Studies on the Constituents of Aceraceae Plants. XII.[1] Two New Diarylheptanoid Glycosides from *Acer triflorum*

Satoru SHIRATORI,[a] Seiji NAGUMO,[a] Takao INOUE,[a] Masahiro NAGAI,*[,a] and Hyung Joon CHI[b]

Faculty of Pharmaceutical Sciences, Hoshi University,[a] Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan, and Natural Products Research Institute, Seoul National University,[b] Seoul, Korea.
Received November 9, 1993; accepted December 24, 1993

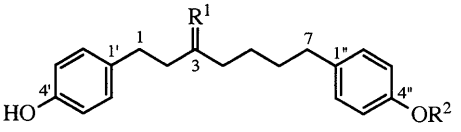
Two new diarylheptanoid glycosides, named aceroside XII (7), $C_{30}H_{42}O_{12}$, $[\alpha]_D$ −70.3°, and aceroside XIII (8), $C_{25}H_{34}O_8$, $[\alpha]_D$ −40.2°, were isolated along with triterpenoids and caffeoyl esters from the branches of *Acer triflorum* KOM. (Aceraceae). On acid hydrolysis, 7 yielded (−)-centrolobol (9), glucose and apiose. On partial hydrolysis, 7 gave 8 and apiose. Acerosides XII (7) and XIII (8) were determined to be the 4″-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside and the 4″-*O*-β-D-glucopyranoside of (−)-centrolobol, respectively, on the basis of chemical and spectral evidence.

Keywords *Acer triflorum*; aceroside (XII, XIII); diarylheptanoid; (−)-centrolobol; Aceraceae; chemotaxonomy

From *Acer triflorum* KOM. (Aceraceae) we previously reported the isolation of two glycosides of a diarylheptanoid, acerosides IX (**1**) and X (**2**), and of catechin.[2] In a continuation of our chemical investigation on the same plant, six compounds (**3**—**8**) including two new diarylheptanoid glycosides were newly isolated. This paper deals with their isolation and structure determination.

A methanol extract of the branches of *A. triflorum* was partitioned between ethyl acetate and water. The ethyl acetate-soluble portion was successively subjected to silica gel and Sephadex LH-20 column chromatographies, affording compounds **3**—**6**. The water-soluble portion yielded two new diarylheptanoid glycosides, named acerosides XII (**7**) and XIII (**8**), after successive chromatographic separation. Each of compounds **3** and **4** was isolated as a mixture, though each showed one spot on the TLC plates. Their compositions were elucidated as follows: **3**, a mixture of oleanolic acid and ursolic acid; **4**, a mixture of α- and β-amyrin. Compound **5** showed IR absorptions at 1690, 1660, 1280 cm^{-1} (unsaturated ester(s)) and ^{1}H-NMR signals assignable to 1,2,4-trisubstituted benzene protons at δ 6.87 (d), 7.01 (dd) and 7.08 (d) ppm, and two olefinic protons at δ 6.26 and 7.56 ppm (each d), along with signals attributable to alkyl chains. These spectral data were almost identical with the reported data for a mixture of caffeoyl esters of higher alcohols[3] and this substance was finally concluded to be a mixture of docosyl caffeate, tetracosyl caffeate, and hexacosyl caffeate, since the higher alcohols were identified by gas-liquid chromatography (GLC) after alkaline hydrolysis. Compound **6** was suggested to be a caffeoyl ester of a triterpenoid from its ^{1}H-NMR spectrum. The methyl ester of **6** dimethyl ether was partially hydrolyzed with alkali to afford the methyl ester of betulinic acid. Therefore, **6** was determined to be identical with pyracrenic acid (caffeoyl betulinic acid).[4]

Acerosides XII (**7**), $[\alpha]_D$ −70.3° and XIII (**8**), $[\alpha]_D$ −40.2° were both obtained as amorphous white powder and showed positive colorations with ferric chloride reagent (blue). Their molecular formulae were determined as $C_{30}H_{42}O_{12}$ and $C_{25}H_{34}O_8$, respectively, by high-resolution FAB-MS analysis. In the UV spectrum, **7** showed absorption maxima at 222, 275 and 279 nm, which exhibited bathochromic shifts on addition of alkali. The ^{13}C-NMR spectra of **7** and **8** showed signals due to their common genin, namely signals ascribable to two benzene rings, six methylenes, and a hydroxy methine of a diarylheptanoid. The carbon signals due to their sugar moieties were in good agreement with those of the β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl group in aceroside IX (**1**) and the β-D-glucopyranosyl group in aceroside X (**2**), respectively. On acid hydrolysis, **7** yielded apiose, glucose and a genin (**9**), mp 128—130 °C, $[\alpha]_D$ −8.8°. The genin (**9**) was identified by direct comparison with (−)-centrolobol, the genin of acerosides VII and VIII from *A. nikoense*.[5] Partial hydrolysis of aceroside XII (**7**) with 50% acetic acid gave apiose and a glucoside, of which the latter was found to be identical with aceroside XIII (**8**) on the basis of TLC, $[\alpha]_D$ and NMR comparisons. In the ^{13}C-NMR spectrum of **7** compared with that of its genin (**9**), a glycosylation shift of about +3 ppm was observed for one of the quaternary aromatic carbons: the shifted signal (δ 136.7 ppm) is assignable to the *para*-carbon of the glycosylated phenol, and the other quaternary carbon (δ 133.7 ppm), to the *para*-carbon of the free phenol in **7**.[6] In order to determine the linking site of the sugar to the genin, NMR analysis of **7** was carried out in more detail. In the ^{1}H-NMR spectrum of **7**, the geminal protons of one of the two benzyl positions were observed at δ 2.88 (1-H_a) and 3.02 (1-H_b) ppm. The former proton 1-H_a was correlated with the aromatic carbon signal at δ 133.7 ppm assigned to the *para*-carbon (C-1′) of the free phenol, in the correlation spectroscopy (COSY) *via* long-range coupling (COLOC) spectrum. On the other hand, a two-proton multiplet at δ 1.96 ppm (2-H_2) attributable to geminal protons in the ^{13}C–^{1}H COSY spectrum, gave cross peaks with 1-H_a, -H_b and the hydroxy methine proton at δ 3.85 ppm (3-H), in the ^{1}H–^{1}H COSY spectrum. This finding means that the methylene, of which the protons were observed at δ 1.96 ppm (2-H_2), is located between the hydroxy methine (C-3) and the carbon (C-1) substituted with the *p*-hydroxyphenyl radical. Consequently, the structures of acerosides XII (**7**) and XIII (**8**) were elucidated as 4″-*O*-β-D-apiofuranosyl-(1→6)-β-D-



1 : R^1 = O, R^2 = β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl
2 : R^1 = O, R^2 = β-D-glucopyranosyl
7 : R^1 =  , R^2 = β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl
8 : R^1 =  , R^2 = β-D-glucopyranosyl
9 : R^1 =  , R^2 = H
aceroside VII : R^1 =  O-β-D-glucopyranosyl, R^2 = H
aceroside VIII : R^1 = O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl, R^2 = H

Chart 1

glucopyranoside and 4″-*O*-β-D-glucopyranoside of (−)-centrolobol as illustrated in Chart 1.

We have already reported the isolation of acerosides VII and VIII from *A. nikoense*.[5,7] Acerosides XII and XIII from *A. triflorum* in this paper are composed of the same genin and sugars as the former two glycosides. The only difference between them comes from the position of the glycosidic linkage.

Experimental

Details of the instruments and TLC procedures used in this work were essentially the same as described in our previous paper.[1] GLC analysis was done on a Shimadzu gas chromatograph (model GC-4CM) with a flame ionization detector using a 2% OV-17 column (on Gas-Chrom Q, 1.5 m × 3 mm i.d. glass tube). Nitrogen was used as the carrier gas. The flow rate of carrier gas (ml/min) and the temperature of the column (°C) are shown in parenthesis. Column chromatography was performed on Kieselgel 60 (Merck, 230—400 mesh), Sephadex LH-20 (Pharmacia Fine Chemical Co.) and Amberlite XAD-II (Organo Co., Ltd).

Extraction and Separation The dried branches of *Acer triflorum* collected in Korea (2.5 kg) was cut into small pieces and extracted with MeOH (3 l × 3, 3 h) under reflux. The MeOH extract (360 g) was partitioned between AcOEt and water. The AcOEt extract (65 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH (9 : 1 → 0 : 1) to give fractions 1—8. Fraction 6 was chromatographed on Sephadex LH-20 ($CHCl_3$–MeOH (19 : 1)) and silica gel ($CHCl_3$–MeOH (1 : 0 → 0 : 1)) to give compounds **3** (65 mg), **4** (18 mg) and **5** (28 mg). Fraction 7 was chromatographed on Sephadex LH-20 (acetone) to give compound **6** (32 mg). The water layer was applied to an Amberlite XAD-II column. After being washed with water, the column was eluted with MeOH. The eluate was chromatographed on Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$–MeOH–AcOMe–H_2O (5 : 3 : 6 : 1)) to give compounds **7** (870 mg) and **8** (15 mg).

Compound 3 EI-MS *m/z*: 456 (M^+), 411 (M^+ − COOH), 248. Compound **3** was esterified with diazomethane in ether, and the products were subjected to GLC analysis. GLC t_R (min): 39.9, 46.8 (oleanolic acid methyl ester and ursolic acid methyl ester; intensity ratio 4 : 1) (60 ml/min, 250 °C).

Compound 4 EI-MS *m/z*: 426 (M^+), 218. GLC t_R (min): 17.7, 20.3 (β-amyrin and α-amyrin; intensity ratio 2 : 3) (60 ml/min, 250 °C).

Compound 5 Pale yellow powder, mp 99—102 °C (acetone). $FeCl_3$ reaction: positive (blue). IR ν_{max}^{KBr} cm^{-1}: 3480, 3320, 1690, 1660, 1600, 1520, 1470, 1280, 1180. EI-MS *m/z*: 544, 516, 488, 180, 163. ^{1}H-NMR (acetone-d_6) δ: 0.88 (3H, t, *J* = 6.7 Hz, –CH_3), 1.25—1.42 (s-like, –CH_2–), 4.18 (2H, t, *J* = 6.7 Hz, –O–CH_2–), 5.73 (2H, br s, –OH × 2), 6.26, 7.56 (each 1H, d, *J* = 15.9 Hz, –CH=), 6.87 (1H, d, *J* = 7.9 Hz), 7.01 (1H, dd, *J* = 1.8, 7.9 Hz), 7.08 (1H, d, *J* = 1.8 Hz).

TABLE I. ^{13}C-Chemical Shifts in C_5D_5N

^{13}C	**7**[a]	**8**	Aceroside IX[a]	**9**
Genin				
1	31.8	31.9	29.4	31.9
2	40.8	40.7	44.7	40.7
3	70.3	70.2	209.6	70.3
4	38.3	38.3	42.7	38.4
5	25.9	26.0	23.6	26.0
6	32.1	32.2	31.4	32.5
7	35.4	35.4	35.1	35.5
1′	133.7	133.7	132.0	133.4[d]
1″	136.7	136.5	136.0	133.7[d]
2′,6′	129.7	129.7	129.7	129.8[e]
2″,6″	129.9	130.0	129.9	129.9[e]
3′,5′	116.2	116.2	116.3	116.1
3″,5″	117.3	116.9	117.3	116.1
4′	156.9	156.8[b]	157.0[c]	156.8
4″	156.9	156.9[b]	157.2[c]	156.8
Glucosyl				
1	102.9	102.4	102.9	
2	74.9	75.0	74.9	
3	78.5	78.5	78.6	
4	71.7	71.3	71.7	
5	77.3	78.8	77.3	
6	69.0	62.4	69.0	
Apiosyl				
1	111.2		111.2	
2	77.8		77.8	
3	80.3		80.4	
4	75.0		75.0	
5	65.6		65.6	

a) The signal assignments were based on ^{13}C–^{1}H COSY and COLOC methods. *b*—*e*) Assignments in each column may be reversed.

Alkaline Hydrolysis of 5 A solution of **5** (3 mg) in 5% aqueous KOH–EtOH (1 : 1) was left standing at room temperature for 24 h. The reaction mixture was concentrated to remove EtOH, then diluted with water, and extracted with hexane. The extract was subjected to GLC analysis. GLC t_R (min): 4.4, 7.9, 14.5 (docosanol, tetracosanol, and hexacosanol; intensity ratio 1 : 9 : 4) (50 ml/min, 250 °C).

Compound 6 Pale yellow powder, mp > 300 °C (dec.) (MeOH). $FeCl_3$ reaction: positive (blue). Liebermann–Burchard reaction: positive (reddish purple). IR ν_{max}^{KBr} cm^{-1}: 3300, 1700, 1660, 1620, 1520, 1450, 1290, 1180, 970, 800. EI-MS *m/z*: 618 (M^+), 456, 438. ^{1}H-NMR (acetone-d_6) δ: 0.89, 0.92, 0.93, 0.98, 1.05, 1.71 (each 3H, s, –CH_3 × 6), 4.56 (1H, dd, *J* = 6.1, 10.2 Hz, 3-H), 4.60, 4.73 (each 1H, s, end methylene), 6.28, 7.52 (each 1H, d, *J* = 15.9 Hz, –CH=), 6.86 (1H, d, *J* = 8.2 Hz), 7.02 (1H, dd, *J* = 1.8, 8.2 Hz), 7.15 (1H, d, *J* = 1.8 Hz).

Methylation Followed by Alkali Hydrolysis of 6 Compound **6** (3 mg) was treated with diazomethane in ether for 1 h and a solution of the reaction product in 5% aqueous KOH–EtOH (1 : 1) was kept for 24 h at room temperature. The EtOH in the solution was evaporated off and the remaining solution was diluted with water, and then extracted with ether. After concentration of the ether layer, the residue was subjected to GLC analysis. GLC t_R (min): 36.8 (betulinic acid methyl ester) (60 ml/min, 250 °C).

Aceroside XII (7) Amorphous white powder, $[\alpha]_D$ −70.3° (*c* = 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 222 (4.21), 275 (3.39), 279 (3.39). UV $\lambda_{max}^{MeOH+NaOH}$ nm: 239, 273, 280, 295 (bathochromic shifts). ^{13}C-NMR (C_5D_5N): Table I. ^{1}H-NMR (C_5D_5N : Table II. FAB-MS *m/z*: 617 $[M+Na]^+$, 595 $[M+H]^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{30}H_{42}NaO_{12}$, 617.2574; Found 617.2573. TLC *Rf*: 0.28 ($CHCl_3$–MeOH–AcOMe–H_2O (5 : 3 : 6 : 1)).

Aceroside XIII (8) Amorphous white powder, $[\alpha]_D$ −40.2° (*c* = 0.5, MeOH). UV λ_{max}^{MeOH} nm (log ε): 222 (4.19), 275 (3.46), 279 (3.46). UV $\lambda_{max}^{MeOH+NaOH}$ nm: 239, 273, 280, 295 (bathochromic shifts). ^{13}C-NMR (C_5D_5N): Table I . ^{1}H-NMR (C_5D_5N): Table II. FAB-MS *m/z*: 485 $[M+Na]^+$, 463 $[M+H]^+$. High resolution FAB-MS *m/z*: Calcd for $C_{25}H_{34}NaO_8$, 485.2151; Found 485.2138. TLC *Rf*: 0.40 ($CHCl_3$–MeO-

TABLE II. ^{1}H-NMR Data in C_5D_5N

	7	8
Genin		
1-H_a	2.88 m	2.90 m
1-H_b	3.02 m	3.05 m
2-H_2	1.96 m[a]	1.98 m[a]
3-H	3.85 m	3.87 m
4,5,6-H_2	1.48—1.66 m[b]	1.53—1.70 m[b]
7-H_2	2.49 t-like (7.0)[a]	2.54 t-like (7.1)[a]
2′,6′-H	7.29 d (8.4)[a]	7.30 d (8.4)[a]
2″,6″-H	7.18 d (8.8)[a]	7.13 d (8.4)[a]
3′,5′-H	7.17 d (8.4)[a]	7.19 d (8.4)[a]
3″,5″-H	7.37 d (8.8)[a]	7.33 d (8.4)[a]
Glucosyl		
1-H	5.46 d (7.0)	5.61 d (7.0)
2-H	4.26—4.29 m[a]	4.26—4.37 m[c]
3-H	4.26—4.29 m[a]	4.26—4.37 m[c]
4-H	4.06—4.15 m[a]	4.26—4.37 m[c]
5-H	4.22 m	4.11 m
6-H_a	4.72 dd (1.5, 10.3)	4.55 dd (2.2, 12.1)
6-H_b	4.06—4.15 m[a]	4.41 dd (5.1, 12.1)
Apiosyl		
1-H	5.71 d (2.2)	
2-H	4.76 d (2.2)	
4-H_a	4.58 d (9.3)	
4-H_b	4.32 d (9.3)	
5-H_2	4.17 br s[a]	

The signal assignments were based on ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and NOESY methods. The coupling constants (J values) in parentheses are in Hz. *a*) Two-proton signal. *b*) Six-proton signal. *c*) Three-proton signal.

H–AcOMe–H_2O (5:3:6:1)).

Acid Hydrolysis of 7 A mixture of **7** (55 mg), MeOH (5 ml) and 10% HCl (5 ml) was heated for 1 h under reflux. The reaction mixture was concentrated to remove MeOH, diluted with water, and extracted with AcOEt. The AcOEt extract was chromatographed on silica gel with benzene–AcOEt (5:1) to give a genin (9) (22 mg). Compound **9**, white crystalline powder, mp 128—130 °C (benzene–acetone), $[\alpha]_D$ −8.8° (c=0.5, MeOH), was identical with an authentic sample of (−)-centrolobol (lit.[5] mp 129—130 °C, $[\alpha]_D$ −10.2° (EtOH)) by TLC and IR comparison, and mixed melting point determination. The water layer was passed through Amberlite MB-3 and the eluate was concentrated *in vacuo*. Glucose and apiose were detected on TLC (BuOH–AcOH–H_2O (6:1:2); *Rf* 0.24 (glucose), 0.41 (apiose)).

Partial Hydrolysis of 7 A mixture of **7** (100 mg) and 50% acetic acid (10 ml) was refluxed for 1 h. The reaction mixture was diluted with water, and extracted with AcOEt. The AcOEt extract was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (200:55:7) to give a partial hydrolyzate which was identical with aceroside XIII (**8**) on the basis of ^{13}C-NMR, ^{1}H-NMR, $[\alpha]_D$ and TLC comparisons. The aqueous layer was treated in the same way as in the case of acid hydrolysis of **7**, and apiose was detected on TLC (BuOH–AcOH–H_2O (6:1:2), *Rf* 0.41).

Acknowledgement We thank Misses Y. Takahashi and Y. Nagayama for measurements of NMR and MS. We are also grateful to Dr. Shigihara for measurements of FAB-MS.

References and Notes

1) Part XI: S. Nagumo, N. Kaji, T. Inoue, M. Nagai, *Chem. Pharm. Bull.*, **41**, 1255 (1993).
2) M. Nagai, E. Matsuda, T. Inoue, M. Fujita, H. J. Chi, T. Ando, *Chem. Pharm. Bull.*, **38**, 1506 (1990).
3) *a*) S. Kudo, F. Yoshizaki, Y. Kondo, *Shoyakugaku Zasshi*, **36**, 170 (1982). *b*) M. Komatsu, T. Tomimori, K. Hatayama, Y. Makiguchi, *Yakugaku Zasshi*, **90**, 459 (1970).
4) H. Otsuka, S. Fujioka, T. Komiya, M. Goto, Y. Hiramatu, H. Fujimura, *Chem. Pharm. Bull.*, **29**, 3099 (1981).
5) M. Nagai, N. Kenmochi, M. Fujita, N. Furukawa, T. Inoue, *Chem. Pharm. Bull.*, **34**, 1056 (1986).
6) I. Sakamoto, T. Tanaka, O. Tanaka, T. Tomiori, *Chem. Pharm. Bull.*, **30**, 4088 (1982).
7) Recently, acerosides VII and VIII were isolated from the inner bark of *Betula pendula* (Betulaceae). E. Smite, L. N. Lundgren, R. Andersson, *Phytochemistry*, **32**, 365 (1993).