Special Collection of Papers

This article is dedicated to Professor Satoshi Ōmura in celebration of his 2015 Nobel Prize.

Note

Acylated Triterpene Saponins from the Stem Bark of *Acer nikoense* (Aceraceae)

Shin-ichiro Kurimoto,*[,a] Yu F. Sasaki,[a,b] Yoshihiro Suyama,[c] Naonobu Tanaka,[c] Yoshiki Kashiwada,[c] and Takanori Nakamura[a]

[a] *Faculty of Pharmaceutical Sciences, Himeji Dokkyo University; 7–2–1 Kamiohno, Himeji, Hyogo, 670–8524, Japan:* [b] *Laboratory of Genotoxicity, Faculty of Chemical and Biological Engineering, Hachinohe National College of Technology; 16–1 Tamonoki Uwanotai, Hachinohe, Aomori, 039–1192, Japan: and* [c] *Graduate School of Pharmaceutical Sciences, Tokushima University; 1–78 Shomachi, Tokushima 770–8505, Japan.*

Received February 15, 2016; accepted March 16, 2016

Three new acylated triterpene saponins, acernikoenosides A–C (1–3), were isolated from the stem bark of *Acer nikoense*, together with a known sterol glucoside. Their structures were elucidated on the basis of extensive spectroscopic analyses. This study provided the first example of triterpene saponins isolated from this plant. The anti-genotoxic activity of 1, 3 and 4 against ultraviolet irradiation was evaluated by comet assay.

Key words *Acer nikoense*; triterpene saponin; Aceraceae

Acer nikoense Maxim. (Japanese name: Megusurino-ki) is an Aceraceous plant native to Japan, of which the stem bark has been used traditionally for the treatment of eye disorders and hepatic disease.[1)] A variety of phenolic compounds including diarylheptanoids,[2–11)] phenylbutanoids,[4,12)] coumarinolignans,[13)] neolignans,[14)] tannins[15)] and flavonoids[12)] have been reported as the constituents of *A. nikoense*. Some of these compounds show various biological activities such as anti-oxidant,[16)] anti-inflammatory,[9)] hepatoprotective,[17)] osteogenic,[18)] vasorelaxant,[19)] melanogenesis inhibitory,[11,16)] nitric oxide (NO) production inhibitory[8,20)] and sodium glucose cotransporter (SGLT) inhibitory activities.[10)]

We have been investigating edible and medicinal plants effective for the suppression of genotoxicity to discover new chemopreventive agents. Since the *n*-BuOH-soluble fraction from the MeOH extract of the stem bark of *A. nikoense* exhibited an anti-genotoxic effect against UV-irradiation in screening, we explored the chemical constituents of this fraction, resulting in the isolation of three new acylated olean-type triterpene saponins (**1**–**3**), together with a known sterol glucoside (**4**). Herein, we describe the isolation, structure elucidation and biological activity evaluation of the isolated compounds (Fig. 1).

The stem bark of *A. nikoense* was extracted with MeOH at room temperature. After removal of the solvent, the MeOH extract was partitioned successively between EtOAc, *n*-BuOH and H_2O. These fractions were evaluated for their anti-genotoxic activity by comet assay.[21)] The *n*-BuOH-soluble fraction exhibited significant activity in a dose-dependent manner (data not shown). Therefore, repeated column chromatography of this fraction over silica gel, octadecyl silica (ODS) and purification by reversed-phase (RP) HPLC was carried out to afford compounds **1** (38 mg), **2** (11 mg), **3** (7 mg) and **4** (45 mg). Compound **4** was identified as *β*-sitosterol-3-*O*-*β*-D-glucopyranoside[22)] by comparison of its spectroscopic data with those reported in the literature.

Compound **1** was obtained as an optically active amorphous powder. The molecular formula of **1** was determined as $C_{51}H_{80}O_{21}$ (*m/z* 1051.5087 $[M+Na]^+$) by the high-resolution electrospray ionization (HR-ESI)-MS. The ^{1}H-NMR spectrum exhibited the signals due to seven tertiary methyls (δ_H 0.77, 0.85, 0.89, 0.94, 1.08, 1.08, 1.19), two acetyl groups (δ_H 1.96, 2.00), one trisubstituted olefin [δ_H 5.32 (brt, 3.2)], two oxygenated methines [δ_H 4.95 (d, 10.5), 5.28 (d, 10.5)], along with signals ascribable to three anomeric protons [δ_H 4.30 (d, 7.2), 4.47 (d, 7.8), 4.70 (d, 7.7)]. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra showed the presence of nine methyl carbons (δ_C 15.9, 16.9, 17.7, 19.8, 20.7, 20.7, 26.6, 28.5, 29.3), eight sp^3 methylene carbons (δ_C 19.3, 19.3, 24.6, 27.1, 28.2, 33.9, 39.8, 46.8), three sp^3 methine carbons (δ_C 43.0, 48.9, 57.0), six sp^3 quaternary carbons (δ_C 36.9, 37.8, 40.4, 40.6, 42.9, 53.2), three oxygen-bearing sp^3 methines (δ_C 74.9, 77.3, 91.5), one sp^2 methine (δ_C 125.1), one sp^2 quaternary carbon (δ_C 142.9), and three carbonyl carbons (δ_C 171.8, 172.3, 177.5). Furthermore, it represented 17 carbon resonances assignable to a pentose and two hexoses. Acid hydrolysis of **1** with 5% H_2SO_4 provided D-glucose and L-arabinose, which were identified by HPLC analysis.[23)] These data were similar to those of dipterosides A–D, acylated olean-type triterpene saponins previously isolated from *Dipteronia dyeriana* (Aceraceae),[24)] implying **1** was also an acylated olean-type triterpene saponin. This was further confirmed based on the two-dimensional (2D)-NMR correlations shown in Fig. 2. The ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum revealed the connectivity of C-2 to C-3, C-5 to C-6, C-9 to C-12, C-18 to C-19, and C-21 to C-22. The locations of

* To whom correspondence should be addressed. e-mail: kurimoto@gm.himeji-du.ac.jp

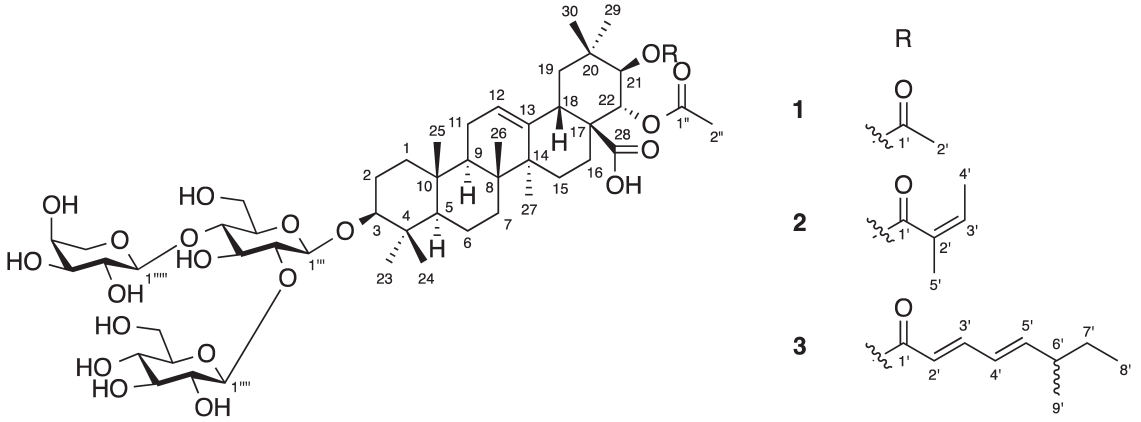

Fig. 1. The Structures of New Compounds

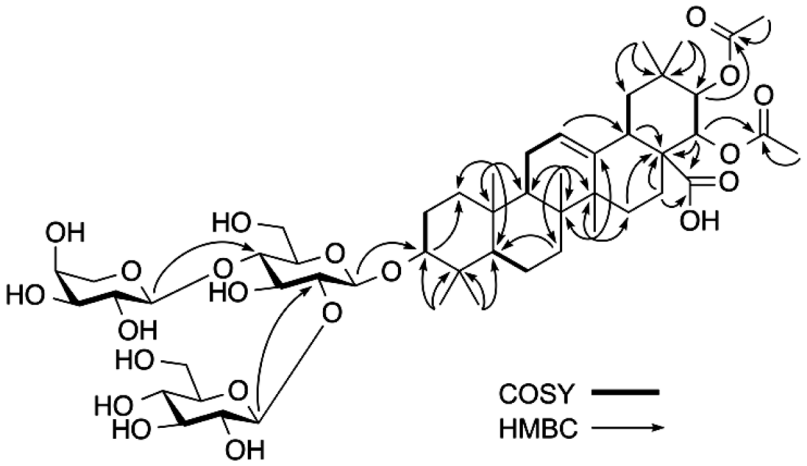

Fig. 2. Key COSY and HMBC Correlations of **1**

hydroxyl groups were elucidated to be at C-3, C-21 and C-22 from the heteronuclear multiple bond coherence (HMBC) correlations of H_3-23 (δ_H 1.08) and H_3-24 (δ_H 0.85) with C-3 (δ_C 91.5), C-4 (δ_C 40.4), C-5 (δ_C 57.0), and of H_3-29 (δ_H 0.89) and H_3-30 (δ_H 1.08) with C-19 (δ_C 46.8), C-20 (δ_C 36.9) and C-21 (δ_C 77.3). The relative configuration of the C-3 hydroxyl group was assigned as β equatorial from the *J*-value of the H-3 signal observed in pyridine-d_5 [δ_H 3.19 (dd, 3.9, 11.7)]. The chair conformation of E-ring and axial orientations of H-21 and H-22 were determined from the large coupling constant ($J_{21,22}$=10.5 Hz) and the rotating frame Overhauser effect, and exchange spectroscopy (ROESY) correlation of H-21 with H-16α, H-19α and H_3-29, and of H-22 with H-18 and H_3-30. The HMBC correlations of H-21 with C-1′ (δ_C 172.3), and of H-22 with C-1″ (δ_C 171.8) suggested that two acetyl groups were attached at C-21 and C-22 hydroxyl groups. The sequence of α-L-arabinopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl and its attachment to the C-3 hydroxyl group were deduced from the HMBC correlation of H-1‴ (inner glucose H-1) with C-3, H-1⁗ (terminal glucose H-1) with C-2‴ (inner glucose C-2), and of H-1⁗ (arabinose H-1) with C-4‴ (inner glucose C-4), coupled with the *J*-values of anomeric protons (H-1‴: 7.8 Hz, H-1⁗: 7.7 Hz, H-1⁗: 7.2 Hz). From these findings, the structure of **1**, designated acernikoenoside A, was determined as shown (Fig. 1).

A pseudo-molecular ion peak at *m/z* 1091.5420 $[M+Na]^+$ (calcd for 1091.5403) observed in the HR-ESI-MS of **2** indicated a molecular formula of $C_{54}H_{84}O_{21}$. The ^{1}H- and ^{13}C-NMR spectra of **2** were correlated with those of **1** (Tables 1, 2), but differ in the occurrence of signals ascribable to an angeloyl group {a carbonyl carbon (δ_C 168.6), a sp^2 quaternary carbon (δ_C 128.8), a sp^2 methine [δ_H 6.14 (dq, 1.4, 7.2); δ_C 139.9], a secondary methyl [δ_H 1.94 (brdd, 1.4, 7.2); δ_C 16.0], and a tertiary methyl [δ_H 1.82 (brt, 1.4); δ_C 20.7] instead of signals due to an acetyl group seen in **1**. The location of the angeloyl group at C-21 was determined from the HMBC correlation of H-21 [δ_H 5.07 (d, 10.4)] with the carbonyl carbon. The remaining structure including the relative configuration of triterpene unit and the absolute configuration of sugar units were elucidated to be the same as **1** by the interpretation of 1D and 2D-NMR data of **2** and acid hydrolysis of **2** followed by the HPLC analysis. Therefore, the structure of **2** was characterized as shown in Fig. 1.

Compound **3** was found to possess a molecular formula of $C_{58}H_{90}O_{21}$ by the HR-ESI-MS experiment. The analyses of the 1D and 2D-NMR data of **3** revealed that **3** had the same structure as **1** and **2** except for the acyl moiety attached at the C-21 hydroxyl group. The acyl moiety at C-21 was clarified as a (2*E*,4*E*)-6-methylocta-2,4-dienoyl moiety from the ^{1}H–^{1}H COSY correlations of H-2′–H-3′–H-4′–H-5′–H-6′–(H_3-9′)–H_2-7′–H_3-8′ and the coupling constants of H-2′ [δ_H 5.78 (d, 15.3)], H-3′ [δ_H 7.23 (dd, 10.9, 15.3)], H-4′ [δ_H 6.23

Table 1. ^{1}H-NMR Data for **1**–**3** in CD_3OD

Position	**1**	**2**	**3**
1	1.61 (m)	1.62 (m)	1.62 (m)
	0.99 (m)	1.00 (m)	0.99 (m)
2	1.92 (m)	1.93 (m)	1.93 (m)
	1.70 (m)	1.70 (m)	1.70 (m)
3	3.18 (m)	3.19 (m)	3.19 (m)
5	0.78 (m)	0.78 (m)	0.78 (m)
6	1.56 (m)	1.56 (m)	1.59 (m)
	1.40 (m)	1.40 (m)	1.40 (m)
7	1.50 (brd, 12.5)	1.52 (brd, 12.5)	1.52 (brd, 12.4)
	1.31 (m)	1.32 (m)	1.32 (m)
9	1.57 (m)	1.59 (m)	1.59 (m)
11	1.90 (m)	1.90 (m)	1.91 (m)
12	5.32 (brt, 3.2)	5.33 (brs)	5.33 (brs)
15	1.84 (m)	1.87 (m)	1.86 (m)
	1.10 (m)	1.13 (m)	1.13 (m)
16	2.12 (m)	2.15 (m)	2.13 (m)
	1.84 (m)	1.86 (m)	1.85 (m)
18	3.05 (dd, 2.3, 13.3)	3.08 (brd, 13.7)	3.08 (brd, 14.0)
19	2.03 (m)	2.07 (t, 13.7)	2.05 (t, 14.0)
	1.30 (dd, 4.7, 14.3)	1.33 (m)	1.32 (m)
21	4.95 (d, 10.5)	5.07 (d, 10.4)	5.01 (d, 10.5)
22	5.28 (d, 10.5)	5.34 (m)	5.34 (m)
23	1.08 (s)	1.08 (s)	1.08 (s)
24	0.85 (s)	0.85 (s)	0.85 (s)
25	0.94 (s)	0.94 (s)	0.94 (s)
26	0.77 (s)	0.79 (s)	0.79 (s)
27	1.19 (s)	1.21 (s)	1.20 (s)
29	0.89 (s)	0.90 (s)	0.89 (s)
30	1.08 (s)	1.11 (s)	1.11 (s)
Acyl-21			
2′	2.00 (s)	—	5.78 (d, 15.3)
3′	—	6.14 (dq, 1.4, 7.2)	7.23 (dd, 10.9, 15.3)
4′	—	1.94 (brdd, 1.4, 7.2)	6.23 (dd, 10.9, 15.2)
5′	—	1.82 (brt, 1.4)	6.08 (dd, 7.9, 15.2)
6′	—	—	2.18 (sept, 6.9)
7′	—	—	1.39 (m)
8′	—	—	0.88 (t, 7.5)
9′	—	—	1.03 (d, 6.8)
Ac-22			
2″	1.96 (s)	1.90 (s)	1.88 (s)
Glucosyl (inner)			
1‴	4.47 (d, 7.8)	4.47 (d, 7.7)	4.47 (d, 7.8)
2‴	3.64 (dd, 7.8, 9.0)	3.64 (m)	3.64 (dd, 7.8, 9.0)
3‴	3.73 (dd, 9.0)	3.72 (dd, 9.0)	3.73 (dd, 9.0)
4‴	3.54 (m)	3.54 (m)	3.55 (m)
5‴	3.39 (ddd, 2.7, 3.6, 9.7)	3.39 (ddd, 2.6, 3.7, 9.7)	3.40 (ddd, 3.1, 3.6, 9.5)
6‴	3.86 (dd, 2.7, 12.0)	3.86 (dd, 2.6, 12.1)	3.86 (m)
	3.83 (m)	3.82 (m)	3.83 (m)
Glucosyl (terminal)			
1⁗	4.70 (d, 7.7)	4.70 (d, 7.7)	4.70 (d, 7.7)
2⁗	3.20 (m)	3.19 (m)	3.20 (m)
3⁗	3.36 (dd, 9.0)	3.35 (dd, 8.8)	3.36 (dd, 8.7)
4⁗	3.22 (m)	3.22 (m)	3.22 (m)
5⁗	3.23 (m)	3.23 (m)	3.23 (m)
6⁗	3.82 (m)	3.82 (m)	3.82 (m)
	3.61 (m)	3.61 (m)	3.61 (m)
Arabinosyl (p)			
1⁗′	4.30 (d, 7.2)	4.30 (d, 7.3)	4.30 (d, 7.3)
2⁗′	3.55 (m)	3.55 (m)	3.55 (m)
3⁗′	3.51 (dd, 3.3, 9.5)	3.50 (dd, 3.3, 9.6)	3.51 (dd, 3.3, 9.5)
4⁗′	3.81 (m)	3.80 (m)	3.81 (m)
5⁗′	3.91 (dd, 2.1, 12.6)	3.91 (dd, 2.1, 12.7)	3.91 (dd, 2.0, 12.6)
	3.61 (m)	3.61 (m)	3.61 (m)

δ ppm (mult., J in Hz), 500 MHz.

Table 2. ^{13}C-NMR Data for **1**–**3** in CD_3OD

Position	1	2	3
1	39.8	39.8	39.8
2	27.1	27.1	27.1
3	91.5	91.5	91.5
4	40.4	40.4	40.4
5	57.0	57.0	57.0
6	19.3	19.3	19.3
7	33.9	33.9	33.9
8	42.9	43.0	43.0
9	48.9	48.9	48.9
10	37.8	37.9	37.9
11	24.6	24.6	24.6
12	125.1	125.2	125.1
13	142.9	142.9	142.9
14	40.6	40.7	40.7
15	28.2	28.2	28.2
16	19.3	19.3	19.3
17	53.2	53.2*	53.2
18	43.0	43.1	43.1
19	46.8	46.8	46.7
20	36.9	37.0	37.1
21	77.3	76.7	77.2
22	74.9	75.0	75.1
23	28.5	28.5	28.5
24	16.9	16.9	16.9
25	15.9	15.9	15.9
26	17.7	17.8	17.8
27	26.6	26.6	26.6
28	177.5	nd	177.5
29	29.3	29.3	29.3
30	19.8	20.1	19.9
Acyl-21			
1′	172.3	168.6	168.4
2′	20.7	128.8	119.5
3′	—	139.9	147.7
4′	—	16.0	128.1
5′	—	20.7	152.3
6′	—	—	40.2
7′	—	—	30.3
8′	—	—	12.1
9′	—	—	19.9
Ac-22			
1″	171.8	171.7	171.8
2″	20.7	20.7	20.8
Glucosyl (inner)			
1‴	105.3	105.3	105.3
2‴	80.4	80.5	80.5
3‴	76.7	76.8	76.8
4‴	80.5	80.5	80.5
5‴	76.1	76.1	76.1
6‴	62.0	62.0	62.0
Glucosyl (terminal)			
1⁗	104.4	104.4	104.4
2⁗	76.4	76.4	76.4
3⁗	77.8	77.8	77.7
4⁗	71.9	71.9	71.9
5⁗	78.2	78.3	78.2
6⁗	63.1	63.1	63.1
Arabinosyl (p)			
1⁗′	105.3	105.3	105.3
2⁗′	72.5	72.5	72.5
3⁗′	74.3	74.4	74.3
4⁗′	69.9	70.0	70.0
5⁗′	67.8	67.8	67.9

δ ppm, 125 MHz. nd: not detected. *Detected by the HMBC experiment.

(dd, 10.9, 15.2)] and H-5′ [δ_H 6.08 (dd, 7.9, 15.2)], together with the HMBC cross peaks of H-21, H-2′ and H-3′ with C-1′ (δ_C 168.4). The identification of D-glucose and L-arabinose were carried out in the same manner as **1** and **2**. Thus, the structure of **3** was elucidated as shown (Fig. 1), although the stereochemistry of C-6′ remained to be determined.

The anti-genotoxic activity against UV-irradiation was tested for **1**, **3** and **4** by comet assay, but none displayed positive activity.

Conclusion

Our investigation on the constituents of the *n*-BuOH-soluble fraction of *A. nikoense* has led to the isolation of three new acylated olean-type triterpene saponins (**1**–**3**), named acernikoenosides A–C. Although several triterpene saponins have been isolated from Aceraceous plants,[24–26] this appears to be the first example of the isolation of triterpene saponins from *A. nikoense*. The anti-genotoxic activity against UV-irradiation was evaluated for compounds **1**, **3** and **4**, but unfortunately they were inactive. Therefore, further study on the chemical constituents of this plant is necessary to clarify the active components.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO P-2200 polarimeter. IR spectra were recorded on a JASCO FT-IR-6200. HR-ESI-MS were obtained on a Waters PREMIER2695. NMR spectra were measured on a Bruker AVANCE-500 instrument using residual solvent signals as internal standards. Column chromatography was carried out with silica gel 60 (0.040–0.063 mm; Merck, Germany), silica gel PSQ 100B (Fuji Silysia Chemical, Japan) and Chromatorex ODS (100-200 mesh; Fuji Silysia Chemical). Analytical HPLC for identification of the sugar was carried out on a Shimazu apparatus consisting of a LC-6AD pump, a DGU-20 A_3 degasser, and a SPD-M20AP photodiode array detector (at a wavelength of 250 nm) with COSMOSIL 5C_{18}-AR-II (5 μm, 4.6 i.d.×250 mm, Nacalai Tesque, Japan). Preparative HPLC was performed on a JAi apparatus LC-9201R/U (Japan Analysis Industry Ltd., Japan) consisting of a S-310A model II UV detector (at a wavelength of 210 nm) and a RI-50s refractive index detector with COSMOSIL 5C_{18}-AR-II (5 μm, 20 i.d.×250 mm, Nacalai Tesque) or COSMOSIL πNAP (5 μm, 20 i.d.×250 mm, Nacalai Tesque).

Extraction and Isolation The stem bark of *A. nikoense* (1.9 kg), purchased from Tochimoto Tenkaido Co., Ltd. (Lot. 122614002), was extracted with MeOH (5 L), three times at room temperature for 96 h each time. After removal of solvents, the MeOH extract (64 g) was successively partitioned with EtOAc, *n*-BuOH and H_2O. The *n*-BuOH-soluble fraction (17 g) was chromatographed over silica gel [$CHCl_3$: MeOH : H_2O (10 : 1 : 0.1→6 : 4 : 1)] to afford sixteen fractions. Fraction 5 was recrystallized from MeOH to furnish *β*-sitosterol-3-*O*-glucoside (45 mg). Fraction 10 was separated by a Chromatorex ODS column [MeOH : H_2O (1 : 9→1 : 0)] to give fractions 10.1–10.14. Fraction 10.10 was purified by HPLC on a COSMOSIL πNAP (MeOH : H_2O=7 : 3; 9.0 mL/min) to give **1** (38 mg). The purification of fr. 10.11 by HPLC on COSMOSIL 5C_{18}-AR-II (MeOH : H_2O=3 : 1; 9.0 mL/min) gave **2** (11 mg). Fraction 10.13 was purified by HPLC on COSMOSIL πNAP (MeOH : H_2O=4 : 1; 9.0 mL/min), yielding

compound **3** (7 mg).

Acernikoenoside A (=(3*β*,21*β*,22*α*)-21,22-Di(acetyloxy)-3-{{*O*-*α*-L-arabinopyranosyl-(1→4)-*O*-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl}oxy}-olean-12-en-28-oic Acid) (**1**)

White amorphous powder; $[\alpha]_D^{26}$ +20.7 (*c*=0.74, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD) see Tables 1, 2. IR (KBr) cm^{-1}: 3443, 3395, 2934, 2880, 1740, 1654, 1568, 1466, 1375, 1257, 1161, 1075, 1038. HR-ESI-MS *m*/*z* 1051.5087 $[M+Na]^+$ (Calcd for $C_{51}H_{80}O_{21}Na$, 1051.5090).

Acernikoenoside B (=(3*β*,21*β*,22*α*)-22-(Acetyloxy)-3-{{*O*-*α*-L-arabinopyranosyl-(1→4)-*O*-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl}oxy}-21-{[(2*Z*)-2-methyl-1-oxobut-2-en-1-yl]oxy}-olean-12-en-28-oic Acid) (**2**)

White amorphous powder; $[\alpha]_D^{25}$ +19.6 (*c*=0.47, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD) see Tables 1, 2. IR (KBr) cm^{-1}: 3389, 2939, 1729, 1654, 1568, 1461, 1381, 1247, 1161, 1069. HR-ESI-MS *m*/*z* 1091.5420 $[M+Na]^+$ (Calcd for $C_{54}H_{84}O_{21}Na$, 1091.5403).

Acernikoenoside C (=(3*β*,21*β*,22*α*)-22-(Acetyloxy)-3-{{*O*-*α*-L-arabinopyranosyl-(1→4)-*O*-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl}oxy}-21-{[(2*E*,4*E*)-6-methyl-1-oxoocta-2,4-dien-1-yl]oxy}-olean-12-en-28-oic Acid) (**3**)

White amorphous powder; $[\alpha]_D^{27}$ +21.3 (*c*=0.72, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD) see Tables 1, 2. IR (KBr) cm^{-1}: 3443, 3362, 2961, 2928, 2875, 1723, 1643, 1562, 1461, 1391, 1262, 1140, 1075, 1032. HR-ESI-MS *m*/*z* 1145.5865 $[M+Na]^+$ (Calcd for 1145.5872, $C_{58}H_{90}O_{21}Na$).

Acid Hydrolysis of 1–3 and Identification of the Sugar Compounds **1**–**3** (each at 1.0 mg) were separately hydrolyzed with 5% H_2SO_4 (2 mL) at 80°C for 4 h. Each solution was diluted with H_2O, and extracted with EtOAc. The H_2O layer was neutralized with Amberlite IRA-900 resin and evaporated *in vacuo*. The residue was reacted with L-cysteine methyl ester hydrochloride (1.5 mg) in pyridine (1 mL) at 60°C for 1 h, and then *o*-tolyl isothiocyanate (50 μL) was added to the reaction mixture and reacted at 60°C for 1 h. The reaction mixture was directly analyzed by HPLC on a COSMOSIL 5C_{18}-AR-II (CH_3CN : 50 mM H_3PO_4=25 : 75; 0.8 mL/min; 17°C).[23] The t_R values of the two peaks detected at 18.6 min and 21.0 min were consistent with those of *o*-tolylthiocarbamoyl-thiazolidine derivatives synthesized from D-glucose and L-arabinose.

Biological Activity

Cell Culture

TK6 human lymphoblastoid cells were cultured in RPMI1640 medium containing 10% horse serum, 200 μg/mL sodium pyruvate and 200 μg/mL streptomycin, and incubated at 37°C in a humidified atmosphere with 5% CO_2–95% air.

UV-Irradiation to Cells

Cells were suspended in saline in a 6 cm dish to prepare 5×10^5 cells/mL suspension. This suspension was exposed to UVC radiation at 80 J/m^2 using a germicidal lamp.

Comet Assay

After UV-irradiation, cells were post-treated with each sample for 30 min. Treated cells were suspended in 1% agarose-low gelling temperature (LGT) at 5×10^5 cells/75 μL, and 75 μL of the cell suspension was immediately deposited on a fully frosted slide, which was coated with 1% agarose GP-42 and then covered with another slide glass. The slides were placed in order to allow the agarose to gel. The samples on the slides were then immediately exposed to a lysing solution (pH 10) of 2.5 M NaCl, 100 mM ethylenediaminetetraacetic acid (EDTA) disodium (Na_2EDTA), 10 mM Trizma, 1% sarkosyl, 10% dimethyl sulfoxide (DMSO) and 1% Triton X-100, and left at 4°C for 1 h. The slides were then placed on a horizontal gel electrophoresis platform and covered with a pH >13 alkaline solution consisting of 300 mM NaOH and 1 mM Na_2EDTA. The slides were left at 0°C for 20 min to allow unwinding of the DNA and expression of alkali-labile sites to occur. The power supply was set at 1 V/cm and 250 mA. The DNA was subject to electrophoresis at 0°C for 20 min and the slides were rinsed with 400 mM Trizma (pH 7.5) to neutralize the excess alkalinity. Each slide was stained with 50 μL of 20 μg/mL ethidium bromide and covered with a cover slip. Fifty cells on one slide per slide (one slide was prepared for each plate and 2 plates were prepared for each dose) were examined and photographed at ×200 magnification using a fluorescence microscope equipped with a G filter. The whole length of the whole comet (head to tail) was measured manually using a scale for 50 nuclei.

Acknowledgment This work was partially supported by a Himeji Dokkyo University Special Research Grant.

Conflict of Interest The authors declare no conflict of interest.

References

1) Inoue T., *Yakugaku Zasshi*, **113**, 181–197 (1993).
2) Nagai M., Kubo M., Fujita M., Inoue T., Matsuo M., *Chem. Pharm. Bull.*, **26**, 2805–2810 (1978).
3) Kubo M., Inoue T., Nagai M., *Chem. Pharm. Bull.*, **28**, 1300–1303 (1980).
4) Nagai M., Kubo M., Takahashi K., Fujita M., Inoue T., *Chem. Pharm. Bull.*, **31**, 1923–1928 (1983).
5) Nagai M., Kenmochi N., Fujita M., Furukawa N., Inoue T., *Chem. Pharm. Bull.*, **34**, 1056–1060 (1986).
6) Nagumo S., Kaji N., Inoue T., Nagai M., *Chem. Pharm. Bull.*, **41**, 1255–1257 (1993).
7) Nagumo S., Ishizawa S., Nagai M., Inoue T., *Chem. Pharm. Bull.*, **44**, 1086–1089 (1996).
8) Morikawa T., Tao J., Toguchida I., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **66**, 86–91 (2003).
9) Akihisa T., Taguchi Y., Yasukawa K., Tokuda H., Akazawa H., Suzuki T., Kimura Y., *Chem. Pharm. Bull.*, **54**, 735–739 (2006).
10) Morita H., Deguchi J., Motegi Y., Sato S., Aoyama C., Takeo J., Shiro M., Hirasawa Y., *Bioorg. Med. Chem. Lett.*, **20**, 1070–1074 (2010).
11) Akihisa T., Takeda A., Akazawa H., Kikuchi T., Yokokawa S., Ukiya M., Fukatsu M., Watanabe K., *Chem. Biodivers.*, **9**, 1475–1489 (2012).
12) Inoue T., Ishidate Y., Fujita M., Kubo M., Fukushima M., Nagai M., *Yakugaku Zasshi*, **98**, 41–46 (1978).
13) Furukawa N., Nagumo S., Inoue T., Nagai M., *Shoyakugaku Zasshi*, **42**, 163–165 (1988).
14) Morikawa T., Tao J., Ueda K., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 62–67 (2003).
15) Okuda T., Yoshida T., Hatano T., Ikeda Y., Shingu T., Inoue T., *Chem. Pharm. Bull.*, **34**, 4075–4082 (1986).
16) Akazawa H., Akihisa T., Taguchi Y., Banno N., Yoneima R., Yasukawa K., *Biol. Pharm. Bull.*, **29**, 1970–1972 (2006).
17) Shinoda M., Ohta S., Kumasaka M., Fujita M., Nagai M., Inoue T., *Shoyakugaku Zasshi*, **40**, 177–181 (1986).
18) Yonezawa T., Lee J.-W., Akazawa H., Inagaki M., Cha B.-Y., Nagai K., Yagasaki K., Akihisa T., Woo J.-T., *Bioorg. Med. Chem. Lett.*, **21**, 3248–3251 (2011).

19) Iizuka T., Nagumo S., Yotsumoto H., Moriyama H., Nagai M., *Biol. Pharm. Bull.*, **30**, 1164–1166 (2007).
20) Deguchi J., Motegi Y., Nakata A., Hosoya T., Morita H., *J. Nat. Med.*, **67**, 234–239 (2013).
21) Tice R. R., Agurell E., Anderson D., Burlinson B., Hartmann A., Kobayashi H., Miyamae Y., Rojas E., Ryu J.-C., Sasaki Y. F., *Environ. Mol. Mutagen.*, **35**, 206–221 (2000).
22) Kojima H., Sato N., Hatano A., Ogura H., *Phytochemistry*, **29**, 2351–2355 (1990).
23) Tanaka T., Nakashima T., Ueda T., Tomii K., Kouno I., *Chem. Pharm. Bull.*, **55**, 899–901 (2007).
24) Guo R., Luo M., Long C.-L., Li M.-L., Ouyang Z.-Q., Zhou Y.-P., Wang Y.-H., Li X.-Y., Shi Y.-N., *Helv. Chim. Acta*, **91**, 1728–1735 (2008).
25) Glénsk M., Włodarczyk M., Bassarello C., Pizza C., Stefanowicz P., Świtalska M., *Z. Naturforsch.*, **2009**, 1081–1086 (2009).
26) Kupchan S. M., Takasugi M., Smith R. M., Steyn P. S., *J. Org. Chem.*, **36**, 1972–1976 (1971).