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FLAVONOL GLYCOSIDES IN THE ROOTS OF *EPIMEDIUM DIPHYLLUM*

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Key Word Index—*Epimedium diphyllum*, Berberidaceae, noranhydroicaritin glycoside; 8-γ,γ-dimethylallylkaempferol 3-*O*-β-D-glucopyranosyl (1→2)-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside; 8-γ,γ-dimethylallylkaempferol 3-*O*-α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside, diphylloside A, diphylloside B

Abstract—Two new 8-γ,γ-dimethylallylkaempferol glycosides, diphyllosides A and B, were isolated from the roots and rhizomes of *Epimedium diphyllum* in addition to four known flavonol glycosides Their structures were established by spectroscopic methods. The difference in chemical constituents between aerial and underground parts of the plant is also described

INTRODUCTION

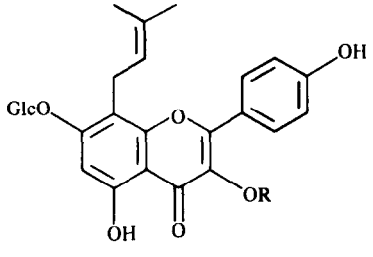
In *Epimedium* species, the aerial parts are mainly utilized as tonic, robust agents, whereas the underground parts are used for treating asthamatic fits and menstrual irregularity. In recent studies of the aerial parts of the plant, 12 flavonol glycosides have been found [1–6]. The constituents of the underground parts (roots and rhizomes) of *E. diphyllum* (Morr. et Decne) Lodd. have now been investigated.

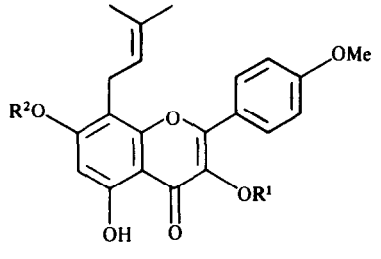
RESULTS AND DISCUSSION

The butanol-soluble portion of the 70% methanolic extract was separated by use of medium pressure liquid chromatography on silica gel to give seven compounds (**1**–**7**).

Compound **1**, mp 197–199°, obtained as a pale yellow powder, was a flavonol glycoside. The aglycone moiety was deduced to be 8-γ,γ-dimethylallylkaempferol (noranhydroicaritin) by the fragment *m/z* 354 ($C_{20}H_{18}O_6$) in the EI mass spectrum, which was supported by the ^{1}H NMR spectral data as follows; a one-proton singlet (6.64 ppm) assignable to the proton at C-6 because of a glycosylation at C-7 [5, 6] and two two-protons doublets (6.96 and 7.86 ppm, J=8.8 Hz) those of C-3′,5′ and C-2′,6′ of kaempferol, the presence of γ,γ-dimethylallyl group substituted at C-8 was confirmed by the peaks of two three-protons singlets at 1.63 and 1.71 ppm ($-CMe_2$), a two-protons multiplet at 3.04 ppm ($C\underline{H}_2$–CH=C), a one-proton triplet at 5.2 ppm (J=6.2 Hz) (CH_2–$C\underline{H}$=C<).

In the ^{1}H NMR spectrum, the conspicuous differences of **1** compared with sagittatoside A (anhydroicaritin 3-*O*-β-D-glucosyl-(1→2)-α-L-rhamnoside [6] were that the chemical shift of H-6 shifted towards downfield by 0.3 ppm and no peak due to methoxyl group was observed. The data showed that sugars are substituted at both C-3 and at C-7 of noranhydroicaritin. The UV spectrum also supported the location of the sugars, since bathochromic shifts indicated that the C-5 and C-4′ hydroxyls were unsubstituted.





1 R = Rha—(2)—Glc (diphylloside A)
2 R = Rha—(2)—Rha (diphylloside B)
3 R = Rha (epimedoside A)
4 R = Rha—(2)—Xyl (epimedoside E)

5 R^1 = R^2 = H (anhydroicaritin)
6 R^1 = Rha, R^2 = Glc (icarin)
7 R^1 = Rha—(2)—Rha R^2 = Glc (epimedin C)

Concerning the sugar moieties, three anomeric protons based on two β-glucose and one α-rhamnose were observed in two one-proton doublets at 4.28 ($J = 7.7$ Hz) and 5.18 ppm ($J = 6.8$ Hz), and a one proton broad singlet at 5.60 ppm. Assignment of these sugars, and their interlinkage were determined on the basis of the ^{13}C NMR spectral data by comparison with those of sagittatoside A and epimedin A (anhydroicaritin 3-*O*-β-D-glucosyl-(1→2)-α-L-rhamnoside-7-*O*-β-D-glucoside [4] (Table 1). The result was that one β-glucose is attached at C-7-OH and the other two at C-3-OH as β-D-glucosyl-(1→2)-α-L-rhamnose. By hydrolysis with β-glucosidase, **1** gave sagittatoside A after cleavage of β-glucose of C-7. Consequently **1** is 8-γ,γ-dimethylallylkaempferol 3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside-7-*O*-β-D-glucopyranoside and is named diphylloside A.

Compound **2**, mp 190–191°, was obtained as a pale yellow powder. The EI mass and the UV spectral data were closely similar to those of diphylloside A. In the ^1H NMR, three anomeric protons based on two α-L-rhamnoses and one β-D-glucose were observed in two one-proton broad singlets at 4.93 and 5.44 ppm, and a one-proton doublet at 5.03 ppm ($J = 6.2$ Hz). As in the case of **1**, the sugar moieties were determined by comparison of the ^{13}C NMR spectra with those of epimedin C (anhydroicaritin-3-*O*-L-rhamnosyl-(1→2)-α-L-rhamnoside-7-*O*-β-D-glucoside (**7**) [4, 6]. Therefore **2** is 8-γ,γ-dimethylallyl-kaempferol 3-*O*-α-L-rhamnopyranosyl (1→2)-α-L-rhamnoside-7-*O*-β-D-glucopyranoside and is named diphylloside B.

Compound **3** (233–233°), **4** (248–250°), **6** (224–226°) and **7** (141°) were obtained as pale yellow powders from the butanol soluble portion. From spectroscopic evidence, **3**–**7** were established as the known flavonol glycosides, epimedoside A [2], epimedoside E [3], icariin [1] and epimedin C [4], respectively. Furthermore, from the chloroform-soluble portion, **5** (232–233°) was obtained as yellow needles. The structure was determined to be anhydroicaritin by spectroscopic evidence. Compound **5** corresponds to the aglycone of **6** and **7**. The flavonol HPLC glycosides of the underground organs were compared by HPLC with those of the aerial parts (Table 2) and these are clear differences in content.

EXPERIMENTAL

Details of the spectral and chromatographic apparatus used were described in our previous paper [6].

Table 1. ^{13}C NMR chemical shifts of flavonol glycosides (**1**–**4**)

C	1	2	3	4
2	157.6	157.9	157.8	157.6
3	134.7	134.4	134.3	134.3
4	178.3	178.3	178.3	178.3
5	160.5	160.5	160.2	160.4
6	98.2	98.3	98.1	98.1
7	160.5	160.6	160.5	160.5
8	108.4	108.5	108.3	108.4
9	153.1	153.1	152.0	152.9
10	106.2	105.7	105.6	105.5
1′	122.3	120.5	122.3	122.3
2′	130.7	130.8	130.7	130.6
3′	115.6	115.7	115.5	115.5
4′	159.2	159.3	159.1	159.1
5′	115.6	115.7	115.5	115.5
6′	130.7	130.8	130.7	130.6
11	21.6	21.6	21.5	21.5
12	122.3	122.3	122.0	122.0
13	131.1	131.3	131.1	131.1
14	25.6	25.6	25.5	25.5
15	17.5	15.0	17.5	17.4
1″	101.1	100.7	101.9	100.9
2″	81.4	75.9	70.4	80.6
3″	70.5	70.9	70.8	70.6
4″	71.8	72.2	71.2	70.5
5″	70.8	71.6	70.1	70.0
6″	18.0	17.8	17.9	17.7
1‴	105.6	105.7		105.5
2‴	73.9	70.3		73.8
3‴	77.3	70.8		76.2
4‴	69.4	71.6		69.3
5‴	76.7	70.0		65.8
6‴	60.6	17.7		
1⁗	100.7	100.8	100.6	100.6
2⁗	73.4	73.5	73.4	73.4
3⁗	76.7	76.8	76.6	76.6
4⁗	69.8	69.0	69.7	69.7
5⁗	77.3	77.3	77.0	77.2
6⁗	60.8	60.8	60.7	60.7

All spectra were measured in DMSO-d_6. The carbons shown with two, three and four primes represent those of the endo- and exo-sugar at C-3, and of the β-D-glucose at C-7 respectively.

Table 2. Concentrations (area %) and retention times (min) of flavonol glycosides **1**–**9**

Compound	Underground parts		Aerial parts	
1	6.3	(12.6)		
2	16.0	(14.2)	—	
3	18.5	(14.9)	1.6	(14.9)
4	17.3	(13.4)	1.2	(13.4)
5	—		—	
6			10.2	(25.0)
7	14.9	(23.5)	15.5	(23.5)
8	1.7	(21.3)	6.6	(21.3)
9	1.4	(22.3)	10.6	(22.3)

*Anhydroicaritin (**5**) could not be detected with this solvent system.

Plant material The roots and rhizomes of *Epimedium diphyllum* were collected in November, 1986 at Chisyanoki, Miyazaki prefecture, Japan, and a voucher specimen is deposited in the Herbarium of Gifu Pharmaceutical University, Gifu-city, Japan

Extraction and isolation The dried and cut underground parts (4 3 kg) of *E. diphyllum* were extracted ×3 with 70% MeOH (18 1) at room temp. for one week The combined extracts were concd The residue (630 g) was suspended in H_2O, and extracted successively with $CHCl_3$, EtOAc and *n*-BuOH The *n*-BuOH fraction (70 g) was chromatographed on silica gel (eluent: $CHCl_3$–MeOH–H_2O 13 7 2, lower phase) Recryst gave **1** (200 mg), **2** (300 mg), **3** (1 5 g), **4** (500 mg), **6** (700 mg) and **7** (150 mg), respectively The $CHCl_3$ fraction (42 g) was also subjected to chromatography on silica gel using EtOAc–*n*-hexane (1 1) From an earlier fraction (*ca* 300 ml), **5** (150 mg) was obtained

Compound **1** (*diphylloside A*) A yellow powder (MeOH), mp 197–199°, $C_{38}H_{48}O_{20}$, UV λ_{max}^{MeOH} nm 269, 322, 352, +NaOMe 269, 394, +$AlCl_3$. 280, 307, 352, 410, +$AlCl_3$–HCl 280, 308, 346, 409, +NaOAc 269, 375, +NaOAc–H_3BO_3 269, 321, 352 EIMS (*m/z*) (rel. int) 354 (100), 339 (90), 299 (54), 286 (48), 165 (13), 121 (44) ^{1}H NMR (DMSO-d_6, 270 MHz): δ 0 89 (3H, d, J = 5 9 Hz, rham-Me), 1 63, 1.71 (each 3H, s, $C_{14,15}$-Me), 3 00–3 90 (sugar protons), 3 04 (1H, *m*, H-11), 4 14 (1H, *s*, *br d*, rham H-2), 4.28 (1H, *d*, J = 7 7 Hz, glc H-1), 5.02 (1H, *d*, J = 6 2 Hz, glc H-1), 5.18 (1H, *t*, J = 6 8 Hz, H-12), 5 60 (1H, *br s*, rham H-1), 6 64 (1H, *s*, H-6), 6.96 (2H, *d*, J = 8 8 Hz, H-3′,5′), 7 83 (2H, *d*, J = 8 8 Hz, H-2′,6′), 12 45 (1H, *s*, C_5-OH)

Compound **2** (*diphylloside B*) A yellow powder (MeOH), mp 190–191°, $C_{38}H_{48}O_{19}$ UV λ_{max}^{MeOH} nm. 269, 320, 350, +NaOMe· 269, 392, +$AlCl_3$ 279, 352, 408, +$AlCl_3$–HCl. 280, 347, 406, +NaOAc 269, 385, +NaOAc–H_3BO_3 269, 318, 349. EIMS (*m/z*) (rel int.) 354 (100), 339 (87), 299 (53), 286 (44), 165 (12), 121 (43). ^{1}H NMR (DMSO-d_6, 270 MHz) δ 0 87 (3H, *d*, J = 4.8 Hz, rham-Me), 1 15 (3H, *d*, J = 5 9 Hz, rham-Me), 1.63, 1 72 (each 3H, s, $C_{14,15}$-Me), 3 18 (2H, *m*, H-11), 3.35–4 80 (sugar protons), 4 17 (1H, *br s*, rham H-2), 4.93 (1H, *br s*, rham H-1), 5.03 (1H, *d*, J = 6.2 Hz, glc H-1), 5 19 (1H, *br t*, J = 6 9 Hz, H-12), 5.44 (1H, *br s*, rham H-1), 6 66 (1H, *s*, H-6), 6 97 (2H, *d*, J = 8 5 Hz, H-3′,5′), 7 83 (2H, *d*, J = 8 5 Hz, H-2′,6′), 12 69 (1H, *br s*, C_5-OH)

Compound **3** (*epimedoside A*) A pale yellow powder (EtOH), mp 233–235°, $C_{32}H_{38}O_{15}$ UV λ_{max}^{MeOH} nm 270, 320, 352, +NaOMe 270, 390, +$AlCl_3$ 280, 352, 410, +$AlCl_3$–HCl. 279, 345, 410, +NaOAc 269, 386, +NaOAc–H_3BO_3: 269, 319, 350. EIMS (*m/z*) (rel int) 354 (100), 339 (53), 299 (36), 286 (29), 165 (9), 121 (31). ^{1}H NMR (DMSO-d_6, 270 MHz) δ 0 79 (3H, *d*, J = 5 1 Hz, rham-Me), 1 60, 1 68 (each 3H, *s*, $C_{14,15}$-Me), 3 17 (2H, *m*, H-11), 3.99 (1H, *br s*, rham H-2), 3 20–5 40 (sugar protons), 4 97 (1H, *d*, J = 6 1 Hz, glc H-1), 5 15 (1H, *br t*, J = 6.2 Hz, H-12), 5.29 (1H, *br s*, rham H-1), 6 61 (1H, *s*, H-6), 6 93 (2H, *d*, J = 8 8 Hz, H-3′,5′), 7 79 (2H, *d*, J = 8 8 Hz, H-2′,6′), 10.26 (1H, *s*, C_4-OH), 12 60 (1H, *s*, C_5-OH)

Compound **4** (*epimedoside E*). A pale yellow powder (EtOH), mp 248–250°, $C_{37}H_{46}O_{19}$ UV λ_{max}^{MeOH} nm 269, 321, 349, +NaOMe· 269, 391, +$AlCl_3$ 280, 352, 408, +$AlCl_3$–HCl· 280, 346, 405, +NaOAc 269, 383, +NaOAc–H_3BO_3 269, 319, 348 EIMS (*m/z*) (rel int). 354 (27), 339 (24), 299 (13), 286 (11), 165 (2), 121 (9), 73 (100) ^{1}H NMR (DMSO-d_6, 270 MHz) δ 0 90 (3H, *d*, J = 6 2 Hz, rham-Me), 1 63, 1 71 (each 3H, *s*, $C_{14,15}$-Me), 3 00–4 90 (sugar protons), 3 10 (2H, *m*, H-11), 4 04 (1H, *br s*, rham H-2), 4 20 (1H, *d*, J = 7 7 Hz, xyl H-1), 4 99 (1H, *d*, J = 7 3 Hz, glc H-1), 5 19 (1H, *br t*, J = 6 9 Hz, H-12), 5 40 (1H, *br s*, rham H-1), 6 63 (1H, *s*, H-6), 6 96 (2H, *d*, J = 8 8 Hz, H-3′,5′), 7 81 (2H, *d*, J = 8 8 Hz, H-2′,6′), 12 64 (1H, *s*, C_5-OH)

Compound **5** (*anhydroicaritin*) Yellow needles (EtOAc–C_6H_{14}), mp 232–233°, $C_{21}H_{20}O_6$ UV λ_{max}^{MeOH} nm 222sh, 272, 313, 354, 430, +NaOMe 291, 325sh, 430, +$AlCl_3$ 242, 315, 361, 420sh, +$AlCl_3$–HCl· 242, 315, 361, 420sh, +NaOAc 288, 434 EIMS (*m/z*) (rel int) 368 (100), 353 (78), 313 (59), 300 (44), 165 (11), 135 (33) ^{1}H NMR (DMSO-d_6, 270 MHz) δ 1 64, 1 75 (each 3H, *s*, $C_{14,15}$-Me), 3 43 (2H, *m*, H-11), 3 84 (3H, *s*, OMe), 5 17 (1H, *br t*, J = 6 6 Hz, H-12), 6 29 (1H, *s*, H-6), 7 12 (2H, *d*, J = 9 2 Hz, H-3′,5′), 8.13 (2H, *d*, J = 9 2 Hz, H-2′,6′), 9 45 (1H, s, C_7-OH), 10 73 (1H, *s*, C_3-OH), 12 36 (1H, *s*, C_5-OH)

Compound **6** (*icarin*) *and* **7** (*epimedin C*) The spectroscopic data were described in refs [1, 4, 6]

Enzymatic hydrolysis of **1** A boric acid buffer soln (pH 5.0) containing **1** (12 mg) and β-glucosidase (500 units) (3 mg) was incubated at 38° for 24 hr. After filtration, the soln was compared on HPLC with sagittatoside A isolated from *E sagittatum* [6]

A quantitative analysis of the flavonol glycosides in E diphyllum. According to the same conditions of HPLC as our previous paper [6], the flavonol glycosides both in the underground and in the aerial parts were quantitatively determined. The results are shown in Table 2

During the preparation of this article, the same structure as diphylloside A (**1**) was reported as a constituent of *Epimedium glandiflorum*, and named ikarisoside C [7]

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