Acerogenin M, a Cyclic Diarylheptanoid, and Other Phenolic Compounds from *Acer nikoense* and Their Anti-inflammatory and Anti-tumor-Promoting Effects

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A new cyclic diarylheptanoid, acerogenin M (1), has been isolated along with nine known diarylheptanoids, 2—10, and two known phenolic compounds, 11 and 12, from a MeOH extract of the stem bark of *Acer nikoense* MAXIM. (Aceraceae). The structure of 1 was determined on the basis of a spectroscopic method. Upon evaluation of the inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1 μg/ear) in mice of nine of the compounds (2—6, 8, 10—12), six (2, 4—6, 8, 10) showed a marked anti-inflammatory effect with a 50% inhibitory dose (ID_{50}) of 0.26—0.81 mg per ear. In addition, upon an evaluation against the Epstein–Barr virus early antigen (EBV-EA) activation induced by TPA for all of the compounds, all exhibited moderate inhibitory effects against EBV-EA induction (IC_{50} values of 356—534 mol ratio/32 pmol TPA).

Key words *Acer nikoense*; diarylheptanoid; anti-tumor-promoter; 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema; Epstein–Barr virus early antigen

The Aceraceae plant *Acer nikoense* MAXIM. is indigenous to Japan (Japanese name, Megusurino-ki) and its stem bark has been used as a folk medicine for the treatment of hepatic disorders and eye disease.[1)] The MeOH extract of its stem bark was reported to have hepatoprotective effects and (+)-rhododendrol (**11**) was isolated as the active principle.[2)] The stem bark is reported to contain various diarylheptanoids and phenolic compounds,[3—5)] and some principal diarylheptanoid constituents have been reported to exhibit inhibitory effects on the release of β-hexosaminidase in RBL-2H3 cells[6)] and on the nitric oxide production in lipopolysaccharide-activated macrophages.[7)] In the course of our search for potential anti-tumor promoters from natural sources,[8,9)] we were especially interested to undertake the investigation of *A. nikoense*. In this paper, we present the isolation and structure elucidation of a new diarylheptanoid of the cyclic biphenyl ether type, **1**, along with nine known diarylheptanoids, **2**—**10**, and two known phenolic compounds, **11** and **12**, from a MeOH extract of its stem bark, as well as their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice, and on Epstein–Barr virus early antigen (EBV-EA) activation induced by TPA, as primary screenings for anti-tumor promoters.[8,9)]

The *n*-hexane and MeOH extracts of *A. nikoense* stem bark and the EtOAc-, *n*-BuOH-, and H_2O-soluble fractions obtained from the MeOH extract were examined against TPA (1.7 nmol)-induced inflammation in mice and TPA (32 pmol)-induced EBV-EA activation in Raji cells. As shown in Table 1, the EtOAc-soluble fraction exhibited the most potent inhibitory activities in both assay systems [66% inhibition of inflammation in mice at a dose of 1.0 mg/ear; and 48.5% inhibition of TPA (32 pmol)-induced EBV-EA activation at a dose of 10 μg/ml]. The EtOAc-soluble fraction was further investigated for the active constituents in this study. Chromatography of this fraction on normal-phase and reversed-phase silica gel column and reversed-phase prepara-

Table 1. Inhibitory Effects of the Extracts of *Acer nikoense* on TPA-Induced Inflammation in Mice and on the Induction of Epstein–Barr Virus Early Antigen

Extract and fraction	Inhibition of inflammation		Percentage of EBV-EA induction[c)]		
	Mean±S.D.[a)] ($\times 10^{-2}$ mm)	I.R.[b)] (%)	Concentration (μg/ml)[d)]		
			100	10	1
Control A (MeOH–$CHCl_3$–H_2O=2 : 1 : 1)	27.8±1.71	—			
n-Hexane extract	12.3±1.50[e,f)]	56	8.5±0.5 (60)	56.2±1.4 (>80)	96.2±0.9 (>80)
MeOH extract	18.3±1.71[e,f)]	34	6.9±0.4 (50)	52.7±1.2 (>80)	94.0±0.6 (>80)
EtOAc-soluble fraction	9.5±1.29[e,f)]	66	6.0±0.4 (50)	51.5±1.1 (>80)	93.3±0.6 (>80)
Control B (MeOH–H_2O=1 : 1)	28.5±1.73	—			
n-BuOH-soluble fraction	23.0±2.45[g,h)]	19	15.4±0.6 (50)	66.3±1.8 (>80)	100.0±0.3 (>80)
H_2O-soluble fraction	25.5±1.29[g,h)]	11	13.7±0.4 (50)	64.9±1.7 (>80)	100.0±0.4 (>80)

a) Ear thickness measured. *b*) I.R.: inhibitory ratio at 1.0 mg/ear. *c*) Each value represents the relative percentage to the positive control value. TPA (32 pmol, 20 ng)=100%. Data are expressed as mean±S.D. Values in parentheses are the percentage viability of Raji cells. *d*) The values of all extracts at 10 μg/ml were different from the control value ($p<0.05$) without cytotoxicity. *e*) $p<0.01$ by Student's *t*-test compared with the control group. *f*) Solvent used: MeOH–$CHCl_3$–H_2O=2 : 1 : 1 (control A). *g*) $p<0.05$ by Student's *t*-test compared with the control group. *h*) Solvent used: MeOH–H_2O=1 : 1 (control B).

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Table 2. ^{13}C- (150 MHz) and ^{1}H-NMR (600 MHz) Spectral Data for Compounds **1**, **3**, and **7** (C_5D_5N)

Atom No.	**1**		**3**		**7**	
	δ_C	δ_H [a]	δ_C	δ_H [a]	δ_C	δ_H [a]
1	150.6 s		152.3 s		150.9 s	
2	152.4 s		145.3 s		146.0 s	
3	118.1 d	7.30 (d, 8.4)	117.4 d	7.53 (d, 8.3)	117.8 d	7.23 (d, 8.0)
4	123.0 d	7.92 (dd, 2.0, 8.4)	122.4 d	6.68 (dd, 2.0, 8.3)	123.4 d	6.78 (dd, 2.0, 8.0)
5	128.3 s		136.2 s		132.5 s	
6	119.7 d	6.58 (d, 2.0)	117.2 d	5.93 (d, 2.0)	118.0 d	5.97 (d, 2.0)
7	197.3 s		32.2 t	2.39 (ddd, 3.0, 7.3, 15.6)	28.7 t	2.61 (m)
				2.45 (ddd, 3.0, 7.8, 15.6)		2.98 (ddd, 2.4, 11.3, 13.7)
8	49.9 t	2.63 (dd, 9.2, 12.6)	28.7 t	1.26 (m)	36.5 t	1.50 (ddt, 11.3, 14.4, 2.4)
		3.05 (dd, 1.7, 12.6)		1.50 (m)		1.77 (dddd, 2.4, 6.5, 8.9, 14.4)
9	70.4 d	3.76 (br q, 6.9)	25.5 t	1.00 (m)	65.2 d	3.57 (br t, 8.9)
				1.26 (m)		
10	40.0 t	1.15 (dddd, 6.0, 6.3, 6.6, 13.2)	40.0 t	1.12 (m)	53.5 t	2.04 (dd, 2.4, 16.5)
		1.54 (dddd, 6.3, 7.3, 7.3, 13.2)		1.39 (m)		2.47 (dd, 8.9, 16.5)
11	22.6 t	1.24 (dddd, 6.3, 6.9, 6.9, 13.7)	69.8 d	3.51 (br dt, 9.2, 6.2)	212.5 s	
		1.35 (dddd, 6.3, 6.6, 7.2, 13.7)				
12	31.4 t	1.61 (2H, br q, 6.3)	41.2 t	1.71 (ddt, 13.2, 13.9, 4.4)	44.8 t	2.61 (m)
				2.12 (dq, 4.1, 13.9)		2.70 (ddd, 5.5, 9.6, 13.7)
13	35.1 t	2.57 (dd, 6.3, 12.9)	32.9 t	2.74 (dt, 3.9, 12.7)	31.6 t	2.89 (2H, m)
		2.61 (dd, 6.3, 12.9)		2.93 (dt, 12.7, 3.9)		
14	140.3 s		140.1 s		137.7 s	
15	132.6[b] d	7.20 (br d, 8.4)[b]	132.0[b] d	7.21 (dd, 2.4, 8.2)[b]	132.2[b] s	7.07 (dd, 2.4, 8.2)[b]
16	123.6[c] d	6.88 (br d, 8.4)[c]	123.3[c] d	6.97 (dd, 2.4, 8.2)[c]	123.8[c] d	6.85 (dd, 2.4, 8.2)[c]
17	155.8 s		156.8 s		157.9 s	
18	123.7[c] d	7.33 (br s)[c]	124.4[c] d	7.20 (dd, 2.4, 8.2)[c]	123.8[c] d	7.22 (dd, 2.4, 8.2)[c]
19	131.2[b] d	7.33 (br s)[b]	130.6[b] d	7.28 (dd, 2.4, 8.2)[b]	130.4[b] d	7.35 (dd, 2.4, 8.2)[b]
1′			102.8 d	5.81 (dt, 7.3, 1.9)		
2′			75.0 d	4.40 (dd, 7.8, 7.8)		
3′			78.6 d	4.39 (dd, 7.8, 7.8)		
4′			71.4 d	4.37 (dd, 7.8, 7.8)		
5′			78.9 d	4.15 (ddd, 2.4, 5.1, 7.8)		
6′			62.5 t	4.42 (dd, 5.1, 12.0)		
				4.56 (dd, 2.4, 12.0)		

a) Figures in parentheses denote *J* values (Hertz). *b*, *c*) Values bearing the same superscript may be interchangeable within the same column.

tive HPLC eventually yielded a new cyclic diarylheptanoid, **1**, nine known diarylheptanoids, **2**—**10**, and two known phenolic compounds, **11** and **12**.

The high-resolution electron-ionization (HR-EI)-MS of compound **1** furnished a M^+ at *m*/*z* 312.1363 according with the molecular formula $C_{19}H_{20}O_4$. The ^{13}C-NMR spectrum of **1** (Table 2) exhibited 19 signals assignable to two benzene rings (five singlets and seven doublets), five methylenes, a carbonyl, and an oxymethine carbon. These findings, in combination with its IR spectrum which showed absorption bands at 3389, 1704, 1595, 1517, 1503, and 1053 cm^{-1} assignable to hydroxyl, carbonyl, aromatic ring, and ether functions, and its UV spectrum which exhibited an absorption at 278 nm (log ε 3.14), suggested that **1** is a biphenyl ether-type cyclic diarylheptanoid.[3,4] Compound **1** was shown to possess a β-ketol system at C-7 (C=O) and C-9 (>CHOH) in the heptane chain by observation of ^{13}C–^{1}H long-range couplings from H-4 to C-7, from H-6 to C-7, from H-8 to C-7, from H-10 to C-8, and from H-8, H-10 and H-11 to C-9, in the heteronuclear multiple-quantum coherence (HMBC) spectrum (Fig. 1). Furthermore, the presence of a hydroxyl group at C-2 was shown by ^{13}C–^{1}H long-range couplings from H-3, H-4, and H-6 to C-2 observed in the HMBC spectrum of **1**. The above evidence coupled with analysis of ^{1}H–^{1}H correlation spectroscopy (COSY) (Fig. 1), ^{1}H-detected multiple-quantum coherence (HMQC), and HMBC data indicated that **1** possesses a structure as formulated in Chart 1 which has been called acerogenin M.[10] Stereochemistry at C-9 remain undetermined. Comparison of the ^{13}C- and ^{1}H-NMR data of **1** with those of the structurally related cyclic diarylheptanoids[4,5,7] supported the proposed structure.

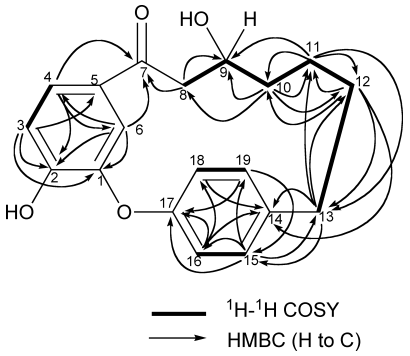

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations of Compound **1**

Identification of nine known compounds, acerogenin A (**2**),[11] aceroside III (**4**),[12] (*R*)-acerogenin B (**5**),[7] aceroside B_1 (**6**),[7] aceroside IV (**8**),[13] acerogenin K (**9**),[5] (−)-centrolobol (**10**),[14] (+)-rhododendrol (**11**),[15] and (+)-catechin (**12**),[16] was made by spectral comparison with corresponding compounds in the literature. Two other known compounds, aceroside I (**3**)[11] and acerogenin D (**7**),[4] were identified

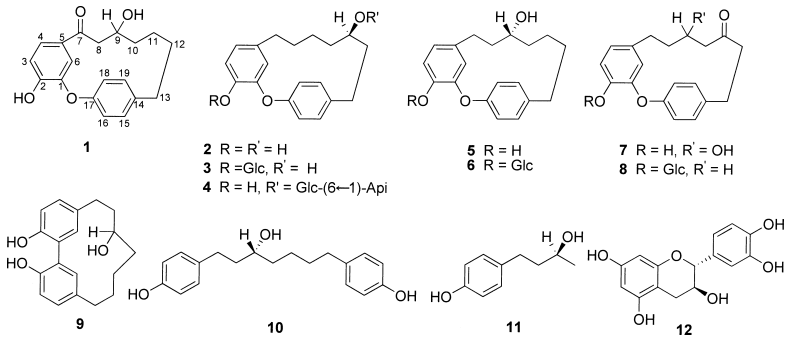

Chart 1. Structures of Compounds **1**—**12**

A conventional numbering system of acerosides[5] was adopted for the cyclic diarylheptanoids.

Table 3. Inhibitory Effects of Compounds from *Acer nikoense* and Reference Compounds on TPA-Induced Inflammation in Mice and on the Induction of Epstein–Barr Virus Early Antigen

Compound	Inhibition of inflammation[a]	Percentage of EBV-EA induction[c]				IC_{50}[e]
		Concentration (mol ratio/32 pmol TPA)[d]				(mol ratio/32 pmol TPA)
	ID_{50}[b] (mg/ear)	1000	500	100	10	
1 Acerogenin M		13.3±0.4 (70)	52.4±1.5 (>80)	76.4±2.1 (>80)	100.0±0.3 (>80)	479
2 Acerogenin A[f]	0.32	18.2±0.6 (60)	47.0±1.5 (>80)	83.5±2.5 (>80)	100.0±0.3 (>80)	488
3 Aceroside I	>1.0	15.4±0.5 (70)	53.5±1.5 (>80)	76.3±2.0 (>80)	100.0±0.4 (>80)	485
4 Aceroside III	0.81	17.8±0.8 (70)	57.1±1.6 (>80)	79.0±2.3 (>80)	100.0±0.3 (>80)	489
5 (*R*)-Acerogenin B	0.26	11.3±0.5 (70)	44.3±1.4 (>80)	79.1±2.2 (>80)	100.0±0.2 (>80)	480
6 Aceroside B_1	0.32	15.0±0.5 (70)	59.3±1.7 (>80)	80.0±2.5 (>80)	100.0±0.2 (>80)	491
7 Acerogenin D		13.5±0.4 (70)	45.1±1.5 (>80)	81.3±2.3 (>80)	100.0±0.2 (>80)	483
8 Aceroside IV	0.49	19.3±0.6 (70)	61.5±1.7 (>80)	82.7±2.8 (>80)	100.0±0.2 (>80)	534
9 Acerogenin K[f]		7.8±1.2 (60)	37.4±1.1 (>80)	76.8±2.0 (>80)	100.0±0.3 (>80)	384
10 (−)-Centrolobol[f]	0.66	5.0±0.5 (60)	36.6±1.2 (>80)	74.0±2.0 (>80)	96.0±0.5 (>80)	356
11 (+)-Rhododendrol[f]	>1.0	8.7±0.5 (60)	39.8±1.2 (>80)	75.1±1.9 (>80)	100.0±0.3 (>80)	392
12 (+)-Catechin[g]	>1.0	11.3±0.3 (60)	41.3±1.4 (>80)	75.8±0.9 (>80)	100.0±0.2 (>80)	471
Reference compounds						
Quercetin	1.6	21.6±1.1 (60)	55.7±1.8 (>80)	82.7±2.1 (>80)	100.0±0.2 (>80)	560
Indomethacin	0.30					
β-Carotene		8.6±0.5 (70)	34.2±1.4 (>80)	82.1±2.0 (>80)	100.0±0.3 (>80)	397

a) Solvent sytem used: MeOH–$CHCl_3$–H_2O=2 : 1 : 1. *b*) ID_{50}: 50% inhibitory dose. *c*) Values represent relative percentage to the positive control value (100%). Data are expressed as mean±S.D. Values in parentheses are the percentage viability of Raji cells. *d*) The values of all compounds at 500 mol ratio/TPA were different from the control value ($p<0.05$) without cytotoxicity. *e*) IC_{50} represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol TPA. *f*) EBV-EA induction data taken from literature.[21] *g*) EBV-EA induction data taken from literature.[28]

based on the MS and NMR spectral analysis since the spectral data were unavailable in the literature.

Seven diarylheptanoids, **2**—**6**, **8**, and **10**, and two phenolic comopounds, **11** and **12**, were evaluated with respect to their anti-inflammatory activity against TPA-induced inflammation in mice, and the inhibitory effects were compared with those of quercetin (3,3′,4′,5,7-pentahydroxyflavone), a known inhibitor of TPA-induced inflammation in mice, and indomethacin, a commercially available anti-inflammatory drug, as shown in Table 3. All of the diarylheptanoids tested except for compound **3** inhibited the TPA-induced inflammation [ID_{50} (50% inhibitory dose) 0.26—0.81 mg/ear], making them more inhibitory than quercetin (ID_{50} 1.6 mg/ear). Three compounds, **2**, **5**, and **6**, exhibited the strongest inhibitory effects (ID_{50} 0.26—0.32 mg/ear) among those tested, which being comparable with that of indomethacin (ID_{50} 0.30 mg/ear). The inhibitory effect against TPA-induced inflammation has been demonstrated to closely parallel that of the inhibition of tumor promotion in two-stage carcinogenesis initiated by 7,12-dimethylbenz[*a*]anthracene (DMBA) and then by TPA, a well-known promoter, in a mouse skin model.[17] Thus, these diarylheptanoids can be expected to possess a high anti-tumor-promoting effect in the same animal model. Curcumin (diferuloylmethane),[18—22] the major yellow pigment in turmeric (*Curcuma longa* L.), and other naturally occurring diarylheptanoids[19—21,23,24] have also been demonstrated to possess marked anti-inflammatory[18,19,22,23] and anti-tumor-promoting effects.[20,21,24]

The inhibitory effect on EBV-EA activation induced by TPA was further examined as a preliminary evaluation of the potential anti-tumor-promoting effects of the ten diarylheptanoids, **1**—**10**, and the two phenolic compounds, **11** and **12**. The results are shown in Table 3, together with comparable data for quercetin as well as *β*-carotene, a vitamin A precursor that has been intensively studied in cancer chemoprevention by using animal models.[25] All of the compounds tested showed inhibitory effects with IC_{50} values of 356—534 mol ratio/32 pmol TPA, which were more inhibitory than quercetin (IC_{50} 560 mol ratio/32 pmol TPA). Among them, three compounds, **9**—**11**, exhibited potent inhibitory effects

(IC_{50} 356—392 mol ratio/32 pmol TPA) almost comparable with β-carotene (IC_{50} 397 mol ratio/32 pmol TPA).

From the results of *in vivo* anti-inflammatory test and *in vitro* EBV-EA induction test, it may be suggested that diarylheptanoids from the EtOAc-soluble fraction of the MeOH extract of *A. nikoense* stem bark are useful as agents that inhibit inflammation and chemical carcinogenesis.

Experimental

Optical rotations were measured on a JASCO P-1030 polarimeter in MeOH at 25 °C. UV spectra on a Shimadzu UV-2200 spectrometer and IR spectra on a JASCO FTIR-300E spectrometer were recorded in MeOH and KBr disks, respectively. NMR spectra were recorded with a JEOL ECA-600 spectrometer at 600 MHz (^{1}H-NMR) and 150 MHz (^{13}C-NMR) in C_5D_5N with tetramethylsilane as internal standard. EI-MS (70 eV) and HR-EI-MS were recorded on a JEOL JMS-BU20 spectrometer using a direct inlet system. FAB-MS were obtained with a JEOL JMS-BU20 spectrometer using glycerol as a matrix. Silica gel (Silica gel 60, 220—400 mesh, Merck) and C_{18} silica (Chromatorex-ODS, 100—200 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for open column chromatography. Reversed-phase preparative HPLC was carried out on a C_{18} silica column (Pegasil ODS II column, 5 μm; 25 cm×10 mm i.d.; Senshu Scientific Co., Ltd., Tokyo, Japan; flow rate of the mobile phase: 3.0 ml/min) at 25 °C. A refractive index detector was used for the HPLC.

Material and Chemicals The stem bark obtained from a 25 year old tree of *Acer nikoense* in the summer of 2002 was purchased from Sirakami Fruit Park (Gunma, Japan). The plant was authenticated by Mr. Hiroshi Sato (Sirakami Fruit Park), and the voucher of the plant is on file in the laboratory of the College of Science and Technology, Nihon University. Compounds were purchased as follows: TPA from ChemSyn Laboratories (Lenexa, KS, U.S.A.), quercetin, indomethacin, and β-carotene from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and the EBV cell culture reagents and *n*-butanoic acid from Nacalai Tesque, Inc. (Kyoto, Japan).

Extraction and Isolation The dried stem bark of *A. nikoense* (450 g) was finely cut and extracted three times with *n*-hexane under reflux for 3 h which yielded the extract (3.4 g). The residue was then extracted three times with MeOH under reflux for 3 h giving the MeOH extract (63.6 g) which was partitioned in an EtOAc–H_2O (1 : 1, v/v) mixture. The aqueous layer was extracted with *n*-BuOH, and removal of the solvent *in vacuo* from the EtOAc-, *n*-BuOH-, and H_2O-soluble portions yielded 26.2, 26.6, and 4.5 g of the residue, respectively.

Column chromatography on silica gel (800 g) of the EtOAc-soluble fraction (25.2 g) eluted with *n*-hexane–EtOAc (1 : 0→0 : 1) and then with EtOAc–MeOH (7 : 3→0 : 1) gave five fractions, fractions 1 (0.17 g), 2 (0.44 g), 3 (1.98 g), 4 (0.88 g), and 5 (20.64 g), arranged in the increasing order of polarity. Fraction 3 (1.98 g) was further separated by column chromatography on silica gel (100 g) [*n*-hexane–EtOAc (2 : 3→0 : 1) and then EtOAc–MeOH (95 : 5→0 : 1)] to furnish six fractions, fractions 3-1 (1113 mg), 3-2 (416 mg), 3-3 (96 mg), 3-4 (69 mg), 3-5 (53 mg), and 3-6 (49 mg). Preparative HPLC [MeOH–H_2O–acetic acid (AcOH) (65 : 35 : 0.1, v/v/v)] of fraction 3-1 (140 mg) gave **2** [14.1 mg, retention time (t_R) 14.5 min], **5** (24.5 mg, t_R 15.3 min), and **10** (3.0 mg, t_R 12.2 min). In addition, preparative HPLC of fraction 3-2 (416 mg) under the same conditions as above yielded compounds **1** (3.0 mg, t_R 11.1 min), **2** (8.2 mg), **5** (3.2 mg), **7** (4.6 mg, t_R 7.3 min), **9** (5.9 mg, t_R 17.7 min), and **10** (2.8 mg), along with a highly polar fraction (237.9 mg) eluted with retention times within 6 min. The latter fraction was further subjected to HPLC [MeOH–H_2O–AcOH (50 : 50 : 0.1, v/v/v)] which afforded **11** (111.4 mg, t_R 8.8 min). Chromatography of fraction 4 (528 mg) on ODS column (45 g) [MeOH–H_2O (1 : 4→1 : 0)] gave four fractions, fractions 4-1 (82 mg), 4-2 (150 mg), 4-3 (188 mg), and 4-4 (66 mg). Upon HPLC [acetonitrile (MeCN)–H_2O (13 : 7, v/v)], fraction 4-2 (150 mg) afforded **12** (59.8 mg, t_R 7.6 min). Fraction 5 (20.53 g) was subjected to further chromatography on silica gel (800 g) [EtOAc–MeOH (4 : 1→0 : 1)] which yielded five fractions, fractions 5-1 (2.42 g), 5-2 (12.52 g), 5-3 (0.44 g), 5-4 (1.44 g), and 5-5 (1.23 g). Fraction 5-1 (141 mg) was subjected to HPLC [MeOH–H_2O (11 : 9, v/v)] to give **8** (8.8 mg, t_R 15.2 min). Chromatography of fraction 5-2 (452 mg) on ODS column (35 g) [MeOH–H_2O (1 : 1→1 : 0)] gave four fractions, fractions 5-2a (32 mg), 5-2b (27 mg), 5-2c (145 mg), and 5-2d (210 mg). Further HPLC [MeCN–H_2O (3 : 7, v/v)] was performed on fraction 5-2b which yielded **3** (6.7 mg, t_R 16.0 min); fraction 5-2c afforded **3** (23.2 mg), **4** (30.8 mg, t_R 22.9 min), and **6** (11.1 mg, t_R 25.3 min); and fraction 5-2d gave **4** (64.4 mg).

Some spectral data for compounds **1**, **3**, and **7** are given below. ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H COSY, HMQC, HMBC, and nuclear Overhauser enhancement and exchange spectroscopy (NOESY) experiments were used for structure elucidation and to assign the ^{13}C- and ^{1}H-NMR spectra of these compounds.

Acerogenin M (**1**): Amorphous solid. $[\alpha]_D^{25}$ +4.0° (c=0.10, MeOH). IR (KBr) cm^{-1}: 3389 (OH), 1704 (C=O), 1595, 1517, 1503 (aromatic C=C), 1053 (C–O–C). UV λ_{max} (MeOH) nm (log ε): 213 (3.35), 231 (3.36), 278 (3.14), 303 (2.95). HR-EI-MS m/z: 312.1363 [Calcd for $C_{19}H_{20}O_4$ (M^+): 312.1361]. EI-MS m/z (%): 312 (M^+, 100), 294 (27), 284 (16), 269 (16), 256 (23), 241 (50), 225 (15), 212 (15), 198 (31), 152 (17), 121 (16), 117 (28), 107 (56). ^{13}C- and ^{1}H-NMR data, see Table 2.

Aceroside I (**3**): FAB-MS m/z: 483 $(M+Na)^+$. ^{13}C- and ^{1}H-NMR data, see Table 2.

Acerogenin D (**7**): FAB-MS m/z: 335 $(M+Na)^+$. ^{13}C- and ^{1}H-NMR data, see Table 2.

Animals The experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the College of Pharmacy, Nihon University, Chiba, Japan. Specific pathogen-free female ICR mice were obtained from Japan SLC (Shizuoka, Japan). The animals were housed, five per polycarbonate cage, in an air-conditioned specific pathogen-free room at 24±2 °C. Food and water were available *ad libitum*.

Assay of TPA-Induced Inflammation Ear Edema in Mice TPA (1 μg, 1.7 nmol) dissolved in acetone (20 μl) was applied to the right ear of mice by a micropipette. A volume of 10 μl was delivered to both the inner and outer surfaces of the ear. The samples or their vehicles, MeOH–$CHCl_3$–H_2O (2 : 1 : 1, v/v/v; 20 μl) or MeOH–H_2O (1 : 1, v/v; 20 μl), as control, were applied topically about 30 min before TPA treatment. For ear thickness determinations, a pocket thickness gauge with a range of 0—9 mm, graduated at 0.01 mm intervals and modified so that the contact surface area was increased to reduce the tension, was applied to the tip of the ear. The ear thickness was measured before treatment (a), and 6 h after TPA treatment (b=TPA alone; b'=TPA plus sample). The following values were then calculated:

Edema A is induced by TPA alone ($b-a$).

Edema B is induced by TPA plus sample ($b'-a$).

Inhibitory ratio (%)=[(edema A−edema B)/edema A]×100.

Each value was the mean of individual determinations from five mice. The 50% inhibitory dose (ID_{50}) values were determined by the method of probit-graphic interpolation for four dose levels. A statistical analysis was carried out by Student's t-test. Details of the *in vivo* anti-inflammatory test have been described previously.[26]

***In Vitro* EBV-EA Activation Experiment** The inhibition of EBV-EA activation was assayed using Raji cells (EBV genome-carrying human lymphoblastoid cells; non-producer type), cultivated in 10% fetal bovine serum RPMI-1640 medium (Sigma, St. Louis, MO, U.S.A.). The indicator cells (Raji cells; 1×10^6 cells/ml) were incubated in 1 ml of the medium containing 4 mmol *n*-butanoic acid as an inducer, 32 pmol of TPA (20 ng/ml in dimethyl sulfoxide), and a known amount (32, 16, 3.2, 0.32 nmol) of the test compound at 37 °C in a CO_2 incubator. After 48 h, cell suspensions were centrifuged at 1000 rpm for 10 min, and the supernatant was removed. The activated cells were stained with high-titer EBV-EA-positive sera from nasopharyngeal carcinoma patients, and the conventional indirect immunofluorescence technique was employed for detection. In each assay, at least 500 cells were counted and the experiments were repeated three times. The average extent of EA induction was determined and compared with that on positive control experiments in which the cells were treated with *n*-butanoic acid plus TPA where the extent of EA induction was ordinarily more than around 40%. The viability of treated Raji cells was assayed by the Trypan Blue staining method. Details of the *in vitro* assay of EBV-EA induction have been reported previously.[27]

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10) Whereas compound **1** can be referred to as 4,10-dihydroxy-2-oxatricyclo[13.2.2.1^{3,7}]eicosa-3,5,7(20),15,17,18-hexaen-8-one when followed by the IUPAC nomenclature, a conventional numbering system of acerosides[5] was adopted for **1** and the other diarylheptanoids of the cyclic biphenyl ether type in this study.
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