Dimeric Flavonol Glycoside and Galloylated *C*-Glucosylchromones from *Kunzea ambigua*

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A novel dimeric flavonol glycoside linked through a methylene group, kunzeagin A (**1**), and six new chromone *C*-glucosides, kunzeachromones A−F (**2**−**7**), were isolated along with seven known compounds from the leaf extract of *Kunzea ambigua*. The structures of these compounds were elucidated on the basis of spectroscopic analyses and chemical properties. Kunzeachromones A−F provided additional examples of galloylated *C*-glucosidic chromones occurring in the Myrtaceae. Kunzeagin A (**1**) and major constituents of this plant (6-*C*- and 8-*C*-glucosylchromones and their monogallates) exhibited potent inhibitory effects on activation of Epstein−Barr virus early antigen induced by 12-*O*-tetradecanoylphorbol 13-acetate in Raji cells.

Kunzea ambigua (SM.) Druce, which is a plant of the Myrtaceae family, is a bushy shrub that is endemic to eastern Australia (Tasmania, New South Wales, and Victoria). Its essential oil, which contains α-pinene, α-terpinene, and 1,8-cineole, has been used as an insecticide and antiseptic and as a remedy for the treatment of wounds.[1] Although myrtaceous plants are known to be rich in both polyphenols and terpenoids,[2,3] there have been no reports on the polyphenol constituents of *K. ambigua*. As part of our studies on the polyphenolics of the Myrtaceae family, we have investigated the constituents of this plant and isolated 14 polyphenols, including seven newly discovered phenolic compounds. In this report, we describe the elucidation of the structures of these novel compounds. Some of the major constituents of the plant were also evaluated for an in vitro inhibitory effect on activation of Epstein−Barr virus early antigen (EBV-EA) induced by tumor promoter.

Results and Discussion

A concentrated aqueous acetone homogenate of dried leaves was extracted successively with ether, ethyl acetate, and *n*-butanol, thereby producing an extract and a water-soluble portion at each step. The ethyl acetate and *n*-butanol extracts were each subjected to a series of column chromatography steps, which involved Diaion HP-20, Toyopearl HW-40, MCI GEL CHP-20P, and Sephadex LH-20 columns, resulting in the purification of a new flavonoid named kunzeagin A (**1**), six new chromones, kunzeachromones A−F (**2**−**7**), and seven known compounds. The known compounds were identified as quercitrin, pinoquercetin (6-methylquercetin),[4] myricetin 3-*O*-*β*-galactopyranoside,[5,6] 6-*β*-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (**8**),[6] 6-*β*-*C*-(2′-*O*-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**9**),[7] 8-*β*-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (**10**),[8] and 8-*β*-*C*-(2′-*O*-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**11**).[7]

Kunzeagin A (**1**) was obtained as yellow needles, and its molecular formula of $C_{44}H_{42}O_{22}$ was established by high-resolution electrospray ionization (HRESI) MS [*m*/*z* 923.2233 (M + H)$^+$]. Its UV spectrum (λ_{max} 271 and 352 nm) was indicative of its flavonol skeleton. The ^{1}H NMR spectrum of **1** in DMSO-d_6 contained six hydrogen-bonded hydroxyl proton signals at δ 13.17, 13.02, 9.66, 9.64, 9.31, and 9.28; the first two signals were characteristic of a 5-OH group. The spectrum exhibited two ABX system sets [δ 7.49/7.27 (each d, J = 2.0 Hz); 6.86/6.83 (each d, J = 8.5 Hz); 7.37/7.23 (each dd, J = 2.0, 8.5 Hz)] due to B-ring protons, and only one A-ring proton appeared as a singlet at δ 6.35. The presence of two rhamnose residues was also suggested by two secondary methyl signals (δ 0.83/0.80, each d, J = 6.0 Hz) and two anomeric proton signals [δ 5.23/5.25 (each br s)]. These spectral features, together with that of an isolated methylene (δ 4.05, br s) and a methyl signal (δ 1.98, s), imply that **1** is a dimer of quercitrin, in which each glycoside is linked through a methylene bridge between the A-rings, with one of the A-rings containing a methyl group at C-6 or C-8. This assumption was consistent with the ^{13}C NMR data (Table 1). The HMBC measurement indicated

Table 1. ^{1}H and ^{13}C NMR Spectral Data for Kunzeagin A (**1**) (δ_H, 500 MHz; δ_C, 126 MHz, in DMSO-d_6)

position	δ_H	δ_C
2		157.14, 157.08
3		134.2, 130.0
4		178.2, 177.9
5		158.7(U), 156.3(L)
5-OH	13.17 (1H, s, U),[a] 13.02 (1H, s, L)	
6		110.0(U), 106.3(L)
7		164.0(U), 160.7(L)
8	6.35 (1H, s, U)	93.3(U), 105.0(L)
9		154.6(U), 152.1(L)
10		103.8(2C)
11	4.05 (2H, br s, U), 1.98 (3H, s, L)	16.3(U), 8.1(L)
1′, 6′	7.37, 7.23 (each 1H, dd, 2.0, 8.5, H-6′)[b]	121.5, 121.0, 121.3(2C)
2′, 5′	7.49, 7.27 (each 1H, d, 2.0, H-2′) 6.86, 6.83 (each 1H, d, 8.5, H-5′)	116.2, 115.8, 115.62, 115.56
3′		145.4, 145.3
4′		148.54, 148.50
rhamnose		
1″	5.25, 5.23 (each 1H, br s)	102.1, 102.0
2″-5″	3.1−3.6	71.43, 71.35, 70.8(2C), 70.6, 70.5, 70.3, 70.2
6″	0.83, 0.80 (each 3H, d, 6)	17.69, 17.67

[a] U: upper unit, L: lower unit. [b] J values (Hz) are in parentheses.

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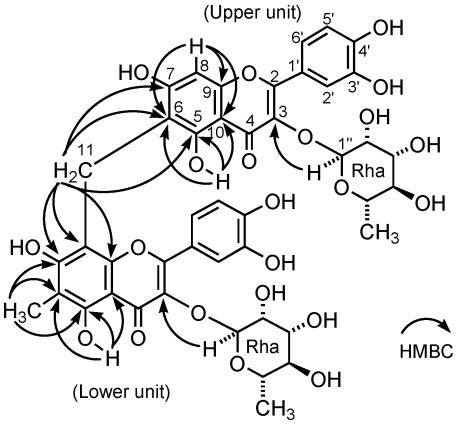
Table 2. ^{1}H NMR Spectral Data for Kunzeachromones A−F (**2**−**7**) (500 MHz, DMSO-d_6)

proton	2	3	4	5	6	7
OH-5	13.49 (1H, s)[a]	12.98 (1H, s)	13.41 (1H, s)	13.51 (1H, s)	12.92 (1H, s)	12.94 (1H, s)
H-3	6.07 (1H, s)	6.21 (1H, s)	6.10 (1H, s)	6.11 (1H, s)	6.08 (1H, s)	6.11 (1H, s)
H-6		6.10 (1H, s)			6.20 (1H, s)	6.24 (1H, s)
H-8	6.29 (1H, s)		6.26 (1H, s)	6.28 (1H, s)		
H-11	2.81 (1H, hept, 7)	3.02 (1H, hept, 7)	2.28 (3H, s)	2.28 (3H, s)	2.40 (3H, s)	2.47 (3H, s)
H-12, 13	1.18 (6H, d, 7)	1.44, 1.39 (each 3H, d, 7)				
glucose						
C-1′	5.03 (1H, d, 10)	5.06 (1H, d, 10)	4.85 (1H, d, 10)	5.02 (1H, d, 10)	4.86 (1H, d, 10)	5.04 (1H, d, 10)
C-2′	5.97 (1H, br t, 10)	5.88 (1H, t, 10)	5.67 (1H, m)	5.97 (1H, m)	5.56 (1H, t, 10)	5.81 (1H, t, 10)
C-3′	5.25 (1H, t, 10)	5.34 (1H, t, 10)	3.42−3.46	5.25 (1H, t, 10)	3.52 (1H, m)	5.32 (1H, t, 10)
C-4′	3.55 (1H, m)	3.63 (1H, t, 10)	3.25 (1H, m)	3.42−3.54	3.52 (1H, m)	3.65 (1H, t, 10)
C-5′	3.43 (1H, m)	3.51 (1H, m)	3.25 (1H, m)	3.42−3.54	3.25 (1H, m)	3.46 (1H, m)
C-6′	3.75 (1H, br d, 11.5) 3.50 (1H, dd, 6.5, 11.5)	3.78 (1H, br d, 11) 3.51 (1H, m)	3.73 (1H, br d, 11.5) 3.42−3.46	3.75 (1H, br d, 11) 3.42−3.54	3.72 (1H, br d, 11.5) 3.46 (1H, m)	3.75 (1H, br d, 11) 3.55 (1H, dd, 5, 11)
galloyl						
C-2″, 6″	6.87, 6.69 (each 2H, s)	6.84, 6.63 (each 2H, s)	6.77 (2H, s)	6.87, 6.64 (each 2H, s)	6.75 (2H, s)	6.88, 6.63 (each 2H, s)

[a] *J* values (Hz) are in parentheses.

that each 5-OH proton correlated through two- and three-bond couplings with C-5, C-6, and C-10 of each A-ring. The signals assignable to each C-6 (δ 110.0 and 106.3) were also correlated with the methylene and methyl protons, respectively, which establishes the location of those alkyl substituents. The location of the rhamnosyl residues was deduced by the correlation of each anomeric proton with the C-3 signals of upper and lower units, respectively. The other long-range correlations are illustrated in the formula.



1

Acid hydrolysis of **1** yielded an aglycone whose ^{1}H NMR and HRESIMS spectra were coincident with the proposed structure. A liberated sugar from **1** was identified as L-rhamnose by co-chromatography with an authentic sample on an HPLC system that was equipped with an optical rotation detector. The L-series of rhamnose was also confirmed by enzymatic hydrolysis of **1** with naringinase. On the basis of these data, structure **1** was assigned to kunzeagin A.

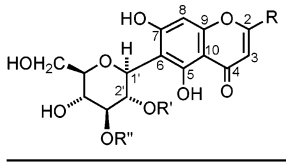
Numerous flavonoids, including prenylated flavonoids, biflavonoids, and flavan-3-ol oligomers (condensed tannins), have been reported to date. To the best of our knowledge, kunzeagin A constitutes a new class of naturally occurring bisflavonol glycoside that is bonded through a methylene bridge. The existence of this class of flavonol in the Myrtaceae is not surprising, because many formylated phloroglucinol derivatives and methylated flavonol monomers have been found among the plants of this family.[8,9]

Kunzeachromone A (**2**), which is a pale yellow amorphous powder, exhibited an $[M + H]^+$ ion peak at *m*/*z* 687.1359 in the HRESIMS, corresponding to the molecular formula $C_{32}H_{30}O_{17}$. The ^{1}H NMR spectrum of **2** revealed the presence of a hydrogen-bonded OH at δ 13.49, two 2H singlets due to galloyl groups at δ 6.87 and 6.69, two 1H singlets at δ 6.29 and 6.07, a methine proton signal at δ 2.81 (1H, hept, J = 7 Hz) coupled with two methyl signals at δ 1.18 (6H, d, J = 7 Hz), and aliphatic proton signals arising from a β-glucopyranosyl moiety (Table 2). These data were similar to those for 6-β-*C*-(2′-*O*-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**9**),[7] except for the presence of the extra galloyl group. In the ^{13}C NMR spectrum of **2**, resonances that were ascribable to two galloyl, isopropyl, chromone, and glucose moieties were also observed (Table 3). Treatment of **2** with tannase furnished 6-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (**8**) and **9**, as well as gallic acid. The location of the extra galloyl group at C-3′ was determined by comparison of the ^{1}H NMR spectrum of **2** with that of **9**, which revealed a remarkable downfield shift (ca. Δ 1.8 ppm) of H-3′ in **2**. On the basis of these findings, the structure of kunzeachromone A was determined as **2**.

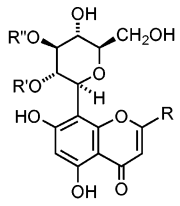
Kunzeachromone B (**3**) has the molecular formula $C_{32}H_{30}O_{17}$, which is the same as that of **2**, as revealed by HRESIMS, which showed an $[M + H]^+$ ion peak at *m*/*z* 687.1360. The ^{1}H and ^{13}C NMR spectral data for **3** (Tables 2 and 3) were similar to those for 8-β-*C*-(2′-*O*-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**11**), except for resonances due to the extra galloyl group, which suggests that compound **3** is 8-*C*-digalloylglucosyl-5,7-

Table 3. ^{13}C NMR Spectral Data for Kunzeachromones A−F (**2**−**7**) (126 MHz, DMSO-d_6)

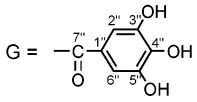
carbon	2	3	4	5	6	7
C-2	174.9	174.5	167.8	167.7	167.6	167.7
C-3	105.3	105.6	107.0	106.3	107.8	108.0
C-4	182.2	182.4	182.0	182.0	182.2	182.2
C-5	160.9	161.0	160.4	161.1	160.8	161.0
C-6	106.4	97.9	108.1	108.1	97.6	98.0
C-7	163.2	162.6	162.4	163.1	162.2	162.4
C-8	93.4	102.1	93.9	93.4	103.9	103.9
C-9	157.0	157.2	157.0	157.1	157.1	157.1
C-10	103.1	104.0	102.9	103.0	102.8	102.0
C-11	32.4	33.1	20.0	19.9	20.0	20.1
C-12	19.78	20.3				
C-13	19.75	20.1				
glucose						
C-1′	70.7	70.8	70.8	70.7	70.6	70.7
C-2′	69.7	70.2	72.0	69.7	72.5	70.1
C-3′	77.6	77.0	76.7	77.6	76.1	77.0
C-4′	68.6	68.9	70.8	68.7	70.8	68.5
C-5′	81.7	81.9	82.0	81.8	81.7	81.5
C-6′	61.2	61.3	61.6	61.2	61.5	61.0
galloyl						
C-1″	119.7, 119.0	119.6, 118.7	119.9	119.7, 119.1	119.7	119.6, 118.7
C-2″, 6″	108.9 (2C), 108.7 (2C)	108.9 (2C), 108.7 (2C)	108.9 (2C)	108.9 (2C), 108.7 (2C)	108.8 (2C)	108.9 (2C), 108.7 (2C)
C-3″, 5″	145.4 (2C), 145.3 (2C)	145.5 (2C), 145.4 (2C)	145.4 (2C)	145.5 (2C), 145.3 (2C)	145.5 (2C)	145.6 (2C), 145.5 (2C)
C-4″	138.4, 138.3	138.7, 138.6	138.2	138.5, 138.4	138.8	138.7, 138.5
C-7″	165.4, 164.4	165.4, 164.8	164.8	165.3, 164.6	165.0	165.5, 164.8



	R	R'	R"
2	-CH(CH$_3$)$_2$ (11, 12,13)	G	G
4	-CH$_3$	G	H
5	-CH$_3$	G	G
8	-CH(CH$_3$)$_2$	H	H
9	-CH(CH$_3$)$_2$	G	H
12	-CH$_3$	H	H



	R	R'	R"
3	-CH(CH$_3$)$_2$	G	G
6	-CH$_3$	G	H
7	-CH$_3$	G	G
10	-CH(CH$_3$)$_2$	H	H
11	-CH(CH$_3$)$_2$	G	H
13	-CH$_3$	H	H



dihydroxy-2-isopropylchromone. The galloylation at C-3′ of the glucosyl residue of **3** was substantiated by a notable downfield shift of H-3′ (ca. Δ 1.8 ppm) relative to **11**. Enzymatic hydrolysis of **3** with tannase afforded 8-*β*-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (**10**), **11**, and gallic acid. On the basis of these data, the structure of kunzeachromone B was established to be **3**.

Kunzeachromones C (**4**) and D (**5**) had molecular formulas $C_{23}H_{22}O_{13}$ and $C_{30}H_{26}O_{17}$, respectively, by HRESIMS. The ^1H and ^{13}C NMR spectra of **5** (Tables 2 and 3) were very similar to those of **2**, except for the presence of a methyl signal (δ_H 2.28 and δ_C 19.9) in place of the isopropyl group at C-2 in **2**, which indicates that **5** is the 2′,3′-digallate of 6-*β*-*C*-glucopyranosyl-5,7-dihydroxy-2-methylchromone (**12**; biflorin).[10] The ^1H NMR spectrum of **4** exhibited a 2H singlet due to one galloyl group (δ 6.67) and an upfield shift in H-3′ compared with that of **5** (δ 5.25 in **5** → δ 3.42−3.46 in **4**); the remaining signals were similar to those of **5**. The structural relationship between **4** and **5** was confirmed chemically by enzymatic hydrolysis of **5** with tannase, which afforded **4** and biflorin (**12**), along with gallic acid. The ^{13}C NMR spectra of **4** and **5** were also consistent with the proposed structures.

Kunzeachromones E (**6**) and F (**7**) were isomers of **4** and **5** at the position of the *C*-glucosyl residue, on the basis of their respective molecular formulas of $C_{23}H_{22}O_{13}$ and $C_{30}H_{26}O_{17}$ and their NMR spectra, which were similar, except for the chemical shifts of H-6 to H-8 and C-6 to C-8, respectively. Therefore, compounds **6** and **7** were assumed to be the mono- and digallate forms of 8-*β*-*C*-glucopyranosyl-5,7-dihydroxy-2-methylchromone (**13**; isobiflorin).[11] Treatment of **6** and **7** with tannase afforded isobiflorin (**13**) and gallic acid. The locations of the galloyl groups in each compound were evident from the ^1H NMR spectra that were assigned by COSY (Table 2). Consequently, the structures of kunzeachromones E and F were represented by **6** and **7**.

Galloylated *C*-glucosylchromones have been identified only in *Baeckea frutescens*[7] and *Syzygium aromaticum*[11] in Myrtaceae. Isolation of kunzeachromones from the genus *Kuzea* would thus be of chemotaxonomical significance. It is also noteworthy that kunzeachromones A (**2**), B (**3**), D (**5**), and F (**7**) are the first examples of *C*-glucosylchromone digalloyl esters.

Polyphenols possessing galloyl unit(s) represented by (−)-epigallocatechin gallate (EGCG) from green tea and many hydrolyzable tannins have been reported to be possible chemopreventive agents of cancer on the basis of their remarkable suppression of tumor promotion in multistep mechanisms of chemical carcinogenesis.[12−14] To develop a beneficial function of the *K. ambigua* leaves, some major constituents obtained in the present study were assessed for their inhibitory effects on the activation of EBV-EA using Raji cells, which has been frequently used as an in vitro preliminary screening test for searching possible anti-tumor-promoting agents in nature.[15] Tested samples were 6-*C*- and 8-*C*-glucosidic chromones (**8**, **10**) and their monogallates (**9**, **11**) in addition to bisflavonoid (**1**). The results are shown in Table 4. All of them significantly inhibited (67−76%) EBV-EA activation induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) at a concentration of 500 mol ratio/TPA without exhibiting cytotoxicity. These activities were more potent or equivalent to that (65.1% inhibition) of EGCG. It is noteworthy that 6-*C*-glucosylchromones were more potent than 8-*C* congeners, and

Table 4. Relative Ratio of EBV-EA Activation[a] with Respect to Positive Control (100%) in the Compounds from *K. ambigua*

	concentration (mol ratio/TPA)[b]			
	1000	500	100	10
1	4.3 (70)[c]	32.5	66.1	90.5
8	4.6 (70)	23.7	65.0	91.0
9	5.9 (70)	26.8	70.4	94.8
10	9.1 (70)	29.6	72.5	100
11	11.5 (70)	31.6	74.7	100
EGCG[d]	6.4 (70)	34.9	68.1	87.7

[a] Values represent percentages relative to the positive control value (100%). [b] TPA concentration was 20 ng (32 pmol)/mL. [c] Values in parentheses are the viability percentage of Raji cells; unless otherwise stated, the viability percentage of Raji cells was more than 80%. [d] Reference compound.

galloylation on these skeletones resulted in reducing the inhibitory effect on EBV-EA activation. Further in vivo anti-tumor-promoting effects and other biological activity of the compounds in *K. ambigua* are under investigation.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanaco micro-melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for ^{1}H, and 126 MHz for ^{13}C), and the chemical shifts are given in δ (ppm) values relative to that of the solvent [DMSO-d_6 (δ_H 2.49; δ_C 39.7), acetone-d_6 (δ_H 2.04; δ_C 29.8)] and tetramethylsilane. The standard pulse sequences that were programmed into the instrument (VXR-500) were used for each two-dimensional measurement. The J_{CH} value was set at 6 Hz in the HMBC spectra. Optical rotations were measured with a Jasco DIP-1000 polarimeter. UV spectra were measured with a Hitachi U-2000 spectrophotometer. ESIMS was recorded on a Micromass Auto Spec OA-TOF mass spectrometer (solvent: 50% aqueous MeOH containing 0.1% NH_4OAc; flow rate: 0.02 mL/min). Normal-phase HPLC was conducted on a YMC-Pack SIL A-003 column (4.6 i.d. × 250 mm; YMC Co. Ltd.) and developed at room temperature with a solution of *n*-hexane/MeOH/tetrahydrofuran/formic acid (55:33:11:1) that contained 450 mg/L oxalic acid (flow rate: 1.5 mL/min; detection: UV 280 nm). Reversed-phase HPLC was performed with a YMC-Pack ODS-A A-302 column (4.6 mm i.d. × 150 mm) and developed at 40 °C with 10 mM H_3PO_4/10 mM KH_2PO_4/EtOH/EtOAc (45:45:8:2, solvent A) or 10 mM H_3PO_4/10 mM KH_2PO_4/MeCN (42.5:42.5:15, solvent B; 40:40:20, solvent C). Column chromatography was carried out on Diaion HP-20, MCI GEL CHP-20P (Mitsubishi Kasei Co.), Toyopearl HW-40 (coarse grade; Tosoh Co.), YMC-GEL ODS AQ 120-50S (YMC Co., Ltd.), Sephadex LH-20 (Pharmacia Fine Chemicals Co., Ltd.), and Mega Bond Elut C_{18} (Varian Inc.) columns.

Plant Materials. *K. ambigua* (SM.) Druce was collected in April 1998 from the herbal garden of Pola Co. Ltd., Japan. The voucher specimen has been deposited in the herbarium of Pola Co. Ltd and in the Medicinal Herbal Garden of Okayama University (specimen no. OKP-MY98003).

Extraction and Isolation. Dry leaves (700 g) of *K. ambigua* were homogenized in 70% aqueous acetone (10 L), and the homogenate was filtered and concentrated. The concentrated solution was extracted successively with ether, EtOAc, and water-saturated *n*-BuOH. A 2.0 g portion of the 11.0 g EtOAc extract was chromatographed over a Toyopearl HW-40 column (2.2 cm i.d. × 42 cm) with H_2O containing increasing amounts of MeOH in a stepwise gradient. The 20% and 30% MeOH eluates were subjected separately to column chromatography over an MCI GEL CHP-20P column (1.1 cm i.d. × 25 cm) with aqueous MeOH, to yield 6-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (**8**) (13 mg) and 8-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (**10**) (21 mg). The 40% MeOH eluate was further purified by column chromatography over an MCI GEL CHP-20P column (1.1 cm i.d. × 25 cm) to yield 6-β-(2′-*O*-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**9**) (39 mg) and 8-β-(2′-*O*-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**11**) (31 mg). The 50% MeOH eluate was similarly chromatographed over an MCI GEL CHP-20P column (1.1 cm i.d. × 25 cm) with aqueous MeOH, and a Sephadex LH-20 column (1.1 cm i.d. × 28 cm) with EtOH/MeOH, to afford kunzeagin A (**1**) (44 mg) and pinoquercetin (4 mg). The other portion (3.3 g) of the EtOAc extract was subjected to column chromatography over a Toyopearl HW-40 column (2.2 cm i.d. × 42 cm) with aqueous MeOH in a stepwise gradient. The 30% and 40% MeOH eluates were purified by column chromatography over a YMC-GEL ODS-A 120-S50 column (1.1 cm i.d. × 44 cm) with aqueous MeOH to afford **9** (44 mg), **11** (71 mg), and myricetin 3-*O*-β-galactopyranoside (10 mg), respectively. The 50% and 60% MeOH eluates were separately rechromatographed over a YMC-GEL ODS-A 120-S50 column (1.1 cm i.d. × 44 cm) with aqueous MeOH, yielding kunzeachromones A (**2**) (23 mg), B (**3**) (17 mg), D (**5**) (31 mg), and F (**7**) (25 mg). A 10.1 g portion of the 24.6 g *n*-BuOH extract was fractionated by column chromatography over a Diaion HP-20 column (5.0 cm i.d. × 50 cm) and developed with H_2O and increasing amounts of MeOH in a stepwise gradient. The 30% MeOH eluate was purified by column chromatography over a Toyopearl HW-40 column (2.2 cm i.d. × 40 cm) and/or a Mega Bond Elut C_{18} cartridge with aqueous MeOH to afford myricetin 3-*O*-β-galactopyranoside (3 mg). The 100% MeOH eluate was further purified on a Toyopearl HW-40 column (2.2 cm i.d. × 35 cm) and an MCI GEL CHP-20P column (1.1 cm i.d. × 23 cm) with aqueous MeOH and a Sephadex LH-20 column (1.1 cm i.d. × 21 cm) with EtOH−MeOH to yield **1** (11 mg) and **8** (14 mg). The remainder of the *n*-BuOH extract was pooled and further purified by column chromatography over Diaion HP-20, MCI GEL CHP-20P, and YMC-GEL ODS AQ 120-50S columns and a Mega Bond Elut cartridge with aqueous MeOH, and/or a Sephadex LH-20 column with EtOH-MeOH, to afford kunzeachromones C (**4**) (24 mg) and E (**6**) (19 mg).

Kunzeagin A (1): yellow needles, mp 243−245 °C (from MeOH); $[\alpha]^{23}_D$ −282° (*c* 1, MeOH); UV (MeOH) λ_{max} (log ϵ) 271 (4.67), 352 (4.53) nm; for the ^{1}H and ^{13}C NMR spectral data, see Table 1; ESIMS *m/z* 923 $[M + H]^+$, 940 $[M + NH_4]^+$; HRESIMS *m/z* 923.2233 $[M + H]^+$ ($C_{44}H_{42}O_{22}$+H, 923.2246).

Hydrolysis of 1. A 10 mg solution of **1** in 1 M HCl (5 mL) was heated for 1 h in a boiling water bath. After cooling, the reaction mixture was separated using a Mega Bond Elut C_{18} cartridge column to yield the aglycone of **1** (5 mg) from the MeOH eluate.

To a 1 mL solution of **1** (0.6 mg) in phosphate buffer (pH 3.8) was added 1.0 mg of naringinase (N-1385, Sigma), and the mixture was incubated at 40 °C for 48 h. The HPLC analysis revealed the completion of hydrolytic cleavage of the glycosidic linkage.

Aglycone of 1: yellow powder; ^{1}H NMR (500 MHz, acetone-d_6+D_2O) δ 8.03, 7.73 (each 1H, d, J = 2.0 Hz, H-2′), 7.89, 7.62 (each 1H, dd, J = 2.0, 8.5 Hz, H-6′), 6.98, 6.94 (each 1H, d, J = 8.5 Hz, H-5′), 6.71 (1H, s, U-H-8), 4.15 (2H, s, U-H-11), 2.01 (3H, s, L-H-11); ESIMS *m/z* 631 $[M + H]^+$; HRESIMS *m/z* 631.1313 $[M + H]^+$ ($C_{32}H_{22}O_{14}$+H, 631.1089). The water eluate was neutralized and analyzed by reversed-phase HPLC with an optical rotation detector (Shodex OR-2; Showa Denko Co., Ltd.) [column, TSK-gel Amide-80 (4.6 mm i.d. × 250 mm) (Tosoh Co.); solvent, MeCN/H_2O (75:25); column temp, 35 °C], to detect L-rhamnose (t_R 6.5 and 8.6 min) as the negative peaks identical with that of an authentic specimen.

Kunzeachromone A (2): pale yellow amorphous powder; $[\alpha]^{23}_D$ +30° (*c* 1, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.83), 258 (4.50), 277 (4.41) nm; for ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; ESIMS *m/z* 687 $[M + H]^+$; HRESIMS *m/z* 687.1359 $[M + H]^+$ ($C_{32}H_{30}O_{17}$+H, 687.1361).

Kunzeachromone B (3): pale yellow amorphous powder; $[\alpha]^{23}_D$ −23° (*c* 1, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.84), 258 (4.48), 277 (4.42) nm; for ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; ESIMS *m/z* 687 $[M + H]^+$; HRESIMS *m/z* 687.1360 $[M + H]^+$ ($C_{32}H_{30}O_{17}$+H, 687.1361).

Kunzeachromone C (4): colorless fine needles, mp 212−214 °C (from MeOH); $[\alpha]^{23}_D$ −87° (*c* 1, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.73), 258 (4.45), 274 (4.23) nm; for ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; ESIMS *m/z* 507 $[M + H]^+$, 529 $[M + Na]^+$; HRESIMS *m/z* 507.1193 $[M + H]^+$ ($C_{23}H_{22}O_{13}$+H, 507.1139).

Kunzeachromone D (5): pale yellow amorphous powder, $[\alpha]^{23}_D$ −23° (*c* 1, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.74), 259 (4.33), 277 (4.32) nm; for ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; ESIMS *m/z* 659 $[M + H]^+$; HRESIMS *m/z* 659.1238 $[M + H]^+$ ($C_{30}H_{26}O_{17}$+H, 659.1248).

Kunzeachromone E (6): colorless needles, mp 204−205 °C (from MeOH); $[\alpha]^{23}_D$ −135° (*c* 1, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.72), 257 (4.40), 281 (4.25) nm; for ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; ESIMS *m/z* 507 $[M + H]^+$, 529 $[M + Na]^+$; HRESIMS *m/z* 507.1085 $[M + H]^+$ ($C_{23}H_{22}O_{13}$+H, 507.1139).

Kunzeachromone F (7): pale yellow amorphous powder, $[\alpha]^{23}_D$ −4° (*c* 1, MeOH); UV λ_{max} (MeOH) nm (log ϵ) 216 (4.78), 257 (4.39), 277 (4.34); for ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; ESIMS *m/z* 659 $[M + H]^+$; HRESIMS *m/z* 659.1263 $[M + H]^+$ ($C_{30}H_{26}O_{17}$+H, 659.1248).

Partial Hydrolyses of 2−7 with Tannase. A solution of each compound (0.4−0.5 mg) in H_2O (0.4 mL) was treated at 37 °C for an appropriate time with two drops of tannase, which was obtained from *Aspergillus niger*, as described previously.[16] After the addition of EtOH, the reaction mixture was evaporated to dryness. The residue was analyzed by normal- and reversed-phase HPLC. The liberated compounds in addition to gallic acid from each compound were as follows and identified respectively by co-chromatography with authentic specimens: Biflorin (**12**) was obtained by hydrolysis of **4** (5 mg) with tannase and identified by comparison of the spectral data with those reported in the literature.[10] **12**: pale yellow amorphous powder; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.39 (1H, s, 6-OH), 6.37 (1H, s, H-8), 6.16 (1H, s, H-3), 4.55 (1H, d, J = 10.0 Hz, Glc-H-1′), 3.0−4.1 (6H, m, Glu-H-2′-6′), 2.34 (3H, s, CH_3); ESIMS *m/z* 335 $[M + H]^+$. Isobiflorin (**13**), which was previously isolated from *Eucalyptus cypellocarpa*,[17] was used as standard sample upon comparison on HPLC.

2: reaction time 8 h; normal- and reversed-phase HPLC (solvent C) resulted in peaks for 6-*β*-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (**8**) and 6-*β*-*C*-(2′-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**9**).

3: reaction time 24 h; normal- and reversed-phase HPLC (solvent C) resulted in peaks for 8-*β*-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (**10**) and 8-*β*-*C*-(2′-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**11**).

4: reaction time 48 h; normal- and reversed-phase HPLC (solvent B) resulted in peaks for biflorin (**12**).

5: reaction time 48 h; normal- and reversed-phase HPLC (solvent B) resulted in peaks for biflorin (**12**) and kunzeachromone C (**4**).

6: reaction time 150 h; normal- and reversed-phase HPLC (solvent B) resulted in peaks for isobiflorin (**13**).

7: reaction time 48 h; normal- and reversed-phase HPLC (solvent B) resulted in peaks for isobiflorin (**13**) and kunzeachromone E (**6**).

EBV-EA Assay. The inhibition of EBV-EA activation was assayed using Raji cells (virus nonproducer), the EBV genome-carrying lymphoblastoid cells derived from Burkitt's lymphoma, which were cultured in 10% fetal bovine serum (FBS) in RPMI-1640 medium (Nissui). The indicator cells (Raji, 1×10^6/mL) were incubated at 37 °C for 48 h in the medium (1 mL) containing *n*-butyric acid (4 mM), TPA [20 ng (32 pM) in DMSO 2 μL] as inducer, and a known amount of test compound in 5 μL of DMSO. Smears were made from the cell suspension, and the activated cells that were stained by EBV-EA positive serum were detected by a conventional indirect immunofluorescens technique. In each assay, at least 500 cells were counted, and the number of stained cells was recorded. Triplicate assays were carried our for each compound. The average EBV-EA induction of the test compound was expressed as a relative ratio to the positive control experiment (100%) with *n*-butyric acid plus TPA in which EA induction was ordinarily 35%.

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