mg of residues (IC_{50}: 11 and 10 μg/mL, respectively). The aqueous MeOH extract (30 mg) was inactive. The CH_2Cl_2 extract was selected due to its relatively greater quantity and potency as compared with the hexane extract. The CH_2Cl_2 extract was dissolved in MeOH, and the MeOH solution was filtrated through an SPE cartridge over C18 before being injected into the HPLC (85% MeOH−H_2O). Four fractions were collected (I, II, III, and IV). Fraction II yielded compound **1** (20 mg, t_R 33 min). Further purification of fraction I was carried out by C8 HPLC with 85% MeOH as the eluent to yield compound **2** (0.6 mg, t_R 24 min).

Guttiferone K (1): yellow oil; $[\alpha]_D^{23}$ −2 (*c* 0.35, $CHCl_3$); IR (film) ν_{max} 3348, 2968, 2916, 2857, 1728, 1670, 1640, 1602, 1542, 1519, 1440, 1376, 1289, 1191, 1116 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 241 (sh), 255 (4.54), 325 (4.14) nm; ^{1}H NMR (500 MHz, CD_3OD and pyridine-d_5) and ^{13}C NMR (125 MHz, CD_3OD and pyridine-d_5), see Table 1; HRFABMS *m*/*z* 603.3712 (calcd for $C_{38}H_{51}O_6$, 603.3686).

16-Hydroxyguttiferone K (2): yellow oil; $[\alpha]_D^{23}$ −8 (*c* 0.06, $CHCl_3$); IR (film) ν_{max} 3450, 2966, 2922, 2856, 1732, 1672, 1604, 1494, 1461, 1384, 1292, 1267, 1191 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 251 (4.0), 285 (sh), 364 (3.6) nm; ^{1}H NMR (500 MHz, CD_3OD and pyridine-d_5) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; HRFABMS *m*/*z* 601.3562 [M − OH] (calcd for $C_{38}H_{49}O_6$, 601.3529).

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

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