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Saponins from the Fructus of *Kochia scoparia*

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Abstract □ Two new triterpenoidal saponins, B(**1**) and C(**2**) were isolated from the fructus of *Kochia scoparia*. On the basis of chemico-spectral evidences, the structures of **1** and **2** were elucidated as oleanolic acid 3-*O*-β-D-ribopyranosyl-(1→2)-β-D-glucuronopyranoside and 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucuronopyranosyl-olean-12-en-28-*O*-β-D-glucopyranosyl ester, respectively.

Keywords □ *Kochia scoparia*, Chenopodiaceae, two new oleanolic acid saponins, monodesmoside, bidesmoside, *Kochiae* fructus.

Kochiae fructus (Zi Bu Za) has been primarily used in the treatment of gonorrhea and dermatitis, and as a diuretics[1]. The isolation and characterization of *Kochia spp.* saponins have been conducted by several researchers[2-5]. However, no detailed chemical investigation of *Kochia scoparia* fructus appears to have been done. The present study deals with the isolation and structural elucidation of two new triterpenoidal saponins from the fructus of *Kochia scoparia* (L.) SCHRAD (Chenopodiaceae).

The water extract of the fructus of *Kochia scoparia* was fractionated on the Amberlite XAD-2 column using 95% MeOH as an eluent. Two new olean type saponins, compounds **1** and **2** have been isolated by HPLC using reverse phase column.

Compound **1** was obtained as colorless powder, gave positive reactions in Lieberman-Burchard, and Molish test, and showed carboxyl (1690 cm^{-1}) and glycosidic (1,100-1,000 cm^{-1}) absorption band in its IR spectrum. Acid hydrolysis of **1** afforded **1a** as the sapogenin, and glucuronic acid and ribose at the molar ratio of 1:1 as a sugar moiety. Compound **1a** was identified as oleanolic acid by direct comparison of its physicochemical constants with those of authentic oleanolic acid[6-14].

The ^{1}H-NMR spectrum of **1** showed seven quaternary methyl signals at δ 0.8-1.1 (3H, s, $CH_3\times7$) with two anomeric proton signals at 4.3 and 4.7 ppm.

The ^{13}C-NMR analysis (Table I) of **1** showed two anomeric carbon signals at 104.7, 104.1 ppm and two carboxyl carbon signals at 180.2 (C-28), 173.8 (glucuronic) ppm. The glucuronic acid and ribose units in **1** were suggested to be linked to the 3-C hydroxyl group of **1a** by comparison with ^{13}C-NMR spectral data of **1**. Compound **1** was methylated according to the method of Hakomori to give permethylate whose anomeric proton signals were observed at 4.36 and 4.72 ppm.

Partial hydrolysis of **1** affforded **1b** with ribose, and **1b** gave oleanolic acid and glucuronic acid by acid hydrolysis. The mode of linkage of the glucuronic acid and ribose units were examined on the basis of the coupling constants (d, J=3.9 Hz, J=8.0 Hz) of the anomeric proton signals in the ^{1}H-NMR spectrum of **1**.

Based on these evidences, the structure of **1** was elucidated as oleanolic acid 3-*O*-β-D-ribopyranosyl-(1→2)-β-D-glucuronopyranoside.

Compound **2** was obtained as colorless powder, gave positive reactions in Lieberman-Burchard, and Molish test, and showed ester (1730 cm^{-1}) and glycosidic (1,100-1,100 cm^{-1}) absorption band in its IR spectrum. The ^{1}H-NMR of **2** showed seven quaternary methyl signals at 0.82-1.30 ppm and three anomeric proton signals at 5.03, 5.41, 6.34 ppm, respectively. The ^{13}C-NMR spectrum (Table I) of **2** showed three anomeric carbon signals at δ 106.3 (28-C, in-

Table I. ^{13}C-NMR chemical shifts (δ) of compounds 1 and 2 in C_5D_5N

Carbon No.	Oleanolic acid	1	2	C-3 sugar	1	2	C-28 sugar	2
3	78.1	88.2	89.3	GlcUA-1	104.7	106.3	Glc-1	95.8
12	122.6	121.3	122.9	GlcUA-2	85.0	74.2	Glu-2	74.2
13	144.9	143.8	144.2	GlcUA-3	76.0[a]	86.4[a]	Glu-3	79.4[a]
23	28.8	27.4	28.3	GlcUA-4	73.1	71.4	Glu-4	71.2
24	16.0	16.3	17.0	GlcUA-5	74.2[a]	78.2[a]	Glu-5	78.9[b]
28	180.2	180.0	176.5	GlcUA-6	173.8	172.2	Glu-6	62.3
				Rib-1	104.1			
				Rib-2	73.4			
				Rib-3	70.2[a]			
				Rib-4	69.3			
				Rib-5	65.8			
				Xyl-1		106.9		
				Xyl-2		74.4		
				Xyl-3		77.6[a]		
				Xyl-4		71.1		
				Xyl-5		67.4		

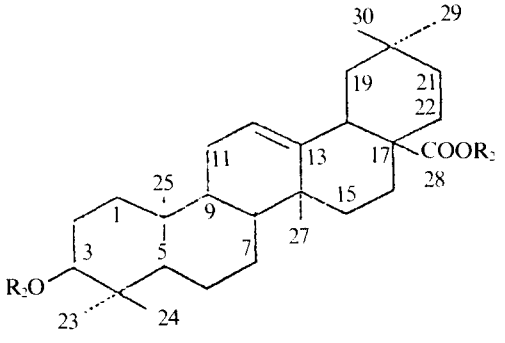
[a,b]may be reversed.
GlcUA: β-D-glucuronic acid, Glu: β-D-glucopyranose

ner carboxyl carbon) signals at δ 172.2 (3-C, inner glucuronic acid), 176.5 (28-C, carboxyl). Acid hydrolysis of **2** afforded oleanolic acid as the sapogenin and glucuronic acid, xylose and glucose at the molar ratio of 1:1:1 as a sugar moiety.

Alkali saponification of **2** gave **2a** and glucose. Compound **2a**, colorless powder gave positive reactions in Lieberman-Burchard, and Molish test, and showed carboxyl residues (1725, 1655 cm^{-1}) glycosidic (1,100-1,000 cm^{-1}) absorption band in its IR spectrum. The ^{1}H-NMR spectrum of **2a** showed seven quaternary methyl signals at δ 0.8-1.1 with two anormeric proton signals at 4.3, 4.77 ppm, respectively. ^{13}C-NMR analysis (Table I) of **2a** showed two anomeric carbon signals at 104.7, 104.1 ppm and two carboxyl signals at δ 180.2 and 173 ppm.

Acid hydrolysis of **2a** afforded oleanolic acid as the sapogenin, and glucuronic acid and xylose at the molar ratio of 1:1 as a sugar moiety. The glycosylation shift around 3-C as well as two anomeric carbon signals at 106.3 and 106.9 ppm in the ^{13}C-NMR spectrum of **2a** indicates that **2a** is 3-*O*-glycoside of oleanolic acid which has two monosaccharide units.

Negative FAB-Mass of **2a**, ion at *m/z* 763 (M^+), 631 (M^+-xylose), 455 (M^+-xylose+glucuronic acid)



	R_1	R_2
1.	-GlcUA—Rib	H
2.	-GlcUA—Xyl	Glc
1a.	H	H
2a.	-GlcUA—Xyl	H

indicates that sugar moiety of **2a** is consisted of a linear glycosyl unit with terminal xylose and inner glucuronic acid.

The structural correlativity between **2a** and **1** confirms that the terminal moiety of ribose in **1** is replaced by xylose in **2a** by comparing the physical constants and spectral data of these two compounds.

The aqueous layer obtained from the alkali hy-

drolysis of **2** was acidified[16] and analyzed by GC and TLC, showing the presence of glucose.

These experimental results suggest that **2** is an ester composed of **2a** as the acid part, and glucose as the alcohol part.

The signal at 6.3 ppm (1H, d, J=8.1 Hz) in ^{1}H-NMR spectrum of **2** can be attributed to the anomeric proton of glucose linked to the 28-carboxyl group of **2a** in the ester form. The signal of the anomeric carbon of the glucose was observed at 95.8 ppm, supporting the glucose was linked to C-28 as an ester form.

Based on the above results, the structure of **2** was elucidated as 3-*O*-β-xylopyranosyl-(1→3)-β-D-glucuronopyranosyl-olean-12-en-38-*O*-β-glucopyranosyl ester.

EXPERIMENTAL METHODS

General procedure

Acid hydrolysis of **1** and **2** was carried out by refluxing with 2N-HCl : dioxane (1:1) at 90℃ for 4 h and partial hydrolysis was performed by heating in the sealed tube with 1.5% H_2SO_4 at 70℃ for 7 h. Alkaline saponification of **2** was carried out using 0.05 N KOH in MeOH for 1 h. Acid or alkali in the hydrolyzed solution was neutralized with Amberites MB 3. Melting points were measured with Mitamura Riken Kogyo MELT TEMP and uncorrected. IR spectra were taken by Shimadzu IR 408, 435. GC was performed on Shimadzu GC-9A gas chromatograph. ^{1}H-NMR spectra were measured on a Brucker AM-200, JEOL JNN-GX-500 spectrometer and ^{13}C-NMR spectra were measured on a Brucker AM-200, JEOL JNN-GX-500 spectrometer, using tetramethylsilan as an internal standard. Chemical shifts are given in ppm. Mass spectra were recorded on a JMS-DX300 (FAB-Mass), Hewlett Packard 5985B, Finnigan automated gas chromatography spectrosystem (EI-CI, GC-Mass). Elemental analysis was performed by Perkin Elmer 240 EA. Optical rotation was measured with Union automatic digital polarimeter PM-101. Preparative liquid chromatography was carried out on a column of YMC packed column ODS-5, S-5, 120A ODS with Japan TOSOH system. For TLC, silica gel 60 F_{254} (thickness 0.2 mm, Merck) and RP-δ (thickness 0.25 mm, Merck) were used. Solvent system for silica gel column was consisted of $CHCl_3$: MeOH : H_2O (70 : 30: 4, v/v/v). Solvent system for HPLC was 73% MeOH (0.05% TFA H_2O).

Extraction and isolation

Air dired fructus of *Kochia scoparia* (1 kg) collected in Seoul, Korea[17-19], was extracted with hot water. The water extract was passed through an Amberlite XAD-2 column and the adsorbed material was eluted with 95% methanol. The methanol eluates were concentrated to dryness to give a crude saponin (30 g), which was passed through a silica gel column employing a solvent system of $CHCl_3$: MeOH : H_2O (70 : 30 : 4). The resulting two fractions showed the presence of four different saponins by TLC. The four different saponins were named as saponins A-D according to their R_f values.

Among them, compounds **1** (R_f 0.55) and **2** (R_f 0.35) were separated by using preparative HPLC according to their t_R (41.4, 20.3).

Saponin B(**1**)-A white powder, mp. 220-222℃ $[\alpha]_D^{20}$ +13.4 (C=0.60, MeOH) *Anal. calcd.* for $C_{41}H_{68}O_{15}$ $2H_2O$: C, 61.7; H, 8.5, Found: C, 61.1; H, 8.3. IR ν_{max}^{KBr} cm^{-1} 3400 (brs, OH), 1727, 1651 (COOH), 1450 (C=C), 1055, 1042 (CHOH). ^{1}H-NMR (pyridine-d_5): 0.71 (3H, s), 0.74 (3H, s), 0.87 (6H, s), 0.97 (3H, s), 1.09 (3H, s), 1.20 (3H, s), 4.31 (1H, d, anomeric H, ribose), 4.72 (1H, d, anomeric, H, glucuronic acid), 5.15 (brs, 12-H). ^{13}C-NMR data are listed in Table I.

Saponin C(**2**)-A white powder, mp. 197-201℃ (uncorr.) $[\alpha]_D^{20}$+32.5 (C=0.05, MeOH). *Anal. calcd.* for $C_{47}H_{78}O_{20}H_2O$: C, 57.6; H, 8.2, Found: C, 58.6; H, 8.1, IR. ν_{max}^{KBr} cm^{-1} 3400 (brs, OH), 1730 (COO-R), 1635 (C=C), 1100-1000 (CHOH). ^{1}H-NMR δ: 0.82, 0.89, 0.91, 1.00, 1.01, 1.09, 1.29, 1.30, (each 3H, s), 5.03, 5.41 6.34, (1H, anomeric H, glucoronic acid, xylose and glucose), 5.42 (s, 12-H). ^{13}C-NMR data are listed in Table I.

Aglycone of compound 1 and 2

Compounds **1** and **2** (each 100 mg) were hydrolyzed, respectively by using the mothod described in general procedure. The hydrolysate was diluted with H_2O and extracted with $CHCl_3$. The residue from the $CHCl_3$ extract was chromatographed on silica gel column using an eluting solvent system of $CHCl_3$: MeOH (7 : 3). The eluates (from **1** and **2**) were concentrated, respectively, and the resulted residues were recrystallyzed, respectively in MeOH to give

the same aglycone of **1a**, colorless needle, mp. 307-309℃ (uncorr.), which was identified as oleanolic acid by direct comparison with the authentic sample. After being neutralized with Amberlite MB-3, the filtrate was concentrated to a small volume and examined by TLC and GC, to show the presence of glucuronic acid, ribose (t_R, 17.8, 6.8) from **1**, glucuronic acid, xylose and glucose (t_R, 20.5, 9.5, 15.3) from **2**.

Alkali saponification of compound 2

Compound **2** (100 mg) was hydrolyzed by alkali using the method, described in general procedure. The reaction mixture was diluted with H_2O then, neutralized with Amberlite MB-3, and extracted with EtOAc : MeOH (2 : 1). The organic layer thus obtained was washed with H_2O and concentrated to give **2a**.

Compound **2a**, colorless powder (MeOH), 225-227 ℃ (uncorr) $[\alpha]_D^{30}$+15.5 (0.5, MeOH). *Anal. calcd.* for $C_{41}H_{68}O_{15}$: C, 61.5; H, 8.2. Found: C, 61.1; H, 8.3. IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1727, 1651 (COOH), 1450 (C=C), 1055, 1042 (CHOH). ^{1}H-NMR 0.82, 0.89, 0.92, 1.00, 1.09, 0.29, 1.03 (3H, s, CH_3), 5.02 (1H, d, J=7.9, anomeric H), 5.36 (1H, d, J=7.5, anomeric H).

Permethylate 3 of compound 1

Compound **1** was methylated by the method of Hakomori[15]. Compound **1** (50 mg), DMSO 6.0 m*l* and NaH 350 mg were reacted with streaming N_2 for 2 h, in the ultrasonicator. After cooling, 30 m*l* of CH_3I was added and allowed to stand for another 1 h in the ultrasonicator. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and recrystallized from MeOH to give **3** as a colorless needles, mp. 124-126℃ (uncorr.).

IR ν_{max}^{KBr} cm^{-1} 1723, 1745 (COOR), 1460 (C=C), 1096, 1025 (OCH_3). *Anal. calcd.* for $C_{48}H_{80}O_{14}$: C, 65.5; H, 9.1 *Found*: C, 65.0; H, 9.3. ^{1}H-NMR ($CDCl_3$) 0.71, 0.83, 0.88, 0.89, 0.92, 1.01, 1.11 (3H, s, CH_3), 3.25, 3.45, 3.48, 3.60, 3.61 (3H, s, OCH_3), 4.36, 4.72 (1H, d, anomeric H), 5.26 (brs, 12-H).

Methanolysis of compound 3

Twenty mg of **3** was boiled with 8% HCl-MeOH (3 m*l*) on water bath for 3 h. The hydrolysate was neutralized and the filtrate was evaporated. The methylated sugar was identified as 3,4-dimethyl-6-carboxymethylglucuronopyranoside and 2,3,4-trimethylribopyranoside by GC (t_R, 6.36, 14.73).

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