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NEW FLAVONOL GLYCOSIDE FROM THE LEAVES OF *EPIMEDIUM SEMPERVIRENS*

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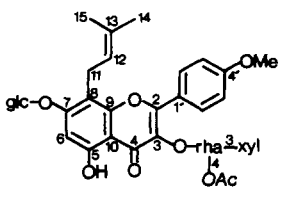
ABSTRACT.—A new flavonol glycoside, sempervirenoside B [**1**], was isolated from the leaves of *Epimedium sempervirens*. The structure was established to be 3-*O*-[β-D-xylopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl]-7-*O*-β-D-glucopyranosyl-3,5,7-trihydroxy-4′-methoxy-8-(3-methyl-2-butenyl) flavone, or anhydroicaritin 3-*O*-[β-D-xylopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside 7-*O*-β-D-glucopyranoside] by means of uv, fabms, eims, and ^{1}H, ^{13}C, ^{1}H-^{13}C, and ^{1}H-^{13}C long-range COSY nmr spectra.

In an earlier paper (1), we discussed the structure of a new flavonol glycoside, sempervirenoside A, with two acetyl groups on its sugar moieties, which was isolated from the leaves of *Epimedium sempervirens* Nakai (Berberidaceae). Among some unidentified peaks in a 70% MeOH extract of the leaves observed upon hplc, another flavonol glycoside, named sempervirenoside B [**1**], which helped to characterize the species chemotaxonomically, has been isolated. We describe the structure elucidation of **1** in this paper.

Sempervirenoside B [**1**], $C_{40}H_{50}O_{20}$, a yellow amorphous powder, was separated, from a much earlier fraction than that which contained sempervirenoside A, by using medium pressure liquid chromatography on reversed-phase C_{18} gel. The uv spectrum showed absorption bands at 271, 314, and 350 nm, and bathochromic shifts were observed on addition of certain reagents ($AlCl_3$ and NaOMe), which indicated that **1** was a flavonol glycoside with a hydroxyl group at C-5. The negative ion fabms data showed that **1** contained one glucose unit, one xylose unit, and a rhamnose unit possessing an acetyl group. The xylose unit was attached to rhamnose. The presence of these sugars was further confirmed by acid hydrolysis. The structure of the aglycone moiety was determined to be anhydroicaritin (8-$_{\gamma,\gamma}$-dimethylallyl-3,5,7-trihydroxy-4′-methoxyflavone) by means of eims and ^{1}H nmr.



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Three anomeric protons in the ^{1}H nmr of **1** were observed and assigned to those of xylose δ 4.22 (d, J = 7.3 Hz), glucose 5.14 (d, J = 6.6 Hz), and rhamnose 5.32 (br s). The glycosyl anomeric proton showed a cross peak with that of C-6 (δ 6.63) of anhydroicaritin in the ^{1}H-^{1}H long range COSY, indicating that the glucose was attached by a β-glycosidic linkage to the phenoxyl group at C-7. On the other hand, the anomeric proton of the rhamnose moiety caused a cross peak with a carbon assigned to C-3 (δ 134.2), which also indicated that the rhamnose was attached to the phenoxyl group at C-3 through a glycosidic linkage. The interlinkage of rhamnose and xylose and the position of the acetyl group on the rhamnose were determined by a carbon signal (δ 76.6) assignable to C-3 of the rhamnose, which shifted downfield by ca. 5 ppm. The glycosylation shift is usually observed to be 7–10 ppm (2). In this case, the β effect of the acetyl group attached to C-4 diminished the shift value.

This finding showed the interlinkage of the biose to be β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranoside and that

the acetyl group was esterified at C-4 of the rhamnose. These results were also supported by other evidence: a proton at C-3 (δ 3.73) of the rhamnose had a cross peak with an anomeric carbon of xylose (δ 105.6) in the ^1H-^{13}C long range COSY, and a proton at C-4 of the rhamnose (δ 4.83) not only had a cross peak with protons at C-6 (δ 0.72) of the rhamnose in the proton relay COSY, but also caused a cross peak with a carbonyl carbon (δ 169.7) of the acetyl group. From the spectral data described above, the structure of sempervirenoside B is concluded to be 3-*O*-[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 [**1**], or anhydroicaritin 3-*O*-[β-D-xylopyranosyl-(1↦2)-4-*O*-acetyl-α-L-rhamnopyranoside 7-*O*-β-D-glucopyranoside].

EXPERIMENTAL

PLANT MATERIAL.—The leaves of *E. sempervirens* were collected at Tonami City in Toyama prefecture, Japan, in June 1986. A voucher specimen has been deposited in the Herbarium of Gifu Pharmaceutical University.

EXTRACTION AND ISOLATION.—According to our previous procedures (1), a 70% MeOH extract of the leaves of *E. sempervirens* was prepared. The extract was analyzed by hplc (solvent system MeCN/H_2O) (3). One of the unidentified peaks, consisting of sempervirenoside B [**1**], appeared at about 28 min. By medium pressure liquid chromatography over Lichrosorb RP-18 (Nakalai Tesque, Inc., Japan), **1** (35 mg) was obtained in pure form.

SEMPERVIRENOSIDE B [**1**].—A yellow amorphous powder: negative ion fabms (emission current = 30 mA, collision chamber = −3 kV, acceleration voltage = 2 kV, matrix triethanolamine, gas = Xe, ion mult. voltage = 2.00 kV) *m/z* $[M-H]^-$ 849, $[849-glc]^-$ 687, $[687-xyl]^-$ 555, $[555-(rha\text{-}Ac)]^-$ 367, $[849-(Ac\text{-}rha\text{-}xyl)]^-$ 529, $[529-glc]^-$ 367, $[aglycone-H]^-$ 367; eims (rel. int.) *m/z* 353 (42), 313 (29), 300 (27), 165 (9), 135 (27), 60 (25), 43 (100); uv λ max (MeOH) 271, 314, 350 nm, +NaOMe 283, 382, +$AlCl_3$ 281, 306, 340, 410, +$AlCl_3$/HCl 282, 306, 339, 410, +NaOAc 273, 302 sh, 340 sh, +NaOAc/H_3BO_3 272, 302 sh, 340 sh; ^1H nmr (270 MHz, DMSO-d_6 after addition of D_2O) δ 0.72 (3H, d, J = 5.9 Hz, rha-Me), 1.61, 1.68 (3H, s × 2, Me-14 and -15), 1.97 (3H, s, Ac), 3.88 (3H, s, OMe), 4.14 (1H, br s, rha-H-2), 4.22 (1H, d, J = 7.3 Hz, xyl-H-1), 4.83 (1H, t, J = 9.9 Hz, rha-H-4), 5.14 (1H, d, J = 6.6 Hz, glc-H-1), 5.18 (1H, m, *CH*=C<), 5.32 (1H, br s, rha-H-1), 6.63 (1H, s, H-6), 7.14 (2H, d, J = 8.8 Hz, H-3′ and -5′), 7.86 (2H, d, J = 8.8 Hz, H-2′ and -6′); before D_2O addition, a chelated hydroxyl group (OH-5) was observed at 12.50; ^{13}C nmr see Table 1. For acid hydrolysis, a 3% H_2SO_4 solution (10 ml) containing sempervirenoside B (5 mg) was warmed on an H_2O bath for 2 h. Yellow precipitates were removed by filtration. The filtrate was subjected to paper partition chromatography (eluent 80% phenol) after neutralization with $BaCO_3$ to result in identification of glucose, rhamnose, and xylose by the usual procedures.

TABLE 1. ^{13}C-nmr Data of Sempervirenoside B [**1**]a,b.

Aglycone moiety		Sugar moiety	
C-2	153.1	glucose	
C-3	134.2	C-1″	100.6
C-4	178.2	C-2″	73.4
C-5	159.1	C-3″	76.6
C-6	98.3	C-4″	69.6
C-7	160.6	C-5″	77.2
C-8	108.5	C-6″	60.7
C-9	157.5	rhamnose	
C-10	105.6[c]	C-1‴	101.5
C-11	21.4	C-2‴	69.7
C-12	122.1[d]	C-3‴	76.6
C-13	131.2	C-4‴	71.3
C-14	25.5	C-5‴	68.4
C-15	17.9	C-6‴	17.0
C-1′	122.1[d]	xylose	
C-2′	130.6	C-1⁗	105.6[c]
C-3′	114.1	C-2⁗	73.0
C-4′	161.6	C-3⁗	76.7
C-5′	114.1	C-4⁗	69.5
C-6′	130.6	C-5⁗	65.8
OMe	55.6	CO-*Me*	20.8
		CO-Me	169.7

[a]Measured in DMSO-d_6.

[b]All carbons were assigned by ^1H-^{13}C and ^1H-^{13}C long-range COSY.

[c,d]Peaks having the same superscripts overlapped.

LITERATURE CITED

1. M. Mizuno, M. Iinuma, T. Tanaka, N. Sakakibara, T. Nakanishi, A. Inada, and M. Nishi, *Chem. Pharm. Bull.*, **37**, 2241 (1989).
2. J.B. Harborne and T.J. Mabry, "The

Flavonoids: Advances in Research," Chapman and Hall, New York, 1983, pp. 37–43.

3. M. Mizuno, M. Iinuma, T. Tanaka, N. Sakakibara, S. Hanioka, and X. Liu, *Chem. Pharm. Bull.*, **36**, 3487 (1988).

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