A Novel Flavonol Glycoside in the Leaves of *Epimedium sempervirens*

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A new flavonol glycoside with two acetyl groups, sempervirenoside A, was isolated from the leaves of *Epimedium sempervirens* NAKAI. The structure of sempervirenoside A was established to be 3-*O*-[3-*O*-acetyl-β-D-xylopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl]-7-*O*-β-D-glucopyranosyl-3,5,7-trihydroxy-4′-methoxy-8-(3-methyl-2-butenyl) flavone by ultraviolet, fast atomic bombardment mass, and proton and carbon-13 nuclear magnetic resonance spectral studies.

Keywords *Epimedium sempervirens*; Berberidaceae; flavonol glycoside; sempervirenoside A

About 25 flavonol glycosides have been isolated from the genus *Epimedium* (Berberidaceae). All the peaks of the glycosides in high-performance liquid chromatography (HPLC) were identified and the relationship of their structures and retention times was discussed.[1] In a continuation of the study of flavonol glycosides in *Epimedium*, a few unidentified peaks were observed on the chromatograph of the extract of *Epimedium sempervirens* NAKAI (Syn. *E. grandiflorum* MORR. subsp. *sempervirens* (NAKAI) KITAM.), which is an evergreen herb, and grows in districts along the Japan Sea. In this paper, the structure elucidation of a new flavonol glycoside with two acetyl groups in the sugar moiety is described.

Results and Discussion

An unidentified peak of the 70% MeOH extract of *Epimedium sempervirens* appeared at about 32 min under the same HPLC conditions as reported previously.[1] By the use of medium-pressure liquid chromatography, sempervirenoside A was preparatively purified. Compound **1**, $C_{42}H_{52}O_{21}$, mp 149—150 °C, $[\alpha]_D^{20}$ −99.6° (c=0.51, MeOH) gave absorption bands at 272, 315 and 351 nm in the ultraviolet (UV) spectrum. The responses to some test reagents suggested that **1** was a flavonol glycoside with a free hydroxyl group at C-5. In addition, the negative ion fast atomic bombardment mass spectral (FAB-MS) data (Chart 1) indicated that **1** was composed of a hexose unit and a disaccharide unit, *i.e.*, a mono acetyl aldopentose in the terminal chain, and a mono-acetyl deoxy-hexose in the inner chain. These results indicated **1** to be a bisdesmosyl triglycoside of a flavonol.

The structure of the aglycone part was investigated by analysis of the proton nuclear magnetic resonance (^{1}H-NMR) (Table I) and ^{13}C-NMR (Table II) spectral data compared with the published data,[2,3] and the aglycone was concluded to be 3,5,7-trihydroxy-4′-methoxy-(3-methyl-2-butenyl) flavone (anhydroicaritin). On the other hand, the structures of the sugar parts were clarified as follows. In the ^{1}H-NMR spectrum measured at room temperature and at 50 °C, all protons including hydroxy protons were assigned with the aid of ^{1}H–^{1}H chemical shift correlation spectroscopy (COSY), nuclear Overhauser effect correlation spectroscopy (NOESY), and ^{1}H–^{13}C COSY as shown in Table I. An anomeric proton at δ 5.30 (in measurement at 50 °C, δ 5.30 (d, J=1.6 Hz)), a methine on carbon bearing an acetoxy group at δ 4.84 (t, J=10.0 Hz) assignble to H-4, and the other proton signals due to the inner sugar of a disaccharide suggested the presence of a 4-*O*-acetyl-α-L-rhamnopyranosyl (C1 conformation)[4,5] moiety. Similarly, the signal of an anomeric proton at δ 4.34 with a large coupling constant (d, J=7.6 Hz), that of a methine on carbon bearing an acetoxy group at δ 4.71 (t, J=9.4 Hz) assignable to H-3, and those of the other protons due to the terminal sugar of the disaccharide are in good agreement with those of a 3-*O*-acetyl-β-D-xylopyranosyl (C1 conformation) moiety.[4] In addition, the other anomeric proton at δ 5.00 (d, J=7.6 Hz) (see annotation *h*) in Table I) and other proton signals due to the hexose are consistent with β-D-glucopyranoside (C1 conformation).[4]

A consideration of all the hydroxy proton signals attributable to the disaccharide moiety revealed that the hydroxy group at C-3α of 4-*O*-acetyl-α-L-rhamnopyranose gave no hydroxy proton signal, inasmuch as it was linked with 3-*O*-acetyl-β-*O*-D-xylopyranose by an ether band *via* the ano-

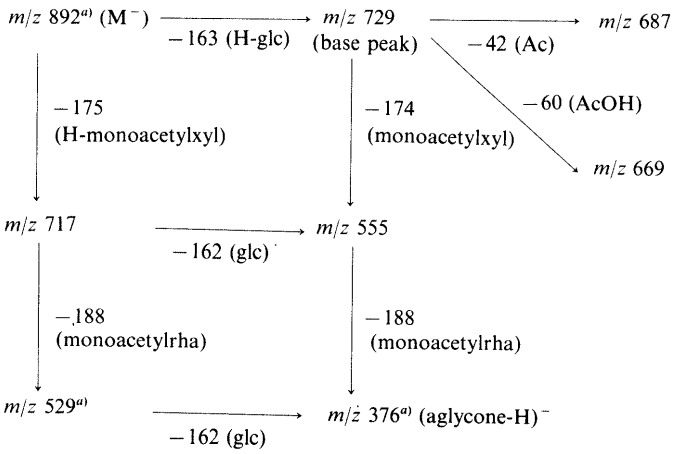
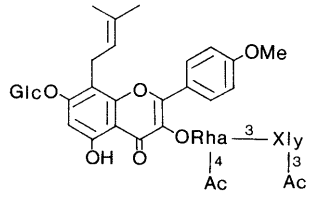


Chart 1. Negative Ion FAB-MS Spectrum

(Emission current=30 mA, collision chamber=−3 kV, acceleration voltage=2 kV, matrix triethanolamine, gas=Xe, ion mult. voltage=2.00 kV). *a*) Abundant fragment ion.



sempervirenoside A (**1**)

Chart 2

TABLE I. ^{1}H-NMR Data for Sempervirenoside A (**1**) (400 MHz, DMSO-d_6)[a]

Aglycone moiety	6-H 6.65 (s), 11-H_2 3.56 (dd, 14.5, 7.5) 3.44 (m),[b] 12-H 5.18 (m),[c] 14 and 15-H_3 1.68 (s) 1.61 (s), 2′(6′)-H_2 7.88 (d, 8.9), 3′(5′)-H_2 7.17 (d, 8.9), 4′-OCH_3 3.89 (s)		
Sugar moiety	3-*O*-Inner rhamnose	3-*O*-Terminal xylose	7-*O*-Glucose
1-H	5.30 (m)[d]	4.34 (d, 7.6)	5.00 (m)[h]
2-H	4.16 (m)	3.13 (m)[g]	3.31 (m)[e]
3-H	3.79 (dd, 10.0, 2.5)	4.71 (t, 9.4)	3.31 (m)[e]
4-H	4.84 (t, 10.0)	3.47 (m)[b]	3.18 (m)[g]
5-H	3.26 (m)[e]	3.84 (dd, 11.3, 5.3)	3.43 (m)[b]
		3.47 (m)	
6-H	0.71 (d, 6.2)	—	3.72 (ddd, 11.0, 5.2, 2.5)
			3.50 (m)[b]
2-OH	5.30 (m)	5.24 (d, 5.6)	5.30 (m)[d,i]
3-OAc		2.20 (s)[f]	
3-OH		—	5.08 (br d, 4.0)[i]
4-OAc	1.95 (s)		
4-OH		5.18 (m)	5.00 (m)[h]
6-OH			4.58 (t, 5.2)

a) Measured at room temperature. Chemical shifts are in δ values from internal tetramethylsilane (TMS) and coupling constants in parentheses are in Hz. *b*—*e*, *g*, *h*) Signals with the same superscript overlapped each other. *c*) When the ^{1}H-NMR measured at 50 °C, this overlapped signal changed to two discrete signals at δ 5.20 (m, 12-H) and 5.08 (d, J=5.3 Hz, xylosyl 4-OH). *d*) When the ^{1}H-NMR was measured at 50 °C, this overlapped signal changed to three discrete signals at δ 5.32 (d, J=1.6 Hz, rhamnosyl 1-H), 5.18 (d, J=5.5 Hz, glucosyl 2-OH), and 5.16 (d, J=4.9 Hz, rhamnosyl 2-OH). *f*) Assignments may be interchanged. However, an NOE cross peak was observed between an acetyl methyl at δ 2.02 and the xylosyl proton at C-3 in the NOESY spectrum and thus, this methyl signal must be assigned to the acetyl group, xylosyl-3-OAc. *h*) When the ^{1}H-NMR was measured at 50 °C, this overlapped signal changed to two discrete signals at δ 5.00 (d, J=7.6 Hz, glucosyl 1-H) and 4.90 (d, J=5.2 Hz, glucosyl 4-OH). *i*) Assignments for these hydroxyl protons maybe interchanged. However, the COSY peak observed between 2-OH (at δ 5.30) and 1-H on glucose was more intense than that between 3-OH (at δ 5.08) and 1-H on the glucose. On the contrary, the NOESY peak observed between 3-OH (at 5.08) and 1-H on the glucose was more intense than that between 2-OH (at 5.30) and 1-H on glucose. These findings allow the hydroxyl signal at 5.30 to be assigned to 2-OH and the other at 5.08 to 3-OH.

meric hydroxy group of the xylose. Furthermore, in the NOESY spectrum, intense cross peaks were confirmed between 3β-H of the inner rhamnose and the anomeric proton of the terminal xylose. These findings clarified that a 3-*O*-acetyl-β-D-xylopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl residue existed as a disaccharide moiety of **1**.

The full structure of **1** was made clear on the basis of the following NOESY experiments. Two significant NOE cross peaks between the anomeric protons of rhamnose (inner sugar of the disaccharide) and the protons at C-2′ and 6′ of the aglycone and between the anomeric proton of glucose and a proton at C-6 of the aglycone were observed. These findings suggested that the disaccharide and the glucosyl residues were respectively connected to the hydroxy groups at C-3 and C-7 through a glycosidic linkage. These lines of evidence led us to conclude that the structure of sempervirenoside A is 3-*O*-[3-*O*-acetyl-β-D-xylopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl]-7-*O*-β-D-glucopyranosyl-3,5,7-trihydroxy-4′-methoxy-8-(3-methyl-2-butenyl) flavone. The ^{13}C-NMR data (Table II) were also consistent with this structure.

TABLE II. ^{13}C-NMR Data for Sempervirenoside A (100.5 MHz, DMSO-d_6)[a]

Aglycone moiety		Sugar moiety	
C-2	153.01	3-*O*-Inner rhamnose	
C-3	134.14	C-1	101.40
C-4	178.04	C-2	69.47
C-5	160.52	C-3	76.87
C-6	98.16	C-4	71.08
C-7	161.55	C-5	68.26
C-8	108.36	C-6	16.89
C-9	157.38	4-*O*-$\underline{C}OCH_3$	169.55[d]
C-10	105.53	4-*O*-CO$\underline{C}H_3$	20.67
C-11	21.34	3-*O*-Terminal xylose	
C-12	122.01[b]	C-1	105.21
C-13	131.01	C-2	70.67
C-14	25.35[c]	C-3	77.78
C-15	17.74[c]	C-4	67.36
C-1′	122.01[b]	C-5	65.42
C-2′	130.51	3-*O*-$\underline{C}OCH_3$	169.70[d]
C-3′	114.04	3-*O*-CO$\underline{C}H_3$	21.04
C-4′	158.99	7-*O*-Glucose	
C-5′	114.04	C-1	100.53
C-6′	130.51	C-2	73.27
OCH_3	55.50	C-3	76.53
		C-4	69.61
		C-5	77.13
		C-6	60.58

a) Chemical shifts are shown in δ values from internal TMS. All primary, secondary, and tertiary carbons were assigned with the aid of ^{1}H–^{13}C COSY data measured at room temperature and at 50 °C. Assignments for the aglycone moiety were made according to the previous paper.[2,3] *b*) Signals overlapped. *c*, *d*) Assignments for signals with the same superscripts may be interchanged.

Experimental

Plant Material The leaves of *Epimedium sempervirens* NAKAI were collected at Tonami City in Toyama prefecture, Japan, in June 1986. A sample has been deposited in the Herbarium of Gifu Pharmaceutical University.

Extraction and Isolation of Sempervirenoside A (1) The dry and pulverized leaves (400 g) of *E. sempervirens* were extracted three times with 70% MeOH (each 10 l) under reflux. The combined extract was concentrate *in vacuo* and the residue (150 g) was suspended in H_2O, and then extracted successively with $CHCl_3$, EtOAc and *n*-BuOH. The EtOAc-soluble layer was chromatographed on silica gel with $CHCl_3$–MeOH solution. Fractions that contained **1** (monitored by HPLC) were further separated by octadecyl silica (ODS) column chromatography developed with 28% CH_3CN under moderately high pressure to give **1** (25 mg).

Sempervirenoside A (1) mp 149—150 °C, a pale yellow amorphous powder, $[\alpha]_D^{20}$ −99.6° (c=0.51, MeOH). EI-MS *m/z* (rel. int.): 368 (100), 353 (69), 313 (44), 300 (40), 165 (11), 135 (49). FAB-MS is shown in Chart 1. IR ν_{max}^{KBr} cm^{-1}: 3400, 1725, 1645, 1595. UV λ_{max}^{MeOH} nm: 272, 315, 351; λ+NaOMe: 283, 384; λ+$AlCl_3$: 280, 306, 341, 412; λ+$AlCl_3$/HCl: 281, 305, 339, 412; λ+NaOAc: 272, 310, 346 sh; λ+NaOAc/H_3BO_3: 272, 310, 346 sh. ^{1}H and ^{13}C-NMR spectral data are shown in Tables I and II.

References and Notes

1) M. Mizuno, M. Iinuma, T. Tanaka, N. Sakakibara, S. Hanioka and X. Liu, *Chem. Pharm. Bull.*, **36**, 3487 (1988).
2) M. Mizuno, N. Sakakibara, S. Hanioka, M. Iinuma, T. Tanaka, X. Liu and D. Shi, *Phytochemistry*, **27**, 3641 (1988); M. Mizuno, M. Iinuma, T. Tanaka, N. Sakakibara, M. Nishi, A. Inada and T. Nakanishi, *ibid.*, accepted.
3) K. R. Markham, B. Ternai, R. Stanley, H. Geiger and T. J. Mabry, *Tetrahedron*, **34**, 1389 (1978).
4) With respect to the configurations of rhamnose, xylose and glucose in **1**, the L, D and D forms may be preferable from the viewpoint of natural occurrence of these sugars.
5) Coupling constants $J_{1,2}$ (1.6 Hz) and $J_{2,3}$ (2.5 Hz) may be more reasonably assigned to diequatorial ($J_{1,2}$) and equatorial–axial ($J_{2,3}$) relations for α-anomeric orientation of L-rhamnose rather than axial–equatorial ($J_{1,2}$) and equatorial–axial ($J_{2,3}$) relations for β-anomeric orientation, because, in the latter orientation, it is assumed from Dreiding model inspection that $J_{1,2}$ and $J_{2,3}$ would have the same axial–equatorial relation and then identical J-values would be expected.