Polyprenylated Benzophenones from *Garcinia assigu* and Their Potential Cancer Chemopreventive Activities

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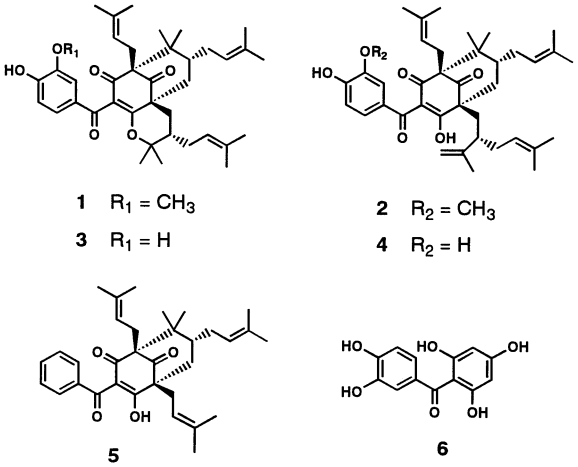
In a further study on the chemical constituents of *Garcinia assigu*, two new benzophenones corresponding to the 13-*O*-methyl ethers (**1** and **2**) of the known isogarcinol and garcinol, respectively, were isolated and characterized, along with known benzophenones (**3**−**6**). Inhibitory effects of the benzophenones isolated from this plant on Epstein−Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells and their radical-scavenging ability against 1,1-diphenyl-2-picrylhydrazyl (DPPH) were demonstrated. The cyclized polyprenylbenzophenones (**1**−**5**) showed comparable or stronger potential cancer chemopreventive activity when compared to glycyrrhetic acid, a known anti-tumor promoter.

Previously, we reported the first example of isolation of a depsidone, garcinisidone, from a higher plant, *Garcinia assigu* Lantb. (Guttiferae), along with some new xanthones.[1] Our recent research has shown that some depsidones and xanthones isolated from *Garcinia* species are potential cancer chemopreventive agents as a result of their activity in a short-term in vitro assay to assess 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein−Barr virus early antigen (EBV-EA) activation in Raji cells. We reported that the presence of a prenyl moiety in the xanthone[2] and depsidone[3] molecules isolated from *Garcinia* species appears to play an important role in mediating their tumor-promoting inhibitory activity. Moreover, a polyprenylated benzophenone derivative, garcinol (**4**), has been reported to possess antioxidative,[4] antibiotic,[5] and anti-human immunodeficiency virus (HIV) activities[6] and suppressed colonic aberrant crypt foci (ACF) formation.[7]

In further studies of constituents of this plant, two new polyprenylated benzophenones (**1** and **2**) were isolated and characterized, along with four known benzophenones (**3**−**6**). Here we also describe the inhibitory effect of these benzophenones on EBV-EA activation induced by TPA in Raji cells and their radical-scavenging ability against 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Results and Discussion

The EtOH extract of dried stem bark of *G. assigu* collected in the Central Province of Papua New Guinea was fractionated chromatographically, as been previously reported,[1] leading to the isolation of two new compounds, isogarcinol 13-*O*-methyl ether (**1**) and garcinol 13-*O*-methyl ether (**2**), and the known compounds isogarcinol (**3**),[6,8−11] garcinol (**4**),[6,8,9,11] clusianone (**5**),[12] and macurin (**6**).[13] The structures of these known compounds were identified by comparison with literature spectroscpic data.



Isogarcinol 13-*O*-methyl ether (**1**) was obtained as a yellow oil, $[\alpha]_D$ −199° (EtOH). The molecular formula $C_{39}H_{52}O_6$ was determined by HREIMS. The IR spectrum showed bands due to a hydroxy group (3525 cm^{-1}) and both isolated (1730 cm^{-1}) and conjugated (1674 cm^{-1}) carbonyl groups. The ^1H NMR spectrum (Table 1) revealed signals assignable to three 3-methyl-2-butenyl (prenyl) units, a methoxy, four quaternary methyls, and two methylenes attached to methine carbons. Aromatic ^1H NMR ABC type signals (δ_H 7.46, 6.76, 7.11) and typical ^{13}C NMR signals for substituted aromatic carbons at δ_C 130.6 (s), 111.9 (d), 149.3 (s), 154.0 (s), 115.7 (d), 126.6 (d) and a conjugated carbonyl at δ_C 194.8, respectively, were indicative of a 3,4-dioxygenated benzoyl moiety. The close resemblance of chemical shift values and multiplicities of each signal in the ^1H and ^{13}C NMR spectra (Table 1) of **1** with those of isogarcinol (**3**, also named cambogin),[6,8−11] except for the appearance of a methoxy signal in the spectra of **1**, suggested a formula corresponding to the *O*-methyl ether of **3**. Location of the methoxy group at C-13 was indicated by observation of a NOE between a methoxy and a *meta*-coupled aromatic proton at δ_H 7.46 (1H, d, J = 1.8 Hz). Together with results of HMBC and HMQC analyses, the structure was determined as shown by formula **1**, including the relative stereochemistry. The absolute stereochemistry

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Table 1. NMR Data of Compounds **1** and **2** from *G. assigu*[a]

	isogarcinol 13-*O*-methyl ether (**1**)			garcinol 13-*O*-methyl ether (**2**)		
	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
1	173.1 (s)		H-7, H-29	194.6 (s)		H-7, H-29
2	127.9 (s)			119.0 (s)		
3	196.0 (s)		H-17	*b*		
4	71.7 (s)		H-17, H-22, H-23	71.2 (s)		H-17, H-22, H-23
5	47.0 (s)		H-7, H-22, H-23	49.2 (s)		H-22, H-23
6	42.5 (d)	1.98 (m)	H-22, H-23	43.9 (d)	1.55[b]	H-22, H-23
7	43.4 (t)	2.17 (m), 1.58 (t, 13.9)		45.9 (t)	2.06 (m), 1.44 (t, 13.2)	
8	54.3 (s)		H-7, H-29	61.5 (s)		H-29
9	207.1 (s)		H-17, H-7, H-29	209.6 (s)		H-17
10	194.8 (s)		H-12, H-16	196.0(s)		H-12, H-16
11	130.6 (s)		H-15	129.9 (s)		H-15
12	111.9 (d)	7.46 (d, 1.8)		113.6 (d)	7.35 (d, 1.8)	H-16
13	149.3 (s)		H-12, H-15, OCH_3	148.8 (s)		H-15, OCH_3
14	154.0 (s)		H-12, H-15, H-16	153.6 (s)		H-12, H-16
15	115.7 (d)	6.76 (d, 8.4)		115.1 (d)	6.70 (d, 8.1)	
16	126.6 (d)	7.11 (dd, 8.4, 1.8)	H-12	126.8 (d)	7.06 (dd, 8.1, 1.8)	H-12
17	25.8 (t)	2.62 (m), 2.44 (m)		26.1 (t)	2.65 (2H, m)	
18	121.4 (d)	4.85 (m)	H-17, H-20, H-21	121.7 (d)	4.98 (m)	H-17, H-20, H-21
19	135.1 (s)		H-17, H-20, H-21	135.4 (s)		H-20, H-21
20	26.5 (q)	1.59 (3H, s)	H-18, H-21	26.4 (q)	1.69 (3H, s)	H-18, H-21
21	18.2 (q)	1.59 (3H, s)	H-18, H-20	18.2 (q)	1.68 (3H, s)	H-18, H-20
22	22.6 (q)	1.09 (3H, s)	H-23	23.9 (q)	1.08 (3H, s)	H-23
23	16.3 (q)	0.76 (3H, s)	H-22	16.3 (q)	0.77 (3H, s)	H-22
24	28.7 (t)	2.20 (m), 1.81 (m)		29.2 (t)	2.07 (m), 1.65 (m)	
25	123.8 (d)	5.14 (m)	H-27, H-28	123.7 (d)	5.01 (m)	H-27, H-28
26	134.2 (s)		H-27, H-28	134.4 (s)		H-27, H-28
27	26.0 (q)	1.72 (3H, s)	H-25, H-28	25.9 (q)	1.67 (3H, s)	H-25, H-28
28	18.0 (q)	1.62 (3H, s)	H-25, H-27	18.0 (q)	1.55 (3H, s)[c]	H-25, H-27
29	28.5 (t)	3.03 (m), 1.06 (t, 13.6)		36.7 (t)	1.96 (2H, m)	H-30, H-34
30	44.8 (d)	1.36 (m)	H-32, H-33, H-34	45.3 (d)	2.61 (m)	H-32, H-34
31	88.6 (s)		H-29, H-32, H-33	149.6 (s)		H-29, H-32, H-34
32	29.0 (q)	0.96 (3H, s)	H-33	113.1 (t)	4.46 (2H, s)	H-30, H-33
33	21.5 (q)	1.23 (3H, s)	H-32	18.4 (q)	1.54 (3H, s)	H-30, H-32
34	30.5 (t)	2.05 (m), 1.81 (m)		33.4 (t)	1.99 (2H, m)	H-29, H-30
35	122.9 (d)	5.19 (m)	H-37, H-38	124.1 (d)	4.99 (m)	H-34, H-37, H-38
36	134.7 (s)			132.7 (s)		H-34, H-37, H-38
37	18.0 (q)	1.63 (3H, s)	H-35, H-38	18.2 (q)	1.57 (3H, s)	H-35, H-38
38	26.0 (q)	1.78 (3H, s)	H-35, H-39	26.0 (q)	1.65 (3H, s)	H-35, H-37
OCH_3	56.5 (q)	3.89 (3H, s)		56.8 (q)	3.88 (3H, s)	

[a] Values in (δ_H and δ_C) ppm. All signals correspond to 1H, unless otherwise stated. Figures in parentheses are coupling constants (*J*), in Hz. Spectra were taken in deuterated MeOH with 0.1% TFA. [b] No signal was detected for overlapping or broadening of the signal. [c] Overlapped signals.

of **1**, the same as that of **3** reported by Krishnamurthy et al.,[10] was indicated by observation of the same type of Cotton effect of **1** as that of **3** by CD analysis. This compound is being reported for the first time as a natural product.

Garcinol 13-*O*-methyl ether (**2**) was obtained as a yellow oil, $[\alpha]_D$ −117°. The molecular formula was established as $C_{39}H_{52}O_6$ by HREIMS. The ^{1}H NMR spectrum showed signals assignable to a vinyl methyl and two *exo*-methylene protons along with signals due to a methoxy, three prenyls, two *gem*-dimethyls on quaternary carbons, two methylenes bonded to methine carbons, and a 3,4-dioxygenated benzophenone moiety. The ^{1}H and ^{13}C NMR features (Table 1) of **2** were found to be similar with those of garcinol (**4**, also named camboginol),[6,8,9,11] isolated from the same plant, except for the appearance of a methoxy signal in the spectrum of **2**. Consideration of these data coupled with the observation of a NOE between the 3H singlet at δ_H 3.88 (OCH_3) and a doublet at δ_H 7.35 and the same Cotton effects of **2** as those of **4** in the CD spectrum suggested the structure of **2** to correspond to the *O*-methyl ether of garcinol (**4**). This is the first report of the *O*-methyl ether of **4** from a natural source.

In the present study, we tested the inhibitory effects of compounds **1**−**6** in a short-term in vitro assay involving TPA-induced EBV-EA activation in Raji cells. Their effects on the activation of the virus-genome and the viabilities of Raji cells are summarized in Table 2. Compounds **1**−**5** showed a detectable inhibition on EBV-EA activation at a concentration of greater than 1×10^2 mol ratio/TPA (11.0−15.1%) and a strong effect (80.3−85.6%) at a higher concentration (1×10^3 mol ratio/TPA) without causing a decrease in viability of the Raji cells. Among these compounds, garcinol (**4**) exhibited the strongest activity. The inhibitory effects of all compounds (**1**−**5**) were similar to or stronger than that of glycyrrhetic acid, a potent antitumor promoter.[14] However, the inhibitory effects of compounds **1**−**5**, with a cyclized polyprenyl-benzophenone nucleus, was found to be weaker than that of the nonprenylated **6** and also than certain xanthones[2] and depsidones[3] having one or more directly bonded prenyl side chain to the aromatic nuleus.

Accumulated evidence demonstrates that oxidative stresses are tightly associated with carcinogenic processes by their capacity of causing DNA mutation and protein modification. Garcinol (**4**) is known to be an antioxidant, and its radical-scavenging ability against 1,1-diphenyl-2-picrylhydrazyl (DPPH) is nearly 3 times greater than that of DL-α-tocophenol.[4] To investigate whether their potential cancer chemopreventive activity could be attributed to their antioxidative effects, we assessed the free-radical-scavenging capacities of the five natural polyprenylated benzophenones (**1**−**5**). Table 1 summarizes the DPPH free-radical-scavenging activity (IC_{50}) of benzophenones **1**−**5** as compared

Table 2. Inhibitory Effects on TPA-Induced EBV-EA Activation and DPPH Free-Radical-Scavenging Activities of Benzophenones **1**−**6**

compound	EBV-EA-positive cells (% viability)[a] compound concentration (mol ratio/32 pmol TPA) 1000	500	100	10	DPPH radical-scavenging activity[d] (μM)
isogarcinol 13-*O*-methyl ether (**1**)	18.5 ± 1.1 (60)	60.1 ± 2.1 (>80)	89.0 ± 1.9 (>80)	100.0 ± 0.3 (>80)	>100
garcinol 13-*O*-methyl ether (**2**)	19.7 ± 0.9 (60)	54.9 ± 2.6 (>80)	88.1 ± 1.2 (>80)	100.0 ± 0.3 (>80)	>100
isogarcinol (**3**)	17.7 ± 1.0 (60)	56.0 ± 1.9 (>80)	87.1 ± 1.8 (>80)	100.0 ± 1.1 (>80)	13.3 ± 1.3
garcinol (**4**)	14.4 ± 0.9 (60)	47.0 ± 2.5 (>80)	84.9 ± 1.8 (>80)	100.0 ± 1.0 (>80)	10.2 ± 1.4
clusianone (**5**)	17.4 ± 1.2 (60)	53.7 ± 2.3 (>80)	87.7 ± 1.3 (>80)	100.0 ± 0.9 (>80)	>100
macurin (**6**)	0.0 ± 1.1 (70)	52.7 ± 2.3 (>80)	80.2 ± 2.1 (>80)	94.3 ± 1.2 (>80)	
glycyrrhetic acid[b]	15.6 ± 1.1 (>80)	54.3 ± 2.3 (>80)	100.0 ± 1.9 (>80)	100.0 ± 0.2 (>80)	
vitamin E[c]					22.8 ± 0.2

[a] Mole ratio/TPA (32 pmol = 20 ng/mL), 1000 mol ratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol, and 10 mol ratio = 0.32 nmol. Values are EBV-EA activation (%) ± SD in the presence of the test compound relative to the positive control (100%). Values in parentheses represent the surviving Raji cells measured through Trypan Blue staining. At least 60% surviving Raji cells 2 days after treatment with the compounds is required for an accurate result. [b] Positive control substance on TPA-induced EBV-EA activation. [c] Positive control substance on DPPH radical-scavenging activities. [d] IC_{50} were mean ± SD of five replicates.

to that of vitamin E. Garcinol (**4**) and isogarcinol (**3**), with a catechol (1,2-dihydroxy) group in their structures, caused rapid decolorization of the DPPH solution, indicating marked radical-scavenging potencies, whereas garcinol 13-*O*-methyl ether (**2**), clusinone (**5**), and isogarcinol 13-*O*-methyl ether (**1**) had almost no effect. The EC_{50} (μM) values for free-radical-scavenging effects of garcinol (**4**) and isogarcinol (**3**) were 10.2 ± 1.4 and 13.3 ± 1.3 (n = 5), respectively. The relative free-radical-scavenging activity of garcinol (**4**) was 2 times stronger than that of vitamin E (IC_{50}, 22.8 ± 0.2). On the other hand, latisxanthone C (a xanthone)[2] and garcinisidone E (a depsidone)[3] showed remarkable inhibitory effects on EBV-EA activation and had almost no effect on the radical-scavenging activity of DPPH.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in MeOH, and IR spectra on an IR-230 (JASCO) spectrometer in $CHCl_3$. ^1H and ^{13}C NMR, COSY, HMQC, HMBC (J = 8 Hz), and NOE spectra were measured using JNM A-400, A-600, and/or ECP-500 (JEOL) spectrometers. Chemical shifts are shown in δ (ppm) with tetramethylsilane (TMS) as the internal reference. All mass spectra were taken under EI conditions, unless otherwise stated, using HX-110 (JEOL) and/or JMS-700 (JEOL) spectrometers having a direct inlet system. Preparative TLC was done on Kieselgel 60 F_{254} (Merck).

Plant Material. The plant material used in this study, *Garcinia assigu* Lantb., was collected in the Central Province of Papua New Guinea in February 1993. A voucher specimen (PNGSR034) has been deposited at the herbarium of the University of Papua New Guinea.

Extraction and Isolation. The dried stem bark (560 g) of *G. assigu* was extracted with EtOH under reflux. The EtOH extract was evaporated under reduced pressure to give an oily residue, which was fractionated with hexane, CH_2Cl_2, EtOAc, CH_2Cl_2−MeOH (3:1), and MeOH. The hexane-soluble portion was subjected to silica gel chromatography eluted with hexane−acetone (9:1, 17:3, 4:1, 7:3, 1:1), acetone, CH_2Cl_2−MeOH (3:1), and MeOH, successively. The hexane−acetone (4:1) eluate was further subjected to preparative TLC with hexane−acetone (4:1) and benzene−MeOH (19:1) as developing solvents to obtain isogarcinol (**3**, 10.2 mg) and clusianone (**5**, 11.7 mg) along with garcinisidone A (2.5 mg). The CH_2Cl_2-soluble portion was subjected to silica gel column chromatography. Successive elution with hexane−EtOAc (9:1, 4:1, 7:3, 3:2, 1:1, 3:7), EtOAc, acetone, CH_2Cl_2−MeOH (3:1), and MeOH gave 10 fractions. Each fraction was further subjected to silica gel column chromatography and preparative TLC with appropriate combinations of hexane, benzene, $CHCl_3$, *i*-Pr_2O, EtOAc, acetone, and MeOH as developing solvents to give the following compounds. Isogarcinol (**3**, 17.4 mg) was isolated from the hexane−EtOAc (7:3) fraction, and garcinol (**4**, 29.7 mg), garcinol 13-*O*-methyl ether (**2**, 18.2 mg), isogarcinol 13-*O*-methyl ether (**1**, 5.3 mg), and 1,3,5-trihydroxyxanthone (2.0 mg) were obtained from the hexane−EtOAc (1:1) fraction. From the hexane−EtOAc (3:7) fraction, macurin (**6**, 9.8 mg) was afforded. The EtOAc-soluble portion was treated in the same manner as the CH_2Cl_2-soluble portion described above, and from the hexane−acetone (7:3) fraction, isogarcinol 13-*O*-methyl ether (**1**, 5.3 mg), garcinol (**4**, 8.5 mg), and garcinol 13-*O*-methyl ether (**2**, 11.1 mg) were obtained.

Isogarcinol 13-*O*-methyl ether (1): yellow oil; $[\alpha]_D$ −199° (c 0.300, EtOH); UV (MeOH) λ_{max} (log ϵ) 232 (4.0), 276 (4.1), 311 (3.8) nm; CD (MeOH) $[\theta]_{342}$ +2412, $[\theta]_{326}$ 0, $[\theta]_{318}$ −921, $[\theta]_{310}$ 0, $[\theta]_{298}$ +3054, $[\theta]_{290}$ 0, $[\theta]_{264}$ −29720, $[\theta]_{239}$ 0, $[\theta]_{220}$ +22790; IR ($CHCl_3$) ν_{max} 3525, 1730, 1674 cm^{-1}; differential NOE, irradiation of OCH_3 (δ 3.89) −16% enhancement of H-12 (δ 7.46); EI-MS m/z 616 [M^+] (47), 547 (16), 479 (83), 463 (26), 355 (85), 231 (49), 151 (100); HREIMS m/z 616.3760 (calcd for $C_{39}H_{52}O_6$, 616.3761).

Garcinol 13-*O*-methyl ether (2): pale yellow oil; $[\alpha]_D$ −117° (c 1.011, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.1), 279 (4.1), 350 nm (3.6); CD (MeOH) $[\theta]_{390}$ −563, $[\theta]_{368}$ 0, $[\theta]_{337}$ +2381, $[\theta]_{322}$ 0, $[\theta]_{315}$ −2087, $[\theta]_{308}$ 0, $[\theta]_{294}$ +6984, $[\theta]_{282}$ 0, $[\theta]_{265}$ −7507, $[\theta]_{248}$ 0, $[\theta]_{239}$ +2528, $[\theta]_{230}$ 0; IR ($CHCl_3$) ν_{max} 3529, 1722, 1657 cm^{-1}; differential NOE, irradiation of OCH_3 (δ 3.88) −15% enhancement of H-12 (δ 7.35); EI-MS m/z 616 [M^+] (21), 547 (16), 479 (44), 463 (11), 355 (27), 231 (61), 151 (100); HREIMS m/z 616.3742 (calcd for $C_{39}H_{52}O_6$, 616.3760).

Isogarcinol (3): CD (MeOH) $[\theta]_{344}$ +3050, $[\theta]_{325}$ 0, $[\theta]_{318}$ −795, $[\theta]_{311}$ 0, $[\theta]_{297}$ +3917, $[\theta]_{289}$ 0, $[\theta]_{264}$ −25340, $[\theta]_{239}$ 0, $[\theta]_{220}$ +18300.

Garcinol (4): CD (MeOH) $[\theta]_{386}$ −1000, $[\theta]_{366}$ 0, $[\theta]_{340}$ +2321, $[\theta]_{322}$ 0, $[\theta]_{317}$ −650, $[\theta]_{311}$ 0, $[\theta]_{297}$ +4553, $[\theta]_{288}$ 0, $[\theta]_{264}$ −7933, $[\theta]_{246}$ 0, $[\theta]_{237}$ +2000, $[\theta]_{223}$ 0.

In Vitro EBV-EA Activation Experiments. The inhibition of EBV-EA activation was assayed for compounds **1**−**6** using the same method described previously.[2,3] In brief, Raji cells were grown to a density of 10^6 cells/mL, harvested by centrifugation, and resuspended in RPMI 1640 medium (Sigma, St. Louis, MO) with 10% FCS containing 4 mM *n*-butyric acid as inducer, 32 pmol of TPA (20 ng/mL in DMSO), and 32, 3.2, or 0.32 nmol of the test compound (DMSO solutions). The cells were incubated at 37 °C for 48 h, and cell number and viability were determined after 48 h by means of a hemocytometer (Trypan Blue staining method). EBV-EA inhibitory activity of the test compounds was estimated on the basis of the percentage of positive cells compared to that observed in the case of a control without the test product. In each assay, at least 500 cells were counted, and the results were read blind.

DPPH Free-Radical-Scavenging Activity. The free-radical-scavenging capacities of benzophenones **1**−**5** were measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH)

method as described previously.[15] Briefly, each benzophenone dissolved in ethanol was mixed with 2 mL of a 0.1 mM DPPH solution. After incubation at room temperature for 30 min, absorbance at 520 nm was measured with a V-550 spectrophotometer (JASCO). To evaluate the effects of benzophenones **1**−**5** and vitamin E on free-radical-scavenging capacity, the EC_{50} values were estimated by probit-transformation technique.

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