Studies on the Constituents of Aceraceae Plants. VII.[1] Diarylheptanoids from *Acer griseum* and *Acer triflorum*

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A diarylheptanoid glycoside, named aceroside IX (1), $C_{30}H_{40}O_{12}$, $[\alpha]_D$ −63.5°, was isolated from the stem bark of *Acer griseum* and *A. triflorum*. Another diarylheptanoid glycoside, named aceroside X (2), $C_{25}H_{31}O_8$, mp 121°, $[\alpha]_D^{21}$ −29.7°, and catechin were isolated from the stem bark of *A. griseum* and detected by thin layer chromatography in the case of *A. triflorum*. On acid hydrolysis 1 yielded a new diarylheptanoid, acerogenin G (3), $C_{19}H_{22}O_3$, which was identified as a ketonic derivative of (−)-centrolobol (5), and sugars, glucose and apiose. On partial hydrolysis, 1 gave aceroside X (2) and apiose. The sugar moiety of aceroside IX is bound to the phenolic hydroxyl at C-4″ of acerogenin G (3) from the results of mass spectral analysis of 3 and its monomethyl ether (6), the latter of which was formed on methylation of 1 followed by acid hydrolysis. Acerosides (1) and X (2) were determined to be the 4″-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside and the 4″-*O*-β-D-glucopyranoside of acerogenin G, respectively, on the basis of carbon-13 nuclear magnetic resonancence spectral analyses in comparison with the spectrum of aceroside VIII (7).

Keywords *Acer griseum*; *Acer triflorum*; diarylheptanoid; aceroside (IX, X); acerogenin G

Acer griseum (FRANCH.) PAX is distributed in the central part of China, *A. triflorum* KOM. (Japanese name: onimegusuri) in north-eastern China and Korea, and *A. nikoense* MAXIM. (Japanese name: megusurinoki) in Japan. All these Aceraceae plants belong to the Section *Trifoliata*, the Series *Grisea* POJARK,[3] and are deciduous trees having triple compound leaves with many small hairs on the back and the leaf-stalks. The red-brown periderm of *A. griseum* and *A. triflorum* characteristically drops away in all seasons. Several glycosides such as acerosides I,[1,4,5] III,[1,6] IV,[7] VI,[1,6] VII,[1] and VIII[1] have been reported as diarylheptanoid constituents of the stem bark of *A. nikoense*. This paper deals with two new glycosides of a linear-type diarylheptanoid, aceroside IX (**1**) and aceroside X (**2**), isolated from the stem bark of *A. griseum* and *A. triflorum* together with catechin.

Aceroside IX (**1**), $C_{30}H_{40}O_{12}$, was isolated from both plants as a white powder, $[\alpha]_D$ −63.5°, and aceroside X (**2**) was obtained in a small amount from the stem bark of *A. griseum* as colorless needles, mp 120—121°. $[\alpha]_D^{21}$ −29.7°. The latter glycoside (**2**) is less polar than the former, and was also detected in the methanolic extract of *A. triflorum* on thin layer chromatography (TLC). Both compounds gave positive colorations with ferric chloride reagent (blue) and 2,4-dinitrophenylhydrazine reagent (yellow). In the infrared (IR) spectrum, aceroside IX (**1**) showed absorptions due to hydroxyl groups at 3100—3650 cm^{-1}, aromatic rings at 1620 and 1515 cm^{-1}, and a carbonyl group at 1710 cm^{-1}. It has ultraviolet (UV) spectral maxima at 222, 274 and 279 nm, which exhibited bathochromic shifts on addition of alkali. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of aceroside IX (**1**) and aceroside X (**2**) were very similar to each other except for several signals due to their sugar moieties (Table I). Aceroside X (**2**) showed six sugar carbons, probably due to a glucose on the basis of their chemical shifts, while aceroside IX (**1**) showed eleven sugar signals, two of which at 102.9 ppm (d) and 111.2 ppm (d) seemed to be anomeric carbons of its sugar moiety, consisting of a pentose and a hexose. On partial hydrolysis with 50% acetic acid, aceroside IX (**1**) yielded apiose and a glucoside (**4**). The latter product **4** was found to be identical with aceroside X (**2**) by mixed melting point determination and TLC comparison. The five signals due to the pentose, especially two triplets at δ 65.6 and 74.9 ppm and a singlet at δ 80.4 ppm in the ^{13}C-NMR spectrum of **1**, were consistent with the detection of apiose in the partial hydrolysate. Furthermore, partial hydrolysis caused an upfield shift by 6.6 ppm for another triplet at δ 69.0 ppm (C-6 of glucose), indicating the presence of a disaccharide, apiofuranosyl (1→6)-hexose as the sugar moiety of **1**.

In the ^{13}C-NMR spectra, **1** and **2** showed signals due to

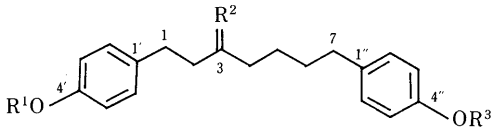
TABLE I. ^{13}C-Chemical Shifts (δ ppm) in C_5D_5N

	Carbon	1	2	7
Genin	1′ and 1″	136.0 s 132.0 s	136.0 s 132.2 s	
	2′,6′ and 2″,6″	129.9 d×2 129.7 d×2	129.9 d×2 129.7 d×2	
	3′,5′ and 3″,5″	117.3 d×2 116.3 d×2	116.9 d×2 116.3 d×2	
	4 and 4″	157.2 s 157.0 s	157.2 s 156.9 s	
	1—7	23.6 t 29.4 t 31.4 t 35.1 t 42.7 t 44.7 t 209.6 s	23.6 t 29.4 t 31.4 t 35.0 t 42.7 t 44.7 t 209.5 s	
Glucosyl	1	102.9 d	102.4 d	103.4 d
	2	75.0	75.0	75.1
	3	78.6	78.8	78.6
	4	71.7	71.3	71.5
	5	77.3	78.5	76.7
	6	69.0 t	62.4 t	68.5 t
Apiosyl	1	111.2 d		110.8 d
	2	77.8		77.7
	3	80.4 s		80.3 s
	4	74.9 t		74.9 t
	5	65.6 t		65.6 t

their common genin portion, namely signals ascribable to a carbonyl, six methylenes, and several aromatic carbons. The aromatic carbon signals comprised four singlets and four doublets. The four singlets are probably assignable to two oxygenated carbons and two alkylated ones from their chemical shifts. Each of the four doublets seems to be due to two methine carbons which have an equivalent chemical shift: in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, **2** (=**4**) showed two sets of AA'BB' patterns centered at δ 7.19 and 7.21 ppm (J=8.4 Hz) [two *p*-substituted benzenes], in addition to four methylene triplets and two methylene multiplets. These findings suggested that aceroside IX (**1**) is the apiofuranosyl (1→6) hexoside of a diarylheptanoid which has two *p*-substituted benzene structures and a carbonyl group on its heptane chain.

On complete hydrolysis with 10% sulfuric acid, **1** yielded a genin (**3**), glucose and apiose. The genin (**3**), $C_{19}H_{22}O_3$, designated acerogenin G, was established as 1,7-bis-(4-hydroxyphenyl)-heptan-3-one, since it was shown to be identical with the Oppenauer oxidation product of (−)-centrolobol (**5**), the genin of acerosides VII and VIII (**7**), by direct comparison.

Since acerosides IX (**1**) and X (**2**) have a phenolic hydroxyl group in the molecule, one of the two phenolic hydroxyls of acerogenin G (**3**) must be free from glycosidation and the other hydroxyl must bear the sugars. In order to determine the linking site of apiosylglucose to the genin (**3**), **1** was methylated with dimethyl sulfate and then hydrolyzed with 10% sulfuric acid. A methylated genin (**6**) was obtained



1 : R^1=H, R^2=O,
R^3=β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl
2(=**4**) : R^1=H, R^2=O,
R^3=β-D-glucopyranosyl
3 : R^1=R^3=H, R^2=O
5 : R^1=R^3=H, R^2= 
6 : R^1=CH_3, R^2=O, R^3=H
7 : R^1=R^3=H,
R^2= 
O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl

Chart 1

as a white powder, $C_{20}H_{24}O_3$. The mass spectrum (MS) of **6** was compared with that of acerogenin G (Chart 2). In the case of the methylated genin (**6**) an ion at m/z 163.0758 corresponding to $C_{10}H_{11}O_2$ was observed instead of m/z 149.0601 corresponding not to $C_{10}H_{13}O$ but to $C_9H_9O_2$ in the case of acerogenin G (**3**). This indicates that the methylation had occurred at the phenolic hydroxyl at C-4' of the genin (**3**), the hydroxyl nearer to the carbonyl at C-3. It follows that the apiosylglucose moiety of **1** is bound to the phenolic hydroxyl at C-4'' of th genin **3**.

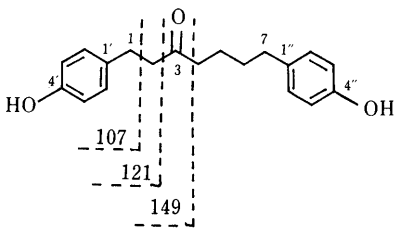
The ^{13}C-NMR spectra of **1** and **2** (=**4**) (Table I) in comparison with that of aceroside VIII (**7**)[1] disclosed that the sugar portion of **2** is a β-D-glucopyranosyl residue while that of **1** is a β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl residue.

Acerosides IX (**1**) and X (**2**) are the glycosides of a linear-type diarylheptanoid, and are common constituents of *A. griseum* and *A. triflorum*, but they have not been found so far in *A. nikoense* in spite of the morphological similarity of the plants (the same section and the same series) to each other. Glycosides of a linear-type diarylheptanoid, acerosides VII[1] and VIII,[1] have been found in *A. nikoense*, but glycosides of cyclic-type diarylheptanoids such as acerosides I, III, IV, and VI are major components in this plant, though they have not been detected in *A. griseum* or *A. triflorum*.

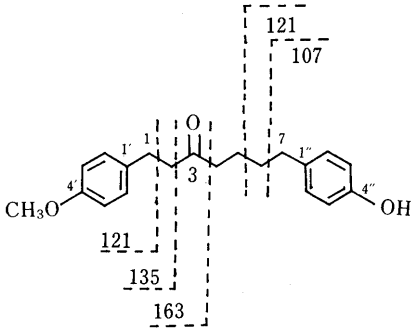
Experimental

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, *p*-anisaldehyde–H_2SO_4 or 2,4-dinitrophenylhydrazine reagent followed by heating. TLC for sugars was run on Cellulose F precoated plates (Merck) and spots were visualized by spraying aniline hydrogen phthalate followed by heating at 105 °C for 5—10 min. Silica gel (Kieselgel 60, 230—400 mesh, Merck), Sephadex LH-20 (Pharmacia Fine Chemical Co.), Amberlite XAD-II (Organo Co., Ltd.), and Diaion HP-20 (Mitsubishi Kasei Co., Ltd.) were used for chromatography. The following instruments were used: melting point, a Yanagimoto micro melting point determination apparatus; IR spectra, a Hitachi 260-10 spectrometer; UV spectra, a Shimadzu UV 250 double-beam spectrometer; MS, a JEOL JMS-D-300 mass spectrometer; optical rotations, a JASCO DIP-181 automatic polarimeter (using a dm tube); ^{1}H- and ^{13}C-NMR spectra, a JEOL JNM FX-400 spectrometer {tetramethylsilane as an internal standard; chemical shifts expressed on the δ scale (ppm) and coupling constants (J values) in Hz; s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet}.

Extraction and Isolation The dried stem bark, including the whole of the cambium in this experiment, of *A. griseum* (80 g) {Introduction Code (Fac. Horticulture, Chiba Univ.): 83S-164} was chopped and extracted with MeOH (1.2 l) in a Soxhlet extractor. The methanolic solution was concentrated to dryness under reduced pressure. The residue (11.93 g), dissolved in 30% MeOH (1 l), was applied to an Amberlite XAD-II column.



acerogenin G (**3**)



6

Chart 2

The 30% MeOH eluate was concentrated to distil off the MeOH, and the remaining solution was washed with $CHCl_3$. The aqueous layer was concentrated to dryness and the residue was repeatedly chromatographed on silica gel column with $CHCl_3$–MeOH–MeOAc–H_2O (10:4:8:1) to give catechin. The MeOH eluate from the Amberlite XAD-II column was concentrated to dryness, and the residue was chromatographed on a Sephadex LH-20 column with MeOH as the solvent. Chromatographic fractions containing the acerosides were collected and concentrated to dryness. Repeated chromatographic separation of the residue over a silica gel column with $CHCl_3$–MeOH–MeOAc–H_2O (5:3:6:1) afforded aceroside IX (**1**) (2 g) and aceroside X (**2**) (0.2 g).

The dried branches of *A. triflorum* from Korea (1840 g) were chopped and extracted with MeOH under reflux. The MeOH extract (144 g), suspended in water, was successively extracted with hexane, ether and EtOAc. The EtOAc-soluble fraction was concentrated *in vacuo* and the residue (20.9 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (200:55:7), and then on a polyamide column (water as the solvent) to give aceroside IX (**1**) (85.2 mg). Aceroside X (**2**) and catechin were detected in the MeOH extract on TLC.

Catechin Light yellow needles from MeOH–H_2O. This was shown to be identical with a standard sample by TLC, IR, ^{1}H-NMR and ^{13}C-NMR comparisons.

Aceroside IX (1) An amorphous white powder, $[\alpha]_D$ −63.5° (c=1.0, EtOH). $FeCl_3$ reagent: positive (blue). 2,4-Dinitrophenylhydrazine reagent: positive (yellow). *Anal.* Calcd for $C_{30}H_{40}O_{12}$: C, 60.80; H, 6.80. Found: C, 60.34; H, 6.89. IR ν_{max}^{KBr} cm^{-1}: 3400 (br OH), 2930, 1710 (CO), 1620, 1515 (aromatic). UV λ_{max}^{EtOH} nm (log ε): 222 (4.23), 274 (3.45), 279 (3.45). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 242, 280, 295 (bathochromic shifts). ^{13}C-NMR (C_5D_5N): Table I. ^{1}H-NMR (C_5D_5N) δ: *ca.* 1.47—1.56 (5-H_2 and 6-H_2, each m), 2.33 (4-H_2, t), 2.48 (7-H_2, t), 2.72 (2-H_2, t), 2.94 (1-H_2, t), 5.48, 5.78 (1H, d, J=6.4 and 1H, m, anomeric H's of glucose and apiose), 7.16, 7.22 (each d, J=8.4, an AA'BB' pattern centered at δ 7.19), 7.17, 7.37 (each d, J=8.4, an AA'BB' pattern centered at δ 7.27). TLC: solvent, $CHCl_3$–MeOH–MeOAc–H_2O (5:3:6:1), *Rf* 0.3.

Aceroside X (2) Colorless needles, mp 120—121 °C. $[\alpha]_D^{21}$ −29.7° (c=1.0, EtOH). $FeCl_3$ reagent: positive (blue). 2,4-Dinitrophenylhydrazine reagent: positive (yellow). ^{13}C-NMR (C_5D_5N): Table I. ^{1}H-NMR (C_5D_5N) δ: *ca.* 1.52—1.57 (5-H_2 and 6-H_2, each m), 2.36 (4-H_2, t), 2.48 (7-H_2, t), 2.72 (2-H_2, t), 2.95 (1-H_2, t), 5.61 (1H, d, J=7.1, anomeric H of glucose), 7.15, 7.22 (each d, J=8.4, an AA'BB' pattern centered at δ 7.19), 7.11, 7.31 (each d, J=8.4, an AA'BB' pattern centered at δ 7.21). This compound (**2**) was shown to be identical with **4** described below by mixed melting point determination and TLC comparisons {solvent, $CHCl_3$–MeOH–MeOAc–H_2O (5:3:6:1), *Rf* 0.5; $CHCl_3$–MeOH (5:1), *Rf* 0.3}.

Hydrolysis of Aceroside IX (1) with Dilute Sulfuric Acid A mixture of **1** (500 mg), 50% MeOH (5 ml) and 10% aqueous sulfuric acid (2.5 ml) was heated for 1 h under reflux. After cooling, the reaction mixture was concentrated to distil off the MeOH, then diluted with water, and extracted with EtOAc. After concentration of the organic layer, the residue was chromatographed over silica gel with benzene–EtOAc (5:1) to give acerogenin G (**3**) as an amorphous white powder. $FeCl_3$ reagent: positive (blue). 2,4-Dinitrophenylhydrazine reagent: positive (yellow). High MS m/z: Calcd for $C_{19}H_{22}O_3$ (M^+), 298.1570; $C_9H_9O_2$, 149.0603. Found: 298.1569; 149.0601. MS m/z: 298 (M^+), 149, 121, 107 (base ion peak) (Chart 2). The aqueous layer was neutralized with $BaCO_3$ and concentrated *in vacuo*. Glucose and apiose were detected in the residue on TLC {solvent, BuOH–AcOH–H_2O (6:1:2); *Rf* 0.18 (glucose), 0.32 (apiose)}.

Partial Hydrolysis of Aceroside IX (1) with Dilute Acetic Acid A mixture of **1** (300 mg) and 50% aqueous acetic acid (15 ml) was heated for 1 h under reflux. The reaction mixture was diluted with water, and extracted with EtOAc. The organic layer was concentrated to dryness and the residue was chromatographed on silica gel with $CHCl_3$–MeOH (5:1) to give a crude product. This product was recrystallized from MeOH–EtOAc–dilute acetic acid to give **4** (10 mg) as colorless needles, mp 120—121 °C. $FeCl_3$ reagent: positive (blue). 2,4-Dinitrophenylhydrazine reagent: positive (yellow). The aqueous layer was concentrated to dryness under reduced pressure. Apiose was detected in the residue on TLC {solvent, BuOH–AcOH–H_2O (6:1:2), *Rf* 0.32}.

Oppenauer Oxidation of (−)-Centrolobol The oxidation of (−)-centrolobol (1.43 g) was carried out by using toluene (total 60 ml), cyclohexanone (1 ml) and aluminium isopropoxide (300 mg) according to the procedure described by Eastham and Teranishi[8] for the preparation of cholest-4-en-3-one. After addition of 2 N HCl (8 ml) to the reaction mixture, the products were extracted with benzene–EtOAc (2:1). The organic layer was extracted with 2 N NaOH. The aqueous layer, after being acidified with 2 N HCl, was extracted with EtOAc. The organic layer was concentrated to dryness under reduced pressure, and the residue was chromatographed over silica gel with benzene–EtOAc (5:1) to give 1,7-bis-(4-hydroxyphenyl)-heptan-3-one as an amorphous white powder. This product was proved to be identical with acerogenin G (**3**) by MS and TLC comparisons. Solvent, benzene–AcOEt (5:1), *Rf* 0.25; cyclohexane–acetone (4:3), *Rf* 0.54.

Methylated Genin (6) of Aceroside IX Dimethyl sulfate (0.53 ml) was added dropwise over 15 min to a solution of aceroside IX (220 mg) in 5% NaOH (4.4 ml) with stirring under ice cooling. The mixture was stirred overnight at room temperature. The reaction mixture, after being diluted with water, was applied to a column of Diaion HP-20. The column was washed with water and then with MeOH. The MeOH eluate was concentrated to dryness. The residue was dissolved in a mixture of MeOH (7 ml) and 10% aqueous H_2SO_4 (2.5 ml), and the solution was heated for 1.5 h under reflux. After cooling, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was concentrated and the residue was chromatographed on silica gel with benzene–EtOAc (8:1) to give **6** as a white powder. $FeCl_3$ reagent: positive (blue). 2,4-Dinitrophenylhydrazine reagent: positive (yellow). High MS m/z: Calcd for $C_{20}H_{24}O_3$ (M^+), 312.1726; $C_{10}H_{11}O_2$, 163.0759. Found: 312.1724; 163.0758. MS m/z: 312 (M^+), 163, 135, 121 (base ion peak), 107 (Chart 2).

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References and Notes

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