Studies on the Constituents of Aceraceae Plants. XI.[1] Two Types of Cyclic Diarylheptanoid from *Acer nikoense*

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Two diarylheptanoid glycosides, acerosides V (1) and XI (2), were isolated from the stem bark of *Acer nikoense* MAXIM. (Aceraceae). On hydrolysis, 1 and 2 yielded new diarylheptanoids, acerogenin D (6) and acerogenin E (7), respectively. Acerogenin D (6) was chemically correlated with acerogenin C (5), and acerogenin E, with alnusone (8). On the basis of the chemical and spectroscopic evidence, the chemical structures of 1 and 2 were established as the 2-*O*-β-D-glucopyranoside of acerogenin D and 3-*O*-β-D-glucopyranoside of acerogenin E, respectively. Aceroside XI is the first example of the biphenyl type of diarylheptanoid isolated from this plant. *A. nikoense* thus contains three types of diarylheptanoid, linear, cyclic biphenyl and cyclic diphenyl ether types.

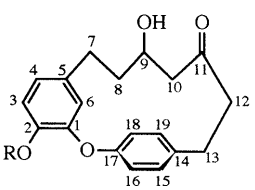
Keywords *Acer nikoense*; aceroside (V, XI); acerogenin (D, E); Aceraceae; diarylheptanoid; biphenyl

Acer nikoense MAXIM. (Aceraceae) is a deciduous tree having triple compound leaves and is indigenous to Japan. Its stem bark has been used as a folk medicine for hepatic disorder and to prepare an eyewash. We have isolated several diarylheptanoids and other phenolics from the stem bark of this plant.[3] The present paper deals with the isolation and structure determination of two new diarylheptanoid glucosides, designated as acerosides V (**1**) and XI (**2**).

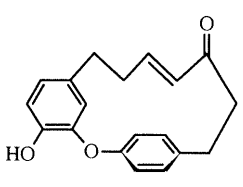
The ethyl acetate-soluble portion of a methanol extract from the stem bark of *A. nikoense* afforded acerosides V (**1**) and XI (**2**) after repeated chromatographic separation.

Aceroside V (**1**), $C_{25}H_{30}O_9 \cdot 3/2H_2O$, mp 130—132 °C, showed absorptions due to hydroxyl group(s) (3350 cm^{-1}), a carbonyl (1700 cm^{-1}) group, and aromatic ring(s) (1500, 1585 cm^{-1}) in the IR spectrum and gave no coloration with ferric chloride reagent. The fast atom bombardment (FAB) mass spectrum (MS) showed the quasi molecular ion at *m/z* 475 ($[M+H]^+$). The ^{13}C-NMR spectrum of **1** exhibited twenty-five signals assignable to two benzene rings (five singlets and seven doublets), five methylenes, a carbonyl, a methine joined to an oxygen atom, and a β-glucopyranosyl group. The aromatic proton signals in the ^1H-NMR spectrum were similar to those of aceroside IV (**3**), a known component of this plant.[3a] These findings suggested that **1** is a diarylheptanoid β-glucopyranoside similar to **3**. Acid hydrolysis of **1** yielded an α,β-unsaturated ketone (**4**) and glucose. The absorption due to the unsaturated carbonyl chromophore was observed at 231 nm (log ε 4.24) in the UV spectrum of **4**. On catalytic hydrogenation in the presence of Pd–C, **4**, afforded a dihydro derivative (**5**) identical with acerogenin C, the aglycone of aceroside IV. Since the conjugated carbonyl is absent in the molecule of **1** itself, **4** was regarded as an artefact formed during the acid hydrolysis. The β-olefinic proton of the α,β-unsaturated ketone system of **4** resonated at δ 6.59 as a multiplet in the ^1H-NMR spectrum, which suggested that the olefinic β-proton exists not on C-13 but on C-9. Taking into consideration that **4** was derived from **1** on acid hydrolysis, a β-ketol system is probably present in the molecule of **1**, because a β-ketol is well known to afford an α,β-unsaturated ketone easily in the presence of acid or alkali. Therefore, the structure of the genuine genin of **1** was presumed to be **6**. The position of the β-ketol system in **1** was confirmed by the ^1H–^1H correlation spectroscopy (COSY) NMR experiment, in which the oxygenated methine proton in the heptane chain at δ 3.47 (9-H) showed a correlation with two pairs of geminal protons at [δ 1.45 (8-H_a) and δ 1.70 (8-H_b)] and [δ 1.99 (10-H_a) and δ 2.44 (10-H_b)]. In order to identify the binding site of the β-glucose, a nuclear Overhauser effect (NOE) experiment was carried out. When the anomeric proton of the glucose at δ 5.71 (d, $J=6.8$) was irradiated, the intensity of the A-proton signal (δ 7.48, 3-H) of the ABX pattern in the aromatic proton signal region was enhanced by 8.4%. Consequently, the glucose is attached to the phenolic hydroxyl at C-2. The lack of coloration of **1** with ferric chloride supported this conclusion and the structure of aceroside V was, thus, established as **1** (Chart 1). The genuine genin (**6**), designated as acerogenin D, mp 171—172 °C, $[\alpha]_D^{17}$ −5.2°, was obtained on enzymatic hydrolysis of **1** with crude hesperidinase.

Aceroside XI (**2**), amorphous powder, $[\alpha]_D^{27}$ −17.7°, showed a quasi molecular ion at *m/z* 457 ($[M-H]^-$) corresponding to $C_{25}H_{30}O_8$ in the negative FAB-MS. The UV spectrum of **2** showed absorption at 245 nm and differs



1 : R = β-D-glucopyranosyl
6 : R = H



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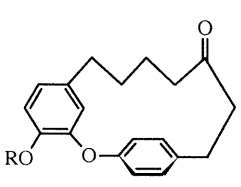


3 : R = β-D-glucopyranosyl
5 : R = H

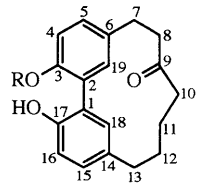
Chart 1

from that of the diphenyl ether type of diarylheptanoid, such as **1**.[3a] The ^{13}C-NMR spectrum of **2** indicated that **2** is a β-glucopyranoside of a diarylheptanoid possessing a carbonyl group on its heptane chain. Acid hydrolysis of **2** gave glucose and a genin, named acerogenin E (**7**). Acerogenin E (**7**), $C_{19}H_{20}O_3$, mp 230—231 °C, optically inactive, gave a positive coloration with 2,4-dinitrophenylhydrazine reagent (orange). Absorptions due to hydroxyl(s) (3300 cm^{-1}), a carbonyl (1685 cm^{-1}), and aromatic ring(s) (1495 and 1600 cm^{-1}) were observed in the IR spectrum. In the ^{13}C-NMR spectrum **7** exhibited nineteen signals, comprising twelve aromatic carbons (six siglets and six doublets), six methylenes and a ketonic carbon. The two singlets at δ 152.87 and 152.90 were assignable to aromatic carbons bearing phenolic hydroxyls. The genin (**7**) has a UV absorption at 247 nm (log ε 3.82) which was assignable to a conjugated biphenyl chromphore.[4] This absorption has not been observed in any diarylheptanoid isolated so far from this plant, indicating that **7** is a diarylheptanoid of the cyclic biphenyl type.[4] The three oxygen atoms in the molecular formula of **7** are accounted for by the two phenolic hydroxyls and the carbonyl. The biphenyl system of **7** is suggested to consist of two 1,2,4-trisubstituted benzene rings, because two sets of ABX signals with similar chemical shifts and coupling constants were observed in the aromatic ^1H-NMR signals of **2**. The NMR experiments on **7** determined the position of the carbonyl group as follows. Two methylene signals in lower magnetic field at δ 42.24 (C-8) and 45.06 (C-10), and those in moderate field at δ 24.48 (C-7) and 31.70 (C-13) were assignable to the two methylene carbons located at alpha positions to the carbonyl and the other two located at benzyl positions, respectively, on the basis of their chemical shifts. The protons on the above four methylene carbons appear at δ 2.78 (8-H_2), 2.67 (10-H_2), 3.06 (7-H_2), and 2.69 (13-H_2), each as a two-proton multiplet. The methylene protons of 7-H_2 and 8-H_2 were correlated with each other but they have no cross peak with other methylene protons in the ^1H–^1H COSY spectrum. These facts indicated that 7-H_2 and 8-H_2 consist of an ethylene moiety between the biphenyl system and the carbonyl group, *i.e.*, the structure of acerogenin E was presumed to be **7**. Two fragment ions at *m/z* 211 (ion a) and 225 (ion b) in the MS also supported this presumption.[4] The proposed structure of **7** corresponds to a dihydro derivative of alnusone (**8**), a constituent of *Alnus japonica*,[5] and catalytic hydrogenation of **8**, in fact, resulted in production of acerogenin E (**7**). Therefore the structure of acerogenin E was determined as **7**. The binding position of the β-glucose in the molecule of **2** was determined by NMR experiments. The geminal protons of one of the benzyl positions, C-7, of **2** appeared at δ 2.89 (7-H_a) and 3.05 (7-H_b). In the correlation spectroscopy *via* long-range coupling (COLOC) spectrum, the former signal (7-H_a) gave a cross peak with an aromatic carbon at δ 134.19 (C-6) and C-6 was further correlated with an aromatic proton at δ 7.43 (4-H) corresponding to an A-proton of one of the two ABX patterns in the aromatic proton signal area. On the other hand, the signal intensity of 4-H was enhanced by 10% when the anomeric proton of the β-glucospyranosyl at δ 5.69 was irradiated. Therefore, the structure of aceroside XI was established as **2** (Chart 2).

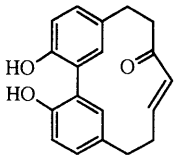
TABLE I. ^{13}C and ^1H-NMR Data for Acerosides V (**1**) and XI (**2**) (in d_5-Pyridine)

Carbon No.	1		2	
	^{13}C	^1H	^{13}C	^1H
1	152.12	—	127.54	—
2	145.67		129.34	—
3	117.45	7.48 (d, J=8.55)	153.02	—
4	122.99	6.69 (dd, J=8.55, 1.71)	114.58	7.43 (d, J=8.55)
5	135.42	—	128.84	7.04 (d, J=8.55, 2.14)
6	118.23	5.88 (d, J=1.71)	134.19	—
7	28.58	H_a 2.52—2.57 (m)[a]	28.52	H_a 2.89 (br dd, J=14.0, 8.3)
		H_b 2.86—2.91 (m)[b]		H_b 3.05 (br dd, J=14.0, 7.5)
8	36.09	H_a 1.45 (br dd, J=14.1, 11.4)	41.97	H_a 2.72—2.82 (m)[a]
		H_b 1.70 (m)		H_b 2.62—2.72 (m)[a]
9	65.16	3.47 (br t, J=9.0)	212.26	—
10	53.34	H_a 1.99 (dd, J=16.60, 2.44)	45.21	H_a 2.62—2.72 (m)[a]
		H_b 2.44 (dd, J=16.60, 9.03)		H_b 2.46—2.61 (m)[a]
11	212.52	—	22.05	1.66—1.86 (m)[b]
12	44.82	H_a 2.52—2.57 (m)[a]	25.88	H_a 1.66—1.86 (m)[b]
		H_b 2.68 (ddd, J=13.43, 9.28, 5.86)		H_b 1.92 (m)
13	31.65	2.86—2.91 (m)[b]	31.73	H_a 2.46—2.61 (m)[a]
				H_b 2.72—2.82 (m)[a]
14	137.56	—	131.42	—
15	130.26[c]	7.00 (dd, J=2.20, 8.30)[c]	130.02	7.08 (dd, J=7.93, 1.83)
16	123.98[d]	6.75 (dd, J=2.45, 8.30)[d]	117.89	7.15 (d, J=7.93)
17	157.69	—	152.82	—
18	123.98[d]	7.17 (dd, J=2.45, 8.30)[d]	134.82	6.99 (d, J=1.83)
19	132.21[c]	7.32 (dd, J=2.20, 8.30)[c]	134.66	6.89 (d, J=2.14)
Glucose				
1′	102.73	5.71 (d, J=6.84)	101.89	5.69 (d, J=7.93)
2′	74.80	4.27—4.36 (m)[b]	74.39	4.18 (m)[a]
3′	78.50	4.27—4.36 (m)[b]	78.14	4.28 (m)
4′	71.34	4.27—4.36 (m)[b]	71.09	4.18 (m)[a]
5′	78.81	4.10 (m)	78.96	4.10 (m)
6′	62.52	H_a 4.38 (dd, J=11.72, 5.13)	62.13	H_a 4.33 (m)
		H_b 4.51 (dd, J=11.72, 2.20)		H_b 4.50 (dd, J=11.90, 1.90)

a) Two-proton signal. *b*) Three-proton signal. *c*—*f*) Assignments may be interchanged in each column.



2: R = β-D-glucopyranosyl
7: R = H



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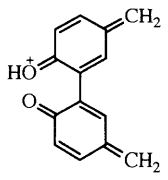
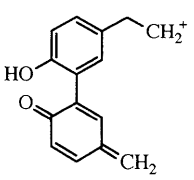


ion a (*m/z* 211)



ion b (*m/z* 225)

Chart 2

Diarylheptanoids possess a C_6 (arom.)–C_7–C_6 (arom.) carbon skeleton and can be structurally classified into three types, linear, cyclic biphenyl and cyclic diphenyl ether types. We have already isolated compounds of the former two types, such as aceroside VII and aceroside **I**, from *Acer nikoense*.[3b] Aceroside XI (**2**) is the first example of the biphenyl type among diarylheptanoids isolated from the plant. *A. nikoense* seems to be the first example of a plant containing all of the three types of diarylheptanoid. We have studied the biosynthesis of acerogenin A and proposed that it is formed *via* (−)-centrolobol, derived from two *p*-coumarate units and one malonate unit.[6] The biphenyl type such as acerogenin E (**7**) must be biosynthesized from (−)-centrolobol as a common intermediate as the diphenyl ether type such as acerogenin A, through oxidative phenol coupling between the two phenol rings. The cooccurrence of these three types in *A. nikoense* demonstrates the biosynthetic significance of diarylheptanoids.

Experimental

All melting points were taken on a Yanagimoto melting point determination apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 automatic polarimeter in a 1 dm cell. IR spectra were obtained with a Hitachi IR 260-10 spectrometer. UV spectra were recorded on a Shimadzu UV 250 spectrometer. NMR spectra were recorded with a JEOL JNM GX-400 or JNM FX-100 spectrometer in d_5-pyridine unless otherwise stated. Chemical shifts are given on the δ scale (ppm), coupling constants (J values) are expressed in hertz (Hz), and the following abbreviations are used: s = singlet, d = doublet, t = triplet, and m = multiplet. MS were recorded with JEOL JMS-D 300 and JMS-SX 102 spectrometers. HPLC was carried out with a Nihon Seimitsu NPG-350L instrument equipped with a Wakosil 5C18-200 column (Wako Pure Chemical Industries, Ltd. 20 mm i.d. × 250 mm). Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} precoated plates (Merck) and detection was carried out by UV irradiation (254 nm) and by spraying 10% H_2SO_4 followed by heating.

Extraction and Isolation The plant material was collected in Gumma prefecture, Japan. The shavings of the stem bark (1.7 kg) were extracted with hot MeOH, and the extract (354 g) dissolved in water (2 l) and partitioned successively with ether, EtOAc and BuOH (each 0.5 l × 3 times). The extracts weighed 21, 91 and 120 g, respectively. The EtOAc extract was chromatographed on silica gel with $CHCl_3$ containing increasing proportions of MeOH (12 : 1, 9 : 1, 6 : 1, and 3 : 1). The eluate with $CHCl_3$–MeOH (9 : 1) was rechromatographed on silica gel using EtOAc–MeOH (9 : 1) as the solvent to afford aceroside V (26 mg). The eluate with the same solvent after elution of **2** was further chromatographed on an ODS column. Elution with MeOH–water (5 : 2) gave three fractions (fr.). Fraction 1 contained predominantly aceroside IV as estimated by visual examination on TLC. Fraction 2 was subjected to HPLC with MeOH–H_2O (5 : 2), affording aceroside XI (16 mg).

Aceroside V (1) Colorless needles (MeOH), mp 130—132 °C, $[\alpha]_D^{20}$ −57.3° (MeOH, c = 1.0). *Anal.* Calcd for $C_{25}H_{30}O_9 \cdot 3/2H_2O$: C, 60.04; H, 6.35. Found: C, 59.69; H, 6.61. FAB-MS m/z: 475 $[M+H]^+$. IR ν_{max}^{KBr} cm^{-1}: 3350, 1700, 1585, 1500. UV λ_{max}^{MeOH} nm (log ε): 278 (4.01); $\lambda^{MeOH+NaOH}$ nm: unchanged.

Aceroside XI (2) Amorphous powder, $[\alpha]_D^{27}$ −17.7° (EtOH, c = 1.0). Negative FAB-MS m/z: 457 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1500. UV λ_{max}^{EtOH} nm (log ε): 245 (sh 4.18), 289 (3.95).

Acid Hydrolysis of 1 A mixture of **1** (20 mg), aqueous MeOH and 5% sulfuric acid (7 ml) was heated for 1 h under reflux. The MeOH was evaporated off, and the residue was diluted with water, then extraced with EtOAc. After concentration of the organic layer, the residue was chromatographed over silica gel with benzene–EtOAc (20 : 1) to give an α,β-unsaturated ketone (**4**, 9 mg). The water-soluble fraction of the above acid hydrolysate was passed through a column of Amberlite MB-3, and the eluate was concentrated to a small volume. Glucose was detected on TLC (Cellulose F_{254} (Merck), Rf 0.36), BuOH–acetone–water (4 : 5 : 1), coloring with aniline–H_3PO_4. **4**, mp 175—177 °C (MeOH), $[\alpha]_D^{20}$ 0° (EtOH, c = 1.0). High MS m/z: Calcd for $C_{19}H_{18}O_3$ (M^+), 294.1256. Found 294.1264. IR ν_{max}^{KBr} cm^{-1}: 3140, 1680, 1610, 1590, 1510. UV λ_{max}^{EtOH} nm (log ε): 208 (4.56, end absorption), 231 (4.24), 269 (3.76); $\lambda_{max}^{EtOH+NaOH}$ nm (log ε): 241 (3.97), 276 (3.73), 301 (3.60). ^{1}H-NMR ($CDCl_3$) δ: 2.33 (2H, m, 8-H_2), 2.66 (4H, m, 7-H_2, 12-H_2), 3.06 (2H, m, 13-H_2), 5.55 (s, Ar-OH), 5.68 (d, J = 16, 10-H), 6.59 (1H, m, 9-H), 5.73 (d, J = 2, 6-H), 6.58 (dd, J = 2, 8, 4-H), 6.82 (d, J = 8, 3-H), 6.98 (2H, d, J = 9, 16-, 18-H), 7.24 (2H, d, J = 9, 15-, 19-H).

Catalytic Hydrogenation of 4 A solution of **4** (5 mg) in EtOH (5 ml) was hydrogenated under atmospheric pressure in the presence of 10% Pd–C. After removal of the catalyst by filtration, the solvent was evaporated off *in vacuo*. The residue was chromatographed over silica gel with benzene–EtOAc (20 : 1) to afford a dihydro derivative (3 mg), mp 116 °C (MeOH), which was identical with acerogenin C (**5**) by TLC, IR and NMR comparisons and mixed melting point determination.

Enzymatic Hydrolysis of 1 A solution of **1** (57 mg) in a mixture of EtOH (28 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (57 ml) was treated with crude hesperidinase (Tanabe Pharm. Co., Ltd.) (57 mg) in water (28 ml), and the total mixture was kept for 24 h with stirring at 38 °C. The EtOH in the mixture was evaporated off and the remaining solution was extracted with ether. After the solvent was evaporated off, the residue was chromatographed over silica gel with benzene–EtOAc (10 : 1) to give acerogenin D (**6**). **6**, colorless needles, mp 171—172 °C (MeOH), $[\alpha]_D^{17}$ −5.2° ($CHCl_3$–MeOH (1 : 1), c = 1.6). High MS m/z: Calcd for $C_{19}H_{20}O_4$ (M^+), 312.1360. Found 312.1355. IR ν_{max}^{KBr} cm^{-1}: 3425, 3190, 2915, 1690, 1585, 1510, 1500.

Acid Hydrolysis of 2 A solution of **2** (5 mg) in 10% H_2SO_4 in 50% MeOH (10 ml) was worked up in the same way as in the case of **1** and yielded a genin (**7**, 2 mg) and glucose. Acerogenin E (**7**): Colorless needles (MeOH), mp 230—231 °C, $[\alpha]_D^{20}$ 0° ($CHCl_3$, c = 2.0). 2,4-Dinitrophenylhydrazine reagent: +(orange). *Anal.* Calcd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.62; H, 6.77. IR ν_{max}^{KBr} cm^{-1}: 3300 (br), 1685, 1600, 1495, 1235, 800. UV λ_{max}^{EtOH} nm (log ε): 207 (4.25, end absorption), 247 (3.82), 299 (3.65). MS m/z (rel. int.): 296 (M^+, 100), 225 (34), 211 (56), 197 (17), 183 (9), 165 (14), 115 (15). ^{1}H-NMR δ : 1.83 (2H, m, 12-H_2), 1.90 (2H, m, 11-H_2), 2.67 (2H, m, 10-H_2), 2.69 (2H, m, 13-H_2), 2.78 (2H, m, 8-H_2), 3.06 (2H, m, 7-H_2), 7.05—7.19 (6H, m, Ar). ^{13}C-NMR δ : 22.42 (C-12), 26.07 (C-11), 28.48 (C-7), 31.70 (C-13), 42.24 (C-8), 45.06 (C-10), 116.82, 117.04, (C-4, -16), 127.23, 127.79 (C-1, -2), 128.99, 129.88 (C-5, -15), 131.59, 132.40 (C-6, -14), 134.15, 134.23 (C-18, -19), 152.87, 152.90 (C-3, -17), 212.40 (C-9).

Catalytic Hydrogenation of Alnusone (8) A solution of **8** (10 mg) in EtOH (5 ml) was hydrogenated in the same way as in the case of **4**. After chromatographic purification of the reaction product, a dihydro derivative of **8**, mp 229—231 °C (MeOH) (lit.[5] mp 240 °C (EtOAc)), was obtained. This product was shown to be identical with acerogenin E (**7**) by IR, NMR and TLC comparisons and mixed melting point determination.

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