Kaempferol Glycosides in *Asplenium scolopendrium* Newm.

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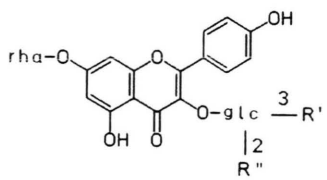
Asplenium scolopendrium, Aspleniaceae, *Phyllitis scolopendrium.* Flavonol Glycoside, Kaempferol 3-O-β-D-glucopyranosyl-(1→3)-β-D-(2-O-caffeoyl)glucopyranoside 7-O-α-L-rhamnopyranoside.

Five kaempferol glycosides in the fronds of *Asplenium scolopendrium* were isolated. The structures of three novel flavonol glycosides were determined to be kaempferol 3-O-β-D-glucopyranosyl-(1→3)-β-D-(2-O-caffeoyl)glucopyranoside 7-O-α-L-rhamnopyranoside, kaempferol 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside and kaempferol 3-O-(2-O-caffeoyl)-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside by means of spectral data (FAB-MS, ^{1}H-^{1}H, ^{1}H-^{13}C COSY and ^{1}H-^{13}C long range COSY), respectively.

Introduction

The morphological similarities of the genus *Asplenium* (Aspleniaceae) to the related genera have disturbed the clear classification of *Asplenium* for others [1]. Our continuous chemotaxonomic studies oriented to the flavonoid compounds on *Asplenium* revealed the existence of two different types; one of which mainly accumulate flavonol glycosides in the fronds such as *A. prolongatum* Hook, *A. incisum* Thunb, etc., the other type produce flavone glycosides in the same part such as *A. normale* Don., *A. normale* var. *boreale* Ohwi ex Kurata [Mizuno *et al.,* unpublished data]. In the present paper, the flavonoid constituents in the fronds of *Asplenium scolopendrium* Newm. which has been once classified in the genus *Phyllitis* are described.



1	R′ = glc	R″ = caffeoyl
2	R′ = glc	R″ = H
3	R′ = H	R″ = caffeoyl
4	R′ = H	R″ = H

Chart 1.

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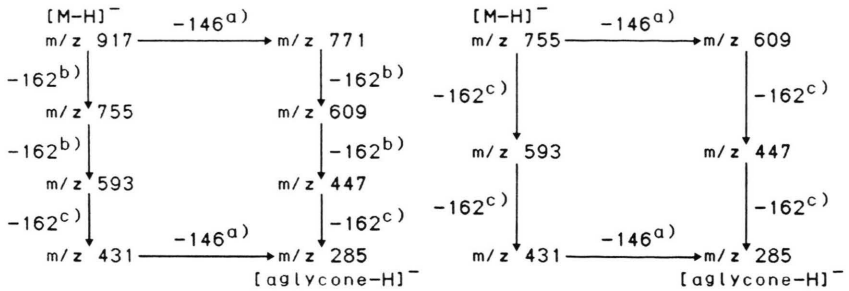
Results and Discussion

By repeated chromatography on silica gel and Sephadex LH 20 of a methanolic extract of *A. scolopendrium,* five compounds **1–5** were isolated.

Compound **1,** m.p. 224–225 °C (decompd), was obtained as a pale yellow powder. The UV spectrum showed absorption bands at 268, 296 and 335 nm. Bathochromic shifts on addition of some diagnostic reagents indicated the presence of free hydroxyl groups at C-5 and C-4′ of a flavonol skeleton [2]. On the basis of a fragment at *m/z* 285 in the FAB-MS, the aglycone moiety of **1** was identified as kaempferol (5,7,4′-trihydroxyflavonol). Negative FAB-MS gave fragment ions shown in Chart 2, which indicated that **1** has a caffeoyl group, and two-glucose and a rhamnose residues. The caffeoyl group is abbreviated afterward as **c** in Experimental. Each component was confirmed by TLC after acid-hydrolysis. An anomeric proton due to rhamnose residue was observed at δ 5.53 as a broad singlet, and due to two-glucose were at δ 4.33 and 5.83 as doublet (each J = 7.7 Hz). The anomeric proton of the rhamnose caused a cross peak with a carbon at δ 160.8 assigned to C-7 of a kaempferol nucleus in the ^{1}H-^{13}C long range COSY, which indicated that the rhamnose is linked with a phenoxyl group at C-7. The glucose-glucose interlinkage was elucidated by the following results; a carbon signal (δ 83.3) assigned to C-3 of an inner glucose showed a down-field shift by *ca.* 7 ppm owing to a glycosylation shift in the ^{13}C NMR, which suggested a terminal glucose shown as (g′) attaches to a hydroxyl group at C-3 of the inner glucose (g). A proton at C-2 of the inner glu-



Compound **1** Compound **3**

a) elimination of rhamnose
b) elimination of glucose or caffeic acid
c) elimination of glucose

Chart 2. The results of the negative ion FAB-MS of compound **1** and **3**.

cose could be assigned to a double doublet like triplet (J = 8.8 and 7.7 Hz) at δ 5.04 in the ^{1}H-^{1}H COSY. In the ^{1}H-^{13}C long range COSY, this signal also caused a cross peak with a carboxyl carbon (δ 165.7) of the caffeoyl moiety, which indicated the caffeoyl group to be attached to the hydroxyl group at C-2 of the inner glucose through an ester bond. The above data showed that a partial structure of **1** was β-D-glucopyranosyl-(1→3)-β-D-(2-O-caffeoyl)glucopyranose and the biose is located at C-3 of the kaempferol nucleus. Therefore, the structure of **1** was concluded to be kaempferol 3-O-β-D-glucopyranosyl-(1→3)-β-D-(2-O-caffeoyl)glucopyranoside.

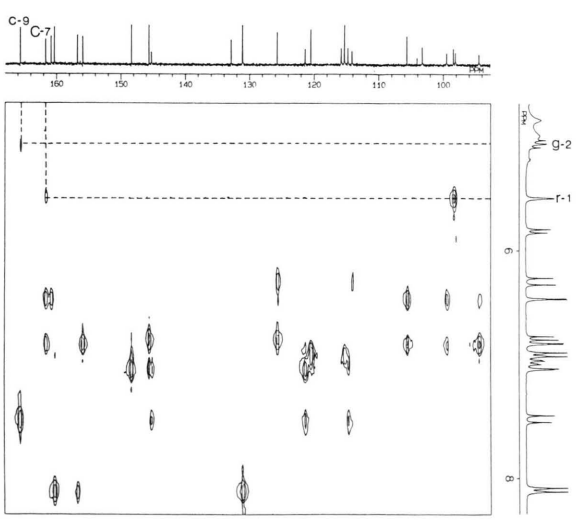


Fig. 1. The ^{1}H-^{13}C long range COSY of compound **1**.

Compound **2,** m.p. 236–237 °C (decompd), obtained as a pale yellow powder was a kaempferol glycoside with free hydroxyl groups at C-5 and C-4′. By its number of aliphatic carbons, the ^{13}C NMR data indicated that **2** possessed two-glucose and a rhamnose residues. Their anomeric protons were observed at δ 5.53 as broad singlet caused by the rhamnose, and at δ 4.38 and 5.58 as doublet (each J = 7.7 Hz) by two-glucose. By comparison of the ^{13}C NMR data of **2** with those of **1,** it was clearly concluded that the rhamnose attached to a phenoxyl group at C-7 and β-D-glucopyranosyl-(1→3)-β-D-glucopyranose, *i.e.* laminaribiose, was at C-3 of the kaempferol nucleus. Therefore, the structure of **2** was determined to be kaempferol 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside.

Compound **3,** $C_{36}H_{36}O_{18}$, obtained as a yellow powder was also a kaempferol glycoside with free hydroxyl groups at C-5 and C-4′. Negative FAB MS gave fragment ions shown in Chart 2, which indicated that **3** has a caffeoyl group, and a glucose and a rhamnose, respectively. Anomeric protons due to the rhamnose and the glucose were observed at δ 5.53 as broad singlet, and δ 5.75 as doublet (J = 8.0 Hz). By comparison of the ^{1}H NMR data of **3** with those of **1,** a proton assignable to H-2 in double doublet like triplet (J = 8.8 and 8.0 Hz) was exhibited at δ 4.87 in a little upper-field than that of **1,** which indicated that a terminal glucose (g′) is lacking in the case of **3.** The above data showed a glycosyl moiety at C-3 was 2-O-caffeoyl-β-D-glucopyranose. Therefore the

structure of **3** was concluded to be kaempferol 3-O-(2-O-caffeoyl)-β-D-glucoside 7-O-α-L-rhamnopyranoside.

Compounds **4** and **5** were the known flavonol glycosides, the structures of which were determined to be kaempferol 3-O-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside and kaempferol 7-O-α-L-rhamnopyranoside, respectively.

A new acylated kaempferol glycoside had been isolated from *Phyllitis scolopendrium* (L.) Newm., the scientific name of which is synonymous to the present material of *Asplenium scolopendrium* [1]. The structure had been determined to be kaempferol 3-O-β-[(4-O-caffeoyl-3-O-β-glucosyl)-glucoside]-7-O-α-L-rhamnoside by means of enzymatic hydrolysis and by analysis of ^{13}C NMR spectral data [3]. The ^{13}C NMR spectral data in the present compound **1,** the chemical shifts of which were absolutely confirmed by ^{1}H-^{1}H and ^{1}H-^{13}C COSY, totally agree with those of the kaempferol glycoside in *P. scolopendrium*. These results mean the structure of **1** must be identical to the glycoside in *P. scolopendrium*. As a conclusion, the structure of the kaempferol gycoside isolated from *P. scolopendrium* is revised to kaempferol 3-O-β-D-glucopyranosyl-(1→3)-β-D-(2-O-caffeoyl)glucopyranoside 7-O-α-L-rhamnopyranoside.

Experimental

The fronds of *Asplenium scolopendrium* Newm. were collected in April, 1987 at Gifu city, Japan, and a voucher specimen has been deposited in the Herbarium of Gifu Pharmaceutical University. Extraction and isolation.

The dried materials of *A. scolopendrium* (70 g) were extracted with MeOH and the extract was concd. The residue was suspended in H_2O, and then extracted successively with EtOAc and *n*-BuOH. The *n*-BuOH layer was concd and chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (45:15:2) as solvent system. The elutes were rechromatographed on silica gel and Sephadex LH-20 (MeOH) for further purification.

Compound **1** *(70 mg) [kaempferol 3-O-β-D-glucopyranosyl-(1→3)-β-D-(2-O-caffeoyl)-glucopyranoside 7-O-α-L-rhamnopyranoside]* EI-MS (rel. int.): *m/z* 286 (100), 258 (9), 153 (7), 121 (22). UV λ_{MeOH}^{max} nm: 268, 296, 335; +$AlCl_3$: 275, 303, 353, 400; +$AlCl_3$ + HCl: 278, 301, 339, 390sh; +AcONa: 268, 296, 337; +AcONa + H_3BO_3: 265, 300sh, 353. ^{1}H NMR (270 MHz, DMSO-d_6) δ: 1.12 (3H, d, *J* = 5.9 Hz, r-Me), 2.9–3.8 (sugar protons), 3.86 (2H, m, g-3 and r-2), 4.33 (1H, d, *J* = 7.7 Hz, g′-1), 5.04 (1H, dd, *J* = 8.8, 7.7 Hz, g-2), 5.52 (1H, br s, r-1), 5.81 (1H, d, *J* = 7.7 Hz, g-1), 6.26 (1H, d, *J* = 15.8 Hz, COC<u>H</u> = CH), 6.42 (1H, d, *J* = 1.8 Hz, H-6), 6.77 (1H, d, *J* = 8.6 Hz, c-3), 6.81 (1H, d, *J* = 1.8 Hz, H-8), 6.92 (2H, d, *J* = 8.8 Hz, H-3′ and 5′), 6.97 (1H, dd, *J* = 8.6, 1.7 Hz, c-H-6), 7.04 (1H, d, *J* = 1.7 Hz, c-H-2), 7.47 (1H, d, *J* = 15.8 Hz, COCH = C<u>H</u>), 8.08 (2H, d, *J* = 8.8 Hz, H-2′ and 6′). ^{13}C NMR (67.5 MHz, DMSO-d_6) δ: 18.0 (r-6), 60.5 (g-6), 61.6 (g′-6), 68.6 (r-5), 69.8 (r-2), 70.0 (r-4), 70.1 (g′-4), 70.3 (g-4), 71.6 (r-3), 72.5 (g-2), 73.3 (g′-2), 76.5 (g′-3), 76.9 (g′-5), 77.3 (g-5), 83.3 (g-3), 94.5 (C-8), 98.2 (g-1), 98.4 (r-1), 99.5 (C-6), 103.3 (g′-1), 105.7 (C-10), 114.1 (c-8), 114.8 (c-2), 115.3 (C-3′ and 5′), 115.8 (c-5), 120.5 (C-1′), 121.4 (c-6), 125.7 (c-1), 131.1 (C-2′ and 6′), 132.9 (C-3), 145.2 (c-7), 145.6 (c-4), 148.4 (c-3), 156.0 (C-2), 156.8 (C-9), 160.3 (C-4′); 160.8 (C-5), 161.7 (C-7), 165.7 (c-9), 177.3 (C-4).

Compound **2** *(20 mg) [kaempferol 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside]* EI-MS *m/z* (rel. int.): 286 (100), 258 (9), 153 (7), 121 (22). UV λ_{MeOH}^{max} nm: 267, 320sh, 347; +NaOMe: 273, 303sh, 345sh, 384; +$AlCl_3$: 276, 302, 350, 394; +$AlCl_3$ + HCl: 276, 302, 347, 394; +AcONa: 267, 349; +AcONa + H_3BO_3: 267, 349. ^{1}H NMR (270 MHz, DMSO-d_6 + D_2O) δ: 1.12 (3H, d, *J* = 5.9 Hz, r-Me), 3.0–4.0 (sugar protons), 4.38 (1H, d, *J* = 7.7 Hz, g′-1), 5.55 (1H, br s, r-1), 5.58 (1H, d, *J* = 7.7 Hz, g-1), 6.45 (1H, d, *J* = 1.8 Hz, H-6), 6.83 (1H, d, *J* = 1.8 Hz, H-8), 6.91 (2H, d, *J* = 8.8 Hz, H-3′ and 5′), 8.07 (2H, d, *J* = 8.8 Hz, H-2′ and 6′). ^{13}C NMR (67.5 MHz, DMSO-d_6) δ: 18.0 (r-6), 60.5 (g-6), 61.1 (g′-6), 68.2 (r-5), 69.8 (r-2), 70.1 (r-4, g′-4 and g-4), 71.6 (r-3), 73.1 (g-2), 73.8 (g′-2), 76.0 (g′-3), 76.9 (g′-5), 77.1 (g-5), 87.2 (g-3), 94.6 (C-8), 98.4 (r-1), 99.5 (C-6), 100.2 (g-1), 104.0 (g′-l), 105.8 (C-10), 115.2 (C-3′ and C-5′), 120.9 (C-1′), 131.0 (C-2′ and C-6′), 133.6 (C-3), 156.1 (C-2), 156.8 (C-9), 160.1 (C-4′), 160.8 (C-5), 161.7 (C-7), 177.6 (C-4).

Compound **3** *(10 mg) [kaempferol 3-O-(2-O-caffeoyl)-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside]* EI-MS *m/z* (rel. int.): 286 (100), 258 (9), 153 (7), 121 (22). ^{1}H NMR (270 MHz, DMSO-d_6 + D_2O) δ: 1.11 (3H, d, *J* = 5.9 Hz, r-Me), 3.0–4.0

(sugar protons), 4.87 (1H, dd, J = 8.0, 8.8 Hz, g-2), 5.52 (1H, br s, r-1), 5.75 (1H, d, J = 8.0 Hz, g-1), 6.28 (1H, d, J = 15.8 Hz, COCH = CH), 6.42 (1H, d, J = 1.8 Hz, H-6), 6.77 (1H, d, J = 8.4 Hz, c-5), 6.81 (1H, d, J = 1.8 Hz, H-8), 6.91 (2H, d, J = 8.8 Hz, H-3′ and 5′), 6.99 (1H, dd, J = 8.4, 1.7 Hz, c-6), 7.05 (1H, d, J = 1.7 Hz, c-2), 7.50 (1H, d, J = 15.8 Hz, COCH = CH), 8.07 (2H, d, J = 8.8 Hz, H-2′ and 6′).

Compound **4** *(15 mg) [kaempferol 3-O-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside]* EI-MS m/z (rel. int.): 286 (100), 258 (12), 153 (8), 121 (22.). UV λ_{MeOH}^{max} nm: 267, 317sh, 350; +NaOMe: 274, 303sh, 344sh, 384; +$AlCl_3$: 275, 300, 349, 397; +$AlCl_3$ + HCl: 276, 300, 347, 397; +AcONa: 267, 320sh, 350; +AcONa + H_3BO_3: 267, 320sh, 350. ^{1}H NMR (270 MHz, DMSO-d_6 + D_2O) δ: 1.12 (3H, d, J = 5.9 Hz, r-Me), 3.0–4.0 (sugar protons), 5.47 (1H, d, J = 7.3 Hz, g-1), 5.56 (1H, br s, r-1), 6.45 (1H, J = 1.8 Hz, H-6), 6.83 (1H, d, J = 1.8 Hz, H-8), 6.90 (2H, d, J = 8.8 Hz, H-3′ and 5′), 8.08 (2H, d, J = 8.8 Hz, H-2′ and 6′).

Compound **5** *(10 mg) [kaempferol 7-O-α-L-rhamnopyranoside]* EI-MS m/z (rel. int.): 286 (100), 258 (9), 153 (7), 121 (22). UV λ_{MeOH}^{max} nm: 268, 327, 360; +NaOMe: 268, 300sh, 364sh, 430; +$AlCl_3$: 268, 305sh, 352, 420; +$AlCl_3$ + HCl: 268, 305sh, 345, 420; +AcONa: 268, 364, 430sh; +AcONa + H_3BO_3: 268, 360, 430sh. ^{1}H NMR (270 MHz, DMSO-d_6) δ: 1.12 (3H, d, J = 5.9 Hz, r-Me), 3.0–4.0 (sugar protons), 5.55 (1H, br s, r-1), 6.43 (1H, d, J = 1.8 Hz, H-6), 6.83 (1H, d, J = 1.8 Hz, H-8), 6.94 (2H, d, J = 8.8 Hz, H-3′ and 5′), 8.09 (2H, d, J = 8.8 Hz, H-2′ and 6′).

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