Cypellocarpins A−C, Phenol Glycosides Esterified with Oleuropeic Acid, from *Eucalyptus cypellocarpa*

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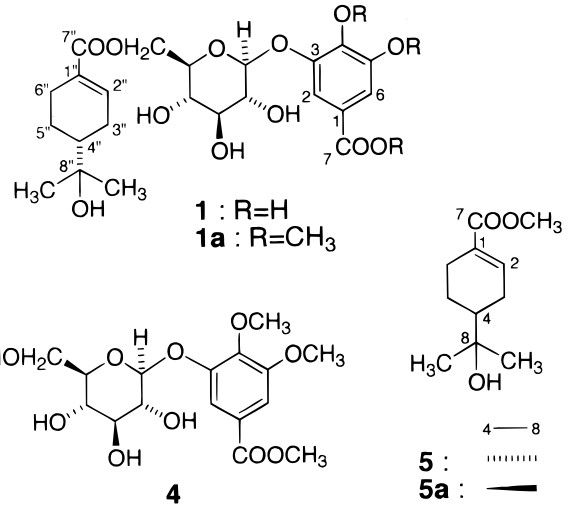
Three new phenol glycosides acylated with (+)-oleuropeic acid, cypellocarpins A (**1**), B (**2**), and C (**3**), along with seven known compounds, were isolated from the dried leaves of *Eucalyptus cypellocarpa*. Structures of the new compounds were determined on the basis of spectroscopic methods, including 2D NMR experiments and chemical degradation. These new compounds and a known related glucoside (**7**) showed potent in vitro antitumor-promoting activity in a short-term bioassay evaluating the inhibitory effect on Epstein−Barr virus early antigen activation induced by 12-*O*-tetradecanoyl phorbol 13-acetate (TPA). These compounds also suppressed an in vivo two-stage carcinogenesis induced with nitric oxide and TPA on mouse skin.

Myrtaceous plants are known to be rich sources of biologically active terpenoids and polyphenols, including flavonoids, phloroglucinol derivatives, and tannins.[1] We previously reported the characterization of several hydrolyzable tannin monomers and oligomers in *Psidium*,[2] *Melaleuca*,[3] *Eucalyptus*,[4] and *Eugenia* species.[5] As a continuation of our studies on polyphenolic constituents of the myrtaceous plants, we have studied extracts of *Eucalyptus cypellocarpa* L. A. S., which is called "grey gum" in Australia due to its easily strippable gray bark and is one of the few feeding plants of the koala. In this paper, we report the isolation and structure elucidation of three novel acylated phenol glucosides named cypellocarpins A (**1**), B (**2**), and C (**3**). In vitro and in vivo antitumor-promoting effects of these new compounds are also described.

Results and Discussion

A concentrated aqueous acetone homogenate of the dried leaves of *E. cypellocarpa* was successively extracted with ether, ethyl acetate, and *n*-BuOH. The ethyl acetate extract was fractionated by column chromatography on Toyopearl HW-40, and fractions eluted with 20% and 30% MeOH were further purified by Si gel chromatography followed by preparative TLC to afford compounds **2** and **3**. Compound **1** was obtained from the *n*-BuOH extract by repeated column chromatography on Diaion HP-20, Toyopearl HW-40, and MCI-gel CHP-20P.

Cypellocarpin A (**1**) was obtained as a pale brown amorphous powder, and its molecular formula, $C_{23}H_{30}O_{12}$, was established from HRESIMS, which gave a pseudomolecular ion peak at *m*/*z* 499.1810 [M + H]$^+$. The ^{1}H NMR spectrum (Table 1) showed two meta-coupled signals (δ 7.35, 7.28) due to a 3-*O*-substituted galloyl unit and an olefinic proton signal (δ 7.00, m), together with sugar proton signals, characteristic of a β-D-glucopyranose. The spectrum also indicated the presence of two tertiary methyl (δ 1.14), one methine (δ 1.51), and three methylene groups (δ 1.16−2.42). The ^{1}H−^{1}H COSY spectrum revealed sequential couplings of the olefinic, methylene, methine, and methylene protons. These data; the ^{13}C NMR spectrum; 10 sp^2 carbons, including two carboxyl carbons; and 13 sp^3 carbons all suggested that **3** was an acylated glycoside composed of galloyl, glucopyranosyl, and a $C_{10}H_{15}O_2$ monoterpenoid acyl units. The constituents of **1** were confirmed by methanolysis of its trimethylated derivative **1a** with NaOMe in MeOH, which yielded methyl di-*O*-methyl-*O*-glucosylgallate (**4**) (ESIMS *m*/*z* 392) and a monoterpene methyl ester (**5**) {$[\alpha]_D$ +83° ($CHCl_3$), ESIMS *m*/*z* 181}. Enzymatic hydrolysis of **4** with β-glucosidase liberated glucose, which gave a positive response to the reaction with D-glucose oxidase.[6] The physicochemical data of **5** were in agreement with those reported for (−)-oleuropeic acid methyl ester (**5a**) {$[\alpha]_D$ −88.7° ($CHCl_3$)},[7] except for the sign of its specific rotation, indicating **5** to be an enantiomer of the known **5a**. The positions of the ester and glycosidic linkages in **1** were established by an HMBC experiment. The olefinic proton (H-2″) showed a three-bond correlation with an ester carbonyl carbon at δ 167.5, which, in turn, correlated through a three-bond coupling with H-6′ of the glucose residue. A correlation between the anomeric proton and the galloyl C-3 was also detected. Based on the above data, structure **1** was assigned to cypellocarpin A.



Cypellocarpin B (**2**) was obtained as a pale yellow amorphous powder. It showed a pseudomolecular ion at *m*/*z* 539 [M + H]$^+$ in ESIMS, corresponding to a molecular

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for **1**−**3** (δ_H, 500 MHz; δ_C, 126 MHz)

	1[a]		**2**[b,c]		**3**[b]	
carbon	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		122.0				
2	7.35 (1H, d, 2)[d]	111.4	1.59, 1.58 (3/2H each, s)	101.54, 101.46	2.07 (1H, d, 1)	168.5
3		146.3	3.10 (1H, d, 17)	47.47, 47.45	6.24 (1H, s)	108.5
			2.67 (1H, d, 17)			
4		140.9		197.0		182.2
5		146.0		162.9, 162.8		161.4
6	7.28 (1H, d, 2)	113.2	6.02, 6.01 (1/2H each, d, 2)	96.1, 95.9	6.61 (1H, d, 2)	94.5
7		167.8		165.2, 165.0		162.8
8			6.12, 6.09 (1/2H each, d, 2)	96.6, 96.1	6.41 (1H, d, 2)	99.5
9				160.4, 160.2		157.6
10				103.14, 103.19		105.3
1′	4.92 (1H, d, 8)	103.3	4.99 (1H, d, 8)	99.6, 99.4	5.11 (1H, d, 8)	99.5
2′	3.56 (1H, dd, 7, 12)	74.3	3.23 (1H, m)	73.12, 73.07	3.30 (1H, m)	76.2
3′	3.62 (1H, t, 9)	76.9	3.30 (1H, m)	76.2	3.28 (1H, m)	73.1
4′	3.44 (1H, t, 9)	71.3	3.16 (1H, m)	70.3, 70.0	3.17 (1H, m)	70.3
5′	3.80 (1H, dd, 2, 7)	75.1	3.72 (1H, m)	74.1	3.78 (1H, m)	73.9
6′	4.54 (1H, dd, 2, 12)	64.4	4.41 (1H, t, 12)	63.9, 63.7	4.43 (1H, br t, 13)	63.8
	4.16 (1H, dd, 7, 12)		3.99, 3.95 (1/2H each, dd, 7, 12)		3.97 (1H, dd, 8, 13)	
1"		130.4		129.5		129.5
2"	7.00 (1H, m)	141.2	6.93 (1H, m)	140.6, 140.5	6.90 (1H, m)	140.4
3" (ax)	1.98 (1H, m)	28.1	1.91 (1H, m)	27.27, 27.26	1.86 (1H, m)	27.2
(eq)	2.32 (1H, m)		2.25 (1H, m)		2.20 (1H, m)	
4"	1.51 (1H, m)	44.8	1.37 (1H, m)	43.8	1.35 (1H, m)	43.8
5" (ax)	1.16 (1H, m)	24.0	1.06 (1H, m)		1.06 (1H, m)	23.1
(eq)	1.98 (1H, m)		1.91 (1H, m)	23.13, 23.11	1.86 (1H, m)	
6" (ax)	2.08 (1H, m)	25.8	2.05 (1H, m)	25.0	1.99 (1H, m)	25.0
(eq)	2.42 (1H, m)		2.38 (1H, m)		2.35 (1H, m)	
7"		167.5		166.4		166.4
8"		72.0		70.4, 70.3		70.3
2-CH_3				27.63, 27.56		20.2
8"-CH_3	1.14 (6H, s)	27.1	1.04 (6H, s)	27.20	1.031 (3H, s)	27.2
		26.7		26.79, 26.75	1.025 (3H, s)	26.7
2-OH			7.12 (1H, br s)			
5-OH			11.98, 11.96 (1/2H each, s)		12.82 (1H, s)	

[a] In acetone-d_6 + D_2O. [b] In DMSO-d_6. [c] Data for a mixture of epimers. [d] J values (Hz) are in parentheses; ax: axial; eq: equatorial.

formula of $C_{26}H_{34}O_{12}$. The ^{1}H and ^{13}C NMR spectra exhibited signals due to a glucose and an oleuropeoyl unit as observed in **1**, suggesting that **2** is a glucoside analogous to **1**, in which only an aglycon is different from **1**. Instead of the galloyl (aglycon) signals in **1**, signals due to two upfield-shifted meta-coupled aromatic protons, a tertiary methyl, and an isolated methylene group, together with a hydrogen-bonded hydroxyl proton, were observed in **2**. Although **2** gave a single peak on both normal and reversed-phase HPLC, most of the aglycon signals in the ^{1}H NMR were duplicated in a ratio of about 1:1, implying that **2** exists as an inseparable mixture. The ^{13}C NMR spectrum revealed that the aglycon had seven sp^2 carbons, including a ketonic carbon, and three sp^3 carbons. The chemical shifts of the sp^2 carbon resonances were indicative of a phloroglucinol system with an acyl side chain. The presence of a hemiacetal carbon, which can be responsible for the formation of an equilibrium mixture, was suggested by a signal at δ 101.54/101.46 (C-2). The HMBC experiment revealed correlations of the tertiary methyl protons (δ 1.59/1.58) with the hemiacetal and the methylene carbons (δ 47.47/47.45) through two- or three-bond coupling, and a similar correlation between the isolated methylene protons (δ 3.10, 2.67, each, d, J = 17 Hz) and the ketone carbonyl carbon (δ 197.0). The position of the glycosidic linkage was determined to be C-7 as manifested by the HMBC correlations observed between the anomeric proton (δ 4.99, H-1′) and C-7 (δ 165.2/165.0), and also by the NOEs between H-1′ and H-8/H-6 (δ 6.01−6.12). The NOESY experiments, together with the analysis of coupling constants of H-3″−H-5″, also indicated the conformation of the oleuropeic acid moiety as shown in Figure 1. Based on these observations,

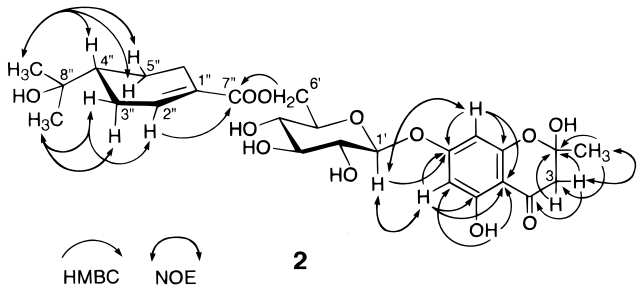
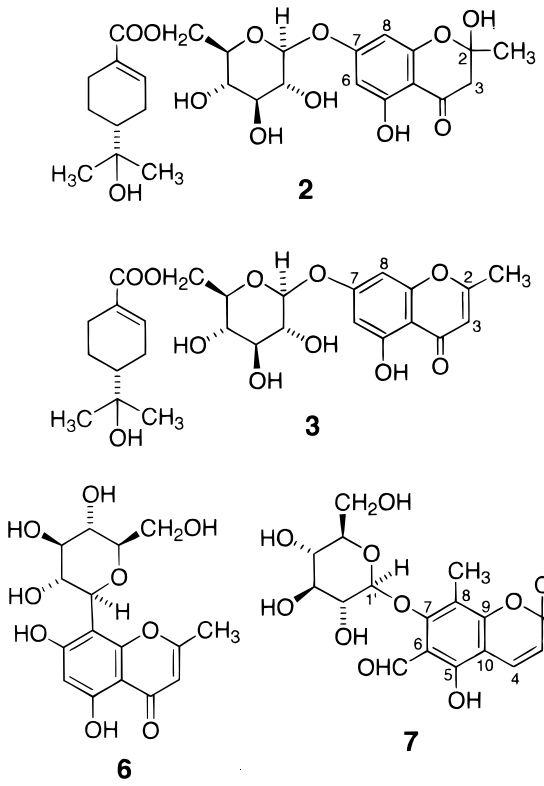


Figure 1. NOE and HMBC correlations of **2**.

cypellocarpin B was deduced to be an equilibrium mixture of C-2 epimers represented by formula **2**. Methanolysis of **2** with NaOMe in MeOH gave oleuropeic acid methyl ester, $[\alpha]_D$ +70°, which was identical with **5** obtained from **1**. Glucose liberated upon acid hydrolysis of **2** was D-series based on a positive reaction to D-glucose oxidase. Consequently, structure **2** was assigned to cypellocarpin B.

Cypellocarpin C (**3**), isolated as colorless needles of mp 229−230 °C, showed a pseudomolecular ion at m/z 521 [M + H]$^+$ in ESIMS, establishing its molecular formula as $C_{26}H_{32}O_{11}$. The ^{1}H and ^{13}C NMR signals were very similar to those of **2**, except for the presence of a double bond (δ_H 6.24; δ_C 168.5, 108.5) instead of the isolated methylene group and hydroxyl proton (δ 7.12) observed in **2** (Table 1). These differences, along with a downfield shift of a tertiary methyl signal (δ 2.07) compared to that of **2**, indicated that **3** is a dehydrated derivative of **2**. This structure is consistent with its UV spectrum (λ_{max} 286, 314 nm), indicative of an extended conjugation system com-



pared to **2**. Conclusive evidence for the structure was obtained by chemical transformation of **2** into **3** upon treatment in an acidic solution. Cypellocarpin C (**3**) thus corresponds to an oleuropeic acid ester of noreugenin-7-*O*-*β*-D-glucoside, which was previously isolated from *Nauclea racemosa* (Rubiaceae).[8]

The known compounds, 2-endo-cineolylol glucoside,[9] chlorogenic acid, 5-*O*-coumaroylquinic acid,[10] rutin, quercetin 3-*O*-*β*-D-glucuronide,[11] isobiflorin (**6**),[12] and chromene glucoside (**7**), were also isolated. Except for **7**, these were identified by comparing their spectral data with those reported in the literature. Compound **7**, a yellowish amorphous powder, $C_{19}H_{24}O_9$ [*m*/*z* 397 $(M + H)^+$ in ESIMS], whose ^1H and ^{13}C NMR data are here fully assigned for the first time (see Experimental Section), corresponds to the known chromene glucoside previously isolated from *Eucalyptus robusta* leaves.[13]

The coexistence of cypellocarpins B (**2**) and C (**3**) is noteworthy from the biosynthesis viewpoint because the aglycon of **2** (equivalent to 2-keto-butyrylphloroglucinol) is the most plausible precursor of chromanone compounds such as **3** and **6**. It is interesting that cypellocarpins not only contain the (+)-oleuropeic acid moiety in the molecules, but also are rare examples of phenol glycosides in which a monoterpenoid acid is esterified with glucose.

Cypellocarpins (**1**−**3**) and the related compound (**7**) were evaluated for their in vitro and in vivo antitumor-promoting activity as follows. First, they were assessed for inhibitory effects on Epstein−Barr virus early antigen (EBV−EA) activation induced by 12-*O*-tetradecanoyl-13-*O*-acetylphorbol (TPA), which is a preliminary in vitro short-term bioassay using Raji cells[14] in a survey of potent antitumor-promoting agents in nature. As shown in Table 2, all of the tested compounds (**1**−**3** and **7**) inhibited EBV−EA activation at 100 mol ratio/TPA, and exhibited more significant inhibitory effects (68.2, 67.4, 63.4, and 54.8% inhibition, respectively) at 500 mol ratio/TPA. These potencies at concentrations preserving high viability of Raji cells

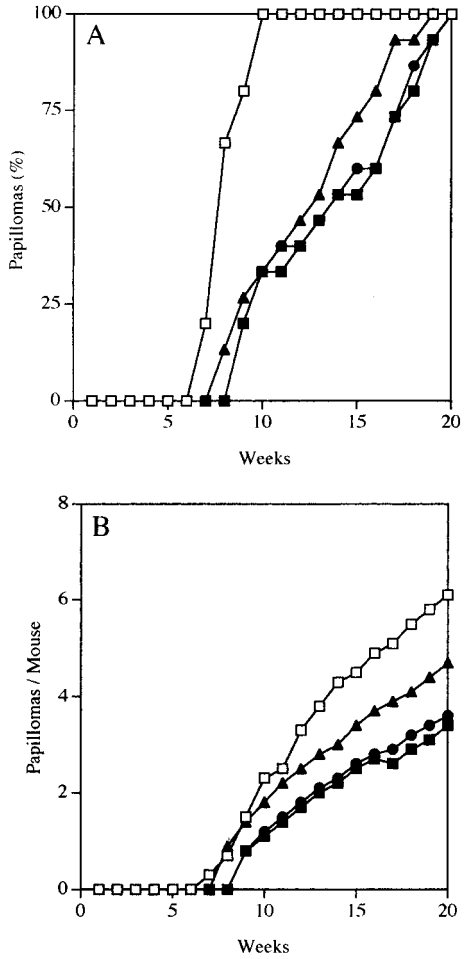


Figure 2. Inhibition of TPA-induced tumor promotion by multiple application of cypellocarpins B (**2**) and C (**3**) and compound **7**. All mice were initiated with NOR1 (390 nmol) and promoted with 1.7 nmol of TPA given twice weekly starting 1 week after initiation. **A**: percentage of mice bearing papillomas. **B**: average number of papillomas per mouse. □, control NOR1 + TPA; ●, NOR1 + TPA + **2** (0.0025%); ▲, NOR1 + TPA + **3** (0.0025%); ■, NOR1 + TPA + **7** (0.0025%); $P<0.01$.

were comparable to that of a positive control, (−)-epigallocatechin gallate (EGCG, the major polyphenol of green tea).[14] Next, we evaluated their effects in the in vivo two-stage carcinogenesis on mouse skin using nitric oxide as an initiator[15,16] and TPA as a promoter. (±)−(*E*)-4-Methyl-2-[(*E*)-hydroxyimino]-6-methoxy-5-nitro-3-hexenamide (NOR1)[15] was used as the releasing agent of nitric oxide. Although cypellocarpin A (**1**) showed potent inhibition of EBV−EA activation, it could not be tested because of the small amount available.

The in vivo experimental results are shown in Figure 2. The control animals showed a 100% incidence of papillomas at 10 weeks after promotion, while treatment with **2**, **3**, and **7** along with the initiator and promoter remarkably reduced the percentage of tumor-carrying mice to 30% after 10 weeks at a concentration of 50 mol ratio/TPA (Figure 2A). Although suppression of carcinogenesis was not observed for all compounds after 20 weeks, the average number of papillomas per mouse in the experimental groups was reduced to 75−58% relative to the control group in week 20 (Figure 2B). As the potencies of these compounds were almost comparable to that of EGCG in this assay system (data not shown), further study on the effect of these compounds in other assay systems would be valuable.

Table 2. Relative Ratio of EBV−EA Activation with Respect to Positive Control (100%) in the Constituents of *E. cypellocarpa*

	relative ratio of EBV−EA Activation[a]			
	concentration (mol ratio/TPA)[b]			
compounds	1000	500	100	10
cypellocarpin A (**1**)	12.4 ± 0.4 (70)[c]	32.6 ± 1.8	70.4 ± 1.8	92.1 ± 0.4
cypellocarpin B (**2**)	16.2 ± 0.5 (70)	36.6 ± 2.0	72.4 ± 1.0	98.3 ± 0.3
cypellocarpin C (**3**)	19.5 ± 0.3 (70)	45.2 ± 1.6	78.6 ± 1.2	100.0 ± 0.5
compound **7**	5.0 ± 0.4 (60)	31.8 ± 1.8	68.9 ± 1.5	90.0 ± 0.4

[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%.

Experimental Section

General Experimental Procedures. Optical rotations were taken with a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Hitachi U-2001 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured 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 referenced to TMS. ESIMS were recorded on a Micromass AutoSpec OA-Tof instrument, using 50% aqueous MeOH containing 0.1% $AcONH_4$ (flow rate 20 μL/min) as solvent. Diaion HP-20, MCI-gel CHP-20P (Mitsubishi Kasei Co. Ltd., Japan), Toyopearl HW-40 (TOSOH, Japan), and YMC-gel ODS AQ-120-S50 (YMC Co., Japan) were used for column chromatography, and a precoated Kieselgel PF_{254} (0.25 mm, Merck) was used for analytical and preparative TLC.

Plant Material. The leaves of *E. cypellocarpa* cultivated at Awaji Flower Park, Hyogo, Japan, were collected in May 1996, and provided by the Park's office. A voucher specimen (OPH-MYR 006) was deposited in the Herbarium, Faculty of Pharmaceutical Sciences, Okayama University.

Extraction and Isolation. Dried leaves of *E. cypellocarpa* (810 g) were homogenized in 70% aqueous acetone (8.5 L), filtered, and concentrated. After removal of a precipitate, the concentrated solution (600 mL) was successively extracted with ether (600 mL × 5), EtOAc (600 mL × 5), and *n*-BuOH (600 mL × 8) to give the respective extracts (11, 40, and 85 g). A part of the EtOAc extract (3 g) was subjected to column chromatography over Toyopearl HW-40 (2.2 cm i.d. × 45 cm) with 20% MeOH → 30%MeOH → 60% MeOH in a stepwise gradient mode and final washing with MeOH−H_2O−acetone (7:2:1). The 20% MeOH eluate gave 2-endo-cineolylol glucoside (4.4 mg) after separation by a combination of chromatography on YMC-gel ODS AQ 120−50S and preparative TLC (toluene−acetone 3:1, multi-development). The 30% MeOH eluate was repeatedly chromatographed over YMC-gel ODS AQ 120−50S with aqueous MeOH to afford cypellocarpins B (**2**) (64 mg) and C (**3**) (22 mg) and compound **7** (10 mg).

A 35-g quantity of the *n*-BuOH extract was first fractionated by chromatography over Diaion HP-20 (5.0 cm i.d. × 40 cm) with H_2O containing increasing amounts of MeOH. A fraction eluted with 30% MeOH was further subjected to chromatography on Toyopearl HW-40 and subsequent purification by YMC-gel ODS AQ 120−50S and/or MCI-gel CHP-20P using aqueous MeOH (10% → 20% → 40% → 50% MeOH) to give isobiflorin (**6**) (64 mg) and chlorogenic acid (70 mg). A fraction eluted with 50% MeOH on the Diaion HP-20 column was similarly rechromatographed over Toyopearl HW-40 eluting with the aqueous MeOH solvent system to afford cypellocarpin A (**1**) (56 mg), 5-*O*-coumaroylquinic acid (20 mg), rutin (49 mg), and quercetin-3-*O*-glucuronide (215 mg).

Cypellocarpin A (1): pale brown amorphous powder; $[\alpha]_D$ −32° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 266 (4.17), 216 (4.76) nm; ^{1}H and ^{13}C NMR spectral data, see Table 1; ESIMS *m/z* 516 $[M + NH_4]^+$, 481 $[M - H_2O + H]^+$; HRESIMS *m/z* $[M + H]^+$ 499.1810 ($C_{23}H_{30}O_{12}$ + H, 499.1816).

Methylation of 1. A solution of **1** (10 mg) in MeOH was treated with an excess of CH_2N_2−Et_2O at room temperature for 2 h. After removal of the solvent, the product was purified by preparative TLC with $CHCl_3$−MeOH (6:1) in a multi-development mode to afford trimethylcypellocarpin A (**1a**) (5.4 mg) as a colorless oil: ESIMS *m/z* 541 $[M + H]^+$, 558 $[M + NH_4]^+$; ^{1}H NMR (acetone-d_6) δ 7.48 (1H, d, *J* = 2 Hz, H-2), 7.34 (1H, d, *J* = 2 Hz, H-6), 6.93 (1H, m, H-2″), 5.08 (1H, d, *J* = 8 Hz, H-1′), 4.52 (1H, dd, *J* = 2, 12 Hz, H-6′), 4.16 (1H, dd, *J* = 7, 12 Hz, H-6′), 4.60, 4.46, 4.45 (1H each, d, *J* = 4 Hz, H-2′, -3′, -4′-OH), 3.88, 3.86, 3.84 (3H each, OCH_3), 3.78 (1H, ddd, *J* = 2, 7, 9 Hz, H-5′), 3.61−3.52 (2H, m, H-2′, -3′), 3.44 (1H, m, H-4′), 2.42 (1H, m, H-6″), 2.30 (1H, m, H-3″), 2.02 (1H, m, H-6″), 1.98 (2H, m, H-3″, H-5″), 1.49 (1H, m, H-4″), 1.18 (1H, m, H-5″), 1.16, 1.15 (3H each, s, 8′-CH_3).

Methanolysis of 1a. A solution of **1a** (20 mg) in 0.5% NaOMe−MeOH (1 mL) was left standing for 2 h. After addition of two drops of HOAc, the concentrated solution was treated with an excess of CH_2N_2 at room temperature for 2 h and evaporated. The precipitate that deposited upon addition of $CHCl_3$ to the residue was collected and washed several times with $CHCl_3$ to give **4** (3.9 mg). The $CHCl_3$-soluble portion was subjected to chromatography over Si gel and subsequently to preparative TLC (toluene−acetone, 3:1) to yield **5** (2.4 mg).

Compound 4: white amorphous powder; ^{1}H NMR (acetone-d_6 + D_2O) δ 7.49 (1H, d, *J* = 2 Hz, H-2), 7.32 (1H, d, *J* = 2 Hz, H-6), 4.99 (1H, d, *J* = 8 Hz, H-1′), 3.87, 3.84, 3.83 (3H each, s, OCH_3), 3.83 (1H, m, H-5′), 3.69 (1H, dd, *J* = 5, 13 Hz, H-6′), 3.85−3.42 (4H in total, H-2′, -3′, -4′, -5′); ESIMS *m/z* 392 $[M + NH_4]^+$.

Compound 5: colorless oil; $[\alpha]_D$ +83° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.99 (1H, m, H-2), 2.00, 2.33 (H-3), 1.55 (H-4), 1.23, 2.00 (H-5), 2.18, 2.54 (H-6), 1.22, 1.21 (8-CH_3), 3.73 (OCH_3); ESIMS *m/z* 181 $[M - H_2O + H]^+$. These NMR data were in agreement with the reported data for (−)-oleuropeic acid methyl ester (**5a**).[7]

Cypellocarpin B (2): A pale brown amorphous powder; $[\alpha]_D$ −45° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 284 (4.01), 210 (4.28) nm; ^{1}H and ^{13}C NMR spectral data, see Table 1; ESIMS *m/z* 556 $[M + NH_4]^+$, 539 $[M + H]^+$; 521 $[M - H_2O + H]^+$; HRESIMS *m/z* $[M + H]^+$ 539.2197 (calcd for $C_{26}H_{34}O_{12}$ + H, 539.2129).

Cypellocarpin C (3): colorless needles, mp 229−230°; $[\alpha]_D$ −138° (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 313 (3.44), 286 (3.59), 255 (4.06), 248 (4.07), 223 (4.22) nm; ^{1}H and ^{13}C NMR spectral data, see Table 1; ESIMS *m/z* 521 $[M + H]^+$, 503 $[M - H_2O + H]^+$; *anal*. C, 59.03%; H, 6.18%; calcd for $C_{26}H_{32}O_{11}$, C, 59.99%; H, 6.20%.

Methanolysis of 2 and 3. A solution of **2** (4 mg) in MeOH (1 mL) containing 1% NaOMe (0.1 mL) was kept standing overnight. After addition of a drop of HOAc, the concentrated solution was directly subjected to preparative TLC (toluene−acetone, 3:1) to yield **5** (1 mg), $[\alpha]_D$ +70° (*c* 0.1, $CHCl_3$), which was identical with the product obtained from **1a** (TLC, ^{1}H NMR, $[\alpha]_D$). Cypellocarpin C (**3**) (3 mg) was similarly subjected to methanolysis to give **5** (0.8 mg), $[\alpha]_D$ +76° (*c* 0.1, $CHCl_3$).

Chemical Conversion of 2 into 3. To an aqueous solution (1 mL) of **2** (5 mg) was added a drop of acetate buffer (pH 5.0). After 48 h, a deposited compound was collected by centrifugation and washed with water to yield **3** (1.6 mg). The starting material (**2**) (3 mg) was recovered by passing the supernatant through BondElut C_{18} followed by elution with MeOH.

Compound 7: yellowish amorphous powder; $[\alpha]_D$ −16° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 314 (3.86), 268 (4.22) nm; ^{1}H NMR (acetone-d_6) δ 12.51 (1H, s, 5-OH), 10.21 (1H, s, 6-CHO), 6.60 (1H, d, *J* = 10 Hz, H-4), 5.68 (1H, d, *J* = 10 Hz, H-3), 4.77 (1H, d, *J* = 8 Hz, H-1′), 3.76 (1H, dd, *J* = 2, 11 Hz, H-6′), 3.66 (1H, dd, *J* = 5, 11 Hz, H-6′), 3.57 (1H, t, *J* = 9 Hz,

H-2′), 3.52−3.46 (2H, m, H-3′, -4′), 3.28 (1H, m, H-5′), 2.13 (3H, s, 8-CH_3), 1.46, 1.45 (3H each, s, 2-CH_3); ^{13}C NMR (acetone-d_6) δ 196.2 (6-CHO), 160.2 (C-9), 159.2 (C-7), 158.1 (C-5), 128.3 (C-3), 115.7 (C-4), 112.0 (C-8), 110.9 (C-6), 106.3 (C-10), 106.0 (C-1′), 79.1 (C-2), 77.9, 71.3 (C-3′, C-4′), 77.8 (C-5′), 75.4 (C-2′), 62.6 (C-6′), 28,6, 28.5 (2-CH_3), 8.76 (8-CH_3); ESIMS *m*/*z* 414 $[M + NH_4]^+$, 397 $[M + H]^+$; *anal.* C 55.77%, H 6.08%, calcd for $C_{19}H_{24}O_9 \cdot 1/2\ H_2O$, C 56.29%, H 6.17%.

***In Vitro* EBV−EA Activation Assay.** The inhibition of EBV−EA activation was assayed using genome-carrying human lymphoblastoid cells [Raji cells (virus nonproducer type) derived from Burkitt's lymphoma] cultured in 10% fetal bovine serum in RPMI 1640 medium (Nissui), according to a reported experimental procedure.[14]

Two-Stage Mouse Skin Carcinogenesis Test by NOR1 and TPA. NOR1 was purchased from Dojindo Laboratories (Kumamoto, Japan). The animals (female, Sencar, 6 weeks old) were divided into two groups, 15 mice each. The back of each mouse was shaved with surgical clippers. The next day the mice were topically treated with NOR1 (90 μg, 390 nmol) in 0.1 mL of acetone for initiation. One week after initiation with NOR1, the mice in the control group were topically treated with TPA (1 μg, 1.7 nmol) in 0.1 mL of acetone twice a week as promotion and provided with drinking water ad libitum during the experimental period. For the sample-treated group, the sample (0.0025%) was given in the drinking water from 1 week before to 1 week after the initiation of treatment with NOR1. Subsequent treatment was identical to that of the control group. The incidence of papillomas was observed weekly for 20 weeks.

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Supporting Information Available: HMBC and NOE correlations of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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