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Aromatic Compounds and Triterpenoidal Saponins from *Clematis koreana* var. *umbrosa*

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From the methanolic extract of aerial parts of *Clematis koreana* var. *umbrosa*, one new triterpenoidal saponin, 3-O-β-D-xylopyranosyl(1-3)-α-L-arabinopyranosyl oleanolic acid 28-O-α-L-rhamnopyranosyl(1-4)-β-D-glucopyranosyl(1-6)-β-D-glucopyranosyl ester, along with five known aromatic compounds and two known triterpenoidal saponins were isolated.

Key words: *Clematis koreana* var. *umbrosa*, Ranunculaceae

INTRODUCTION

Clematidis radix is a very common plant drug of an analgesic and anti-inflammatory agent (Namba *et al.*, 1980). Another member of the genus, *Clematis korana* var. *umbrosa* is a Korean plant, and is not used medicinal plant. In our search for natural products with biological activity, we have invastigated a new Clematis species which occur in Korea (Moon Kyo Bu., 1973).

Several saponins have been isolated from Clematis species (Kizu *et al.*, 1982, 1980, 1979; Kintya *et al.*, 1975). This paper describes the isolation and characterization of the five aromatic compounds and three triterpenoidal saponins from aerial parts of this plant.

MATERIALS AND METHODS

Genaral Experimental Procedures

Mps; uncorr.. ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR were recorded on a JEOL JNN GX-400 NMR spectrometer. FAB-MS was taken on a JEOL JMX SX-102 mass spectrometer by the direct inlet method. Preparative high performance liquid chromatography (HPLC) was carried out on a column of TSK-gel ODS-120T (21.5 mm i.d.×30 cm): detection, ultraviolet (UV) at 210, 254 nm: flow rate, 6 ml/min. Acid and alkali hydrolysis of glycosides and identification of the resulting monosaccarides, methylate sugar by gas-liquid chromatography (GLC) were conducted in the usual manner.

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Plant Material

The plant materials(aerial part) were collected in the Kwang Rung of Kyoung Gi Do. The voucher specimen is deposited at Department of Pharmacognosy, College of Pharmacy, Chung-Ang University. Extraction and isolation The dried aerial parts (1.5 kg) were extracted with MeOH. The MeOH extracts was suspended in H_2O and partitioned with $CHCl_3$ and n-BuOH, successively. 500 g of the n-BuOH extracts was subjected to chromatography on silica gel with $CHCl_3$-MeOH-H_2O (70 : 30 : 4, homogenous) to give five fractions designated as fr.I-V in their order of elution. Further gel filtration of fr.I on Toyopearl HW-40 (TSKgel, TOYO SODA MFG. CO. Ltd., Tokyo, Japan) with MeOH-H_2O (1 : 1) afforded Compound **1** (250 mg). Fr.II (11 g) was chromatographied on Lichroprep. RP-8, Merk (30% MeOH) and subsequent prep. HPLC (19% MeOH, UV 210 nm) to give Compound **2** (230 mg). Fr.III-V were chromatographied on highly porous polymer, DIAION HP-20 (Mithbishi Kasei Co. Ltd., Tokyo, Japan) (H_2O-MeOH 1 : 0, 7 : 3, 5 : 5, 2 : 8 and 0 : 1 successively). The H_2O elute composed of large amount of mono and oligo saccharides. 50% MeOH elute (15 g) of fr.III was separated by HPLC on an octadecylsilica (ODS) column with 17% MeCN and 254 nm to give Compound **3** (124 mg) and Compound **4** (100 mg). 80% MeOH elute (7g) of fr.III was separated by HPLC on ODS column with 73% MeOH and UV 210 nm to give Compound **6** (78 mg) and Compound **7** (23 mg). MeOH elute (5 g) of fr.III was separated by HPLC on an ODS column with 85% MeOH and UV 219 nm to give Compound **8** (53 mg). In addition, 50% MeOH elute of fr.IV was separated by HPLC on an

ODS column with 17% MeCN and UV 254 nm to give Compound **3** (210 mg), Compound **4** (145 mg) and Compound **5** (105 mg). 80% MeOH elute of fr.IV was separated by HPLC on an ODS column with 75% MeOH and UV 210 nm to give Compound **7** (53 mg).

Compound 1: Crystallized from MeOH to yield Compound **1** as colorless needles. mp. 233-235℃; $[\alpha]_D$ =0.4° (c, 0.5, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.9 (2H, d, J=1.5 Hz, H-2,6), 9.2 (3H, brs, 3,4,5 -OH); ^{13}C-NMR: see Table 1; FAB-MS (negative) m/z: 169[M-H]$^-$, 125[(M-H)-COO]$^-$.

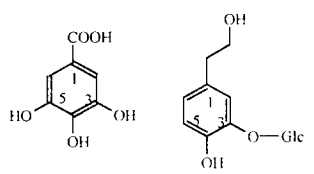
Compound 2: Pale yellow oil; Anal. Calcd. for $C_{14}H_{20}O_8 \cdot H_2O$: C, 47.4: H, 6.96: Found C, 47.37: H, 6.99; $[\alpha]_D$=−7.6°(c, 0.5, pyridine); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.0(1H, s, H-2), 6.7 (2H, s, H-5,6), 4.6 (1H, d, glucose anomeric H, J=7.5 Hz), 2.6 (2H, t, -CH_2, J=7.9 Hz); ^{13}C-NMR: see Table 1; FAB-MS (negative) m/z: 315[M-H]$^-$, 153[(M-H)-glucose]$^-$.

Compound 3: Crystallized from EtOH to yield Compound **3** as pale yellow pellets, mp. 211-213℃; $[\alpha]_D$ =−6.0° (c, 0.5, pyridine); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.55 (1H, s, H-2'), 7.53 (2H, s, H-5',6'), 6.8 (2H, d, J=7.9 Hz, H-2',6'), 6.4, 6.2 (each 1H, d, J=1.8 Hz, H-6,8), 5.3 (1H, d, Glc anomeric, H J=7.0 Hz), 4.4 (1H, s, Rha anomeric H), 1.0 (3H,d, Rha CH_3, J=6.1 Hz); ^{13}C-NMR: see Table I.

Compound 4: Crystallized from EtOH to yield Compound **4** as pale yellow pellets, mp. 208-211℃; Anal. Calcd. for $C_{33}H_{40}O_{19}$ 3/2H_2O: C, 47.27: H, 5.81: Found: C, 47.15: H, 5.86; $[\alpha]_D$=−18.4° (c, 0.5, pyridine); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2H, d, J=9.0 Hz, H-2',6'), 6.9 (2H, d, J=9.0 Hz, H-3',5'), 6.2, 6.4 (each 1H, d, J=2.0 Hz, H-6,8), 5.5 (1H, d, Glc anomer H, J=7.9 Hz), 5.1, 4.4 (each 1H, s, Rha anomeric H), 1.1, 0.8 (each 3H, Rha CH_3, J=6.2 Hz); ^{13}C-NMR: see Table 1; FAB-MS(negative) m/z: 739[M-H]$^-$, 593[(M-H)-Rha]$^-$, 447[(M-H-Rha)-Rha]$^-$, 285[(M-H-Rha-Rha)-Glc]$^-$.

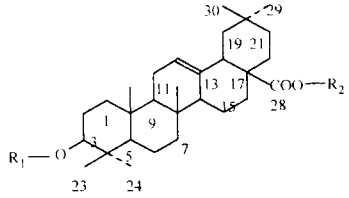
Compound 5: Crystallized from EtOH to yield Compound **5** as pale yellow neddles, mp. 235-237℃; $[\alpha]_D$ =4.7° (c, 1.0, pyridine); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.0 (2H, d, J=8.4 Hz, H-2',6'), 6.9 (2H, J=8.4 Hz, H-3',5'); ^{13}C-NMR: see Table 1; FAB-MS(negative) m/z: 593[M-H]$^-$, 431[(M-H)-Glc]$^-$, 269[(M-H-Glc)-Glc]$^-$.

Compound 6: Crystallized from MeOH to yield Compound **6** as colorless powder, mp. 220-223℃; Anal. Calcd. for $C_{58}H_{94}O_{25} \cdot 5H_2O$: C, 50.92: H, 8.74: Found: C, 50.60: H, 8.71: $[\alpha]_D$=0.6^N (c, 0.5, pyridine); ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 6.3 (1H, d, anomeric H, J=7.9 Hz), 5.9 (1H, s, anomeric H), 5.5 (1H, brs, H-12), 5.3, 5.1, 4.9 (each 1H, d, anomeric H, J=7.3 Hz, 7.9 Hz, 7.5 Hz), 1.7 (3H, d, Rha CH_3, J=6.0 Hz),



Compound **1** Compound **2**

	R1	R2	R3	R4	R5	R6	R7
Compound **3**	H	OH	H	OH	OH	OH	O−Glc^{6}−Rha
Compound **4**	H	OH	H	OH	H	OH	O−Glxc^{6}−Rha^{2}−Rha
Compound **5**	Glc	OH	Glc	OH	H	OH	H



	R1	R2
Compound **6**	Ara^{3}−Xyl	Glc^{6}−Glc^{4}−Rha
Compound **7**	Ara^{2}−Glc	Glc^{6}−Glc^{4}−Rha
Compound **8**	GlcUA^{3}−Xyl	H
Compound **6a**	Ara^{3}−Xyl	H
Compound **7a**	Ara^{2}−Glc	H
Compound **9**	H	H

1.4, 1.3, 1.2, 1.1, 1.0(2), 0.95(each 3H, s, aglycone CH_3); ^{13}C-NMR (100 Mz, pyridine-d_5): see Table II-1, II-2; FAB-MS(negative) m/z: 1189[M-H]$^-$, 1043[(M-H)-Rha]$^-$, 881[(M-H-Rha)-Glc]$^-$, 719[(M-H-Rha-Glc-Glc]$^-$, 587[(M-H-Rha-Glc-Glc)-Xyl]$^-$, 455[(M-H-Rha-Glc-Glc-Xyl)-Ara]$^-$.

Alkali saponification of compound 6: Compound **6** (30 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 resin column, and extracted with EtOAc : MeOH (2 : 1). The organic layer thus obtained was washed with H_2O and concentrated to give 6a.

Compound 6a: Crystallized from MeOH to yield Compound 6a as colorless powder, mp. 252-255℃; $[\alpha]_D$=−10.1° (c, 0.5, pyridine); ^{1}H-NMR (400 MHz, pyridine-d_5): 5.5 (1H, brs, H-12), 5.2, 4.9 (each 1H, d, anomeric H, J=7.3 Hz, 7.5 Hz), 1.12, 1.10, 1.07, 1.05, 0.99, 0.94, 0.88 (each 3H, s, aglycone CH_3), ^{13}C-NMR: see Table2-1, 2-2; FAB-MS(negative) m/z: 719 [M-H]$^-$, 587[(M-H)-Xyl]$^-$, 455[(M-H-Xyl)-Ara].

Permethylate 6a of compound 6a: Compound **6a** was methylated by the method (Hakomori *et al.*, 1964; Kizu *et al.*, 1980). Compound **6a** (15 mg), DMSO 3 ml and NaH 200 mg were reacted with streaming N_2 for 2 hr. After cooling, 15 ml CH_3I was added and

Table I. ^{13}C-NMR data of compound **1-5** (DMSO-d_6, 100 MHz)

Carbon No.	1	2	3	4	5
1	120.4	130.2			
2	109.0	115.4	156.3	156.4	164.0
3	145.3	144.9	133.2	132.7	102.5
4	137.9	144.9	177.3	177.3	182.2
5	145.3	117.4	161.1	161.2	158.5
6	109.0	123.1	98.6	98.7	107.4
7	167.4	38.4	164.0	164.1	161.2
8		60.7	93.5	93.7	105.2
9			156.5	156.4	155.0
10			103.9	104.0	103.6
1′			121.1	121.1	121.4
2′			115.1	130.8	129.0
3′			144.6	115.1	115.9
4′			148.6	155.9	156.0
5′			116.2	115.1	115.8
6′			121.5	130.8	128.6
glc					6-C
1		102.3	101.1	100.6	73.3
2		73.3	74.0	73.8	70.8
3		77.1	76.3	74.9	78.7
4		70.0	70.5	70.4	70.5
5		75.8	75.8	73.9	81.3
6		62.3	67.1	65.2	61.2
rha 1			100.6	99.0	
2			70.3	71.9	
3			70.0	70.4	
4			71.7	70.6	
5			68.1	68.2	
6			17.6	17.3	
rha 1				100.1	
2				70.6	
3				70.7	
4				71.9	
5				68.3	
6				17.9	
glc					8-C
1					74.0
2					71.8
3					78.7
4					70.8
5					81.8
6					61.2

allowed to stand for another 1 hr in ultrasonicator. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ part was evaporated and recrystallized from MeOH to give Permethylate **6a** as a colorless needles.

Permethylate 6a: ^{1}H-NMR (400 MHz, pyridine-d_5): 5.55 (1H, brs, H-12), 5.1, 5.0 (each 1H, d, anomeric H, J=7.3 Hz, 7.5 Hz), 3.21, 3.40, 3.45, 3.48, 3.50, 3.61, 3.63 (each 3H, s, OCH_3), 1.11, 1.10, 1.06, 1.05, 0.98, 0.95, 0.87 (each 3H, s, aglycone CH_3).

Table II-1. ^{13}C-NMR data of aglycone moieties compound **6-9** (pyridine-d_5)

Carbon No.	6	7	8	9	6a	7a
1	38.8	38.7	38.5	39.0	38.8	38.7
2	26.7	26.4	26.5	28.1	26.5	26.4
3	88.7	88.7	89.2	78.1	88.1	88.8
4	39.6	39.5	39.5	39.4	39.5	39.5
5	55.8	55.8	55.6	55.9	55.8	55.9
6	18.5	18.5	18.4	18.8	18.5	18.5
7	33.1	33.1	33.2	33.3	33.2	33.2
8	39.9	40.0	39.7	39.8	39.6	39.7
9	48.0	48.8	47.9	48.2	48.1	48.0
10	37.0	36.9	36.9	37.4	37.0	37.0
11	23.8	23.8	23.8	23.8	23.8	23.7
12	122.8	122.9	122.5	122.6	122.5	122.5
13	144.1	144.1	144.8	144.9	144.8	144.8
14	42.1	42.1	42.1	42.1	42.1	42.1
15	28.3	28.2	28.3	28.4	28.3	28.2
16	23.3	23.3	23.7	23.8	23.7	23.7
17	47.0	47.0	46.6	46.5	46.8	46.6
18	41.6	41.6	41.9	42.0	41.9	42.0
19	46.2	46.2	46.4	46.7	46.3	46.4
20	30.7	30.7	30.9	31.0	30.9	30.9
21	34.0	33.9	34.2	34.3	34.2	34.2
22	32.5	32.5	33.2	33.3	32.5	32.4
23	28.1	28.2	28.0	28.8	28.3	28.3
24	16.9	16.7	16.9	16.6	16.7	16.9
25	15.6	15.5	15.4	15.6	15.4	15.5
26	17.5	17.4	17.3	17.5	17.4	17.4
27	26.0	26.0	26.2	26.2	26.1	26.1
28	176.5	176.5	180.1	180.2	180.1	180.1
29	33.1	33.1	33.3	33.2	33.2	33.2
30	23.7	23.6	23.7	23.8	23.6	23.7

Methanolysis of compound methylate 6a: 10 mg of methylate 6a was boiled with 8% HCl-MeOH (3 ml) on water bath for 3 hr. The hydrolysate was neutralized and the filtrate was evaporated. The methylated sugars were identified as 3-O-linked arabinopyranosyl and terminal xylopyranoside by GC (t_R 16.4, 17.3).

Compound 7: Crystallized from MeOH to yield Compound 7 as colorless powder, mp. 215-216℃; Anal Calcd. for $C_{59}H_{96}O_{26}\cdot 4H_2O$: C, 52.14, H, 8.52: Found: C, 51.86, H, 8.49; $[\alpha]_D=-21.2°$ (c, 0.5, pyridine); ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 6.3 (1H, d, anomeric H, J=8.1 Hz), 5.9 (1H, s, anomeric H), 5.4 (1H, brs, H-12), 5.2, 5.0, 5.0 (each 1H, d, anomeric H, J=7.7, 7.9, 7.8 Hz), 1.7 (3H, d, Rha CH_3, J=6.2 Hz), 1.23, 1.22, 1.10, 1.05, 0.91(2), 0.89 (each 3H, s, aglycone CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): see Table II-1, II-2; FAB-MS(negative) m/z: 1219[M-H]$^-$, 1073[(M-H)-Rha]$^-$, 911[(M-H-Rha)-Glc]$^-$, 749[(M-H-Rha-Glc)-Glc]$^-$, 587[(M-H-Rha-Glc-Glc)-Glc]$^-$, 455[(M-H-Rha-Glc-Glc-Glc)-Ara]$^-$.

Table II-2. ^{13}C-NMR data of sugars moieties compound **6-9** (pyridine-d_5)

Carbon	No.	6	7	8	6a	7a
3-O						
ara	1	106.8	104.8		106.7	104.8
	2	71.8	72.5		71.9	81.0
	3	83.6	72.5		84.5	73.4
	4	69.4	68.2		69.4	68.3
	5	67.0	64.9		67.2	65.4
glc	1		105.9			106.0
	2		76.3			76.4
	3		78.2			78.2
	4		71.6			71.5
	5		78.2			78.2
	6		62.5			62.5
glcUA	1			106.2		
	2			74.6		
	3			86.4		
	4			71.4		
	5			78.1		
	6			172.2		
xyl	1	107.4		106.8	107.5	
	2	75.3		75.3	75.4	
	3	77.1		77.6	77.1	
	4	71.0		70.9	71.1	
	5	67.2		67.4	67.2	
28-COO-						
glc	1	95.6	95.6			
	2	74.0	74.0			
	3	78.7	78.7			
	4	70.8	70.8			
	5	78.0	78.0			
	6	69.2	69.2			
glc	1	104.8	104.7			
	2	75.3	75.1			
	3	77.1	77.1			
	4	78.2	78.3			
	5	76.5	76.5			
	6	61.2	61.3			
rha	1	102.7	102.7			
	2	72.5	72.5			
	3	72.7	72.7			
	4	73.8	73.8			
	5	70.3	70.3			
	6	18.5	18.5			

Alkali saponification of compound 7: Compound **7** (30 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 resin column, and extracted with EtOAc : MeOH (2 : 1). The organic layer thus obtained was washed with H_2O and concentrated to give compound **7a**.

Compound 7a: Crystallized from MeOH to yield Compound **7a** as colorless powder, mp. 235-238℃, $[\alpha]_D = -8.4°$ (c, 0.5, pyridine); ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 5.6 (1H, brs, H-12), 5.2, 5.0 (each 1H, d, anomeric H, J=7.5, 7.7 Hz), 1.11, 1.09, 1.07, 1.04, 0.96, 0.95, 0.91 (each 3H, s, aglycone CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): see Table II-1, II-2; FAB-MS (negative) m/z: 749[M-H]$^-$, 587[(M-H)-Glc]$^-$, 455[(M-H-Glc)-Ara]$^-$.

Compound 8: Crystallized from MeOH to yield Compound **8** as colorless powder, mp. 233-236℃; Anal Calcd. for $C_{41}H_{64}O_{13}\cdot 3H_2O$: C, 57.33, H, 9.16: Found: C, 57.10, H, 9.13; $[\alpha]_D = 0.6°$ (c, 0.5, pyridine); ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 5.5 (1H, brs, H-12), 5.4, 5.0 (each 1H, d, anomeric H, J=7.5, 7.9 Hz), 1.3 (3), 1.0(2), 0.8 (each 3H, s, aglycone CH_3); FAB-MS(negative): 763[M-H]$^-$, 631[(M-H)-Xyl]$^-$, 455[(M-H-Xyl)-GlcUA]$^-$.

Compound 9: Crystallized from MeOH to yield Compound **9** as colorless neddles, mp. 305-307℃; ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 5.5 (1H, brs, H-12), 1.30, 1.25, 1.04, 1.03, 1.02, 0.96, 0.91 (each 3H, s, aglycone CH_3), ; ^{13}C-NMR (100 MHz, pyridine-d_5): see Table II-1.

RESULTS AND DISCUSSION

The n-butanol soluble part of the methanol extract of the aerial part of *Clematis koreana* var. *umbrosa* was separated by silica gel C.C., Diaion C.C.

After removing sugars by eluting with water, the Diaion C.C. was 30% MeOH, 50% MeOH, 80% MeOH and MeOH, successively. Repeated gel filteration and HPLC of the 30% methanol elute afforded two aromatic compounds (Compound **1**, Compound **2**). Repeated HPLC of the 50% methanol elute gave three flavonoid glycosides (Compound **3-5**). Repeated HPLC of the 80, 100% methanol elute gave three triterpenoidal saponins (Compound **6-8**).

Compound **1** was obtained as colorless neddles. the ^{1}H-NMR spectrum (DMSO-d_6) showed a doublet signal at δ 6.9 (2H) and ^{13}C-NMR (DMSO-d_6) showed four signals for an aromatic group and carboxylic acid. These data suggested that compound 1 was 3,4,5-trihydroxy benzoic acid (gallic acid), and melting point and other physical data were identical with those of an authentic sample.

Compound **2** was obtained as pale yellow oil. On the hydrolysis of acid, the compound 1 gave 3,4-dihydroxyphenethyl alcohol and glucose.

^{1}H-NMR spectrum of Compound **2** (DMSO-d_6) demonstrated the presence of three aromatic protons on the 7.0 (1H), 6.7 (2H). This established the aromatic ring as 1,3,4-trisubstitution and showed a sharp doublet of 1'-proton of the D-glucose at 4.6 (J=7.5 Hz, axial/axial coupling indicative of β-D-glucose). ^{13}C-NMR

showed six signals for aromatic ring and two methylene group (Table I). In addition, the DEPT spectrum of **2** in DMSO-d_6 showed the signals at 115.4, 117.4, 123.1 were due to a CH resonace, and δ 102.3 was due to a anomeric carbon of β-D-glucose, and δ 62.3, 60.7, 38.4 were due to a CH_2 resonance. The NMR spectrum of DMSO-d_6 and D_2O were similar to that reported by Pabst *et al.*, and Sakurai *et al.*. The identification was confirmed by ^{13}C-NMR, ^{1}H-NMR, and FAB-MS and by comparison of the reported data (Sakurai *et al.*, 1983; Pabst *et al.*, 1990; Sugiyama *et al.*, 1992). In the results, Compound **2** was 3,4-dihydroxyphenethyl alcohol-3-O-β-D-glucopyranoside.

Compound **3** was obtained as yellow pellets ^{1}H and ^{13}C NMR spectrum of this compound indicated that this is flavonol glycoside. Comparing physical data with the values and authentic sample, Compound **3** was quercetin-3-O-α-L-rhamnopyranosyl(1-6)-α-D-glucopyranoside(Rutin) (Otsuka *et al.*, 1989).

Compound **4** was obtained as yellow pellets. The ^{1}H-NMR spectrum (DMSO-d_6) showed four doublet signals at δ 8.1, 6.9 (each 2H, J=9.0 Hz), 6.4, 6.2 (each 1H, J=2.0) of kaempferol (Yasukawa *et al.*, 1986). The position, sequence and anomeric configuration of the sugar moieties were determined by NMR spectroscopy. ^{13}C-NMR chemical shift data are given in Table I. The ^{1}H-NMR spectrum of compound **4** confirmed the aglycone as kaempferol. The one doublet at δ 5.5 and two singlet at δ 5.1, 4.4 were the anomeric protons. In the FAB-Mass (negative) of compound **4** quasi-molecular ion appeared at m/z 739[M-H]$^-$ signal at 593[(M-H-rha]$^-$ and 447[(M-H-rha)-rha]$^-$ and 285[(M-H-rha-rha)-glc]$^-$ corresponded to the subsequent loss of two rhamnosyl moiety and a glucosyl moiety and indicated clearly that glucose was the inner sugar. The NMR and FAB-Mass data of compound **4**, with the exception of the aglycone moiety, correspond to these of compound **4** is therefore identified as the homologous structure; kaempferol-3-O-α-L-rhamnopyranosyl(1-2)-α-L-rhamnopyranosyl(1-6)-β-D-glucopyranoside.

Compound **5** was obtained as yellow neddles. The ^{1}H-NMR spectrum (DMSO-d_6) showed two doublet signals at δ 8.0, 6.9 (each 2H, J=8.4 Hz) of apigenin.

It was therefore compared with references of 6,8-di-C-β-D-glucopyranosylapigenin (Sakaibara *et al.*, 1977; Bouillant *et al.*, 1975). All data of the ^{1}H-NMR, ^{13}C-NMR, and FAB-MS of Compound **5**, suggested that **5** was 6,8-di-C-β-D-glucopyranosylapigenin (Table 1).

Based on analysis of the ^{1}H-NMR, ^{13}C-NMR spectra (Table II-1, II-2) and FAB-MS, and the results of acid and alkaline hydrolysis, Compound **7** was identified as Ciwujianoside A1, which has already isolated from Leaves of *Acanthopanax senticosus* by direct comparison of the ^{13}C-NMR spectra and optical rotation with those of authentic sample (Shao *et al.*, 1989).

Compound **8** was identified as Molluscidal Saponin 2, which has already isolated from tubers of *Talinum tenuissimum* (Portulaceae) by comparison of the 13C-NMR, optical rotation and FAB-MS with those of reference (Gafner *et al.*, 1985).

On the acid hydrolysis, the new saponin (Compound **6**) gave oleanolic acid (**9**), arabinose, xylose, glucose and rhamnose. In the ^{13}C-NMR spectrum of Compound **6** (Table II-1, II-2), the signals due to the aglycone moiety were in good agreement with those of the 28-glycosylester of 3-O-glycosyl oleanolic acid (bisdesmoside of Compound **9**) and those due to the sugar moiety showed the presence of four monosaccharide units. On the alkaline hydrolysis Compound **6** afforded a prosapogenin (6-a) and L-rhamnose and D-glucose (1 : 2) by comparison of the GLC with that of authentic samples. Acid hydrolysis of Compound **6a** gave 9, arabinose and xylose. The sugar sequence analysis of permethylated Compound 6a showed the 3-O-linked arabinopyranosyl and terminal xylopyranoside(Hakomori *et al.*, 1964; Kizu *et al.*, 1980). The anomeric configuration of each sugar unit was determined by ^{1}H-NMR and ^{13}C-NMR spectroscopy. Based on these results, the structure of Compound **6** was formulated as shown in Chart 1.

REFERENCES CITED

Bouillant, M. L., Favre-Bonvin, J. and Chopin, J., Structural determination of C-glycosylflavones by mass spectrometry of their permethyl ethers. *Phytochemistry*, 14, 2267-2274 (1975).

Gafner, F., Msonthi, J. D. and Hostettmann, K., Molluscidal Saponins from Talinum tenussimum. *Helvetica Chimica Acta*, 68, 555-556 (1985).

Hakomori, S. I., A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbinion in dimethylsulfoxide. *J. Biochemistry*, 55, 205-207 (1964).

Kizu, H. and Tomimori, T., Studies on the constituents of Clematis species V. *Chem. Pharm. Bull.*, 30, 3340-3346 (1982).

Kizu, H. and Tomimori, T., Studies on the constituents of Clematis species VI. *Chem. Pharm. Bull.* 30, 859-865 (1982).

Kizu, H. and Tomimori, T., Studies on the constituents of Clematis species III. *Chem. Pharm. Bull.*, 28, 3555-3560 (1980).

Kizu, H. and Tomimori, T., Studies on the constituents of Clematis species II. *Chem. Pharm. Bull.*, 28, 2827-2830 (1980).

Kizu, H. and Tomimori, T., Studies on the constituents of Clematis species I. *Chem. Pharm. Bull.*, 27, 2388-2393 (1979).

Kintya, P. K., Melnikov, V. N., and Chirva, V. Ya, Structure of vitalvoside G from *Clematis vitalva*. *Khim. Prir.*

Soedin., 6, 802-803 (1974).
Kintya, P. K., Melnikov, V. N., and Chirva, V. Ya, Structure of vitalvoside D from *Clematis vitalva. Khim. Prir. Soedin.*, 6, 803-804 (1974).
Moon Kyo Bu, *Colored illustration of herbaceus plants of Korea*, 1, 176 (1973)
Namba, T., Hikusa publishing Co., Ltd., Coloured illustration of *WAKAN-YAKU*, 1, 112 (1980).
Otsuka, H., Yamasaki, K. and Yamauchi, T., Alangifolioside, A diphenylmethylene dervative and other phenolics from the leaves of *Alangium platanifolium* var. *trilobum*. *Phytochemistry*, 28, 3197-3200 (1989).
Pabst A., Barron D., Adda J. and Schreier P., Phenylbutan-2-one-β-D-glucoside from raspberry fruit. *Phytochemistry*, 29, 3853-3850 (1990).
Sakaibara, M., Mabry, T. J., Bouillant, M. L. and Chopin, J., 6,8-Di-C-Glucosylflavones from *Larrea tridentata* (Zygophyllaceae). *Phytochemistry*, 16, 1113 (1977).
Sakurai, A. and Kato, T.,A new glycoside, kusaginin, Isolated from Clerodendron tricotomum. *Bull. Chem. Soc. Jpn.*, 56, 1573-1574 (1983).
Shao, C. J., Kasai, R., Xu, J. D. and Tanaka, O., Saponins from leaves of *Acanthopanax senticosus*. *Chem. Pharm. Bull.*, 37, 42-45 (1989).
Sugiyama, M. and Kikuchi, M., Structures of Phenolic Glucosides from the leaves of *Osmanthus asiaticus*. *Chem. Pharm. Bull.*, 40(2), 325-326 (1992).
Yasukawa, K. and Takido, M., Studies on the Chemical Constituents of Genus Lysimachia I. *Yakugaku Zasshi*, 106(10), 939-941 (1986).