Guttiferone I, a New Prenylated Benzophenone from *Garcinia humilis* as a Liver X Receptor Ligand

Kithsiri Herath,[†] Hiranthi Jayasuriya,[†] John G. Ondeyka,[†] Ziqiang Guan,[†] Robert P. Borris,[†] Eirik Stijfhoorn,[‡] Dennis Stevenson,[‡] Jianhua Wang,[†] Neelam Sharma,[†] Karen MacNaul,[†] John G. Menke,[†] Aisha Ali,[†] Marvin J. Schulman,[†] and Sheo B. Singh*,[†]

Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065, and Institute of Economic Botany, The New York Botanical Garden, Bronx, New York, 10458

Liver X receptors (LXR) have been implicated in cholesterol homeostasis. Agonists of LXR are expected to increase cholesterol efflux, lower LDL, and raise HDL levels. Screening of a natural product library of plant extracts using a LXR-SPA binding assay and bioassay-guided fractionation of the bark and stem extract of *Garcinia humilis* led to the discovery of a new polyisoprenylated benzophenone named guttiferone I (**1**). The IC$_{50}$ value for this compound in the LXRα-SPA binding assay was 3.4 μM. Details of the isolation, structure elucidation, and ligand binding activity of **1** are described.

Liver X receptors (LXR) are members of a superfamily of nuclear hormone receptors represented by LXRα and LXRβ subtypes.[1−3] The α-subtype is predominantly expressed in the liver, whereas the β-subtype is expressed ubiquitously. Oxysterols have been identified as endogenous ligands for both subtypes.[4−7] These receptors play a role in cholesterol homeostasis.[8] LXRs form heterodimers with the retinoid X receptor, RXR, to regulate direct or indirect expression of a number of genes involved in cholesterol and fatty acid metabolism. LXR agonists increase expression of the ABCA1 gene and raise HDL levels in mice.[9] ABCA1 mediates the efflux of cholesterol from the cells and onto the ApoAI protein of an HDL particle.[10] LXR agonists are expected to increase reverse cholesterol transport, decreasing peripheral cholesterol levels, and therefore have potential to be beneficial therapeutic agents for the control of plasma cholesterol levels.[8]

During the course of our efforts to identify novel natural product agonists of LXR in a ligand binding assay using scintillation proximity assay (SPA) technology, we discovered a new polyprenylated benzophenone named guttiferone I (**1**) by bioassay-guided fractionation of hexane extracts of the bark and stem of *Garcinia humilis* (Vahl) Adams (Clusiaceae). Guttiferone I is a diastereomer of guttiferone G (**2**), which was reported from *Garcinia macrophylla* while the present study was ongoing.[11] The isolation, structure elucidation, and ligand binding activity of guttiferone I (**1**) are described herein.

G. humilis was collected on the island of Dominica in the West Indies. The bark and stem of *G. humilis* were extracted with MeOH and partitioned with hexane. Gel permeation chromatography of the hexane extract on Sephadex LH-20 followed by reversed-phase HPLC afforded guttiferone I (**1**) as a yellow oil. HRFTMS analysis of guttiferone I (**1**) provided a molecular formula of C$_{43}$H$_{58}$O$_6$, indicating the presence of 15 degrees of unsaturation. The molecular formula was supported by the presence of 43 signals in the ^{13}C NMR spectrum. The DEPT spectrum exhibited 10 × CH$_3$, 8 × CH$_2$, and 9 × CH, suggesting the presence of 16 quaternary carbons (Table
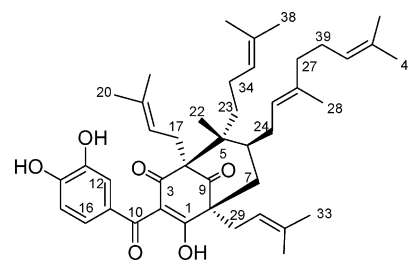
1 (Guttiferone I)
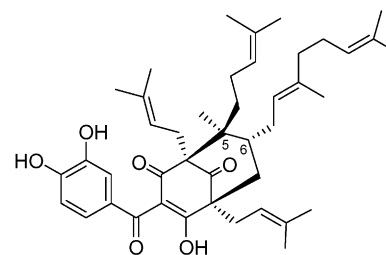
2 (Guttiferone G)

1). The NMR spectral data combined with its UV, IR spectral characteristics and with the known phytochemistry of the genus *Garcinia* suggested that this compound was a member of the guttiferone family. An aromatic trisubstituted AMX system was evident from the proton resonances appearing at δ_H 7.10 (d, *J* = 7.8 Hz), 7.60 (dd, *J* = 7.8, 2.0 Hz), and 7.80 (d, *J* = 2.0 Hz). Characteristic ^{13}C NMR resonances, including those of *ortho*-dihydroxy aromatic carbons (δ_C 147.0, 153.0), and a conjugated carbonyl (δ_C 196.0) indicated the presence of a 3,4-dihydroxy benzoyl group. Further, the presence of a β-hydroxy-1,3-enone (δ_C 191.0, 122.0, and 187.0) in addition to two quaternary carbons (δ_C 70.0 and 63.0) and a cyclohexanone carbonyl (δ_C 210.0) was indicative of the presence of 2,4,6-trisubstituted-3-hydroxycyclohex-2-ene-1,5-dione, typical of guttiferones. The HMBC correlations of H-29, H-17, H-22, H-24, and H-25 to the respective carbons (Table 1) together with the COSY correlations of H7−H6−H24−H25 helped to establish the guttiferone bicyclo[3.3.1]nonane ring system. The ^{1}H and ^{13}C NMR spectra of **1** revealed the presence of five isoprene units accounting for 25 carbons.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Assignments of Guttiferone I (**1**) in C_5D_5N

position	δ_C	δ_H, mult, J in Hz	key HMBC (C → H)
1	191.0		H-29
2	122.0		
3	187.0		
4	70.0		H-17, 18, 22, 23
5	49.9		H-6, 17, 22, 23, 34
6	41.2	2.59, dddd, 13.0, 10.2, 4.2, 2.4	H-22, 23, 25
7	42.0	α: 2.47, dd, 13.0, 4.2	H-29
		β: 1.75, t, 13.0	
8	63.0		H-29, 30
9	210.0		H-17, 29
10	196.0		H-12, 16
11	131.0		
12	117.0	7.80, d, 2.0	
13	147.0		H-15
14	153.0		H-12, 16
15	115.9	7.10, d, 7.8	
16	124.0	7.60, dd, 7.8, 2.0	H-12
17	26.9	3.09, dd, 14, 7.1	
		3.04, dd, 14, 5.4	
18	123.2	5.47, m	H-17
19	132.6		H-17
20	26.4	1.57, s	H-18
21	18.9	1.79, s	H-18
22	16.8	1.01, s	
23	37.3	1.97, m	H-6, 22, 34
		1.97, m	
24	30.0	2.30, m	H-25
		1.96, m	
25	124.2	5.27, m	H-24
26	137.2		H-24, 27, 39
27	40.5	2.00, m	H-25, 39
		2.10, m	
28	16.9	1.67, s	H-24, 25
29	31.8	2.9, d, 6.8	
30	122.4	5.72, heptt, 1.2, 6.6	H-29
31	133.7		H-29
32	18.7	1.75, s	H-30
33	26.6	1.65, s	H-30
34	25.2	2.25, m	
		2.54, m	
35	125.9	5.22, heptt, 1.2, 6.6	H-34
36	131.8		H-34
37	18.4	1.65, s	H-35
38	26.6	1.69, s	H-35
39	27.4	2.11, m	H-27
		2.11, m	
40	125.2	5.18, heptt, 1.8, 7.2	H-27, 39
41	131.8		H-39
42	18.3	1.65, s	H-40
43	26.3	1.71, d, 0.82	H-40

These data suggested that the structure of **1** was similar to guttiferone G (**2**).[11] The ^{1}H and ^{13}C NMR spectra (Table 1) of **1** and **2** were similar, but the two compounds exhibited significant differences in specific rotations, indicating stereochemical differences. Guttiferone I (**1**) showed a positive specific rotation $\{[\alpha]_D + 8.7°$ (c 1.5, CHCl$_3$)$\}$, whereas the specific rotation of guttiferone G was negative $\{[\alpha]_D -25°$ (c 0.04, CHCl$_3$). The H-6 resonance of **1** appeared as a doublet of doublets of doublets of doublets (dddd) at δ_H 2.58 with $J = 13.0, 10.2, 4.2, 2.1$ Hz due to couplings to two flanking methylenes (H$_2$-7 and H$_2$-24). The largest of the four couplings ($J = 13$ Hz) of H-6 was due to the coupling with H-7β (δ_H 1.75, t, $J = 13$ Hz), establishing that these two protons are in a *trans*-diaxial relationship in a flattened, twisted boat conformation, consistent with a dihedral angle of 173° as revealed by the MM2 energy minimized ChemDraw 3D model (Figure 1) and by a Dreiding model. This observation was further supported by the NOESY correlations of H-6 and H-7α indicating their *syn*-relationship (Figure 1). The coupling information of H-6
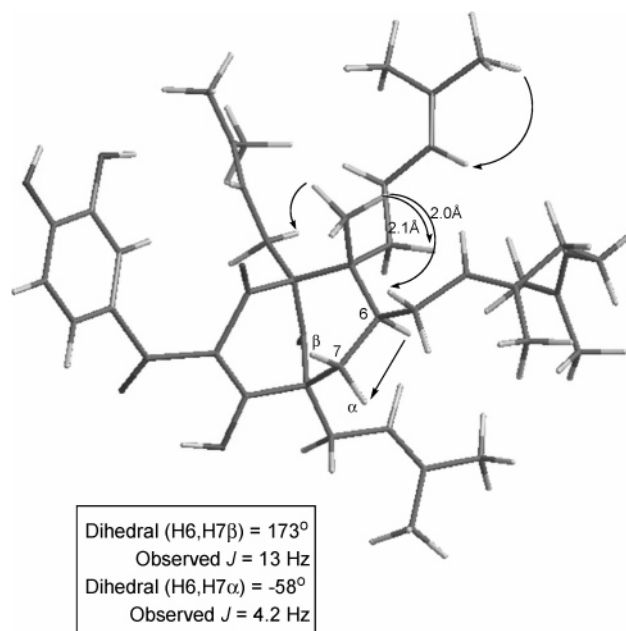
Figure 1. ChemDraw 3D MM2 energy minimized model showing key NOESY correlations.

and H-7 was critical for the stereochemical determination and was easily measured in C_5D_5N at 600 MHz. This coupling information was not reported for guttiferone G (**2**). Further NOESY correlations of guttiferone I (**1**) revealed that it possessed stereochemistry identical to guttiferone A[12] and opposite of the stereochemistry at C-5 and C-6 compared to guttiferone G (**2**). It is interesting to note that guttiferones possessing stereochemistry at C-4, C-5, C-6, and C-8 identical to guttiferones I (**1**) and A (**3**) exhibit positive specific rotations and reversal of stereochemistry at C-6 causes the reversal of the specific rotation to negative values.[12,13]

Guttiferone I (**1**) was first evaluated in a LXR-SPA binding assay using [^{3}H$_2$]-F$_3$-methyl AA {3-chloro-4-(3-(7-(2,3-ditritiopropyl)-3-trifluoromethyl-6-(4,5)-isoxazolyl)-propylthio)phenyl acetic acid}, a compound originally identified in a PPAR program as an agonist in a cell-based transactivation assay using a protocol described earlier.[14] Guttiferone I (**1**) inhibited the binding activity to the LXR α-receptor with an IC$_{50}$ value of 3.4 μM. It was less effective against β-receptor binding (IC$_{50} > 15 \mu$M) and exhibited at least 5-fold selectivity. [^{3}H$_2$]-F$_3$-methyl AA was used as control, which showed IC$_{50}$ values of 35.4 and 24.9 nM, respectively, against α- and β-receptor binding. However, guttiferone I (**1**) did not exhibit any of the desired agonist activities as measured in LXRα and -β HTRF coactivation assays.[14]

In this paper, we have described the discovery of guttiferone I, a new member of the growing family of polyprenylated benzophenones. Guttiferones have been reported to show anti-HIV,[12,15] cytotoxic,[11,16] microtubule disassembly inhibition,[13] and trypanocidal activities.[17] The LXR ligand binding inhibitory activity of guttiferone I is a new addition to the activities reported for this class of compounds. The genus *Garcinia* is one of the richest sources of polyprenylated benzophenones, particularly the guttiferones.[11–13,17,18]

Experimental Section

General Experimental Procedures. All solvents (ACS or HPLC grade) were obtained from Fisher Scientific. Optical rotation was measured on a Perkin-Elmer 241 polarimeter.

The UV spectra were recorded in methanol on a Beckman DU-70 spectrophotometer. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrophotometer. NMR spectra were recorded on a Varian Inova 600 MHz instrument operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C nuclei. LC-MS was performed on a Thermo Quest LCQ instrument using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). High-resolution mass spectral analysis was performed using a Thermo Quest FTMS instrument using electrospray ionization. A Hewlett-Packard HP1100 was used for the analytical HPLC.

Plant Material. Bark and stems of *Garcinia humilis* (Vahl) Adams (Clusiaceae) were collected on the island of Dominica in the West Indies in June 1992. A voucher specimen documenting the collection (Stijfhoorn 839) has been deposited in the permanent collections of the herbarium of the New York Botanical Garden, Bronx, NY.

Extraction and Isolation. Bark and stem of *G. humilis* (approximately 1 kg dry weight) were extracted with MeOH at room temperature. The MeOH extract was concentrated, diluted with water to a concentration of 9:1 MeOH−water, and then extracted with an equal volume of hexane. Solvent was removed from the hexane extract in vacuo using a rotary evaporator to afford a tarry residue. A 90 mg aliquot of the hexane extract was purified on a 75 mL Sephadex LH-20 column eluting with methanol at a flow rate of 1 mL/min to produce a single bioactive cut eluting at 0.58−0.89 column volume. The active cut was concentrated under vacuum to yield 66 mg of an oily material. This was further purified by preparative HPLC on a Zorbax RX C$_8$ (21 × 250 mm) using a 40 min gradient of 30−90% aqueous CH$_3$CN + 0.1% TFA at a flow rate of 8 mL/min. The active fraction containing guttiferone I (**1**) eluted at 31 min and was concentrated under vacuum and lyophilized to afford 15 mg of yellow oily material.

Guttiferone I (1): yellow oily material; $[\alpha]_D^{22}$ +8.7° (*c* 1.5, CHCl$_3$); UV (MeOH) λ max (log ϵ) 205 (4.49), 238 (4.08), 278 (4.03), 349 (3.76) nm; IR (ZnSe) ν_{max} 3345, 1728, 1642 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS *m/z* 671 (M + H)$^+$; HRESIFTMS *m/z* 671.4323 (calcd for C$_{43}$H$_{58}$O$_6$+H, 671.4312).

LXR-SPA Binding Assays. LXR scintillation proximity assay (LXR-SPA) was performed using GST-LXR ligand binding domain (LBD) receptors for α and β using [^{3}H$_2$]F$_3$-methyl AA as detailed by Menke et al.[14]

Coactivator Association Assays. The agonist activity of guttiferone I was measured in an in vitro cofactor association assay. In this assay, the association of recombinant steroid receptor coactivator 1 (SRC1) coactivator protein with recombinant LXRα and -β ligand binding domains was measured using a homogeneous time-resolved fluorescence (HTRF) assay as described earlier.[14]

References and Notes

(1) Willy, P. J.; Umesono, K.; Ong, E. S.; Evans, R. M.; Heyman, R. A.; Mangelsdorf, D. J. *Gene Dev.* **1995**, *9*, 1033−1045.
(2) Miyata, K. S.; McCaw, S. E.; Patel, H. V.; Rachubinski, R. A.; Capone, J. P. *J. Biol. Chem.* **1996**, *271*, 9189−9192.
(3) Shinar, D. M.; Endo, N.; Rutledge, S. J.; R., V.; Rodan, G. A.; Schmidt, A. *Gene* **1994**, *147*, 273−276.
(4) Janowski, B. A.; Willy, P. J.; Devi, T. R.; Falck, J. R.; Mangelsdorf, D. J. *Nature* **1996**, *383*, 728−731.
(5) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Oliver, B. B.; Su, J.-L.; Sundseth, S. S.; Winegar, D. A.; Blanchard, D. E.; Spencer, T. A.; Wilson, T. M. *J. Biol. Chem.* **1997**, *272*, 3137−3140.
(6) Janowski, B. A.; Grogan, M. J.; Jones, S. A.; Wisely, G. B.; Kliewer, S. A.; Corey, E. J.; Mangelsdorf, D. J. *Proc. Natl. Acad. Sci., U.S.A.* **1999**, *96*, 266−271.
(7) Fu, X.; Menke, J. G.; Chen, Y.; Zhou, G.; MacNaul, K. L.; Wright, S. D.; Sparrow, C. P.; Lund, E. G. *J. Biol. Chem.* **2001**, *276*, 38378−38387.
(8) Repa, J. J.; Mangelsdorf, D. J. *Nat. Med.* **2002**, *8*, 1243−1248.
(9) Joseph, S. B.; McKilligin, E.; Pei, L.; Watson, M. A.; Collins, A. R.; Laffitte, B. A.; Chen, M.; Noh, G.; Goodman, J.; Hagger, G. N.; Tran, J.; Tippin, T. K.; Wang, X.; Lusis, A. J.; Hsueh, W. A.; Law, R. E.; Collins, J. L.; Willson, T. M.; Tontonoz, P. *Proc. Natl. Acad. Sci., U.S.A.* **2002**, *99*, 7604−7609.
(10) Sparrow, C. P.; Baffic, J.; Lam, M.-H.; Lund, E. G.; Adams, A. D.; Fu, X.; Hayes, N.; Jones, A. B.; Macnaul, K. L.; Ondeyka, J.; Singh, S.; Wang, J.; Zhou, G.; Moller, D. E.; Wright, S. D.; Menke, J. G. *J. Biol. Chem.* **2002**, *277*, 10021−10027.
(11) Williams, R. B.; Hoch, J.; Glass, T. E.; Evans, R.; Miller, J. S.; Wisse, J. H.; Kingston, D. G. I. *Planta Med.* **2003**, *69*, 864−866.
(12) Gustafson, K. R.; Blunt, J. W.; Munro, M. H. G.; Fuller, R. W.; McKee, T. C.; Cardellina, J. H., II; McMahon, J. B.; Cragg, G. M.; Boyd, M. R. *Tetrahedron* **1992**, *48*, 10093−102.
(13) Roux, D.; Hadi, H. A.; Thoret, S.; Guenard, D.; Thoison, O.; Paies, M.; Sevenet, T. *J. Nat. Prod.* **2000**, *63*, 1070−1076.
(14) Menke, J. G.; Macnaul, K. L.; Hayes, N. S.; Baffic, J.; Chao, Y.-S.; Elbrecht, A.; Kelly, L. J.; Lam, M.-H.; Schmidt, A.; Sahoo, S.; Wang, J.; Wright, S. D.; Xin, P.; Zhou, G.; Moller, D. E.; Sparrow, C. P. *Endocrinology* **2002**, *143*, 2548−2558.
(15) Fuller, R. W.; Blunt, J. W.; Boswell, J. L.; Cardellina, J. H., II; Boyd, M. R. *J. Nat. Prod.* **1999**, *62*, 130−132.
(16) Cuesta-Rubio, O.; Frontana-Uribe, B. A.; Ramirez-Apan, T.; Cardenas, J. *Z. Naturforsch., C: J. Biosci.* **2002**, *57*, 371−378.
(17) Abe, F.; Nagafuji, S.; Okabe, H.; Akahane, H.; Estrada-Muniz, E.; Huerta-Reyes, M.; Reyes-Chilpa, R. *Biol. Pharm. Bull.* **2004**, *27*, 141−143.
(18) Weng, J.-R.; Lin, C.-N.; Tsao, L.-T.; Wang, J.-P. *Chem. Eur. J.* **2003**, *9*, 5520−5527.

NP050045J