Melanogenesis-Inhibitory and Cytotoxic Activities of Diarylheptanoids from *Acer nikoense* Bark and Their Derivatives

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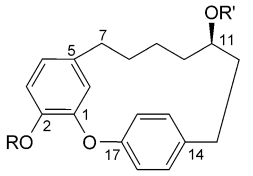
Nine cyclic diarylheptanoids, **1**–**9**, including two new compounds, *i.e.*, 9-oxoacerogenin A (**8**) and 9-*O*-β-D-glucopyranosylacerogenin K (**9**), along with three acyclic diarylheptanoids, **10**–**12**, and four phenolic compounds, **13**–**16**, were isolated from a MeOH extract of the bark of *Acer nikoense* (Aceraceae). Acid hydrolysis of **9** yielded acerogenin K (**17**) and D-glucose. Two of the cyclic diarylheptanoids, acerogenin A (**1**) and (*R*)-acerogenin B (**5**), were converted to their ether and ester derivatives, **18**–**24** and **27**–**33**, respectively, and to the dehydrated derivatives, **25**, **26**, **34**, and **35**. Upon evaluation of compounds **1**–**16** and **18**–**35** for their inhibitory activities against melanogenesis in B16 melanoma cells, induced with α-melanocyte-stimulating hormone (α-MSH), eight natural glycosides, *i.e.*, six diarylheptanoid glycosides, **2**–**4**, **6**, **9**, and **12**, and two phenolic glycosides, **15** and **16**, exhibited inhibitory activities with 24–61% reduction of melanin content at 100 μM concentration with no or almost no toxicity to the cells (88–106% of cell viability at 100 μM). In addition, when compounds **1**–**16** and **18**–**35** were evaluated for cytotoxic activity against human cancer cell lines, two natural acyclic diarylheptanoids, **10** and **11**, ten ether and ester derivatives, **18**–**22** and **27**–**31**, and two dehydrated derivatives, **34** and **35**, exhibited potent cytotoxicities against HL60 human leukemia cell line (IC_{50} 8.1–19.3 μM), and five compounds, **10**, **11**, **20**, **29**, and **30**, against CRL1579 human melanoma cell line (IC_{50} 10.1–18.4 μM).

Introduction. – The bark of the Japanese maple tree, *Acer nikoense* MAXIM. (Aceraceae; Japanese name, *Megusurino-ki*) has been used as a folk medicine for the treatment of hepatic disorders and eye disease [1]. The bark has been reported to contain various diarylheptanoids and phenolic compounds [2–4] possessing several biological properties including inhibitory effects on degranulation in RBL-2H3 cells [5], on nitric-oxide (NO) production in lipopolysaccharide-activated macrophages [6], and on expression of Na^+-glucose co-transporter in COS-1 cells [7], as well as hepatoprotective effects [8]. In our search for potential bioactive compounds from natural sources, we have demonstrated that the diarylheptanoid and phenolic constituents of the extract of *A. nikoense* stem bark exhibited inhibitory effects against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice [9], against *Epstein–Barr* virus early antigen (EBV-EA) activation induced by TPA [9], and against melanogenesis in B16 melanoma cells [10][11], in addition to free radical-scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [10]. In a continuing study on the constituents of *A. nikoense* bark extract, we have isolated twelve diarylheptanoids, **1**–**12**, including two new compounds, **8** and **9**, along with four

phenolic compounds, **13**–**16**, from the extract. In addition, we have prepared 18 derivatives, **18**–**35**, from two cyclic diarylheptanoids, acerogenin A (**1**) and (*R*)-acerogenin B (**5**). Here, we describe structure elucidation of the two new compounds, **8** and **9**, and evaluation of compounds **1**–**16** and **18**–**35** for their inhibitory activities against melanogenesis in B16 melanoma cells induced with α-melanocyte-stimulating hormone (α-MSH), and cytotoxic activities against two human cancer cell lines.

Results and Discussion. – *Isolation, Identification, and Characterization*. The MeOH extract of *A. nikoense* bark was fractionated into AcOEt, BuOH, and H_2O-soluble fractions. Nine cyclic diarylheptanoids, **1**–**9**, three acyclic diarylheptanoids, **10**–**12**, and two phenolic compounds, **13** and **14**, from the AcOEt-soluble fraction, and two phenolic glycosides, **15** and **16**, from the BuOH-soluble fraction were isolated. Two compounds, 9-oxoacerogenin A (**8**) and 9-*O*-β-D-glucopyranosyl acerogenin K (**9**),



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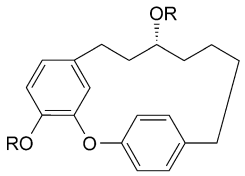
2 R = Glc, R' = H
3 R = H, R' = Api*f*(1→6)Glc
4 R = H, R' = Glc



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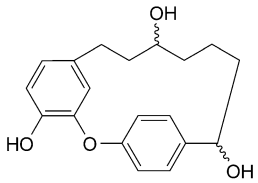

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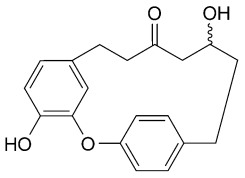

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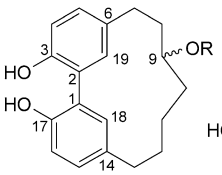

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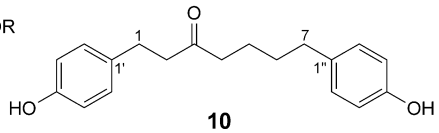

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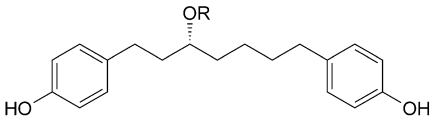

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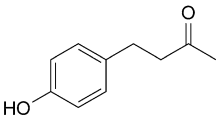

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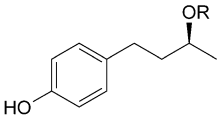

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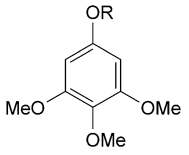
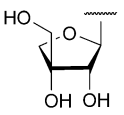
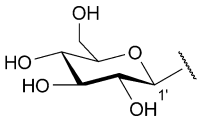


16 R = Api*f*(1→6)Glc



Api*f*



Glc

Table 1. *^{1}H- and ^{13}C-NMR, and HMBC Data* (recorded in C_5D_5N) *for Two Diarylheptanoids* **8** *and* **9** *from* Acer nikoense. Atom numbering as given in the *Formulae.*

Position	**8**			**9**		
	δ(H)[a]) (500 MHz)	δ(C) (125 MHz)	HMBC (H→C)	δ(H)[a]) (400 MHz)	δ(C) (100 MHz)	HMBC (H→C)
1		150.6 (*s*)			128.7 (*s*)	
2		146.0 (*s*)			127.8 (*s*)	
3	7.14 (*d*, *J*=8.1)	117.4 (*d*)	1, 2, 5		152.6 (*s*)	
4	6.70 (br. *d*, *J* = 7.2)	122.4 (*d*)	2, 6, 7	7.25 (*d*, *J*=8.2)	117.4 (*d*)	2, 3
5		132.6 (*s*)		7.15 (*dd*, *J*=1.8, 8.2)	130.0 (*d*)	3, 6, 7, 19
6	5.69 (*d*, *J*=1.5)	114.9 (*d*)	1, 2, 4, 7		130.8 (*s*)	
7	2.49 (*dd*, *J*=10.1, 17.1)	27.2 (*t*)	4, 6, 8	2.48 (br. *t*, *J*=13.8)	30.2 (*t*)	6, 8, 19
	3.30 (*dd*, *J*=11.9, 16.0)		5, 8, 9	2.91 (br. *d*, *J*=18.4)		5, 6, 8, 9
8	2.17 (*dd*-like, *J*=8.0, 16.3)	41.9 (*t*)	7, 9	1.72–1.79 (*m*)	26.9 (*t*)	6
	2.48 (*dd*, *J*=10.1, 17.1)		5, 7, 9	1.99–2.08 (*m*)		7, 10
9		208.3 (*s*)		4.29 (*t*, *J*=9.2)	67.4 (*d*)	8, 11, 1′
10	1.54 (*d*, *J*=18.3)	54.5 (*t*)	9, 11	1.85 (*t*, *J*=13.3)	40.2 (*t*)	9, 11, 12
	2.61–2.68 (*m*)		9, 12	1.95–2.04 (*m*)		9, 11, 12
11	4.43 (*t*, *J*=8.6)	65.2 (*d*)	9, 13	1.40 (*sept.*, *J* = 6.4)	22.9 (*t*)	9, 10, 13
				1.68 (*tt*, *J*=11.4, 11.4)		9, 10, 13
12	1.92 (*ddd*-like, *J*=4.9, 8.0, 13.2)	39.1 (*t*)	10, 11, 13	1.88–1.95 (*m*)	35.2 (*t*)	13, 14
	2.18 (*d*, *J*=16.3)		11	2.36 (br. *t*, *J* = 13.9)		11, 13
13	2.64–2.71 (*m*)	33.4 (*t*)	11, 14, 15, 19	2.96 (br. *d*, *J*=18.4)	27.3 (*t*)	11, 14, 15
	2.93 (*dt*, *J*=3.9, 13.0)		11, 12	3.23 (br. *t*, *J*=13.8)		14, 15, 18
14		138.6 (*s*)			133.0 (*s*)	
15	7.26 (*dd*, *J*=2.0, 8.1)[b])	130.7[b]) (*d*)	13, 17, 19	7.09 (*dd*, *J*=1.8, 8.7)	129.9 (*d*)	13, 16, 17, 18
16	7.14 (*dd*, *J*=2.5, 8.1)[c])	123.9[c]) (*d*)	14, 18	7.48 (*d*, *J*=8.2)	114.5 (*d*)	1, 14, 17
17		155.7 (*s*)			152.9 (*s*)	
18	6.88 (*dd*, *J* = 2.5, 8.1)[c])	124.2[c]) (*d*)	14, 16, 17	7.39 (*d*, *J*=1.8)	135.7 (*d*)	2, 13, 15, 17
19	7.17 (*dd*, *J*=2.0, 8.1)[b])	132.0[b]) (*d*)	13, 15, 17	7.30 (*d*, *J* = 1.8)	135.2 (*d*)	1, 3, 5, 7
1′				5.78 (*d*, *J*=7.8)	101.6 (*d*)	2′, 3′
2′				4.26 (*t*, *J*=8.7)	74.4 (*d*)	3′
3′				4.34 (*t*, *J*=8.7)	78.1 (*d*)	2′, 4′
4′				4.28 (*t*, *J*=9.2)	71.0 (*d*)	5′, 6′
5′				4.19 (*ddd*, *J*=1.8, 5.0, 9.2)	78.9 (*d*)	4′, 6′
6′				4.41 (*dd*, *J*=4.6, 11.5)	62.2 (*t*)	4′
				4.57 (*dd*, *J* = 1.8, 11.9)		4′

[a]) In parentheses, *J* values in Hz. [b]), [c]) Values bearing the same superscript may be interchangeable within the same column.

among the 16 compounds were new ones, and their structures were elucidated on the basis of spectroscopic data and comparison with literature.

The molecular formula of **8** was determined as $C_{19}H_{20}O_4$ on the basis of its HR-ESI-MS (*m/z* 335.1303 ($[M+Na]^+$, $C_{19}H_{20}NaO_4^+$; calc. 335.1259)). The ^{13}C-NMR spectrum of **8** (*Table 1*) exhibited 19 signals assignable to two benzene rings (five *singlet*s and seven *doublet*s), five CH_2 groups, a C=O group, and an CH–O C-atom. These findings, in combination with its IR spectrum which showed absorption bands at 3403, 1705, 1504, and 1055 cm^{-1} assignable to OH, C=O, aromatic ring, and ether functions, and its UV spectrum which exhibited an absorption at 276 nm (log ε 3.52), suggested that **8** is a diphenyl ether-type cyclic diarylheptanoid [2][3][9]. Compound **8** was shown to possess a β-ketol system at C(9) (C(9)=O) and C(11) (>CHOH) in the heptyl chain based on the observation of ^{13}C,^{1}H long-range couplings from H–C(4) and H–C(6) to C(7), from H–C(7) to C(9), from H–C(11) to C(9) and C(13), and from H–C(13) to C(14) and C(15), in the HMBC spectrum (*Table 1* and *Fig.*). Furthermore, the presence of a OH group at C(2) was evidenced by ^{13}C,^{1}H long-range couplings from H–C(3), H–C(4), and H–C(6) to C(2) observed in the HMBC spectrum of **8**. The above findings coupled with analysis of ^{1}H,^{1}H-COSY (*Fig.*), HMQC, and HMBC (*Table 1*, *Fig.*) data indicated that **8** possesses the structure of 4,12-dihydroxy-2-oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-10-one, which has been named 9-oxoacerogenin A. The configuration at C(11) of compound **8** remained undetermined.

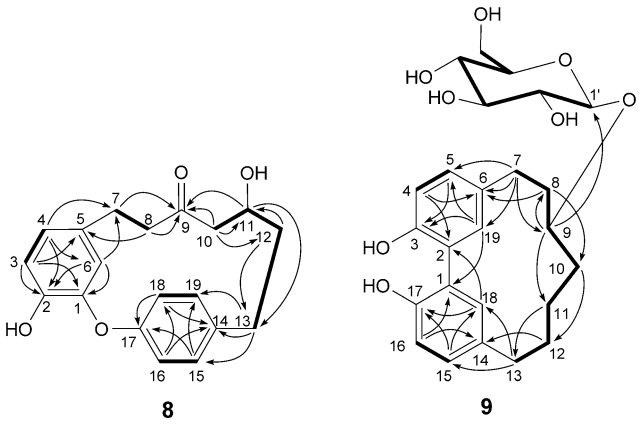


Figure. *^{1}H,^{1}H-COSY* (—) *and major HMBC* (H→C) *correlations for compounds* **8** *and* **9**

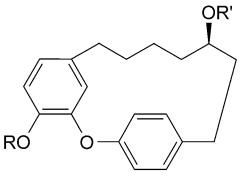
The molecular formula of **9** was determined as $C_{25}H_{32}O_8$, based on its HR-ESI-MS (*m/z* 483.1984 ($[M+Na]^+$, $C_{25}H_{32}NaO_8^+$; calc. 483.1994)). In the UV spectrum of **9**, absorption maxima were observed at 217, 256, and 297 nm, suggesting a biphenyl-type cyclic diarylheptanoid structure [2][4][12]. The IR spectrum of **9** showed absorption bands at 3397, 1506, and 1075 cm^{-1}, ascribable to OH, aromatic ring, and ether functions [12]. Acid hydrolysis of **9** with 1M HCl/MeOH 1:1 furnished compound **17** and D-glucose, of which the latter was identified by GLC analysis of its trimethylsilyl thiazolidine derivative [13]. The ^{13}C- and ^{1}H-NMR of **9** evidenced the presence of two

benzene rings (six *singlet*s and six *doublet*s in the ^{13}C-NMR), six CH_2 groups (six *triplet*s in the ^{13}C-NMR), an CH–O group (δ(C) 67.4 (*d*), δ(H) 4.29 (*t*)), and a β-glucopyranosyl moiety (δ(H) 5.78 (*d*, *J* = 7.8, H–C(1′))) (*Table 1*). The NMR signals of aglycone moiety were very similar to those of acerogenin K (**17**) [4], suggesting that **9** possesses an acerogenin K structure as an aglycone moiety. The HMBC cross-correlation observed for H–C(9) with C(1′) indicated that the sugar moiety is located at C(9) in **9** (*Table 1* and *Fig.*). The above evidences, coupled with analysis of ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra, indicated that **9** has the structure 3,17-dihydroxytricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2,4,6(19),14,16-hexaen-9-yl β-D-glucopyranoside (= 9-*O*-β-D-glucopyranosylacerogenin K). The configuration at C(9) of compounds **9** and **17** remained undetermined.

Identification of 14 known compounds, *i.e.*, acerogenin A (**1**) [7][14], aceroside I (**2**) [9][14], aceroside III (**3**) [15], aceroside VI (**4**) [15], (*R*)-acerogenin B (**5**) [6][7], aceroside B_1 (**6**) [6], acerogenin I (**7**) [4], acerogenin G (**10**) [16], (−)-centrolobol (**11**) [17], aceroside VIII (**12**) [17], raspberry ketone (**13**) [18], (+)-rhododendrol (**14**) [19], apiosylepirhododendrin (**15**) [20], and kelampayoside A (**16**) [21], was achieved by comparison of their MS and ^{1}H-NMR data with those in the literature. Among these, compounds **10** and **16** were isolated from this plant for the first time in this study.

Preparation of Acerogenin A (**1**) *and (*R*)-Acerogenin B* (**5**) *Derivatives.* Acerogenin A (**1**) and (*R*)-acerogenin B (**5**) were obtained from aceroside I (**2**) and aceroside B_1 (**6**), respectively, by acid hydrolysis. Methylation of **1** with CH_2N_2 [14] yielded 2-*O*-methylacerogenin A (**18**). Acetylation of **1** and **18** with Ac_2O gave 2,11-di-*O*-acetylacerogenin A (**21**) and 11-*O*-acetyl-2-*O*-methylacerogenin A (**19**), respectively. On the other hand, treatment of **1** with TsOH in AcOEt yielded 11-*O*-acetylacerogenin A (**20**), which was considered to be formed by transesterification with the solvent AcOEt. Esterification of **1** with crotonic acid and *trans*-cinnamic acid in the presence of *O*,*O*-di(pyridin-2-yl)thiocarbonate (DPTC) and 4-(dimethylamino)pyridine (DMAP) [22] yielded 2,11-di-*O*-crotonylacerogenin A (**22**) and 2,11-di-*O*-cinnamoylacerogenin A (**23**), respectively. Esterification of **1** with succinic anhydride in the presence of DMAP gave 11-*O*-succinylacerogenin A (**24**). Dehydration of **1** with thionyl chloride ($SOCl_2$) [23] yielded (10*E*)-10,11-dehydro-11-deoxyacerogenin A (**25**) and (11*E*)-11,12-dehydro-11-deoxyacerogenin A (**26**). Treatment of compound **5** with the reagents as described above yielded 2-*O*-methylacerogenin B (**27**), 9-*O*-acetyl-2-*O*-methylacerogenin B (**28**), 9-*O*-acetylacerogenin B (**29**), 2,9-di-*O*-acetylacerogenin B (**30**), 2,9-di-*O*-crotonylacerogenin B (**31**), 2,9-di-*O*-cinnamoylacerogenin B (**32**), 9-*O*-succinylacerogenin B (**33**), (8*E*)-8,9-dehydro-9-deoxyacerogenin B (**34**), and (9*E*)-9,10-dehydro-9-deoxyacerogenin B (**35**), respectively. The structures of these derivatives were elucidated based on their ESI-MS, HR-ESI-MS, and ^{1}H-NMR data. In addition, the assigned structures of four dehydration products, **25**, **26**, **34**, and **35**, were confirmed by ^{1}H,^{1}H-COSY spectra.

Melanogenesis Inhibitory Activity. Sixteen compounds, **1**–**16**, isolated from *A. nikoense*, and 18 derivatives, **18**–**35**, synthesized from **1** and **5** were evaluated for their melanogenesis inhibitory activities on α-MSH-stimulated B16 melanoma cells [24], as well as their cytotoxic activities by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay (*Table 2*). At a concentration of 10 μM, eleven natural compounds, **2**–**6**, **8**, **9**, **12**, and **14**–**16**, from *A. nikoense* and three semi-



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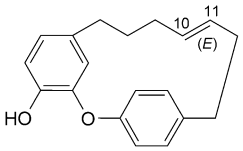
20 R = H, R' = Ac
21 R = R' = Ac
22 R = R' = Cro
23 R = R' = Cin
24 R = H, R' = Suc



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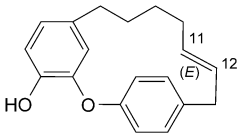

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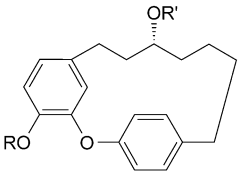
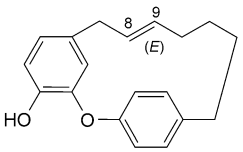


27 R = Me, R' = H
28 R = Me, R' = Ac
29 R = H, R' = Ac
30 R = R' = Ac
31 R = R' = Cro
32 R = R' = Cin
33 R = H, R' = Suc



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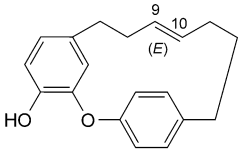

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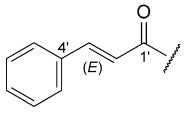

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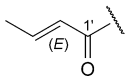

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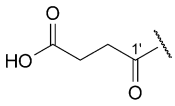

Suc

synthesized compounds, **19**, **27**, and **33**, showed inhibition of melanogenesis (melanin content 58.3–92.5%) with no or almost no toxicity to the cells (cell viability 90.7–111.2%). Their inhibitory activities against melanogenesis were superior to a known melanogenesis inhibitor, arbutin (melanin content 98.4% and cell viability 99.4% at the same concentration), which has been recognized as a useful depigmentation compound for skin whitening in the cosmetic industry [25]. Among the above, eight glycosides, *i.e.*, five cyclic diarylheptanoid glycosides, **2**–**4**, **6**, **9**, one acyclic diarylheptanoid glycoside, **12**, and two phenolic glycosides, **15** and **16**, exhibited no or almost no toxicity to the cells (cell viability 87.9–106.2%) even at a high concentration (100 μM), while these showed inhibition of melanogenesis (melanin content 39.4–76.2%) almost equivalent to or more superior to reference arbutin (melanin content 73.6% and cell viability 88.9% at the same concentration). High melanogenesis inhibitory activity without significant effect on the cell proliferation of three diarylheptanoid glycosides, **2**, **3**, and **6**, was observed also in our previous study, in which they were evaluated in B16 melanoma

cells without α-MSH-stimulation [10]. From the results of the anti-melanogenesis test in B16 melanoma cells in this and in our recent studies [10], it appears that some of the glycosidic diarylheptanoids and phenolic compounds from the bark of *A. nikoense* may be valuable as potential skin-whitening agents.

Cytotoxic Activity. The cytotoxic activities of compounds **1**–**16** and **18**–**35**, and two reference chemotherapeutic drugs, cisplatin and 5-fluorouracil, against two human cancer cell lines, HL60 (leukemia) and CRL1579 (melanoma), were determined by means of MTT assay, and the results are compiled in *Table 2*. Two acyclic diarylheptanoids, **10** and **11**, from *A. nikoense*, and ten esters, **18**–**22** and **27**–**31**, and two dehydroderivatives, **34** and **35**, of compounds **1** and **5** exhibited cytotoxic activities with IC_{50} values in the range of 8.1–19.3 μM, although these were less active than reference cisplatin (IC_{50} 1.9 μM) and 5-fluorouracil (IC_{50} 9.5 μM) against HL60. On the other hand, two acyclic diarylheptanoids, **10** and **11**, and three esters derivatives, **20**, **29**, and **30**, exhibited cytotoxicity against CRL1579 with IC_{50} values in the range of IC_{50} 10.1–18.4 μM, indicating higher activities than reference cisplatin (IC_{50} 21.1 μM) and 5-fluorouracil (IC_{50} >100 μM). On the basis of the results presented in *Table 2*, the following conclusions can be drawn about the structure–activity relationship of the compounds evaluated: *i*) Deglycosylation of cyclic diarylheptanoid glycosides, **1** *vs.* **2**–**4** and **5** *vs.* **6**, and acyclic diarylheptanoid glycoside, **11** *vs.* **12**, increased the activity against both HL60 and CRL1579 cells, which is consistent with the observation of the cytotoxicity against B16 melanoma cells described above. *ii*) Acyclic diarylheptanoids (*i.e.*, **10** and **11**) are more cytotoxic than cyclic diarylheptanoids (*i.e.*, **1**, **5**, **7**, and **8**). *iii*) Methylation of the phenolic OH group (*i.e.*, **18**, **19**, **27**, and **28**), and acetylation and crotonylation of the phenolic (*i.e.*, **21**, **22**, **30**, and **31**) and heptyl-chain OH groups (*i.e.*, **20**–**22** and **29**–**31**) of cyclic diarylheptanoids (*i.e.*, **1** and **5**) increased the activity significantly. *iv*) Cinnamoylation on both the phenolic and heptyl-chain OH groups (*i.e.*, **23** and **32**) and succinylation on the heptyl-chain OH group (*i.e.*, **24** and **33**) of cyclic diarylheptanoids (*i.e.*, **1** and **5**) reduced or exerted almost no influence on the activity. *v*) Dehydration of the heptyl-chain OH group of **1** and **5** into (*E*)-8,9- and (*E*)-9,10-dehydro derivatives **34** and **35**, respectively, increased the activity while that into (*E*)-10,11- and (*E*)-11,12-dehydro derivatives **25** and **26**, respectively, exerted almost no influence or reduced the activity. It may be suggested that acyclic diarylheptanoids and structural modification of natural cyclic diarylheptanoids are useful to develop effective antitumor drugs.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 230–400 mesh; *Merck*), *Diaion HP-20* (*Mitsubishi Chemical Co.*, Tokyo, Japan), and octadecyl silica gel (ODS; *Chromatorex-ODS*, 100–200 mesh; *Fuji Silysia Chemical, Ltd.*, Aichi, Japan). Reversed-phase (RP) prep. HPLC was carried out on an ODS column (*Pegasil ODS-II* 5 μm column (*Senshu Scientific Co., Ltd.*, Tokyo, Japan), 25 cm × 10 mm i.d.) at 25° with MeCN/H_2O/AcOH 45 : 55 : 0.1 (HPLC system *I*; flow rate 2.0 ml/min), MeOH/H_2O/AcOH 55 : 45 : 0.1 (HPLC system *II*; flow rate 2.0 ml/min), or MeOH/H_2O/AcOH 20 : 80 : 0.1 (HPLC system *III*); 30 : 70 : 0.1 (HPLC system *IV*), or 75 : 25 : 0.1 (HPLC system *V*; flow rate 3.0 ml/min) as mobile phase. Gas-liquid chromatography (GLC): *Shimadzu GC-17A* instrument on a *DB-17* fused silica glass cap. column (*Agilent Technologies, Inc.*, Santa Clara, CA, USA; 30 m × 0.32 mm i.d.; column temp. 200°; injection and detector temp. 270°; N_2 flow rate 1.8 ml/min; split ratio 1 : 34). Crystallizations

Table 2. *Melanogenesis Inhibitory Activities and Cytotoxicities of Compounds Isolated from* Acer nikoense *and Their Derivatives*

Compound		Melanogenesis inhibitory activity and cytotoxicity in B16 cells[a])				Cytotoxicity[c])	
		Mean ± S.D. at 10 μM[b])		Mean ± S.D. (%) at 100 μM[b])		IC_{50} [μM][d])	
		Melanin content	Cell viability	Melanin content	Cell viability	HL60	CRL1579
	Control (100% DMSO)	100.1 ± 1.9	99.3 ± 3.5	100.1 ± 1.9	99.3 ± 3.5		
1	Acerogenin A	70.4 ± .24	83.1 ± 2.3	21.6 ± 2.0	37.0 ± 3.7	57.8 ± 1.3	> 100
2	Aceroside I	89.1 ± 3.4	105.0 ± 3.6	64.8 ± 3.1	106.2 ± 3.6	> 100	> 100
3	Aceroside III	91.4 ± 0.7	97.2 ± 3.1	75.8 ± 2.7	91.5 ± 3.6	> 100	> 100
4	Aceroside VI	70.9 ± 1.8	102.9 ± 3.0	39.4 ± 2.6	89.2 ± 3.3	> 100	> 100
5	(*R*)-Acerogenin B	83.7 ± 2.6	94.5 ± 3.8	10.5 ± 0.5	26.6 ± 1.9	25.1 ± 1.6	> 100
6	Aceroside B_1	85.4 ± 2.1	101.7 ± 1.3	76.2 ± 2.7	90.9 ± 2.8	> 100	> 100
7	Acerogenin I	61.7 ± 2.7	79.6 ± 2.4	32.6 ± 2.1	68.2 ± 3.5	> 100	> 100
8	9-Oxoacerogenin A	83.4 ± 2.7	90.7 ± 3.1	17.6 ± 0.6	62.0 ± 0.7	> 100	> 100
9	9-*O*-β-D-Glucopyranosylacerogenin K	67.5 ± 3.0	92.1 ± 1.8	55.8 ± 2.8	87.9 ± 2.4	> 100	> 100
10	Acerogenin G	82.2 ± 3.1	83.3 ± 3.2	8.3 ± 1.7	16.6 ± 1.6	10.2 ± 2.0	10.1 ± 5.7
11	(−)-Centrolobol	63.0 ± 2.5	79.1 ± 2.8	4.2 ± 0.9	14.9 ± 0.1	8.1 ± 0.7	18.4 ± 1.7
12	Aceroside VIII	90.9 ± 2.3	107.4 ± 3.4	54.7 ± 3.1	103.5 ± 3.9	> 100	> 100
13	Rasphberry ketone	38.9 ± 2.7	74.0 ± 2.0	13.8 ± 1.1	56.5 ± 2.0	> 100	> 100
14	(+)-Rhododendrol	58.3 ± 1.9	96.2 ± 2.9	20.2 ± 0.5	71.4 ± 3.7	25.6 ± 2.2	> 100
15	Apiosylepirhododendrin	83.2 ± 3.4	95.0 ± 3.1	64.4 ± 2.3	90.5 ± 3.7	> 100	> 100
16	Kelampayoside A	85.0 ± 3.4	105.0 ± 3.4	65.8 ± 2.0	101.1 ± 3.0	> 100	> 100
18	2-*O*-Methyl acerogenin A	60.3 ± 1.6	70.2 ± 2.3	14.4 ± 1.3	29.2 ± 3.3	18.3 ± 1.7	76.4 ± 0.7
19	11-*O*-Acetyl-2-*O*-methylacerogenin A	92.5 ± 2.3	111.2 ± 2.3	15.5 ± 1.8	24.0 ± 0.7	11.2 ± 3.2	42.4 ± 3.2
20	11-*O*-Acetyl acerogenin A	64.6 ± 2.6	69.2 ± 2.1	1.7 ± 0.1	− 0.1 ± 0.4	14.7 ± 4.7	13.1 ± 4.4
21	2,11-Di-*O*-acetylacerogenin A	80.6 ± 3.4	84.6 ± 2.5	3.7 ± 1.2	0.6 ± 0.2	14.3 ± 1.4	27.8 ± 3.3
22	2,11-Di-*O*-crotonylacerogenin A	49.2 ± 3.0	76.0 ± 6.4	9.2 ± 1.5	2.3 ± 0.3	18.7 ± 0.6	84.8 ± 1.0
23	2,11-Di-*O*-cinnamoylacerogenin A	65.5 ± 3.0	89.2 ± 2.8	19.8 ± 1.9	62.0 ± 2.3	> 100	> 100
24	11-*O*-Succinyl acerogenin A	58.1 ± 1.0	81.5 ± 2.7	34.3 ± 2.2	55.7 ± 5.2	40.0 ± 0.9	94.7 ± 2.8
25	(10*E*)-10,11-Dehydro-deoxyacerogenin A	44.3 ± 1.7	79.6 ± 2.5	14.6 ± 1.8	63.6 ± 1.5	53.3 ± 3.4	67.4 ± 2.7
26	(11*E*)-11,12-Dehydro-deoxyacerogenin A	52.2 ± 2.5	86.0 ± 2.2	49.9 ± 3.0	79.8 ± 1.2	> 100	> 100
27	2-*O*-Methylacerogenin B	71.7 ± 1.9	96.5 ± 2.2	18.4 ± 1.6	44.4 ± 1.0	13.9 ± 1.5	53.3 ± 1.1
28	9-*O*-Acetyl-2-*O*-methylacerogenin B	66.4 ± 0.4	89.1 ± 2.4	13.5 ± 0.5	35.9 ± 2.8	15.2 ± 4.7	> 100
29	9-*O*-Acetyl acerogenin B	54.0 ± 1.9	78.8 ± 3.9	8.7 ± 1.8	16.1 ± 2.0	16.1 ± 2.3	10.1 ± 0.7
30	2,9-Di-*O*-acetylacerogenin B	52.4 ± 2.1	87.6 ± 2.7	20.4 ± 0.3	39.2 ± 3.0	14.9 ± 5.8	11.6 ± 7.3

Table 2 (cont.)

Compound		Melanogenesis inhibitory activity and cytotoxicity in B16 cells[a])				Cytotoxicity[c])	
		Mean ± S.D. at 10 μM[b])		Mean ± S.D. (%) at 100 μM[b])		IC_{50} [μM][d])	
		Melanin content	Cell viability	Melanin content	Cell viability	HL60	CRL1579
31	2,9-Di-*O*-crotonylacerogenin B	47.3 ± 1.6	73.6 ± 3.1	0.3 ± 0.1	0.2 ± 0.1	14.6 ± 6.6	58.5 ± 3.9
32	2,9-Di-*O*-cinnamoylacerogenin B	59.1 ± 3.8	83.8 ± 0.2	45.4 ± 2.4	63.7 ± 2.5	> 100	> 100
33	9-*O*-Succinylacerogenin B	68.6 ± 2.5	107.3 ± 1.9	33.6 ± 2.6	66.8 ± 3.1	85.7 ± 3.8	> 100
34	(8*E*)-8,9-Dehydro-9-deoxyacerogenin B	30.6 ± 0.9	64.1 ± 3.0	5.7 ± 1.9	16.7 ± 1.5	19.3 ± 1.9	64.3 ± 1.8
35	(9*E*)-9,10-Dehydro-9-deoxyacerogenin B	30.6 ± 0.9	70.2 ± 2.5	3.3 ± 1.7	0.6 ± 0.6	16.7 ± 2.8	67.1 ± 1.8
	Arbutin[e])	98.4 ± 3.0	99.4 ± 2.3	73.6 ± 4.0	88.9 ± 3.6		
	Cisplatin[e])					1.9 ± 0.2	21.1 ± 2.1
	5-Fluorouracil[e])					9.5 ± 1.6	> 100

[a]) Melanin content [%] and cell viability [%] were determined based on the absorbances at 405, and 570 (test wavelength) – 630 (reference wavelength) nm, respectively, by comparison with those for DMSO (100%). [b]) Each value represents the mean ± S.D. of three determinations. Concentration of DMSO in the sample solution was 2 μl/ml. [c]) Cells were treated with compounds (1×10^{-4} to 1×10^{-6} M) for 48 h, and cell viability was analyzed by MTT assay. [d]) IC_{50} Value is the concentration of compound required to inhibit the growth of the cells by 50%. Each value represents the mean ± S.D., obtained on the basis of triplicate assay results. [e]) Reference compounds.

were performed in MeOH. M.p.: *Yanagimoto Micro Mp* apparatus; uncorrected. Optical rotations: *JASCO P-1020* digital polarimeter. UV Spectra: *JASCO V-630Bio* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *JASCO FTIR-300E* spectrometer; ν in cm^{-1}. 1D- and 2D-NMR spectra: *JEOL ECX-400* (^{1}H, 400 MHz; ^{13}C, 100 MHz) or *JEOL ECX-500* (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer at r.t.; C_5D_5N or $CDCl_3$ soln.; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-, and HR-ESI-MS: *Agilent 1100 LC/MSD TOF* (time-of-flight) system (positive-ionization mode, cap. voltage 3000 V, fragmentor voltage 225 V).

Chemicals and Materials. The stem bark obtained from a 25-year-old tree of *Acer nikoense* in the summer of 2007 was purchased from *Sirakami Fruit Park* (Gunma, Japan). The plant was authenticated by Mr. *Hiroshi Sato* (*Sirakami Fruit Park*), and voucher specimen has been deposited with the authors' laboratory (*Coll. Sci. Technol., Nihon Univ.*). The chemicals were purchased as follows: 5-fluorouracil from *Nacalai Tesque, Inc.* (Kyoto, Japan), fetal bovine serum (FBS), *RPMI-1640* medium, antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin), and non-essential amino acid (NEAA) from *Invitrogen Co.* (Carlsbad, CA, USA), *Eagle's* minimal essential medium (MEM), and MTT from *Sigma-Aldrich Japan Co.* (Tokyo, Japan), arbutin (4-hydroxyphenyl β-D-glucopyranoside), diazo(trimethylsilyl)methane (Me_3SiCHN_2; 10% in hexane), TsOH, 4-(dimethylamino)pyridine (DMAP), and *O,O*-di(pyridin-2-yl)thiocarbonate (DPTC) from *Tokyo Chemical Industry Co., Ltd.* (Tokyo, Japan), crotonic acid, (*E*)-cinnamic acid, succinic anhydride, cisplatin, L-glucose, and TMS-HT kit (hexamethyldisilazane-trimethylchlorosilane (HMDS-TMCS) in anh. pyridine) from *Wako Pure Chemical Industries Ltd.* (Osaka, Japan), D-glucose, L-cysteine methyl ester hydrochloride, and molecular sieves (3 Å 1/16) from *Kanto Chemical Co., Inc.* (Tokyo, Japan), and *Amberlite MB-4* from *Organo Co.* (Tokyo, Japan). All other chemicals and reagents were of anal. grade.

Cell Cultures. Cell lines HL60 (leukemia), CRL1579 (melanoma), and B16 A45 (murine melanoma) were obtained from *Riken Cell Bank* (Tsukuba, Ibaraki, Japan). Two cell lines, HL60 and CRL1579, were grown in *RPMI-1640* medium. The medium was supplemented with 10% FBS and antibiotics. B16 Cells were cultured in MEM supplemented with 5% FBS and antibiotics. Cells were incubated at 37° in a 5% CO_2 humidified incubator.

Extraction and Isolation. The dried stem bark of *A. nikoense* (1799 g) was finely cut and extracted with hexane (reflux, 3 h, 3 ×) which yielded the extract (9.6 g) containing fatty constituents. The defatted residue was then extracted with MeOH (reflux, 3 h, 3 ×) to give the extract (205.8 g), which was partitioned in an AcOEt/H_2O mixture. The aq. layer was extracted with BuOH, and removal of the solvent under reduced pressure from the AcOEt-, BuOH-, and H_2O-soluble portions yielded 78.2, 84.6, and 30.9 g of the residue, resp.

CC on SiO_2 (990 g) of a portion (57.7 g) of the AcOEt-soluble fraction eluted with hexane/AcOEt 1:1, AcOEt, and then with AcOEt/MeOH 7:3 gave eight fractions, *Frs. 1* (1.14 g), *2* (4.02 g), *3* (0.26 g), *4* (1.06 g), *5* (8.13 g), *6* (1.71 g), *7* (28.55 g), and *8* (7.78 g). A portion (1.00 g) of *Fr. 2* was further separated by CC (SiO_2 (100 g); hexane/AcOEt gradient 1:0 → 0:1) to furnish six fractions, *Frs. 3-1–3-6*. Prep. HPLC (system *I*) of *Fr. 3-2* (105 mg) yielded compounds **5** (22.3 mg; t_R 33.0 min) and **13** (22.3 mg; t_R 11.3 min). *Fr. 3-3* (707 mg) was further subjected to CC (SiO_2 (50 g); hexane/AcOEt gradient 1:0 → 0:1) which yielded seven fractions, *Frs. 3-3a–3-3g*. Prep. HPLC (system *I*) of *Fr. 3-3e* (417 mg) yielded compounds **1** (57.0 mg; t_R 22.0 min), **8** (2.1 mg; t_R 19.3 min), **10** (5.8 mg; t_R 18.8 min), and **14** (258.0 mg; t_R 6.3 min). In addition, prep. HPLC (system *I*) of *Fr. 3-3g* (99 mg) yielded **11** (68.1 mg; t_R 14.3 min). *Fr. 4* was subjected to CC (SiO_2 (53 g); hexane/AcOEt gradient 1:1 → 0:1) to yield purified *Fr. 4* (580 mg), which, upon prep. HPLC (system *I*), yielded compound **7** (5.8 mg; t_R 41.3 min). A portion (23.86 g) of *Fr. 7* was subjected to CC (SiO_2 900 g); AcOEt/MeOH gradient 19:1 → 0:1) to yield nine fractions, *Frs. 7-1–7-9*. Further CC on SiO_2 (37 g) of a portion (1.25 g) of *Fr. 7-1* (10.14 g) yielded four fractions, *Frs. 7-1a–7-1d*. Prep. HPLC (system *II*) of *Fr. 7-1a* (322 mg), *7-1b* (103 mg), and *7-1d* (559 mg) yielded compounds **2** (100.4 mg; t_R 23.0 min) and **6** (202.3 mg; t_R 29.6 min), compounds **4** (10.8 mg; t_R 23.0 min) and **9** (21.4 mg; t_R 39.7 min), and compounds **3** (155.5 mg; t_R 34.8 min) and **12** (154.6 mg; t_R 26.0 min), resp. A portion (52.6 g) of the BuOH-soluble fraction was chromatographed on a *Diaion HP-20* (500 g) column (MeOH/H_2O gradient 1:9 → 10:0) to yield seven fractions, *Fr. B1–B7. Fr. B2* (5.58 g) was further chromatographed on ODS (250 g) column (MeOH/H_2O gradient 0:1 → 1:0) to yield seven fractions, *Fr. B2-1–B2-7*. Prep. HPLC (system *III*) of *Fr. B2-4* (328 mg) yielded **16** (15.4 mg; t_R 24.6 min).

On the other hand, further CC (SiO_2 (75 g); $CHCl_3$/MeOH gradient 1:0→0:1) of a portion (1.50 g) of *Fr. B2-5* (2.86 g) yielded five fractions, *Fr. B2-5a–B2-5e*. Prep. HPLC (system *IV*) of *Fr. B2-5a* (201 mg) yielded **15** (143.4 mg; t_R 16.7 min). The physical characteristics and spectral data of two new compounds, **8** and **9**, are given below.

(11ξ)-9-Oxoacerogenin A (=*4,12-Dihydroxy-2-oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-10-one*; **8**). Fine needles. M.p. 147–150° (MeOH). $[\alpha]_D^{25} = +0.5$ ($c = 0.36$, EtOH). UV (MeOH): 276 (3.52). IR (KBr): 3403 (OH), 1705 (C=O), 1504 (arom. C=C), 1231, 1055 (C–O–C). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 335.1303 ($[M+Na]^+$, $C_{19}H_{20}NaO_4^+$; calc. 335.1259).

*(9ξ)-9-*O*-β-*D*-Glucopyranosylacerogenin K* (=*3,17-Dihydroxytricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2,4,6(19),14,16-hexaen-9-yl β-*D*-Glucopyranoside*; **9**). Fine needles. M.p. 166–169° (MeOH). $[\alpha]_D^{25} = -71.1$ ($c = 0.34$, EtOH). UV (MeOH): 217 (4.03), 256 (3.45), 297 (3.15). IR (KBr): 3397 (OH), 1506 (aromatic C=C), 1075. ^{13}C- and ^{1}H-NMR: *Table 1*. HR-ESI-MS: 483.1984 ($[M+Na]^+$, $C_{25}H_{32}NaO_8^+$; calc. 483.1994).

Acid Hydrolysis of **9** *and Sugar Identification*. A soln. of **9** (12.3 mg) in 1M HCl/MeOH 1:1 (12 ml) was heated under reflux for 2 h. The mixture was diluted with H_2O and extracted with AcOEt (3×). The AcOEt extract was passed through CC (SiO_2 (5 g); hexane/AcOEt) to yield *acerogenin K* (**17**) (5.0 mg). The aq. layer was neutralized by passing through an *Amberlite MB-4*, and the eluate was concentrated. The residue (3.7 mg) was dissolved in pyridine (0.5 ml) and stirred with L-cysteine methyl ester (5 mg) for 1.0 h at 60°. The trimethylsilylation reagent HMDS-TMCS (0.3 ml) was added, and warming at 60° was continued for another 30 min. The mixture was centrifuged, and a portion (1 μl) of the filtrate was analyzed by GLC. The GLC afforded a peak with t_R 18.3 min which was identical with that of authentic D-glucose (t_R 18.2 min). Authentic L-glucose eluted with t_R 16.0 min under the same GLC condition.

(9ξ)-Acerogenin K (=*Tricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2,4,6(19),14,16-hexaene-3,9,17-triol*; **17**). $[\alpha]_D^{24} = -9.0$ ($c = 1.52$, EtOH). ^{1}H-NMR (400 MHz; C_5D_5N): 1.44 (*sept.*, $J = 6.0$, H_a–C(11)); 1.70–1.80 (*m*, H_b–C(11)); 1.74–1.83 (*m*, H_a–C(8)); 1.89 (*dd*-like, $J = 12.1$, 12.1, H_a–C(10)); 1.93–2.05 (*m*, H_b–C(8), H_a–C(12)); 1.98–2.10 (*m*, H_b–C(10)); 2.43 (*dt*, $J = 2.5$, 15.6, H_b–C(12)); 2.49 (*dt*, $J = 3.6$, 13.4, H_a–C(7)); 2.88 (br. *d*, $J = 17.0$, H_b–C(7)); 3.00 (*dt*, $J = 2.4$, 17.4, H_a–C(13)); 3.34 (*ddd*, $J = 2.3$, 13.3, 16.2, H_b–C(13)); 4.47 (br. *t*, $J = 9.6$, H–C(9)); 7.14 (*dd*, $J = 2.3$, 8.2, H–C(5)); 7.19 (br. *s*, H–C(15), H–C(16)); 7.25 (br. *d*, $J = 8.2$, H–C(4)); 7.47 (br. *s*, H–C(19)); 7.57 (br. *s*, H–C(18)). ^{13}C-NMR (100 MHz; C_5D_5N): 23.5 (C(11)); 27.2 (C(8)); 27.6 (C(13)); 30.4 (C(7)); 35.8 (C(12)); 40.7 (C(10)); 68.0 (C(9)); 116.9 (C(4), C(16)); 127.4 (C(2)); 127.5 (C(1)); 129.9 (C(5)); 130.0 (C(15)); 131.1 (C(6)); 131.6 (C(14)); 134.7 (C(19)); 135.2 (C(18)); 152.7 (C(3), C(17)). The structure of **17** was supported by its ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra. The ^{1}H- and ^{13}C-NMR signal assignments for H–C(7), H–C(8), H–C(12), and H–C(13) reported in [4] were revised as described above. ESI-MS: 321 ($[M+Na]^+$, $C_{19}H_{22}NaO_3^+$).

Acid Hydrolysis of **2** *and* **6**. A soln. of **2** (300 mg) in 1M HCl/MeOH (1:1; 24 ml) was heated under reflux for 2 h. After cooling, the mixture was poured into ice-H_2O, and extracted with AcOEt. The AcOEt extract, after usual workup, yielded **1** (185 mg). In a similar procedure of acid hydrolysis, **6** (400 mg) yielded **5** (175 mg).

Acerogenin A (=*(12*R*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaene-4,12-diol*; **1**). $[\alpha]_D^{20} = +59.9$ ($c = 0.49$, EtOH) (lit. [2]: $[\alpha]_D = +57.3$). ^{1}H-NMR (400 MHz; $CDCl_3$): 0.78–0.85 (*m*, H_a–C(9)); 0.90–0.97 (*m*, H_a–C(10)); 0.98–1.06 (*m*, H_b–C(9)); 1.04–1.12 (*m*, H_b–C(10)); 1.18–1.27 (*m*, H_a–C(8)); 1.42–1.52 (*m*, H_b–C(8)); 1.62 (*dddd*, $J = 4.1$, 5.5, 9.6, 10.1, H_a–C(12)); 1.88 (*dq*, $J = 14.2$, 4.1, H_b–C(12)); 2.37–2.49 (*m*, CH_2(7)); 2.68 (*dt*, $J = 2.8$, 12.8, H_a–C(13)); 2.98 (*dt*, $J = 13.3$, 3.7, H_b–C(13)); 3.25 (*tt*, $J = 5.0$, 5.0, H–C(11)); 5.62 (*d*, $J = 1.8$, H–C(6)); 5.72 (br. *s*, OH–C(2)); 6.59 (*dd*, $J = 1.8$, 8.0, H–C(4)); 6.83 (*d*, $J = 8.0$, H–C(3)); 6.92 and 7.14 (*dd*, $J = 2.8$, 8.2, each 1 H, H–C(16), H–C(18)); 7.19 and 7.28 (*dd*, $J = 2.3$, 8.2, each 1 H, H–C(15), H–C(19)). ESI-MS: 321 ($[M+Na]^+$, $C_{19}H_{22}NaO_3^+$).

*(*R*)-Acerogenin B* (=*(10*R*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaene-4,10-diol*; **5**). $[\alpha]_D^{20} = -76.4$ ($c = 0.49$, EtOH) ([2][6]: $[\alpha]_D = -95.0$). ^{1}H-NMR (400 MHz; $CDCl_3$): 0.74–0.82 (*m*, H_a–C(10)); 0.95–1.05 (*m*, H_a–C(11)); 1.18–1.28 (*m*, H_b–C(10)); 1.25–1.35 (*m*, H_b–C(11)); 1.45–1.53 (*m*, CH_2(8)); 1.50–1.58 (*m*, H_a–C(12)); 1.76 (*sept.*-like, $J = 7.2$, H_b–C(12)); 2.52–2.62 (*m*, H_a–C(7)); 2.55–2.67 (*m*, H_b–C(7), H_a–C(13)); 2.80 (*ddd*, $J = 4.4$, 6.0, 13.2, H_b–C(13)); 3.02–3.11 (*m*, H–C(9)); 5.57 (*d*, $J = 1.8$, H–C(6)); 6.63 (*dd*, $J = 1.8$, 8.2, H–C(4)); 6.84 (*d*, $J = 8.2$, H–C(3)); 6.93 and 7.13 (2*dd*, $J = 2.5$, 8.2,

each 1 H, H–C(16), H–C(18)); 7.23 and 7.30 (2*dd*, *J* = 2.1, 8.2, each 1 H, H–C(15), H–C(19)). ESI-MS: 321 ($[M+Na]^+$, $C_{19}H_{22}NaO_3^+$).

CH_2N_2 Methylation of **1** *and* **5**. To a soln. of **1** (10.4 mg) in AcOEt/MeOH (2 : 1; 3.0 ml), Me_3SiCHN_2 (10% in hexane; 0.2 ml) was added, and the mixture was stirred at r.t. for 1 h. Removal of the solvent under reduced pressure yielded **18** (10.3 mg). Similarly, methylation of **5** (9.7 mg) yielded **27** (9.9 mg).

*2-*O*-Methyl Acerogenin A* (=*(12*R*)-4-Methoxy-2-oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-12-ol*; **18**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 3.23–3.31 (*m*, H–C(11)); 3.93 (*s*, MeO). ESI-MS: 335 ($[M+Na]^+$, $C_{20}H_{24}NaO_3^+$).

*2-*O*-Methyl Acerogenin B* (=*(10*R*)-4-Methoxy-2-oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-10-ol*; **27**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 3.02–3.11 (*m*, H–C(9)); 3.93 (*s*, MeO). ESI-MS: 335 ($[M+Na]^+$, $C_{20}H_{24}NaO_3^+$).

Acetylation of **1**, **5**, **18**, *and* **27** *by Ac_2O*. To a soln. of **1** (10.4 mg) in pyridine (0.5 ml), Ac_2O (0.5 ml) was added, and the mixture was left overnight at r.t. The mixture was then poured into ice-H_2O, and extracted with Et_2O. The Et_2O extract, after washing with dil. HCl soln. (HCl/H_2O 1 : 9) and with sat. $NaHCO_3$ aq. soln. followed by usual workup, yielded **21** (10.4 mg). Similarly, acetylation of **5** (5.2 mg), **18** (5.1 mg), and **27** (4.2 mg) yielded **30** (5.1 mg), **19** (5.6 mg), and **28** (4.8 mg), resp.

*11-*O*-Acetyl-2-*O*-methyl Acerogenin A* (=*(12*R*)-4-Methoxy-2-oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-12-yl Acetate*; **19**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.97 (*s*, AcO–C(11)); 3.88 (*s*, AcO–C(2)); 4.32–4.38 (*m*, H–C(11)). ESI-MS: 377 ($[M+Na]^+$, $C_{22}H_{26}NaO_4^+$).

*2,11-Di-*O*-acetyl Acerogenin A* (=*(12R)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaene-4,12-diyl Diacetate*; **21**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 2.02 (*s*, AcO–C(11)); 2.37 (*s*, AcO–C(2)); 4.32–4.38 (*m*, H–C(11)). ESI-MS: 405 ($[M+Na]^+$, $C_{23}H_{26}NaO_5^+$).

*9-*O*-Acetyl-2-*O*-methyl Acerogenin B* (=*(10*R*)-4-Methoxy-2-oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-10-yl Acetate*; **28**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.90 (*s*, AcO–C(9)); 3.88 (*s*, MeO–C(2)); 4.38 (*ddd*, *J* = 3.6, 7.2, 13.8, H–C(9)). ESI-MS: 377 ($[M+Na]^+$, $C_{22}H_{26}NaO_4^+$).

*2,9-Di-*O*-acetyl Acerogenin B* (=*(10*R*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaene-4,10-diyl Diacetate*; **30**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.94 (*s*, AcO–C(9)); 2.27 (*s*, AcO–C(2)); 4.37 (*ddd*, *J* = 3.2, 7.6, 13.2, H–C(9)). ESI-MS: 405 ($[M+Na]^+$, $C_{23}H_{26}NaO_5^+$).

Acetylation of **1** *and* **5** *by AcOEt in the Presence of TsOH*. A soln. of **1** (17.4 mg) and TsOH (17.7 mg), along with a few pieces of molecular sieves in AcOEt (5.0 ml), was heated under reflux for 7 h. The mixture was filtered, and the usual workup of the filtrate yielded a residue (16.0 mg) which, upon CC (SiO_2; hexane/AcOEt gradient 9 : 1 → 7 : 3), yielded **20** (5.4 mg). The similar acetylation of **5** (10.5 mg) yielded **29** (3.4 mg).

*11-*O*-Acetyl Acerogenin A* (=*(12*R*)-4-Hydroxy-2-oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-12-yl Acetate*; **20**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.97 (*s*, AcO–C(11)); 4.31 (*tt*, *J* = 4.0, 6.8, H–C(11)), ESI-MS: 363 ($[M+Na]^+$, $C_{21}H_{24}NaO_4^+$).

*9-*O*-Acetyl Acerogenin B* (=*(10*R*)-4-Hydroxy-2-oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-10-yl Acetate*; **29**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.97 (*s*, AcO–C(9)); 4.38 (*ddd*, *J* = 3.0, 7.2, 14.0, H–C(9)). ESI-MS: 363 ($[M+Na]^+$, $C_{21}H_{24}NaO_4^+$).

Esterification of **1** *and* **5** *with Crotonic Acid*. To a soln. of **1** (20.4 mg) in toluene (3.0 ml), crotonic acid (20.8 mg), DMAP (15.5 mg), and DPTC (30.7 mg) were added, and the mixture was stirred at r.t. for 48 h under N_2. The mixture was poured into H_2O, and extracted with Et_2O. The Et_2O extract (26.2 mg) was passed through CC (SiO_2; hexane/AcOEt gradient 9 : 1 → 1 : 1) to afford **22** (2.8 mg). Similarly, the esterification of **5** (20.5 mg) with crotonic acid yielded **31** (2.7 mg).

*2,11-Di-*O*-crotonyl Acerogenin A* (=*(12*R*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaene-4,12-diyl Bis[(2*E*)-but-2-enoate]*; **22**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.88 (*dd*, *J* = 1.8, 6.9, Me(4″)); 1.98 (*dd*, *J* = 1.8, 6.9, Me(4′)); 4.43 (*ddd*, *J* = 3.7, 7.3, 10.6, H–C(11)); 5.84 (*dq*, *J* = 15.6, 1.4, H–C(2″)); 6.15 (*dq*, *J* = 15.6, 1.8, H–C(2′)); 6.91–6.98 (*m*, H–C(3″)); 7.23–7.29 (*m*, H–C(3′)). ESI-MS: 457 ($[M+Na]^+$, $C_{27}H_{24}NaO_4^+$).

*2,9-Di-*O*-crotonyl Acerogenin B* (=*(10*R*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaene-4,10-diyl Bis[(2*E*)-but-2-enoate]*; **31**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.86 (*dd*, *J* = 1.8, 6.9, Me(4″)); 1.98 (*dd*, *J* = 1.8, 6.9, Me(4′)); 4.45 (*dq*, *J* = 3.2, 7.8, H–C(9)); 5.76 (*dq*, *J* = 15.1, 1.4,

H–C(2″)); 6.15 (*dq*, *J* = 15.6, 1.8, H–C(2′)); 6.85–6.91 (*m*, H–C(3″)); 7.23–7.29 (*m*, H–C(3′)). ESI-MS: 457 ($[M+Na]^+$, $C_{27}H_{24}NaO_4^+$).

Esterification of **1** *and* **5** *with* (E)*-Cinnamic Acid*. To a soln. of **1** (20.5 mg) in toluene (2.0 ml), (*E*)-cinnamic acid (20.6 mg), DMAP (15.8 mg), and DPTC (30.6 mg) were added and stirred at r.t. for 24 h under N_2. The mixture was poured into H_2O, and extracted with Et_2O. The Et_2O extract (30.4 mg) was subjected to CC (SiO_2; hexane/AcOEt gradient 9 : 1 → 1 : 1) to afford **23** (17.8 mg). Esterification of **5** (19.5 mg) with (*E*)-cinnamic acid as described above yielded **32** (11.4 mg).

*2,11-Di-*O*-cinnamoyl Acerogenin A* (= *(12*R*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaene-4,12-diyl Bis[(2E)-3-phenylprop-2-enoate*; **23**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 4.49–4.56 (*m*, H–C(11)); 6.44 (*dt*-like, *J* = 15.6, 2.3, H–C(2″)); 6.75 (*d*, *J* = 16.0, H–C(2′)); 7.39 (*t*-like, *J* = 3.0, H–C(6″), H–C(7″), H–C(8″)); 7.42 (*t*-like, *J* = 3.0, H–C(6′), H–C(7′), H–C(8′)); 7.51–7.55 (*m*, H–C(5″), H–C(9″)); 7.59–7.63 (*m*, H–C(5′), H–C(9′)); 7.68 (*dd*-like, *J* = 1.8, 16.0, H–C(3″)); 7.95 (*d*, *J* = 16.0, H–C(3′)). ESI-MS: 581 ($[M+Na]^+$, $C_{37}H_{34}NaO_5^+$).

*2,9-Di-*O*-cinnamoyl Acerogenin B* (= *(10*R*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaene-4,10-diyl Bis[(2*E*)-3-phenylprop-2-enoate]*; **32**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 4.54 (*ddd*, *J* = 2.8, 8.0, 13.2, H–C(9)); 6.34 (*dt*-like, *J* = 16.0, H–C(2″)); 6.74 (*d*, *J* = 16.0, H–C(2′)); 7.39 (*t*-like, *J* = 3.0, H–C(6″), H–C(7″), H–C(8″)); 7.42 (*t*-like, *J* = 3.0, H–C(6′), H–C(7′), H–C(8′)); 7.49 (*dd*, *J* = 2.0, 6.0, H–C(5″), H–C(9″)); 7.59 (*dd*, *J* = 2.0, 6.0, H–C(5′), H–C(9′)); 7.60 (*d*, *J* = 16.0, H–C(3″)); 7.94 (*d*, *J* = 16.0, H–C(3′)). ESI-MS: 581 ($[M+Na]^+$, $C_{37}H_{34}NaO_5^+$).

Esterification of **1** *and* **5** *with Succinic Anhydride*. To a soln. of **1** (20.8 mg) in pyridine (10.0 ml), succinic anhydride (21.0 mg) and DMAP (10.6 mg) were added, and the mixture was refluxed for 7 h. The mixture was poured into H_2O and extracted with AcOEt. The AcOEt extract was washed with dil. HCl and then with H_2O. The mixture obtained (23.2 mg) was subjected to CC (SiO_2; hexane/AcOEt gradient 8 : 2 → 0 : 1) to afford **24** (2.2 mg). Esterification of **5** (21.0 mg) with succinic anhydride as described above yielded **33** (2.2 mg).

*11-*O*-Succinyl Acerogenin A* (= *Butanedioic Acid Mono[(12*R*)-4-hydroxy-2-oxatricyclo-[13.2.2.1^{3,7}]icosa-3,5,7(20),15,17,18-hexaen-12-yl] Ester*; **24**). Amorphous solid. ^{1}H-NMR (400 MHz; C_5D_5N): 2.86–2.97 (*m*, CH_2(2′), CH_2(3′)); 4.60–4.8 (*m*, H–C(11)). ESI-MS: 421 ($[M+Na]^+$, $C_{23}H_{26}NaO_6^+$).

*9-*O*-Succinyl Acerogenin B* (= *Butanedioic Acid Mono[(10*R*)-4-hydroxy-2-oxatricyclo[13.2.2.1^{3,7}]-icosa-3,5,7(20),15,17,18-hexaen-10-yl] Ester*; **33**). Amorphous solid. ^{1}H-NMR (400 MHz; C_5D_5N): 2.80–2.95 (*m*, CH_2(2′), CH_2(3′)); 4.70–4.79 (*m*, H–C(9)). ESI-MS: 421 ($[M+Na]^+$, $C_{23}H_{26}NaO_6^+$).

Dehydration of **1** *and* **5**. A soln. of **1** (100.6 mg) in pyridine (5.0 ml) was treated with $SOCl_2$ (0.5 ml), and the mixture was stirred at 0° for 5 h. The mixture was poured into ice-H_2O and extracted with AcOEt. The AcOEt extract was washed with sat. aq. $NaHCO_3$ and brine, and then worked up as usual to yield a residue (96.5 mg), which, upon CC (SiO_2; hexane/AcOEt gradient 9 : 1 → 7 : 3), afforded a fraction containing dehydration products. Prep. HPLC (system *IV*) of this fraction yielded **25** (6.3 mg; t_R 46.0 min) and **26** (3.4 mg; t_R 49.6 min). The procedure as described above for **5** (100.3 mg) yielded **34** (9.8 mg; t_R 50.4 min) and **35** (1.7 mg; t_R 48.8 min).

*11-Deoxy-10(11)*E*-dehydroacerogenin A* (= *(11*E*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),11,15,17,18-heptaen-4-ol*; **25**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.47 (*dt*, *J* = 12.0, 6.4, CH_2(8)); 1.77 (*dd*, *J* = 5.7, 12.1, CH_2(9)); 2.25 (*dd*, *J* = 7.1, 12.7, CH_2(12)); 2.44 (*dd*, *J* = 6.4, 6.4, CH_2(7)); 2.78 (*dd*, *J* = 6.4, 6.4, CH_2(13)); 4.90 (*dt*, *J* = 15.4, 5.9, H–C(10)); 5.09 (*dt*, *J* = 14.9, 7.7, H–C(11)); 5.41 (*d*, *J* = 1.7, H–C(6)); 5.52 (br. *s*, HO–C(2)); 6.57 (*dd*, *J* = 2.1, 8.2, H–C(4)); 6.82 (*d*, *J* = 8.3, H–C(3)); 7.00 (*dt*, *J* = 8.5, 2.0, H–C(16), H–C(18)); 7.08 (*dt*, *J* = 8.3, 2.0, H–C(15), H–C(19)). HR-ESI-MS: 303.1364 ($[M+Na]^+$, $C_{19}H_{20}NaO_2^+$; calc. 303.1361).

*11-Deoxy-11(12)*E*-dehydroacerogenin A* (= *(12*E*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),12,15,17,18-heptaen-4-ol*; **26**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.09–1.17 (*m*, CH_2(9)); 1.28–1.37 (*m*, CH_2(8)); 1.91 (*ddd*, *J* = 1.2, 6.4, 10.8, CH_2(10)); 2.46 (*t*, *J* = 6.0, CH_2(7)); 3.29 (*dd*, *J* = 2.0, 5.2, CH_2(13)); 4.48 (*dtt*, *J* = 14.9, 1.6, 6.4, H–C(11)); 5.75 (*dtt*, *J* = 15.6, 1.6, 5.2, H–C(12)); 5.60 (br. *s*, HO–C(2)); 5.69 (*d*, *J* = 1.7, H–C(6)); 6.58 (*dd*, *J* = 1.2, 8.0, H–C(4)); 6.82 (*d*, *J* = 8.3, H–C(3)); 7.07 (*dt*, *J* = 8.5, 2.0, H–C(16), H–C(18)); 7.22 (*dt*, *J* = 8.3, 2.0, H–C(15), H–C(19)). HR-ESI-MS: 303.1369 ($[M+Na]^+$, $C_{19}H_{20}NaO_2^+$; calc. 303.1361).

*9-Deoxy-8(9)*E*-dehydroacerogenin B* (=*(9*E*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),9,15,17,18-heptaen-4-ol*; **34**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$) : 1.09–1.19 (*m*, CH_2(11)); 1.47–1.55 (*m*, CH_2(12)); 1.85 (*dd*, *J* = 7.2, 12.8, CH_2(10)); 2.63 (*dt*, *J* = 10.8, 6.0, CH_2(13)); 3.00 (*d*, *J* = 7.2, CH_2(7)); 5.18 (*dt*, *J* = 14.8, 7.6, H–C(8)); 5.34 (*d*, *J* = 1.6, H–C(6)); 5.39 (*dt*, *J* = 14.8, 7.6, H–C(9)); 5.57 (br. *s*, HO–C(2)); 6.59 (*dd*, *J* = 1.6, 8.0, H–C(4)); 6.82 (*d*, *J* = 7.6, H–C(3)); 7.02 (*dt*, *J* = 8.3, 2.2, H–C(16), H–C(18)); 7.21 (*dt*, *J* = 8.3, 2.2, H–C(15), H–C(19)). HR-ESI-MS: 303.1385 ($[M+Na]^+$, $C_{19}H_{20}NaO_2^+$; calc. 303.1361).

*9-Deoxy-9(10)*E*-dehydroacerogenin B* (=*(10*E*)-2-Oxatricyclo[13.2.2.1^{3,7}]icosa-3,5,7(20),10,15,17,18-heptaen-4-ol*; **35**). Amorphous solid. ^{1}H-NMR (400 MHz; $CDCl_3$): 1.77–1.85 (*m*, CH_2(12)); 2.05–2.15 (*m*, CH_2(11), CH_2(8)); 2.50 (*dt*, *J* = 10.8, 5.6, CH_2(7)); 2.74 (*t*, *J* = 6.3, CH_2(13)); 4.83 (*dtt*, *J* = 16.0, 1.2, 5.6, H–C(10)); 5.00 (*dtt*, *J* = 15.6, 1.6, 6.4, H–C(9)); 5.54 (br. *s*, HO–C(2)); 6.04 (*d*, *J* = 2.4, H–C(6)); 6.54 (*dd*, *J* = 2.0, 8.0, H–C(4)); 6.79 (*d*, *J* = 8.0, H–C(3)); 7.00 (*dt*, *J* = 8.8, 2.0, H–C(16), H–C(18)); 7.28 (*dt*, *J* = 8.8, 2.0, H–C(15), H–C(19)). HR-ESI-MS: 303.1380 ($[M+Na]^+$, $C_{19}H_{20}NaO_2^+$; calc. 303.1361).

Cell Lines and Culture Conditions. B16 4A5 (mouse melanoma), HL60 (human leukemia), and CRL1579 (human melanoma) cell lines were purchased from *Riken Cell Bank* (Tsukuba, Ibaraki, Japan). B16 Cells were cultured in D-MEM medium, while HL60 and CRL1579 cells were grown in RPMI-1640 medium. The medium was supplemented with 10% FBS and antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin). Cells were incubated at 37° in a 5% CO_2 humidified incubator. The cells were cultured according to the method described in [24][26][27].

Cytotoxicity Assay. Cytotoxicity assay was performed according to the method described in [24][26][27].

Assay of Melanin Content. Melanogenesis inhibition assay in the α-MSH-stimulated B16 melanoma cells was performed according to the method described in [27].

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