New Cinnamylphenols from *Dalbergia* Species with Cancer Chemopreventive Activity[1]

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Five new cinnamylphenols (1,3-diphenylpropenes), dalberatins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), were isolated from plants of the *Dalbergia* species. They were characterized and tested for their inhibitory activities against Epstein−Barr virus early antigen activation induced by 12-*O*-tetradecanoylphorbol-13-acetate in Raji cells. The cinnamylphenols were found to show remarkably potent activities. The result of the present investigation indicated that some of these cinnamylphenols might be valuable as potential cancer chemopreventive agents (anti-tumor promoters).

Flavonoids and isoflavonoids are widely distributed in plants, are biogenetically derived from cinnamoyl-CoA and three molecules of malonyl-CoA, and contain a C6 + C3 + C6 structural unit.[2] On the basis of an in vitro primary screening test for inhibition of tumor-promoting agents, we have previously reported that many kinds of flavonoids and isoflavonoids, including flavones, flavanones, flavonols, flavanonols, isoflavones, chalcones, and catechins, showed inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein−Barr virus early antigen (EBV-EA) activation.[3−7]

As part of our continued screening of plant constituents for inhibition of tumor-promoting agents, the EtOH extracts of the stem bark of *Dalbergia cultrata* Grah. and *D. nigrescens* Kurz (Leguminosae) were found to exhibit a significant anti-tumor-promoting activity on TPA-induced EBV-EA activation. We have isolated five new cinnamylphenols (1,3-diphenylpropenes) with structural units (C6 + C3 + C6) biogenetically analogous to flavonoids. Here we describe the structure elucidation of the new compounds dalberatins A (**1**) and B (**2**) from *D. cultrata* Grah. and dalberatins C (**3**), D (**4**), and E (**5**) from *D. nigrescens* Kurz and the results of assays examining the inhibitory effects on TPA-induced EBV-EA activation.

Results and Discussion

The acetone-soluble portions of the EtOH extracts of dried stem bark of *D. cultrata* and *D. nigrescens* were separately fractionated by a combination of silica gel column chromatography and preparative TLC to obtain five new cinnamylphenols.

Dalberatin A (**1**) was obtained as a pale yellow oil, and its molecular formula was determined as $C_{18}H_{20}O_5$ by HREIMS. The IR spectrum exhibited bands at ν_{max} 3531, 3315br, 1604, and 1585 cm^{-1} due to hydroxy and aromatic groups, respectively. In the ^1H NMR spectrum, signals due to three *O*-methyl groups at δ 3.92, 3.86, and 3.76 were observed along with two broad D_2O exchangeable signals at δ 5.71 and 5.68. In the aromatic proton region, ortho-coupled AB-type protons at δ 6.83 and 6.68 (each 1H, d, J = 8.4 Hz) and continuously located ABC-type protons at δ 6.97 (1H, dd, J = 8.1, 2.0 Hz), 6.92 (1H, t, J = 8.1 Hz), and 6.82 (1H, dd, J = 8.1, 2.0 Hz) suggested the presence of 1,2,3,4-tetrasubstituted and 1,2,3-trisubstituted benzene rings in the molecule. The appearance of ABX_2-type proton signals at δ 3.50 (2H, d, J = 6.6 Hz), 6.36 (1H, dt, J = 15.8, 6.6 Hz), and 6.59 (1H, d, J = 15.8 Hz) and the similarity of this signal pattern to those of synthetic 1,3-diarylpropenes reported previously[8] revealed the partial structure [(*E*)-CH═CH−CH_2−]. Further, a lower shift of H-3 (δ 6.59) compared to those (δ 6.30−6.37) in synthetic analogues showed the presence of an O-substituent at C-2″. The arrangement of substituents on two benzene rings was revealed by HMBC analysis. The observation of long-range C−H correlations from C-2′ and C-4′ to H-6′, from C-3′ to H-5′ and 4′-OH, and from C-5′ to 4′-OH suggested the locations of the OH at C-4′ and two OCH_3 at C-2′ and C-3′ on the A ring. The HMBC correlations, from C-2″ and C-4″ to H-6″ and 3″-OH and from C-1″ and C-3″ to H-5″, revealed the locations of the OH group at C-3″ and a OCH_3 group at C-2″ on the B ring. Furthermore, long-range C−H correlations from C-2′ to H-1 attached to an sp^3-carbon and from C-6″ to H-3 on an sp^2-carbon indicated the linkages of C-1 and C-3 with the A and B rings, respectively. Other HMBC correlations are shown in Figure 2. These results, coupled with the appearance of an MS fragment at *m*/*z* 167 due to the cleavage of the benzylically activated C1−C2 bond, and observation of NOE enhancements between H-3/2″-OCH_3 and H-1/H-6′, defined the structure of dalberatin A (**1**).

Dalberatin C (**3**) has the same molecular formula of $C_{18}H_{20}O_5$ as **1**. The ^1H NMR spectrum (Table 1) showed a signal pattern similar to that of **1**, except for differences in the chemical shifts of signals due to the three methoxy and two OH signals. The presence of NOE enhancements between H-1 and H-6′; H-1 and 2′-OCH_3; H-2 and H-6″; and H-3 and 2″-OCH_3 coupled with long-range C-H correlations between 3″-OH and C-4″, 3′-OH and C-2′, and 3′-OH and C-3′ in the HMBC spectrum suggested the

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Table 1. NMR Spectral Data of Cinnamylphenols **1**, **3**, and **5** from *Dalbergia* Species[a]

	dalberatin A (**1**)			dalberatin C (**3**)			dalberatin E (**5**)		
	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
1	33.2 (t)	3.50 (2H, d, 6.6)	H-2, H-3	33.3 (t)	3.54 (2H, d, 6.6)	H-3, H-6′	33.0 (t)	3.53 (2H, d, 6.6)	H-2, H-3, H-6′
2	131.5 (d)	6.36 (dt, 15.8, 6.6)	H-1	131.4 (d)	6.37 (dt, 15.8, 6.6)	H-1	131.0 (d)	6.37 (dt, 15.8, 6.6)	H-1
3	124.6 (d)	6.59 (d, 15.8)		124.6 (d)	6.61 (d, 15.8)	H-1, H-2, H-6″	124.9 (d)	6.61 (d, 15.8)	H-1
1′	125.2 (s)		H-1, H-2, H-5′	126.1 (s)		H-1, H-5′	124.4 (s)		H-2, H-5′
2′	150.7 (s)		H-1, H-6′, 2′-OMe	145.3 (s)		H-1, H-6′, 2′-OMe, 3′-OH	145.7 (s)		H-1, H-6′, 2′-OMe
2′-0Me	60.5 (q)	3.86 (3H, s)		60.7 (q)	3.89 (3H, s)		61.4 (q)	3.84 (3H, s)	
3′	139.8 (s)		H-5′, 3′-OMe, 4′-OH	138.7 (s)		H-5′, 3′-OH	136.4 (s)		H-5′
3′-OH					5.58 (br)			5.48 (br)[b]	
3′-OMe	60.7 (q)	3.92 (3H, s)							
4′	148.2 (s)		H-6′, 4′-OH	146.4 (s)		H-5′, 4′-OMe	143.2 (s)		H-6′
4′-OH		5.68 (br)						5.28 (br)[b]	
4′-OMe				56.2 (q)	3.87 (3H, s)				
5′	110.3 (d)	6.68 (d, 8.4)	4′-OH	106.5 (d)	6.61 (d, 8.4)		111.3 (d)	6.68 (d, 8.4)	
6′	124.8 (d)	6.83 (d, 8.4)		119.6 (d)	6.69 (d, 8.4)	H-1	121.0 (d)	6.66 (d, 8.4)	H-1
1″	130.8 (s)		H-2, H-5″	130.8 (s)		H-2, H-5″	130.7 (s)		H-2, H-5″
2″	144.2 (s)		H-6″, 2″-OMe, 3″-OH	144.2 (s)		H-4″, H-6″, 2″-OMe	144.2 (s)		H-4″, H-6″, 2″-OMe
2″-OMe	61.5 (q)	3.76 (3H, s)		61.5 (q)	3.77 (3H, s)		61.5 (q)	3.76 (3H, s)	
3″	148.9 (s)		H-5″, 3″-OH	148.9 (s)		H-5″	148.9 (s)		H-5″
3″-OH		5.71 (br)			5.71 (br)			5.67 (br)[b]	
4″	114.0 (d)	6.82 (dd, 8.1, 2.0)	H-6″, 3″-OH	113.9 (d)	6.82 (dd, 7.9, 1.8)	H-6″, 3″-OH	114.1 (d)	6.82 (dd, 7.7, 1.8)	H-6″
5″	124.7 (d)	6.92 (t, 8.1)		124.7 (d)	6.93 (t, 7.9)		124.8 (d)	6.94 (t, 7.7)	
6″	118.0 (d)	6.97 (dd, 8.1, 2.0)	H-3, H-4″	118.0 (d)	6.97 (dd, 7.9, 1.8)	H-3, H-4″	118.1 (d)	6.97 (dd, 7.7, 1.8)	H-3, H-4″

[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. [b]Assignments may be reversed.

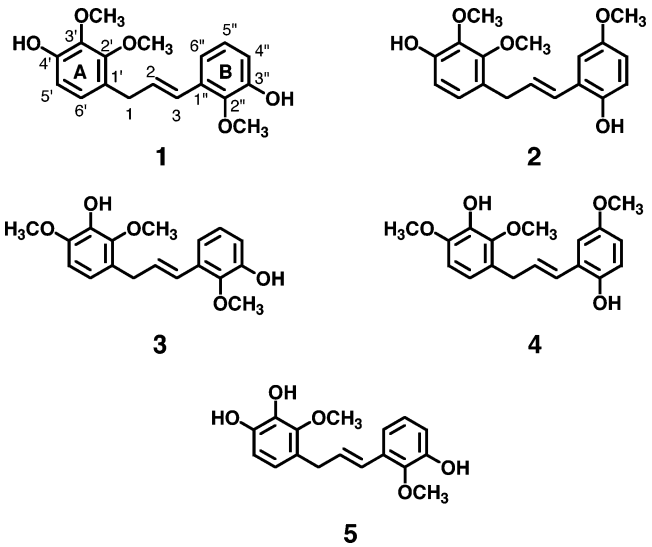

Figure 1. Structures of cinnamylphenols from *Dalbergia* species.

location of three methoxyls at C-2′, C-4′, and C-2″ and OH at C-3′ and C-3″. These spectral data led us to assign the structure **3** to dalberatin C.

Dalberatin E (**5**) was obtained as a colorless oil. The molecular formula $C_{17}H_{18}O_5$ has a difference of CH_2 compared with **1** and was confirmed by HREIMS. The ^{1}H NMR signal pattern of **5** resembled that of **1**, including multiplicities of aromatic proton signals. Differences included the appearance of an additional hydroxy proton signal in place of one of the methoxy signals, observed in **1**, indicating that the structure of **5** corresponded to a demethyl analogue of **1**. Observation of NOE enhancements between H-1 and 2′-OCH_3 and between H-3 and 2″-OCH_3 suggested the presence of a hydroxy at 3′ in **5** instead of the methoxy present in **1**. These data, coupled with the results of ^{13}C NMR and HMBC (Table 1), proved the structure of dalberatin E (**5**).

Dalberatins B (**2**) and D (**4**) were isolated as colorless oils and had the same molecular formula $C_{18}H_{20}O_5$ based on the HREIMS of both compounds. The ^{1}H NMR spectra of **2** and **4** showed signal patterns similar to those of **1** and **3**, due to three methoxyls, two hydroxyls, and an ortho-coupled AB, and ABX_2 protons, respectively. Differences included the appearance of 1,3,4-located ABC-type aromatic protons instead of three adjacent aromatic protons in the spectra of **1** and **3**, indicating the presence of a cinnamylphenol skeleton having three methoxy and two hydroxy groups in the molecule.

Locations of three methoxy and two hydroxy groups on dalberatin B (**2**) were clarified by HMBC, NOE, and MS data as follows: (1) Correlations from C-2′ having OCH_3 to H-1 and H-6′, from C-3′ bonded OCH_3 to H-5′ and 4′-OH, and from C-5′ to 4′-OH indicated the location of two methoxyls at C-2′, C-3′ and a hydroxy at C-4′ on the A ring. (2) Correlation from C-2″ to H-3, H-4″, and H-6″, from C-5″ bearing OCH_3 to H-3″, and an NOE between 5″-OCH_3 and both H-4″ and H-6″ indicated the location of a methoxy at C-5″ and a hydroxy at C-2″ on the B ring. From these spectral data, coupled with results of other HMBC and EIMS, the structure of dalberatin B was determined as **2**.

On the other hand, in NOE experiments of **4**, 7 and 3% NOE enhancements of signals at δ 6.60 (H-3) and 3.75 (5″-OCH_3) were observed on irradiation of the signal at δ 6.86 (H-6″). Irradiation of the signal at δ 3.88 (4′-OCH_3) gave 9% increase of the signal at δ 6.62 (H-5′). Irradiation of H-1 (δ 3.53) gave 4 and 2% increases of the signals at δ 6.69 (H-6′) and 3.89 (2′-OCH_3), respectively. From the aforementioned results, coupled with HMBC results (Table 1), the structure of dalberatin D was concluded to be **4**.

Two known isoflavones, formononetin[9] and biochanin A,[10] were isolated and identified by spectral data derived from the literature.

Inhibitory Effects on EBV-EA Induction. Dalberatins A (**1**), B (**2**), C (**3**), and E (**5**) were tested for their inhibition of tumor-promoting activity by using a short-

Table 2. NMR Spectral Data of Cinnamylphenols **2** and **4** from *Dalbergia* Species[a]

	dalberatin B (**2**)			dalberatin D (**4**)		
	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
1	33.4 (t)	3.49 (2H, d, 6.6)	H-2, H-3, H-6′	33.4 (t)	3.53 (2H, d, 6.6)	H-2, H-3, H-6′
2	132.0 (d)	6.28 (dt, 15.8, 6.6)	H-1	131.8 (d)	6.30 (dt, 15.8, 6.6)	H-1
3	124.9 (d)	6.58 (d, 15.8)	H-6″	124.9 (d)	6.60 (d, 15.8)	
1′	125.4 (s)		H-2, H-5′	125.8 (s)		H-2, H-1, H-5′
2′	150.7 (s)		H-1, H-6′, 2′-OMe	145.3 (s)		H-1, H-6′, 2′-OMe, 3′-OH
2′-OMe	60.5 (q)	3.87 (3H, s)		60.7 (q)	3.89 (3H, s)	
3′	140.0 (s)		H-5′, 3′-OMe, 4′-OH	138.7 (s)		H-5′, 3′-OH
3′-OH					5.50 (br)	
3′-OMe	60.7 (q)	3.93 (3H, s)				
4′	148.2 (s)		H-5′, H-6′, 4′-OH	146.4 (s)		H-6′, 4′-OMe
4′-OH		5.65 (br)				
4′-OMe				56.3 (q)	3.88 (3H, s)	
5′	110.3 (d)	6.68 (d, 8.4)	H-6′, 4′-OH	106.5 (d)	6.62 (d, 8.4)	
6′	124.8 (d)	6.84 (d, 8.4)	H-1	119.7 (d)	6.69 (d, 8.4)	H-1
1″	125.2 (s)		H-2, H-3, H-3″	125.4 (s)		H-2, H-3″, H-6″
2″	146.7 (s)		H-3, H-3″, H-4″, H-6″	146.7 (s)		H-4″, H-6″
2″-OH		4.78 (br)			4.78 (br)	
3″	116.5 (d)	6.71 (d, 8.4)		116.6 (d)	6.71 (d, 8.4)	
4″	113.8 (d)	6.66 (dd, 8.4, 3.3)	H-6″	113.9 (d)	6.66 (dd, 8.4, 3.3)	H-6″
5″	153.7 (s)		H-3″, H-4″, H-6″, 5″-OMe	153.7 (s)		H-3″, 5″-OMe
5″-OMe	55.6 (q)	3.75 (3H, s)		55.8 (q)	3.75 (3H, s)	
6″	112.1 (d)	6.86 (d, 3.3)	H-3, H-4″	112.0 (d)	6.86 (d, 3.3)	H-3, H-4″

[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.

Table 3. Inhibitory Effects of Cinnamylphenols on TPA-Induced EBV-EA Activation[a]

	EBV-EA-positive cells (% viability) compound concentration (mol ratio/32 pmol TPA)				IC_{50}[b]
compound	1000	500	100	10	(mol ratio/32 pmol TPA)
dalberatin A (**1**)	0.0 ± 0.4 (60)	18.5 ± 1.2 (>80)	63.6 ± 2.0 (>80)	85.1 ± 0.3 (>80)	213
dalberatin B (**2**)	0.0 ± 0.5 (70)	31.1 ± 1.5 (>80)	68.3 ± 2.0 (>80)	91.7 ± 0.7 (>80)	303
dalberatin C (**3**)	0.0 ± 0.3 (60)	20.9 ± 1.4 (>80)	65.0 ± 2.2 (>80)	86.7 ± 0.3 (>80)	223
dalberatin E (**5**)	0.0 ± 0.4 (70)	16.4 ± 1.5 (>80)	60.3 ± 2.0 (>80)	82.0 ± 1.4 (>80)	209
β-carotene[c]	9.1 ± 0.5 (60)	34.3 ± 1.1 (>80)	82.7 ± 1.8 (>80)	100.0 ± 0.2 (>80)	400

[a] Mol 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 by Trypan Blue staining. A minimum 60% survival rate of Raji cells 2 days after treatment with the compounds is required for an accurate result. [b] IC_{50} represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA. [c] Positive control substance.

term in vitro assay for TPA-induced EBV-EA activation in Raji cells, and the activation of EBV-EA expression in EBV genome-carrying human lymphoblastoid cells has been used to detect tumor promoter or antitumor promoter. This assay system is composed of EBV-nonproducer cells as the indicator, *n*-butyric acid as the trigger, TPA as the EBV-activator, and the test substance. Their inhibitory effects on the activation of the virus-genome, the viabilities of Raji cells, and the 50% inhibitory concentration (IC_{50}) values are shown in Table 3. All the test compounds showed inhibitory activity on EBV-EA activation, even at 10 mol ratio/TPA (8.3−18.0%) and fully blocked EBV-EA activation at the highest concentration tested (1000 mol ratio/TPA) without causing a decrease in viability of the Raji cells. These values corresponded to an IC_{50} of 209−303 mol ratio/TPA, and dalberatin E (**5**) with a catechol (1,2-dihydroxy) moiety exhibited the most potent activity (IC_{50} 209). The IC_{50} values of all the test compounds were lower than that of β-carotene, a vitamin A precursor commonly used in cancer prevention studies.[11] A study examining the tumor-promoting inhibitory activity of these compounds in vivo is now in progress.

Experimental Section

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

Plant Materials. The plant materials, *Dalbergia cultrata* Grah. and *D. nigrescens* Kurz, were collected in the Sakaerat Environment Research Station, Nakorn Ratsima Province, Thailand, in April 1996. Voucher specimens were authenticated by comparison with those at the herbarium at Royal Forest Department, Ministry of Agriculture and Cooperative, Bangkok. The herbarium specimens (*D. cultrata*; NSR-092511 and *D. nigrescens*; NSR-092512) have been deposited in the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Isolation of Dalberatins A (1) and B (2) from *D. cultrata*. The dried stem bark (5.45kg) of *D. cultrata* was extracted with EtOH at room temperature, and the solvent was evaporated under reduced pressure to give the EtOH extract (284 g). The acetone-soluble portion (17 g) of the EtOH extract was subjected to Si gel column chromatography eluted with hexane−acetone (9:1, 17:3, 4:1, 3:1, 7:3, 3:2, 1:1), acetone, 3:1 CH_2Cl_2−MeOH, and MeOH, successively, to separate 10 fractions. The 7:3 hexane−acetone eluate was subjected to preparative Si gel TLC developed with 9:1 benzene−acetone to afford biochanin A (10.0 mg). The acetone-soluble portion

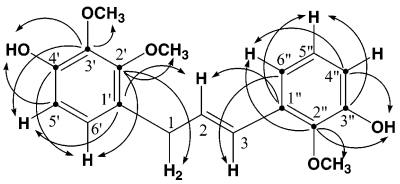

Figure 2. C−H long-range significant correlations in the HMBC spectrum of dalberatin A (**1**).

of the 3:2 hexane−acetone eluate was subjected to preparative Si gel TLC developed with 9:1 benzene−acetone, 98:2 $CHCl_3$−methanol, and 7:3 hexane−acetone, successively, to afford dalberatin A (**1**, 19.4 mg) and dalberatin B (**2**, 7.6 mg). The acetone-insoluble portion of the 3:2 hexane−acetone eluate was subjected to preparative Si gel TLC developed with 96:4 $CHCl_3$−methanol and 9:1 benzene−acetone, successively, to afford formononetin (67.4 mg), dalberatin A (**1**, 5.4 mg), and biochanin A (10.0 mg). The diethyl ether-soluble portion of the 1:1 hexane−acetone eluate was subjected to preparative Si gel TLC developed with 98:2 $CHCl_3$−methanol to afford formononetin (10.2 mg) and dalberatin B (**2**, 9.2 mg).

Dalberatin A (1): pale yellow oil; ^{1}H and ^{13}C NMR (Table 1); UV (MeOH) λ_{max} 206, 220sh, 259 nm; IR ($CHCl_3$) ν_{max} 3531, 3315br, 1604, 1585, 1493 cm^{-1}; HMBC, Figure 2; EIMS *m*/*z* 316 (M^+, 100), 285 ($M^+ - {}^\bullet OCH_3$, 30), 236 (28), 213 (11), 167 ($M^+ - {}^\bullet C_9H_9O_2$, 23); HREIMS *m*/*z* 316.1298 (calcd for $C_{18}H_{20}O_5$, 316.1311).

Dalberatin B (2): colorless oil; ^{1}H and ^{13}C NMR (Table 2); UV (MeOH) λ_{max} 206, 227sh, 258 nm; IR ($CHCl_3$) ν_{max} 3525, 3315br, 1603, 1493 cm^{-1}; EIMS *m*/*z* 316 (M^+, 53), 298 ($M^+ - {}^\bullet OH$, 24), 190 (38), 181 (100), 167 ($M^+ - {}^\bullet C_9H_9O_2$, 65); HREIMS *m*/*z* 316.1297 (calcd for $C_{18}H_{20}O_5$, 316.1311).

Isolation of Dalberatins C (3), D (4), and E (5) from *D. nigrescens*. The dried stem bark (4.26 kg) of *D. nigrescens* was extracted with acetone at room temperature, and the solvent was evaporated under reduced pressure to give the EtOH extract (146 g). The $CHCl_3$-soluble portion (2.8 g) of the EtOH extract was subjected to Si gel column chromatography eluted with $CHCl_3$, $CHCl_3$−acetone (98:2, 96:4, 95:5, 92:8, 9:1, 85:15, 8:2), acetone, CH_2Cl_2−MeOH (3:1), and MeOH, successively, to separate 11 fractions. Successive treatment of each fraction with Si gel column and preparative TLC using appropriate combinations of solvents (hexane, $CHCl_3$, CH_2Cl_2, Et_2O, acetone, benzene, and methanol) as eluting or developing solvents gave the following compounds. From fraction 2 (92:8 $CHCl_3$−acetone): dalberatin C (**3**, 21.8 mg). From fraction 3 (96:4 $CHCl_3$−acetone): dalberatin D (**4**, 3.6 mg), biochanin A (3.0 mg). From fraction 4 (95:5 $CHCl_3$−acetone): formononetin (6.4 mg). From fraction 5 (92:8 $CHCl_3$−acetone): formononetin (2.4 mg). From fraction 6 (9:1 $CHCl_3$−acetone): dalberatin E (**5**, 6.2 mg).

Dalberatin C (3): colorless oil; ^{1}H and ^{13}C NMR (Table 1); UV (MeOH) λ_{max} 206, 224sh, 261 nm; IR ($CHCl_3$) ν_{max} 3537, 3307br, 1616, 1585, 1496 cm^{-1}; EIMS *m*/*z* 316 (M^+, 100), 301 (55), 285 ($M^+ - {}^\bullet OCH_3$, 50), 269 (18), 253 (15), 167 ($M^+ - {}^\bullet C_9H_9O_2$, 24); HREIMS *m*/*z* 316.1291 (calcd for $C_{18}H_{20}O_5$, 316.1311).

Dalberatin D (4): colorless oil; ^{1}H and ^{13}C NMR (Table 2); UV (MeOH) λ_{max} 207, 227sh, 259 nm; IR ($CHCl_3$) ν_{max} 3537, 3350br, 1610, 1496 cm^{-1}; EIMS *m*/*z* 316 (M^+, 100), 257 (24), 236 (34), 180 (51), 167 ($M^+ - {}^\bullet C_9H_9O_2$, 57); HREIMS *m*/*z* 316.1309 (calcd for $C_{18}H_{20}O_5$, 316.1311).

Dalberatin E (5): colorless oil; ^{1}H and ^{13}C NMR (Table 1); UV (MeOH) λ_{max} 205, 221sh, 259 nm; IR ($CHCl_3$) ν_{max} 3547, 3309br, 1608, 1508 cm^{-1}; EIMS *m*/*z* 302 (M^+, 100), 287 (16), 271 (23), 197 (10), 153 ($M^+ - {}^\bullet C_9H_9O_2$, 20); HREIMS *m*/*z* 302.1163 (calcd for $C_{17}H_{18}O_5$: 302.1154).

In Vitro EBV-EA Activation Experiments. The inhibition of EBV-EA activation was assayed using the method previously described.[3−7] 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. Cell number and viability were determined after 48 h by means of a hemocytometer (Trypan Blue staining method). Untreated cultures served as the controls, and EBV-EA inhibitory activity of the test compounds was estimated on the basis of the percentage of the number 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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References and Notes

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