Cancer Chemopreventive Agents. New Depsidones from *Garcinia* Plants

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In a search for cancer chemopreventive agents from natural sources, chemical constituents of two kinds of *Garcinia* plants, *Garcinia neglecta* and *Garcinia puat*, collected in New Caledonia, were examined. Five new depsidones, garcinisidone-B (**2**), -C (**3**), -D (**4**), -E (**5**), and -F (**6**), were isolated, and their structures were determined by spectrometric analyses. Inhibitory effects of these depsidones on EBV-EA activation induced by TPA in Raji cells were also demonstrated.

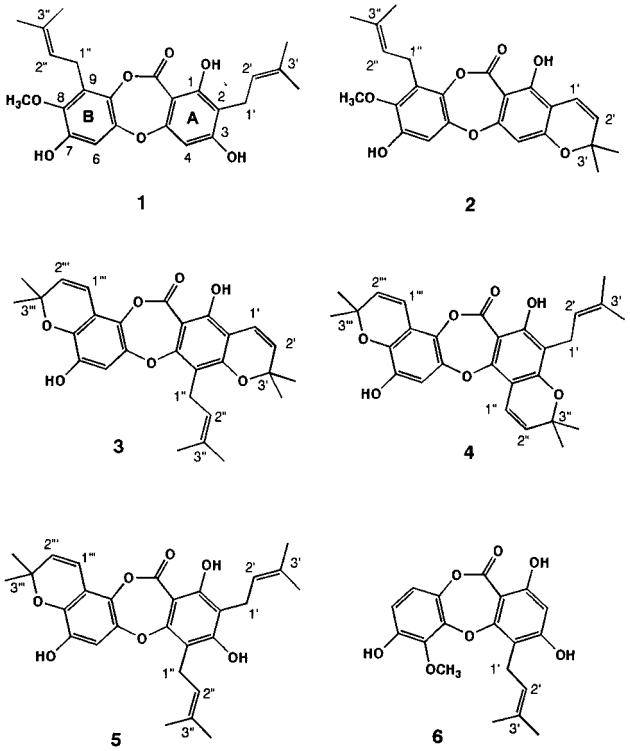
We have previously reported the isolation and characterization of a new depsidone, garcinisidone-A (**1**), along with some new xanthones from *Garcinia assigu* collected in Papua New Guinea, in the course of a program involving a search for cancer chemopreventive agents from plants growing in tropical areas.[1] In this paper,[2] isolation of five new depsidones, named garcinisidone-B (**2**), -C (**3**), -D (**4**), -E (**5**), and -F (**6**), from two additional *Garcinia* plants, *Garcinia neglecta* Vieill. and *Garcinia puat* Guillaumin (Guttiferae), collected in New Caledonia, and elucidation of the structures of these new compounds through spectroscopic analyses are described. We also report here the inhibitory effects of these depsidones on Epstein−Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells.

Results and Discussion

Acetone extracts of dried leaves of *G. neglecta* and *G. puat* were fractionated by a combination of silica gel column chromatography and preparative TLC to obtain five new depsidones, named garcinisidone-B (**2**), -C (**3**), -D (**4**), -E (**5**), and -F (**6**). The presence of the depsidone (11H-dibenzo-[*b*,*e*][1,4]dioxepin-11-one) nucleus, having a hydrogen-bonded 1-hydroxyl group, in all of the new compounds isolated in the present study was suggested by the similarity of the NMR data with those of garcinisidone-A (**1**),[1] taking into account some differences due to the presence of an additional prenyl substituent.

The molecular formula of garcinisidone-B (**2**) was established as $C_{24}H_{24}O_7$ by HRMS. The ^{1}H NMR spectrum showed signals assignable to a 2,2-dimethylpyran ring, a prenyl, a methoxy group, and an additional hydroxyl group, along with two isolated aromatic protons. The location of these substituents on the 1-hydroxydepsidone nucleus was determined by NOE and HMBC experiments. An NOE interaction between the two aromatic singlets (δ_H 6.23 and 6.71) indicated that the locations of these protons were at C-4 and C-6. Observations of NOE between the methoxy signal (8-OCH_3) and H-1″, H-2″, and 7-OH and C−H long-range correlations from C-9a to H-6 and H-1″ in the HMBC spectrum confirmed the location of the prenyl, methoxy, and hydroxyl groups at C-9, -8, and -7, respectively. Further, HMBC correlations from C-2 to the hydrogen-bonded proton (δ_H 11.22), H-2′, and H-4 indicated a [3,2-*b*] orientation of the dimethylpyran ring, as shown in **2**. These data, together with other results of HMBC analysis, shown by arrows in Figure 1, suggested that the structure of garcinisidone-B is **2**.

Scheme 1. Structures of Garcinisidones from *Garcinia* Plants



The ^{1}H NMR spectrum of garcinisidone-C ($C_{28}H_{28}O_7$) (**3**) showed the presence of a prenyl moiety, two dimethylpyran rings, and an additional hydroxyl group, along with a lone isolated aromatic proton. The arrangement of these substituents on the skeleton was revealed by analysis of HMBC data. Correlations from C-2 to the 1-OH and H-2′ and from C-3 to H-1′and H-1″, which also correlated with C-4a, indicated the presence of a [3,2-*b*] oriented dimethylpyran ring and a 4-prenyl substituent in the molecule. On the other hand, HMBC correlations from C-9a to H-6

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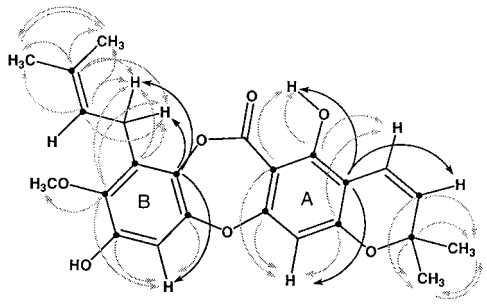

Figure 1. C−H long-range correlations in the HMBC spectrum of garcinisidone-B (**2**). Bold line: more significant correlations in the structure determinations.

and H-1‴ suggested that the orientation of the second dimethylpyran ring is [2,3-*J*] with a phenolic group at the remaining site (7-OH). On the basis of these and other HMBC data, the structure of garcinisidone-C was determined to be **3**.

The molecular formula of garcinisidone-D ($C_{28}H_{28}O_7$) (**4**) was found to be the same as that of **3** by HRMS. The signal pattern of the ^{1}H NMR spectrum was similar to that of **3**, suggesting the presence of two dimethylpyran rings fused to a 1-hydroxydepsidone nucleus having a prenyl moiety and an additional hydroxyl group along with a lone isolated aromatic proton. HMBC and NOE experiments were useful for determination of the locations of these moieties on the skeleton. Assignment of a lone aromatic proton to H-6 was confirmed by C−H correlations both from C-5a and C-9a to this proton. Irradiation of H-6 resulted in NOE enhancement at H-1″ on one of the pyran rings, indicating a [2,3-*c*] orientation of the dimethylpyran ring. In the HMBC experiments, a correlation from C-2 to a hydrogen-bonded 1-OH and H-2′ on the prenyl moiety suggested that the location of the prenyl moiety is at C-2. Further, [2,3-*J*] orientation of another dimethylpyran ring was suggested by C−H long-range correlations from H-6 to C-8, which was further correlated with H-1‴ on the pyran ring. Consequently, the position of the OH group was assigned to the remaining site at C-7. Thus, the structure **4** of garcinisidone-D was established.

The ^{1}H NMR spectrum of garcinisidone-E ($C_{28}H_{30}O_7$) (**5**) showed the presence of a dimethylpyran ring, two prenyl groups, and a non-hydrogen-bonded hydroxyl group on the 1-hydroxydepsidone nucleus (Table 1). The locations of these substituents were elucidated by HMBC and NOE analyses. The locations of the two prenyl groups at C-2 and C-4 and the location of a non-hydrogen-bonded OH group at C-3 were confirmed by observations of C−H long-range correlations from C-2 to the hydrogen-bonded proton and H-1′ on a prenyl moiety and from C-3 to one non-hydrogen-bonded OH, H-1′, and H-1″ on each prenyl group. Further, [2,3-*J*] orientation of the dimethylpyran ring and a 7-OH on the skeleton were confirmed by C−H long-range correlations of C-8 to a lone proton H-6, 7-OH, and H-1‴ on the pyran ring together with NOE enhancement between H-6 and H-1″ on the prenyl group. Thus, structure **5** was proposed for garcinisidone-E.

Garcinisidone-F (**6**) was isolated as a colorless oil from *G. puat*. The molecular formula was established as $C_{19}H_{18}O_7$

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Garcinisidones[a]

	garcinisidone-B (**2**)		garcinisidone-C (**3**)		garcinisidone-D (**4**)		garcinisidone-E (**5**)		garcinisidone-F (**6**)	
	δH	δC	δH	δC	δH	δC	δH	δC	δH	δC
1		159.70		157.77		161.98		160.33		163.29
1-OH	11.22 (s)		11.02 (s)		11.11 (s)		11.00 (s)		10.77 (s)	
2		106.48		106.35		113.80		111.57	6.33 (s)	101.39
3		160.92		158.10		158.19		160.82		162.66
3-OH							6.35 (s)		6.29 (s)	
4	6.23 (s)	100.92		113.56		105.76		111.21		111.47
4a		160.39		158.63		153.66		156.55		158.03
5a		146.92		143.20		143.24		143.41		143.49
6	6.71 (s)	105.41	6.71 (s)	106.86	6.67 (s)	106.44	6.68 (s)	106.60		138.52
6-OCH_3									4.01 (3H, s)	62.68
7		146.59		142.09		142.07		142.04		147.60
7-OH	5.65 (s)		5.39 (s)		5.38 (s)		5.39 (s)		5.62 (s)	
8		142.56		136.39		136.40		136.34	6.78 (d, 8.8)	111.70
8-OCH_3	3.76 (s)	61.78								
9		128.14		113.45		113.75		113.59	6.96 (d, 8.8)	116.40
9a		136.02		132.90		133.00		133.00		138.44
11		168.09		168.44		168.40		168.57		167.82
11a		98.47		98.42		98.25		98.75		99.24
1′	6.63 (d, 10.3)	115.53	6.65 (d, 9.9)	115.97	3.28 (2H, d, 7.3)	21.69	3.37 (2H, d, 7.0)	22.61	3.69 (2H, d, 7.3)	22.47
2′	5.53 (d, 10.3)	127.48	5.53 (d, 9.9)	127.27	5.16 (t, 7.3)	121.69	5.19 (overlapped)	121.78	5.29 (t, 7.3)	121.45
3′		78.39		77.55		131.74		134.87		135.69
3′-CH_3	1.44 (6H, s)	28.63	1.45 (6H, s)	29.69	1.77 (3H, s)	17.86	1.80 (3H, s)	18.06	1.87 (3H, s)	17.97
					1.65 (3H, s)	25.77	1.72 (3H, s)	25.81	1.79 (3H, s)	25.83
1″	3.48 (2H, d, 7.0)	24.06	3.42 (2H, d, 7.0)	22.14	6.81 (d, 9.9)	115.75	3.54 (2H, d, 7.0)	22.13		
2″	5.18 (t, 7.0)	121.20	5.13 (t, 7.0)	122.63	5.64 (d, 9.9)	128.63	5.19 (overlapped)	121.15		
3″		133.17		131.54		78.01		134.92		
3″-CH_3	1.81 (3H, s)	17.99	1.83 (3H, s)	18.10	1.45 (6H, s)	28.44	1.86 (3H, s)	17.89		
	1.68 (3H, s)	25.70	1.71 (3H, s)	25.75			1.78 (3H, s)	25.81		
1‴			6.76 (d, 9.9)	116.24	6.75 (d, 9.9)	116.21	6.74 (d, 9.9)	116.20		
2‴			5.74 (d, 9.9)	131.96	5.73 (d, 9.9)	132.06	5.74 (d, 9.9)	130.02		
3‴				78.22		77.58		77.56		
3‴-CH_3			1.56 (6H, s)	28.26	1.45 (6H, s))	27.86	1.45 (6H, s)	27.67		

[a] Values in (δ_H and δ_C) ppm. All signals correspond to 1H in the ^{1}H NMR spectrum, unless otherwise stated. Numbers in parentheses are coupling constants (*J*) in Hz.

Table 2. Inhibitory Effects of Garcinisidones on TPA-Induced EBV-EA Activation[a]

compound	EBV-EA-positive cells (% viability) compound concentration (mol ratio/32 pmol TPA) 1000	500	100	10	IC_{50}[b] (mol ratio/32 pmol TPA)
garcinisidone-A (**1**)	0.0 ± 0.9 (70)	44.2 ± 2.5 (>80)	78.3 ± 1.0 (>80)	91.6 ± 0.7 (>80)	365
garcinisidone-B (**2**)	0.0 ± 0.6 (70)	50.6 ± 2.4 (>80)	81.7 ± 1.2 (>80)	100.0 ± 0.2 (>80)	410
garcinisidone-C (**3**)	0.0 ± 0.7 (70)	41.8 ± 2.1 (>80)	72.2 ± 1.4 (>80)	93.5 ± 0.9 (>80)	370
garcinisidone-D (**4**)	0.0 ± 0.5 (60)	40.4 ± 2.6 (>80)	72.0 ± 1.0 (>80)	92.8 ± 0.7 (>80)	360
garcinisidone-E (**5**)	0.0 ± 0.3 (70)	40.0 ± 2.1 (>80)	71.1 ± 1.5 (>80)	90.2 ± 0.8 (>80)	350
β-carotene[c]	9.1 ± 0.6 (60)	34.3 ± 1.1 (>80)	82.7 ± 1.8 (>80)	100.0 ± 0.2 (>80)	400

[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 (taken to be 100%). Values in parentheses represent the percent viability of Raji cells as determined through Trypan Blue staining. At least 60% viability of Raji cells 2 days after treatment with the compounds is required for an accurate result. [b] IC_{50} represents mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol TPA. [c] Positive control substance.

by HRMS. The presence of a OCH_3 group, a prenyl moiety, and two OH groups in the molecule was indicated by the ^1H NMR spectrum (Table 1). In the aromatic proton region, *ortho*-coupled AB-type doublets and a 1H singlet were observed. In the HMBC data, C−H long-range correlation between a hydrogen-bonded OH and C-2 linked to a lone aromatic H-2 was observed. Correlation from C-4 to H-2 and H-1′ on the prenyl moiety and from C-3 to H-2 and H-1′, which also correlated with C-4a, showed that the location of the prenyl group is at C-4, and one of the OH groups is at C-3. Reciprocal NOE enhancement between H-1′ and the OCH_3 and C−H correlations from C-6 to the OCH_3 and an additional OH, and from C-7 to *ortho*-coupled H-8 and H-9, indicated the locations of 6-OCH_3 and 7-OH groups. On the basis of these data, the structure of garcinisidone-F was concluded to be **6**.

Inhibitory Effects on EBV-EA Induction. The inhibitory effects of **2**−**5** on TPA-induced EBV-EA activation, their effects on the viability of Raji cells, and the 50% inhibitory concentration (IC_{50}) values are shown in Table 2. All of the garcinisidones tested inhibited EBV activation at more than 1×10^2 mol ratio/TPA (18.3−28.9%) and fully blocked TPA-induced EBV-EA activation at high concentration (1×10^3 mol ratio/TPA) without causing a decrease in viability of the Raji cells. These values corresponded to an IC_{50} of 350−410 mol ratio/TPA. Except for garcinisidone-B (**2**), the IC_{50} values of these compounds were lower than that of β-carotene, a vitamin A precursor commonly used in cancer prevention studies.[3] Garcinisidone-B (**2**), lacking a prenyl moiety in the A-ring, showed no inhibitory effect at 1 × 10 mol ratio/TPA, whereas garcinisidone-A (**1**), -C (**3**), -D (**4**), and -E (**5**), with a prenyl moiety at C-2 and/or C-4 in the A-ring, were effective (6.5−9.8%) even at 1 × 10 mol ratio/TPA. The inhibitory activity of garcinisidone-E (**5**), which has two prenyl groups on C-2 and C-4, was slightly stronger than that of garcinisidone-A (**1**), -C (**3**), or -D (**4**), with one prenyl group on the A-ring, at 1 × 10 mol ratio/TPA. Experiments examining the structure−activity relationship, focusing on the garcinisidone nucleus, demonstrated that the 9-prenyl substituent and the dimethylpyran ring on the A and/or B ring were not essential for the activity. In previous studies, we found that the prenyl side chain on xanthone, 7-methoxycoumarin, phenylpropanoid, and isoflavonoid nuclei plays an important role in anti-tumor-promoting activity.[4−7] In view of the present findings taken together, garcinisidones with one or two prenyl side chains on the A-ring might be valuable anti-tumor-promoting agents effective against chemical-induced carcinogenesis. A study examining the anti-tumor-promoting activity of these compounds in vivo is now in progress.

Experimental Section

^1H and ^{13}C NMR, COSY, HMQC, HMBC (J = 8 Hz), and NOE data were measured on JNM A-400, A-600, and/or ECP-500 (JEOL) spectrometers. Chemical shifts are shown in δ (ppm) with tetramethylsilane (TMS) as an internal reference. All mass spectra were obtained under EI conditions, unless otherwise stated, using M-80 (Hitachi), HX-110 (JEOL), and/or JMS-700 (JEOL) spectrometers having a direct inlet system. UV spectra were recorded using a V-550 UV/VIS spectrophotometer (JASCO) in MeOH, and IR spectra were recorded using an IR-230 (JASCO) in $CHCl_3$. Preparative TLC was performed using Kieselgel 60 F_{254} (Merck).

Plant Materials. The plant materials used in this study, *G. neglecta* Vieill. and *G. puat* Guillaumin (Guttiferae), were collected in New Caledonia. *G. neglecta* was collected at North Province, New Caledonia, in January 1994. A voucher specimen (COGE 1223) is preserved in the herbarium of the Medicinal Plants Laboratory of the CNRS, Noumea, New Caledonia. *G. puat* was collected at Port Bois, New Caledonia, in January 1996. A voucher specimen (LIT 0080) is preserved in the herbarium of the Medicinal Plants Laboratory of the CNRS, Noumea, New Caledonia.

Isolation of Garcinisidone-B (2), -C (3), -D (4), and -E (5) from *G. neglecta*. The dried leaves (260 g) of *G. neglecta* were extracted with acetone at room temperature, and the solvent was evaporated under reduced pressure to obtain the acetone extract (19.4 g). The acetone extract was subjected to silica gel column chromatography eluted with hexane−acetone (10:1, 5:1, 5:2, 2:1, 3:2, 1:1, 1:2), acetone, and MeOH, successively, and nine fractions were obtained. Each fraction was further fractionated by silica gel column chromatography and preparative TLC using appropriate combinations of solvents (hexane, acetone, $^iPr_2O$, benzene, Et_2O, CH_2Cl_2, $CHCl_3$, and MeOH) as eluting or developing solvents, and the following compounds were obtained: in the hexane−acetone (5:1) eluate, **2** (74.6 mg), **3** (6.3 mg), **4** (3.4 mg), and **5** (3.1 mg); in the hexane−acetone (5:2) eluate, **2** (30 mg) and **1** (15.6 mg); in the hexane−acetone (2:1) eluate, **1** (249 mg).

Garcinisidone-A (1): pale yellow oil; HRMS *m*/*z* 426.1661 (calcd for $C_{24}H_{26}O_7$, 426.1676); UV, IR, and ^1H NMR spectra were found to be identical to those of authentic garcinisidone-A (**1**) isolated from *G. assigu*.[1]

Garcinisidone-B (2): pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 264 (4.52), 286 (4.11), 328 (3.65) nm; IR ($CHCl_3$) ν_{max} 3533, 1666, 1618, 1271, 1155 cm^{-1}; differential NOE, irradiation of H-6→2% NOE at H-4; irradiation of 8-OCH_3→3, 2, and 2% NOE at H-1″, H-2″, and 7-OH, respectively; HMBC, Figure 1; EIMS, *m*/*z* 424 (M^+, 95), 409 ($M^+ - CH_3$, 100), 369 ($M^+ - CH{=}C(CH_3)_2$, 8), 353 (20), 229 (17), 203 (56), 191 (16), 177 (23), 135 (18); HRMS, *m*/*z* 424.1484 (calcd for $C_{24}H_{24}O_7$, 424.1519).

Garcinisidone-C (3): pale yellow oil. UV (MeOH) λ_{max} (log ϵ) 224 (4.36), 267 (4.45), 284sh (4.08), 310sh (3.75), 329 (3.61) nm; IR ($CHCl_3$) ν_{max} 3543, 1662, 1612, 1223, 1163 cm^{-1}; HMBC C−H three (or two)-bond correlations, C-1 →(1-OH); C-2→1-OH, H-2′; C-3 →H-1′, H-1″; C-4 →(H-1″); C-4a→H-1″; C-5a→(H-6); C-7→(H-6, 7-OH); C8→H-6, 7-OH, H-1‴; C-9a→H-6,

H-1‴; C-11a→1-OH; C-2′→3′-CH_3; 3′-CH_3→H-2′; C-2″→3″-CH_3, (H-1″); C-3″→H-1″, (3″-CH_3); C-2‴→3‴-CH_3; C-3‴→H-1‴, (H-2‴); 3‴-CH_3→H-2‴; EIMS, *m/z* 476 (M^+, 100), 461 ($M^+ - CH_3$, 59), 421 ($M^+ - CH{=}C(CH_3)_2$, 6), 285 (18), 269 (17), 243 (37), 215 (35), 203 (31), 189 (21), 177 (28), 135 (26); HRMS, *m/z* 476.1839 (calcd for $C_{28}H_{28}O_7$, 476.1833).

Garcinisidone-D (4): pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 224 (4.31), 263 (4.33), 283sh (4.01), 334 (3.55) nm; IR ($CHCl_3$) ν_{max} 3535, 1664, 1620, 1211, 1163 cm^{-1}; differential NOE, irradiation of H-6→5% NOE at H-1″; HMBC C−H three (or two)-bond correlations, C-1→H-1′, (1-OH); C-2→1-OH, H-2′; C-3→H-1′, H-1″; C-4→H-2″; C-4a→H-1″; C-5a→(H-6); C-6→7-OH; C-7→(H-6, 7-OH); C-8 →H-6, H-1‴, 7-OH; C-9→H-2‴; C-9a→H-6; C-11a→1-OH; C-2′→3′-CH_3, (H-1′); C-3′→(H-2′, 3′-CH_3); 3′-CH_3→H-2′; C-2″→3″-CH_3; C-3″→H-1″, (H-2″); C-2‴→3‴-CH_3; C-3‴→H-1‴, (H-2‴); 3‴-CH_3→H-2‴; EIMS, *m/z* 476 (M^+, 100), 461 ($M^+ - CH_3$, 89), 421 ($M^+ - CH{=}C(CH_3)_2$, 14), 363 (18), 285 (17), 243 (42), 215 (39), 203 (24), 135 (22); HRMS, *m/z* 476.1830 (calcd for $C_{28}H_{28}O_7$, 476.1833).

Garcinisidone-E (5): pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 223 (4.52), 272 (4.14), 282 (4.13), 320 (3.73) nm; IR ($CHCl_3$) ν_{max} 3545, 1664, 1616, 1223, 1165 cm^{-1}; differential NOE, irradiation of H-1″→17% NOE at H-6; HMBC C−H three (or two)-bond correlations, C-1→(1-OH); C-2→1-OH, (H-1′); C-3→H-1′, (3-OH), H-1″; C-4→3-OH, (H-1″); C-4a→H-1″; C-5a→(H-6); C-6→7-OH; C-7→(H-6), (7-OH); C-8→H-6, 7-OH, H-1‴; C-9→H-2‴; C-11a→1-OH; C-2′→3′-CH_3, (H-1′); C-3′→H-1′, (3′-CH_3); 3′-CH_3→H-2′; C-2″→3″-CH_3, (H-1″); C-3″→H-1″; 3″-CH_3→H-2″; C-2‴→3‴-CH_3; C-3‴→H-1‴, (H-2‴); EIMS, *m/z* 478 (M^+, 100), 423 ($M^+ - CH{=}C(CH_3)_2$, 23), 366 (16), 203 (53), 189 (26), 177 (39), 135 (46); HRMS, *m/z* 478.1971 (calcd for $C_{28}H_{30}O_7$, 478.1989).

Isolation of Garcinisidone-F (6) from *G. puat*. The dried leaves (600 g) of *G. puat* collected in New Caledonia were extracted with acetone at room temperature. The solvent was evaporated in vacuo to obtain the acetone extract (41.95 g). The extract was subjected to silica gel column chromatography eluted with hexane−acetone (19:1, 9:1, 4:1, 7:3, 3:2, 1:1, 3:7), acetone, and MeOH, successively, and 10 fractions were obtained. The fraction eluted with hexane−acetone (3:2) was further fractionated by silica gel column chromatography and preparative TLC using hexane−acetone (2:1), Et_2O−hexane (3:2), $CHCl_3$−MeOH (98:2), and/or $^iPr_2O$−hexane (4:1) as eluting or developing solvents to obtain **6** (1.6 mg), 1,3,7-trihydroxy-2-(2-butenyl-3-methyl)xanthone (1.4 mg),[8] and 5-hydroxymethyl-2-furaldehyde (2.8 mg).[9] The fraction eluted with hexane−acetone (1:1) was further fractionated by silica gel column chromatography and preparative TLC [solvents: benzene−acetone (4:1), benzene−hexane−acetone (4:4:1), hexane−EtOAc (3:2), benzene−$CHCl_3$−$^iPr_2O$ (1:1:1)] to obtain naringenin (1.8 mg) and apigenin (5 mg). 1,3,7-Trihydroxy-2-(2-butenyl-3-methyl)xanthone and 5-hydroxymethyl-2-furaldehyde were identified through comparison of their spectral data with those in the literature.[8,9] Naringenin and apigenin were spectroscopically identical with authentic samples of commercially available material from Tokyo Kasei Kogyo Co., Ltd., Japan.

Garcinisidone-F (6): colorless oil; UV (MeOH) λ_{max} (log ϵ) 222 (4.28), 276 (3.90), 314 (3.59) nm; IR ($CHCl_3$) ν_{max} 3568 (br), 1669, 1625, 1246, 1155 cm^{-1}; differential NOE, irradiation of 6-OCH_3→2% NOE at H-1′; irradiation of H-1′→3% NOE at 6-OCH_3; HMBC C−H three (or two)-bond correlations, C-1→(1-OH); C-2→1-OH; C-3→H-1′, (H-2); C-4→H-2, (H-1′); C-4a→H-1′; C-5a→H-9; C-6→6-OCH_3, 7-OH; C-7→H-9, (H-8), (7-OH); C-8→7-OH; C-9a→H-8, (H-9); C-11a→1-OH, H-2; C-2′→(H-1′), 3′-CH_3; C-3′→H-1′, (3′-CH_3); EIMS, *m/z* 358 (M^+, 100), 327 (19), 302 (41), 271 (16), 257 (13), 219 (26), 203 (13), 165 (16); HRMS, *m/z* 358.1045 (calcd for $C_{19}H_{18}O_7$, 358.1053).

In Vitro EBV-EA Activation Experiments. Inhibition of EBV-EA activation was assayed by the method described previously.[4−7,10−12] In brief, Raji cells were grown to a density of 10^6 cells/mL, harvested by centrifugation, and resuspended in RPMI 1640 medium (Nakalai Tesque, Kyoto, Japan) supplemented with 10% FCS and containing 4 mM *n*-butyric acid as inducer, 32 pmol of TPA (20 ng/mL in DMSO), and 32, 16, 3.2, or 0.32 nmol of the test compound (added as a DMSO solution). The cells were incubated at 37 °C for 48 h. Cell number and cell viability were determined after 48 h by means of a hemocytometer (Trypan Blue staining method). Untreated cultures served as the controls. 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.

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