Benzophenones and Biflavonoids from *Rheedia edulis*

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Two new polyisoprenylated benzophenones, 32-hydroxy-*ent*-guttiferone M (**1**) and 6-*epi*-guttiferone J (**2**), along with seven known compounds, 6-*epi*-clusianone (**3**), guttiferone A (**4**), xanthochymol (**5**), guttiferone E (**6**), isoxanthochymol (**7**), (+)-volkensiflavone (**8**), and (+)-morelloflavone (**9**), were identified from the seeds and rinds of *Rheedia edulis*. Compounds **1**−**3** and **5**−**9** have been isolated and identified from this species for the first time. The structures of the new compounds were elucidated mainly by analysis of their 1D and 2D NMR spectroscopic data, and their absolute configurations were determined by comparison of their experimental optical rotation and electronic circular dichroism measurements with those values predicted by DFT calculations. Compound **1** showed significant antioxidant activity in both DPPH and ABTS free radical scavenging assays, whereas compound **2** was inactive.

Rheedia edulis Seem. Planch. & Triana (synonym: *Garcinia intermedia* (Pittier) Hammel)[1] is a member of the Clusiaceae family, well known to produce a variety of polyprenylated xanthones and benzophenones that display antioxidant, antiparasitic, antiviral, antifungal, antibacterial, and cytotoxic activity.[2] The species is a canopy tree native to Central American lowland tropical rainforests. The wood contains small gum ducts, and the bark contains yellow latex.[3] The tree produces white flowers and small yellow oval or oblong fruits. The latter are edible with a thin sweet exocarp and contain 1−2 cm long ovoid seeds. Local names for the plant are numerous and include "waiki-plum" in Belize, "arrayán" or "palo de frutilla" in Guatemala, "chaparrón" in El Salvador, "caimito" in Honduras, "jorco" in Costa Rica, "sastra" in Panama, and "limoncillo" in Mexico. This species is also cultivated in Brazil and the Philippines, where it is known as "limão do matto" or "berba", respectively.[3,4]

There was one previous phytochemical study on the species *G. intermedia*,[4] where an organic extract of the leaves showed significant trypanocidal activity against epimastigotes and trypomastigotes of *Trypanosoma cruzi*. The isolated compounds, guttiferone A (**4**), 8-desoxygartanin, garcinixanthone B, podoscarpusflavone A, amentoflavone, and friedelin, showed weak activity.

As part of our ongoing studies of antioxidant/chemopreventive agents from tropical edible fruits, the rinds and seeds of *R. edulis* were subjected to phytochemical investigation for the first time. We report the isolation of two new polyprenylated benzophenones derivatives, 32-hydroxy-*ent*-guttiferone M (**1**) and 6-*epi*-guttiferone J (**2**), along with seven known compounds, 6-*epi*-clusianone (**3**), guttiferone A (**4**), xanthochymol (**5**), guttiferone E (**6**), isoxanthochymol (**7**), (+)-volkensiflavone (**8**), and (+)-morelloflavone (**9**). All isolates were identified using a combination of ^{1}H and ^{13}C NMR spectroscopy and LC-MS time-of-flight (TOF) analyses. The ^{1}H and ^{13}C NMR data allowed us to establish the relative configuration of the new compounds. Absolute configuration was established by means of comparison of experimental optical properties [optical rotation (OR) and electronic circular dichroism (ECD)] with those obtained by molecular modeling calculations. The antioxidant capacity of the extracts and isolates was tested using both DPPH and ABTS assays.

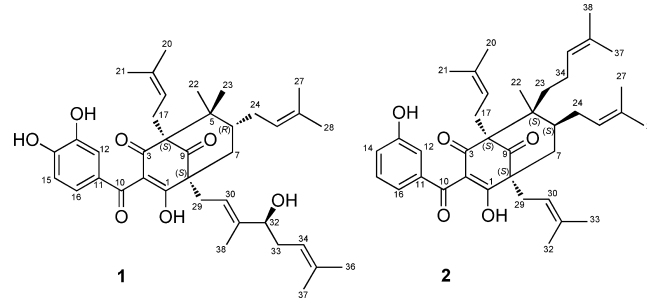

Figure 1. New compounds isolated from *R. edulis*.

Results and Discussion

The antioxidant MeOH-soluble extracts of seeds and rinds of *R. edulis* were fractionated by column chromatography and RP-HPLC on a C_{18}-bonded phase to yield two new compounds, 32-hydroxy-*ent*-guttiferone M (**1**) and 6-*epi*-guttiferone J (**2**) (Figure 1), along with the known compounds 6-*epi*-clusianone (**3**), guttiferone A (**4**), xanthochymol (**5**), guttiferone E (**6**), isoxanthochymol (**7**), (+)-volkensiflavone (**8**), and (+)-morelloflavone (**9**).

Compound **1** was isolated as a yellow oil. The ESIMS spectrum of **1** exhibited a deprotonated molecular ion $[M - H]^-$ at *m*/*z* 617.3472 (calcd 617.3478), consistent with a molecular formula of $C_{38}H_{49}O_7$, corresponding to 14 degrees of unsaturation. The IR spectra showed the typical absorption bands at 3440, 2925, 1733, 1653, 1296, 1120, and 1053 cm^{-1}, implying the presence of hydroxy, carbonyl, and double-bond functionalities. Analysis of the ^{1}H and ^{13}C NMR spectra gave evidence of a 4,6,8-trisubstituted polyprenylated benzophenone skeleton. The ^{13}C and DEPT NMR spectra exhibited 38 signals for carbons consisting of three carbonyls, four double bonds, and nine methyl, five methylene, seven methine (three aromatics), and eight quaternary carbons (three oxygenated), in agreement with the presence of a benzophenone unit and three prenyl groups (Table 1). Moreover, the ^{1}H NMR exhibited signals for a 1,3,4-trisubstituted aromatic ring displayed as a doublet of doublets at δ_H 6.87 (J = 2.1 and 6.9 Hz, H-15), a singlet at δ_H 7.55 (H-12), and another doublet of doublets at δ_H 7.57 (J = 2.1 and 7.2 Hz, H-16); four olefinic triplets at δ_H 4.86, 5.00, and 5.18; and nine methyl singlets between δ_H 1.3 and 1.7. In addition, analysis of the HRESIMS spectrum of **1** confirmed a polyprenylated benzophenone similar to guttiferone M, differing by 16 mass units, attributed to the presence of an additional hydroxy group.[5,6] Comparison of the ^{13}C NMR data indicated a methine at δ_C 40.8 present in guttiferone M, which is replaced by a secondary

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Table 1. ^{13}C and ^{1}H NMR Data of 32-Hydroxy-*ent*-guttiferone M (**1**) and 6-*epi*-Guttiferone J (**2**), in MeOH-d_4 (0.1% TFA)

	1			**2**		
position	δ_C (in ppm), mult.[a]	δ_H (*J* in Hz)[b]	HMBC (H → C)[c]	δ_C (in ppm), mult.[a]	δ_H (*J* in Hz)[b]	HMBC (H → C)[c]
1	194.8, qC			197.3, qC		
2	120.8, qC			118.6, qC		
3	191.6, qC			192.9, qC		
4	69.8, qC			52.1, qC		
5	48.0, qC			49.6, qC		
6	47.5, CH	1.67, m	7, 24	39.9, CH	1.77, m	7, 24
7	39.6, CH_2	1.92, m; 1.87, m	6, 8	35.7, CH_2	1.55, d (13.1); 2.03, dd (13.1, 4.2)	6
8	62.6, qC			67.6, qC		
9	207.0, qC			205.5, qC		
10	197.6, qC			197.3, qC		
11	130.1, qC			133.3, qC		
12	117.0, CH	7.55, s	11, 13, 14	114.8, CH	7.03, bs	11, 13
13	144.7, qC			144.7, qC		
14	150.8, qC			120.3, CH	7.00, bd (7.9)	13, 15
15	114.4, CH	6.87, dd (6.9, 2.1)	13, 16	128.3, CH	7.18, bt (7.9)	14, 16
16	123.3, CH	7.57, dd (7.2, 2.4)	12, 14, 15	123.9, CH	6.97, bd (7.8)	11, 15
17	24.7, CH_2	2.65, m; 2.55, m	4, 9, 18	25.2, CH_2	2.57, d (5.4); 2.50, d (5.1)	4, 9, 18
18	120.0, CH	4.86, t (6.6)	17	120.1, CH	4.92, t (5.1)	17, 19
19	132.5, qC			131.8, qC		
20	26.7, CH_3	1.67, s	19, 21	16.7, CH_3	1.63, s	19, 21
21	16.9, CH_3	1.72, s	19, 20	16.8, CH_3	1.63, s	19, 20
22	16.7, CH_3	1.31, s	5, 23	16.8, CH_3	0.80, s	5
23	24.6, CH_3	1.35, s	5, 22	39.9, CH_2	1.71, m	5
24	29.3, CH_2	2.05, m; 1.88, m	6, 7	29.3, CH_2	1.79, d (13.8); 2.03, dd (15.5, 4.2)	6, 7, 25
25	123.8, CH	5.10, t (7.2)	24	123.9, CH	5.18, t (6.6)	24
26	132.2, qC			131.2, qC		
27	24.7, CH_3	1.69, s	26, 28	16.7, CH_3	1.66, s	26, 28
28	16.9, CH_3	1.62, s	26, 27	24.9, CH_3	1.66, s	26, 27
29	29.2, CH_2	2.28, m; 2.21, m	30	29.3, CH_2	2.50, d (6.9); 2.44, d (6.3)	8, 9, 30
30	120.3, CH	5.18, t (7.2)	29	120.4, CH	5.05, dd (6.6)	29
31	133.2, qC			131.2, qC		
32	65.0, CH_2	3.57, dd (5.1, 0.9)	30, 31, 38	16.7, CH_3	1.31, s	33
33	28.7, CH_2	2.38, m; 2.33, m	32, 34, 35	24.6, CH_3	1.57, s	32
34	124.5, qC	5.00, t (7.2)		22.2, CH_2,	1.95, m	23, 35
35	131.3, qC			124.5, CH	5.18, t (6.6)	34
36	24.8, CH_3	1.67, s	34, 35, 37	124.8, qC		
37	16.3, CH_3	1.63, s	35, 36	16.2, CH_3	1.57, s	37
38	22.3, CH_3	1.31, s	31, 32	24.4, CH_3	1.61, s	36

[a] 75 MHz. [b] 300 MHz. [c] HMBC correlations are from proton(s) stated to the indicated carbons.

oxygenated carbon at δ_C 65.0 in **1**. HMBC correlations between two isoprenyl methylene protons (H_2-17) and C-4 and C-9 and between another two isoprenyl methylene protons (H_2-24) and C-5, C-6, and C-7 (Figure 2) supported the placement of these isoprenyl groups at C-4 and C-6, respectively, which was confirmed by NOESY correlations between H_2-17 and H_3-20 and H_3-21 and between H_2-24 and H_3-27 and H_3-28 (Figure 2). The strong interactions in the NOESY spectrum between H-32 and H_3-38 and between H_2-33 and both H_3-36 and H_3-37, together with the analysis of the HMBC spectrum, were consistent with the placement of a geranyl group at C-8. Finally, the HMBC spectrum of **1** showed long-range correlations between H-32 and C-30, C-31, and C-38; H_2-33 and C-32, C-34, and C-35; and H_3-36 and C-34, C-35, and C-37, which enabled the assignment of the individual hydroxy group at C-32 in the geranyl substituent (Table 1).

Compound **1** showed a positive specific rotation value of $[\alpha]_D^{25}$ +9.6 (MeOH, *c* 0.01), in contrast with the $[\alpha]_D^{25}$ −29.8 (MeOH, *c* 0.15) value reported for guttiferone M,[6] suggesting that these compounds have different absolute configurations, which is in agreement with our NMR observations. Electronic circular dichroism and optical rotatory dispersion are useful measurements to determine the absolute configuration of chiral molecules, particularly in natural products, especially when combined with theoretical calculations of ECD and OR properties using density functional theory (DFT).[7−9] Others have used calculated ECD values, in particular for determination of the absolute configuration of a series of polyisoprenylated benzophenones.[5] Thus, in order to establish the absolute configuration of **1**, a molecular modeling approach was used. First, a Monte Carlo conformational search was performed using the MMFF94 molecular mechanics force field. In this process the energy value was monitored as a convergence criterion to yield global minimum energy structures. Seven global

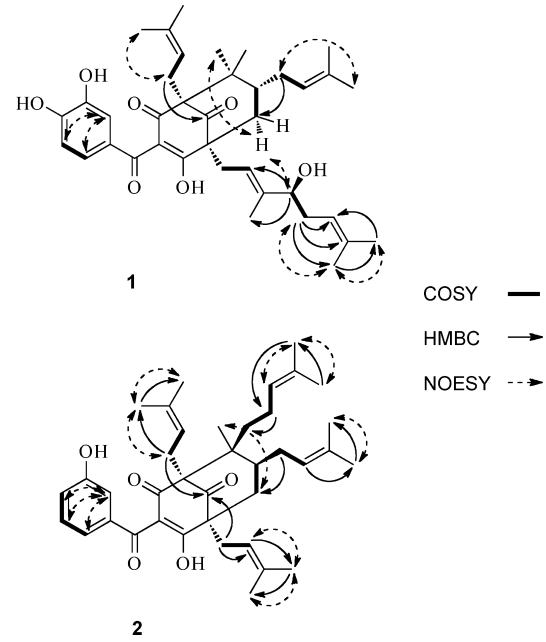

Figure 2. Key COSY, HMBC, and NOESY correlations of **1** and **2**.

Table 2. Relative Free Energies (ΔG),[a] Equilibrium Populations (P),[b] and Specific Rotation ([α])[c] Values of the Conformers of **1** and **2**

	1				**2**		
conformer	ΔG	P (%)	[α]	conformer	ΔG	P (%)	[α]
1a	0.0	84.3	8.45	**2a**	0.0	60.2	15.23
1b	1.2	13.4	3.45	**2b**	0.6	23.8	2.98
1c	2.4	2.2	0.35	**2c**	1.6	4.2	0.51
1d	4.2	0.1	0.02	**2d**	2.0	2.0	1.36
1e	4.9	0.0	0.01	**2e**	2.1	1.7	0.06
1f	8.3	0.0	0.00	**2f**	2.2	1.4	0.06
1g	14.6	0.0	0.00				
conformational average[d]			12.3	conformational average[d]			20.2
experimental			9.5	experimental			10.8

[a] B3LYP/6-31G++(d,p), in kcal/mol. [b] Population percentages based on ΔG, assuming Boltzman statistics at T = 298.15 K and 1 atm. [c] B3LYP/6-31G++(d,p), specific rotation in degrees × [dm × g/cm^3]. [d] Σ_i[α] × P_i, where [α] and P_i are values of [α] and population in percent for the ith conformation.

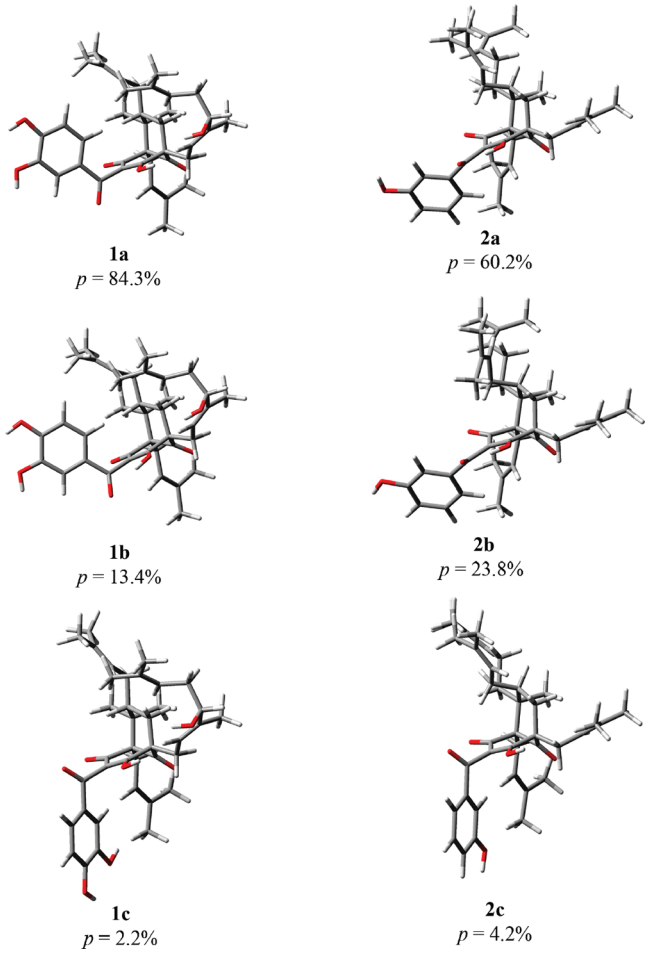

Figure 3. DFT B3LYP/6-31G++(d,p) geometry optimized conformers of **1** (**1a**−**1c**) and **2** (**2a**−**2c**) at 298 K and 1 atm, accounting for ca. 90% of the conformational population of each compound.

minimum conformations of **1** were found (**1a**−**1g**) within a 5 kcal/mol window. Reoptimization of the geometries of **1a**−**1g** using DFT at the B3LYP/6-31G++(d,p) level leads to the relative free energies and equilibrium Boltzmann-weighted populations, also given in Table 2. At this level, **1a**, **1b**, and **1c** constitute over 90% of the equilibrium conformational mixture (Figure 3). After optimization, vibrational frequencies, IR, and optical rotation values were calculated at the same level of theory, as well as thermochemical parameters at 298 K and 1 atm. Theoretical calculation

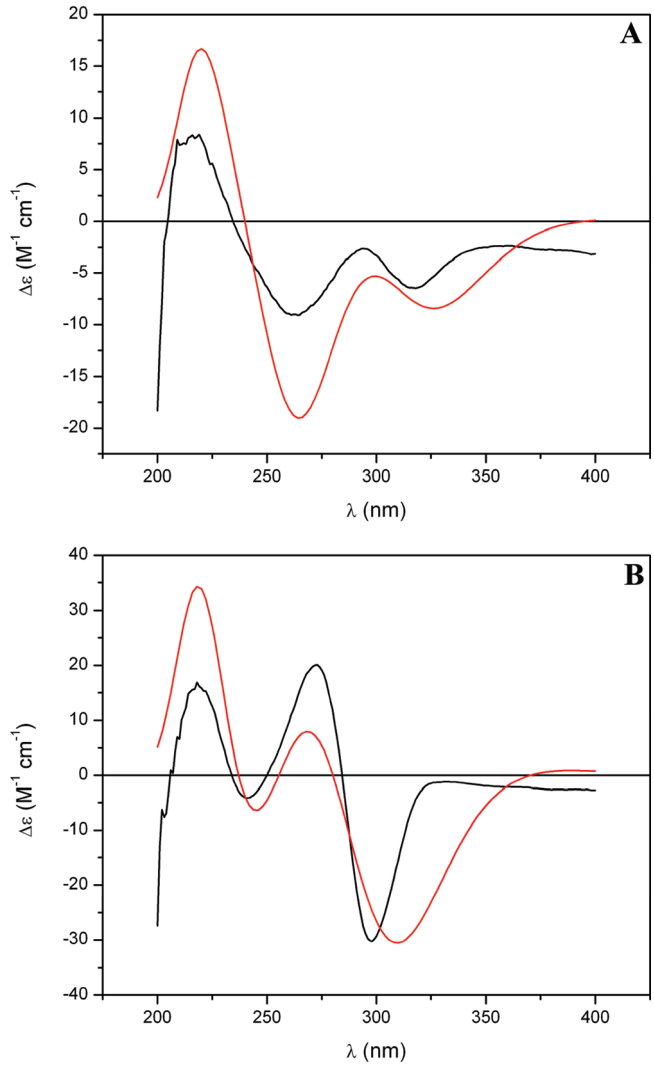

Figure 4. Calculated ECD spectra of **1** (A, red) and **2** (B, red) and its experimental (black) at the B3LYP/6-31G++(d,p) level in MeOH.

of the ECD spectrum of compound **1** based on the previously assigned stereochemistry was performed using time-dependent density functional theory (TDDFT)[10−13] with the 6-31G++(d,p) basis set by the Gaussian03 program package. The results were in agreement with the experimental ECD spectrum: two negative high-amplitude Cotton effects were observed at 265 and 320 nm, along with a positive effect at 220 nm (Figure 4 and Supporting Information). Comparison between OR experimental ($[\alpha]_D^{25}$ +9.6) and the calculated ($[\alpha]_D^{25}$ +12.3) values, as well as the ECD experimental and calculated data, revealed that the established 4*S*,6*R*,8*S*-stereoisomer is in agreement with the stereochemistry proposed by the *cis*-relationship between the substituents at C-4, C-6, and C-8 in the bicyclo[3.3.1]non-3-ene-2,9-dione moiety, observed in the NMR data, and consistent with those of related polyisoprenylated benzophenones reported previously.[5,6] Calculation of the OR for the 4*S*,6*S*,8*S*-stereoisomer (data not shown) showed the opposite sign, supporting that our proposed configuration, 4*S*,6*R*,8*S*, is correct. The relative configuration at C-32 was determined by NOESY data and the comparison of the observed vicinal proton coupling constants (Figure 2). The hydroxy group is oriented above the plane, while the methine protons H-32, H_2-7, and H_3-22 are located below the plane. Thus, compound **1** has been established as (1*S*,5*S*,7*R*)-3-(3,4-dihydroxybenzoyl)-4-hydroxy-5-[(*E*)-4-hydroxy-3,7-dimethylocta-2,6-dienyl)]-8,8-dimethyl-1,7-

bis(3-methylbut-2-enyl)bicyclo[3.3.1]non-3-ene-2,9-dione and given the trivial name 32-hydroxy-*ent*-guttiferone M (**1**).

Compound **2** was assigned the molecular formula $C_{38}H_{49}O_4$ (14 degrees of unsaturation) on the basis of an HRESIMS negative ion (*m/z* 585.3515 $[M - H]^-$, calcd 585.3580). The molecule displayed hydroxy, carbonyl, and double-bond moieties, determined from IR absorptions observed at 3375, 1683, 1558, and 1374 cm^{-1}, respectively. Analysis of the ^1H NMR spectra of **2** (Table 1) showed signals for a 1,3-disubstituted aromatic ring (δ_H 6.97, bd, $J = 7.8$ Hz, H-16; 7.00, bd, $J = 7.9$ Hz, H-14; 7.03, bs, H-12; 7.18, bt, $J = 7.9$ Hz, H-15), four prenyl units at δ_H 4.92 (t, $J = 5.1$ Hz, H-18), 5.05 (t, $J = 6.6$ Hz, H-30), 5.18 (t, $J = 6.6$ Hz, H-25), and 5.17 (t, $J = 6.6$ Hz, H-35), and nine methyl groups between δ_H 0.8 and 1.7. The remaining signals observed between δ_H 1.5 and 2.6 were aliphatic proton multiplets. The ^{13}C NMR spectra showed resonances for three carbonyl groups observed at δ_C 192.9, 197.3, and 205.5, while 15 resonances between δ_C 120 and 135 could be assigned to a phenolic aromatic ring and four double bonds. The remaining carbon resonances were upfield of δ_C 66.0. The $^1H-^1H$ coupling patterns of the four aromatic protons revealed a 1,3-disubstituted aryl ring, which was confirmed by relevant HMBC correlations (Figure 2). In addition, COSY correlations observed between the methylene protons H_2-17, H_2-24, H_2-29, and H_2-34 and the methine protons H-18, H-25, H-30, and H-35, respectively, confirmed the presence of four isoprenyl moieties (Figure 2). HMBC correlations of H_2-17 with C-4, C-9, and C-18; H_2-34 with C-23 and C-35; H_2-24 with C-6, C-7, and C-25; and H_2-29 with C-8, C-9, and C-30 indicated that the isoprenyl groups are attached at C-4, C-23, C-6, and C-8, respectively. These data closely resembled those reported for guttiferone J.[14] However, contrary to guttiferone J, a strong NOESY connectivity was observed between H-7 and H-22, which, by comparison with guttiferone J, appeared further downfield, consistent with an *anti*-relationship between the (pro-*S*) proton at C-7 and the methyl group at C-22 (Figure 2). The most obvious difference between **2** and guttiferone J was the opposite OR value, $[\alpha]_D^{25}$ +10.8 (MeOH, *c* 0.01) and $[\alpha]_D^{25}$ −34.3 (MeOH, *c* 1.75), respectively, suggesting that these compounds are diastereoisomers. The configuration at C-5 and C-6 was unequivocally established by comparing the OR and ECD data with those obtained through molecular modeling calculations following the same protocol described for compound **1**. The agreement between the observed optical rotation ($[\alpha]_D^{25}$ +10.8) and the calculated ($[\alpha]_D^{25}$ +20.2) values (Figure 3 and Table 2), as well as the correlations obtained in the ECD calculations (Figure 4 and Supporting Information), confirmed the *cis*-relationship between the isoprenyl groups at C-5 and C-6 in the benzophenone core, observed by NMR. In particular, as shown in Figure 2, the axial (α) disposition between H-6, H_3-22, and H-7_{ax} in **2** was also consistent with a pseudoequatorial (*β*) disposition of the isoprenyl groups at C-5 and C-6. These data determine the 4*S*,5*S*,6*S*,8*S* absolute configuration for the new compound, named here as 6-*epi*-guttiferone J (**2**).

Benzophenones **3** and **4** were obtained from the seed extract, and their structures were determined by comparing their observed and reported physical data (NMR, MS, and UV).[15,16] Compounds **5**−**7** and **8** and **9** were identified from the seed and rind extracts, respectively, by LC-MS TOF analysis; relative retention times, UV spectra, and MS data were in agreement with authentic standards previously isolated from *G. livingstonei* and *G. xanthochymus* (see Supporting Information).[17,18]

The antioxidant activity of **1**−**4** was evaluated by both DPPH and ABTS radical scavenging activity assays and compared with those of reference antioxidants, gallic acid and Trolox (Table 3 and Supporting Information). The new benzophenone **1** displayed strong antioxidant activity in both DPPH and ABTS assays, with IC_{50} values of 38.32 and 45.58 μM, respectively. This activity was comparable with those of guttiferone A (**4**), a well-known antioxidant polyisoprenylated benzophenone, as well as the positive controls. Compounds **2** and **3** displayed weak antioxidant activity in both assays. Compounds **5**−**9** were previously screened for their antioxidant activity by Baggett et al.[17] in the DPPH assay. Compounds **5**−**7** displayed IC_{50} values in the range 73 to 125 μM, and biflavonoids **8** and **9** at 62 and 298 μM, respectively. Preliminary structure−activity relationship studies revealed a correlation between the number of phenolic functional groups on the aromatic ring and the antioxidant activity. Compounds **1** and **4** contain a 1,3,4-trisubstituted aromatic ring (two hydroxy groups) and display the highest antioxidant activity; compounds **2** and **3**, containing one and no hydroxy groups, respectively, display weak antioxidant activity. These results are consistent with previous reports on the antioxidant activity of benzophenone and biflavonoid derivatives isolated from species in the Clusiaceae family.[17,19,20]

Table 3. DPPH and ABTS Radical Scavenging Activity of Compounds **1**−**4** and MeOH Extracts from *R. edulis*

sample	IC_{50} (μM) ± SD[a]	
	DPPH	ABTS
1	38.32 ± 0.98	45.58 ± 2.00
2	466.07 ± 20.77	252.68 ± 14.77
3	765.60 ± 81.27	286.97 ± 2.91
4	30.99 ± 0.56	12.53 ± 0.11
MeOH seed extract	81.59 ± 1.06	35.27 ± 3.12
MeOH rind extract	352.96 ± 28.25	158.71 ± 16.44
Trolox	70.78 ± 1.00	48.43 ± 1.32
gallic acid	33.92 ± 0.24	19.76 ± 0.55

[a] $n = 4$.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 polarimeter using a 10 mm microcell in MeOH. Electronic circular dichroism spectra were recorded on an Aviv 202-01 spectrophotometer in MeOH. UV spectra were obtained on a Lambda 2 UV/vis spectrophotometer, IR data on a Thermo Scientific Nicolet iS10 spectrophotometer, and NMR data on a Bruker Avance III (300 MHz) instrument with MeOH-d_4 (0.1% TFA). MS analyses were performed on a ThermoFinnigan electrospray LCQ mass spectrometer or on a Waters LCT Premier XE TOF spectrometer equipped with an ESI source in the positive and negative modes. HPLC analyses were carried out on a Waters Alliance series instrument equipped with a PDA detector, using an analytical Synergi Hydro-RP C_{18} (4.6 × 250 mm, 5 μm), a semipreparative Synergi Hydro-RP 80A (10.0 × 250 mm, 4 μm), or a preparative Luna C_{18} (21.2 × 250 mm, 10 μm) column (Phenomenex). Column chromatography (CC) was conducted using Sephadex LH-20 (Pharmacia) and reversed-phase C_{18} silica gel (J.T. Baker). Precoated TLC sheets (Merck) of silica gel 60 GF_{254} and RP F_{254} (0.25 mm) were used, and visualization of plates was carried out using a vanillin (1%) solution in H_2SO_4 (5%).

Plant Material. Rinds and seeds of *R. edulis* were obtained from the Broward County Rare Fruit and Vegetable Council, Florida, and were identified by one of the authors (M.J.B.). A voucher specimen has been deposited in the Lynda Steere Herbarium at the New York Botanical Garden (Bronx, NY).

Extraction and Isolation. The seeds of *R. edulis* (358 g) were ground into powder and extracted with MeOH (2 L × 3). The dried extract (40 g) was further subjected to solvent partition using $CHCl_3$−EtOAc−*n*-BuOH−H_2O. The EtOAc-soluble fraction (7 g) was subjected to RP C_{18} Si gel column chromatography and eluted with MeOH−NH_4OAc (10 mM) (1:0 → 0:1, 50 mL each). Thirteen combined fractions (REE_I−REE_{XIII}) were obtained. Fraction REE_{XIII} (1.8 g) was further resolved by RP-HPLC (Luna C_{18} column; 10 μm; 30% MeOH in NH_4OAc (10 mM) for 15 min; 10 mL/min) to afford compounds **1** (2.5 mg) and **4** (25.0 mg). The $CHCl_3$-soluble fraction (26 g) was subjected to RP-C_{18} Si gel CC eluting with (9:1) MeOH−NH_4OAc (10 mM), to yield five secondary fractions (REC_I−REC_V). Fraction REC_{IV} (890 mg) was separated again by using a RP-C_{18} Si gel CC eluted with MeOH−NH_4OAc (10 mM) from 20% to 80% MeOH, yielding 10 secondary fractions (REC_{IVa}−REC_{IVk}). HPLC purification of fraction REC_{IVb} [Synergi Hydro-RP 80A column; 4 μm; from 40% to 100% MeOH in NH_4OAc (10 mM) for 20 min at room temperature; 4.5 mL/min] led to the isolation of compounds **2**−**4** (2.0,

2.5, and 15.0 mg, respectively). Compounds **5**−**7** were identified from the $CHCl_3$ partition by LC-MS TOF analyses according to the methodology described by Yang and collaborators.[18] Briefly, LC conditions used were a Synergi Hydro RP (4.6 × 250 mm) column; elution gradient schema [10% of MeCN in NH_4OAc (10 mM) for 4 min, from 10% to 100% of MeCN in 34 min, and isocratic until 45 min]; flow rate 1 mL/min. Spike profiles with those of authentic samples previously isolated from the related species *G. xanthochymus* and *G. livingstonei* were employed for the identification, as well as by comparison of their relative retention times, UV spectra, and ESI positive and negative ions in the MS spectra.[17,18]

The rinds of *R. edulis* (2.7 kg) were blended and extracted with MeOH (4 L × 3). The crude extract (72 g) was further subjected to solvent partitioning using $CHCl_3$−EtOAc−*n*-BuOH−H_2O. Compounds **8** and **9** were identified from the $CHCl_3$-soluble fraction through LC-MS TOF analyses using the same method previously indicated for the identification of compounds **5**−**7**.

32-Hydroxy-*ent*-guttiferone M (1): yellow oil; $[\alpha]_D^{25}$ +9.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.65), 263 (3.50), 293 (4.50) nm; IR ν_{max} 3735, 3440, 2925, 2855, 1733, 1653, 1558, 1540, 1457, 1374, 1296, 1120, 1053 cm^{-1}; ^1H (300 MHz) and ^{13}C NMR (75 MHz) data, see Table 1; HRESIMS (negative ion) *m*/*z* 617.3472 $[M - H]^-$ (calcd for $C_{38}H_{49}O_7$, 617.3478).

6-*epi*-Guttiferone J (2): yellow oil; $[\alpha]_D^{25}$ +10.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.89), 220 (3.61), 257 (3.45), 293 (3.12) nm; IR ν_{max} 3375, 2924, 1683, 1558, 1374 cm^{-1}; ^1H (300 MHz) and ^{13}C NMR (75 MHz) data, see Table 1; HRESIMS (negative ion) *m*/*z* 585.3515 $[M - H]^-$ (calcd for $C_{38}H_{49}O_5$, 585.3580).

Computational Methods. Theoretical calculations of optical rotation values and ECD spectra for compounds **1** and **2** were performed with the Gaussian03 program package.[21] Geometry optimizations for both compounds were carried out using the MMFF94 molecular mechanics force field calculations as implemented in the Spartan '08 program. A Monte Carlo search protocol[22] was carried out considering an energy cutoff of 5 kcal/mol, providing seven (**1a**−**1g**) and six (**2a**−**2f**) major conformers, respectively. In each case, the minimum energy structures were filtered and checked for duplicity. No additional minimum energy structures were found. The conformers were optimized by DFT calculations at the B3LYP/6-31++G(d,p) level of theory, and thermochemical properties, optical rotation, IR, and vibrational analysis were done at the same level. The "self-consistent reaction field" method (SCRF) with "conductor-like continuum solvent model" (COSMO) was employed to perform the ECD calculation of major conformers of compounds **1** and **2** in MeOH solution with the same basis set. The calculated excitation energy (in nm) and rotatory strength *R*, in dipole velocity (R_{vel}) and dipole length (R_{len}) forms, were simulated into an ECD curve by using the following Gaussian function:

$$\Delta\varepsilon(E) = \sum_{i=1}^{n} \Delta\varepsilon_i(E) = \sum_{i=1}^{n} \left(\frac{R_i E_i}{2.29 \times 10^{-39} \sqrt{\pi}\sigma} \exp\left[-\left(\frac{E - E_i}{\sigma} \right)^2 \right] \right)$$

where σ is the width of the band at 1/*e* height, and E_i and R_i are the excitation energies and rotatory strengths for transition *i*, respectively. $\sigma = 0.40$ eV and R_{vel} were used.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay. The DPPH radical scavenging activity was investigated according to the method previously described.[23,24] Trolox and gallic acid (Sigma) were used as positive controls. The antioxidant capacity is given as a percent inhibition of DPPH scavenging by samples and comparison with DMSO-treated controls.

ABTS Radical Cation Decolorization Assay. The ABTS assay was performed according to the method of Re et al.,[25] with some modifications.[24] Trolox and gallic acid were used as positive controls. The antioxidant capacity is given as a percent inhibition of ABTS scavenging and was calculated in the same way as previously described by DPPH assay.

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Supporting Information Available: DFT-calculated atomic Cartesian coordinates for each major conformers of **1** and **2**. 1D and 2D data of compounds **1** and **2**. Detailed calculated data of excitation energies, oscillator strengths, and rotational strengths for the six transition states, requiring the lowest excitation energies for compounds **1** and **2**. DPPH and ABTS scavenging activity plots of compounds **1**−**4** and extracts from *R. edulis*. This material is available free of charge via the Internet at http://pubs.acs.org.

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