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FLAVONOL GLYCOSIDES FROM *EPIMEDIUM SAGITTATUM*

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Key Word Index—*Epimedium sagittatum*, Berberidaceae, anhydroicaritin 3-*O*-β-D-glucosyl-(1→2)-α-L-rhamnoside, anhydroicaritin 3-*O*-β-D-xylosyl-(1→2)-α-L-rhamnoside, anhydroicaritin 3-*O*-β-glucosyl-(1→2)-α-L-3-acetylrhamnoside, sagittatoside A, sagittatoside B; sagittatoside C

Abstract—Three new flavonol glycosides, designated sagittatosides A, B and C, were isolated from the aerial parts of *Epimedium sagittatum* in addition to epimedins A, B and C Their structures were established by spectroscopic methods

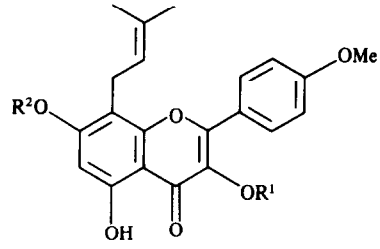
INTRODUCTION

Two new flavonol glycosides, anhydroicaritin 3-*O*-α-rhamnoside and icaritin 3-*O*-α-rhamnoside together with icarisid I and icariin were shown as the constituents of *Epimedium sagittatum* in our previous paper [1]. Further investigation of the aerial parts of *E sagittatum* revealed three new flavonol glycosides (**1**–**3**), sagittatosides A, B and C, in addition to epimedins A, B and C (**4**–**6**). In this paper their structural elucidation is described.

RESULTS AND DISCUSSION

By repeated separation of 35% ethanolic extract of the aerial parts of *Epimedium sagittatum* by use of preparative middle pressure liquid chromatography, compounds **1**–**6** were isolated as pure forms, and deduced to be flavonol glycosides from the Shinoda test

Compound **1**, mp 168–169° was obtained as a pale yellow amorphous powder. The structure of the aglycone moiety was deduced from the ^{1}H NMR spectrum The presence of two two-proton doublets at 7 12 ppm (J = 8.4 Hz) and 7 87 (J = 8.4 Hz) and a one-proton singlet at 6.31 ppm suggested that the aglycone must be based on kaempferol with a substituent carbon linked at C-8. The characteristic signals based on an isopentenyl group as the substituent were observed at 1.62 and 1.81 ppm in each three-proton singlet, 3 13 ppm in a two-proton multiplet and 5 15 ppm in a one-proton broad triplet (J = 5.0 Hz). Since the above ^{1}H NMR spectrum further showed the presence of a methoxy group, the aglycone must be anhydroicaritin. The EI mass spectrum supported the proposed structure from the fragments (*m/z* 368, 353, 313, 165 $[A_1-C_4H_7]^+$ and 135 $[B_2]^+$) formed after retro-Diels–Alder cleavage In the UV spectrum, bathochromic shifts were observed 61 nm of Band I and 10 nm of Band II on addition of aluminium chloride and of sodium acetate, respectively, which indicated not only that the phenolic groups at C-5 and C-7 were unsubstituted, but also that the sugars were substituted only at C-3. The position of the sugars is clear from the ^{1}H NMR spectrum in which the chemical shift of proton at C-6 appeared at *ca* 6.64 ppm in the case of the flavonol glycosides substituted by glucose at C-7 like icarisid I (**8**) and icariin (**9**) [1] On the other hand the relevant shift of **1** was observed at a rather higher field at 6 36 ppm, due to protection of the phenolic group. The ^{13}C NMR spectrum showed that two hexoses were attached at C-3 (Table 1). The chemical shifts as well as those of anomeric protons in the ^{1}H NMR spectrum; a one-proton doublet at 5.53 ppm (J = 1 6 Hz) (rhamnose) and a one-proton doublet at 4 24 ppm (J = 7.1 Hz) indicated that they were α-L-rhamnose and β-D-glucose The nature of sugars was confirmed by acid hydrolysis. The sequence of the sugars was determined as follows, a glycosylation shift was



	R¹	R²	
1	Rha —(2)— Glc	H	(sagittatoside A)
2	Rha —(2)— Xyl	H	(sagittatoside B)
3	Rha —(2)— Glc (3 Ac)	H	(sagittatoside C)
4	Rha —(2)— Glc	Glc	(epimedin A)
5	Rha —(2)— Xyl	Glc	(epimedin B)
6	Rha —(2)— Rha	Glc	(epimedin C)
7	H	Glc	(icarisid I)
8	Rha	H	(icarisid II)
9	Rha	Glc	(icariin)

Table 1 ^{13}C NMR chemical shifts of flavonol glycosides (**1–6**)

C	1	2	3	4	5	6
2	156 4	156 4	156 7	157 8	157 3	157 1
3	134 6	134 4	133 4	134 8	134 8	134 4
4	177 8	177 9	177 8	178 2	178 5	178 1
5	161 6	161 3	161 7	160 4	160 6	160 4
6	98 2	98 4	98 3	98 2	98 5	98 0
7	161 2	162 0	161 4	161 4	161 5	161 3
8	105 9	106 2	105 9	108 2	108 5	108 2
9	153 6	153 6	153 7	152 9	153 1	152 9
10	104 0	103 5	104 0	105 9	105 7	105 4
1′	122 2	122 3	122 1	122 0	122 1	122 0
2′	130 3	130 2	130 4	130 4	130 5	130.4
3′	114 0	114 1	114 1	114 0	114 3	114 0
4′	158 7	158 8	158 7	159 0	159 2	159 0
5′	114 0	114 1	114 1	114 0	114 3	114 0
6′	130 3	130 2	130 4	130 4	130 5	130 4
11	21 0	21 1	21 2	21 3	21 5	21 3
12	122 1	122 3	122 2	122 0	122 3	122 0
13	130 9	130 9	130 9	130 9	131 2	131 0
14	25 3	25 3	25 3	25 3	25 6	26 3
15	17 2	17 7	17 0	17 3	17 9	17 4
1″	100 9	100 9	100 8	100 9	101 2	100 4
2″	81 1	80 5	76 9	81 2	80 8	75 4
3″	70 1	70 2	71 2	70 3	70 6	70 3
4″	71 6	71 6	68 1	71 5	71 8	71 8
5″	71 1	69 2	69 8	70 0	69 8	70 0
6″	17 6	17 3	17 7	17 7	17 5	17 5
1‴	105 8	105 9	104 6	105 5	105 7	105 1
2‴	73 7	73 6	73 0	73 8	73 5	70 3
3‴	76 2	76 1	76 3	77 1	76 3	70 5
4‴	69 2	70 1	69 3	69 1	70 6	71 2
5‴	76 5	65 7	76 8	76 5	65 7	70 1
6‴	60 4		61 0	60 3		17 5
1⁗				100 5	100 7	100 6
2⁗				73 3	73 5	73 2
3⁗				76 5	76 7	76 5
4⁗				69 5	69.8	69 5
5⁗				79 0	77 3	77 1
6⁗				60 5	60 8	60 5
OMe	55 4	55 5	55.5	55 5	55 6	55 4
Ac			169.0			
			20 7			

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

observed at C-2‴ (81 1 ppm) of rhamnose compared with that of icarin (70 4 ppm) which showed β-D-glucose attached to C-2‴ of α-L-rhamnose, i e the sugar moiety is β-D-glucosyl-(1 → 2)-α-rhamnose Thus, **1** is anhydroicaritin 3-*O*-β-D-glucosyl-(1 − 2)-α-L-rhamnoside and is named sagittatoside A

Compound **2**, mp 160°, was obtained as a yellow powder. The EI mass fragments due to the aglycone moiety and the bathochromic shifts on addition to aluminium chloride or sodium acetate were closely similar to those of **1**. In the ^{13}C NMR spectrum, all chemical shifts agreed well with those of **1** except those of the terminal sugar, which appeared as five peaks at 105 9, 76 1, 73 1, 70 1 and 65 7 ppm These chemical shifts correspond to those of β-D-xylose In the ^{1}H NMR spectrum, two anomeric protons were also observed at 4 16 ppm (J = 7 5 Hz) and 5 31 ppm (J = 1 2 Hz), which were assignable to that of β-D-xylose and α-L-rhamnose, respectively Furthermore, the chemical shifts of **2** are coincident to those of epimedin B (**5**) except for the carbons of one glucose substituted at C-7 The carbon of C-8 in **2** appeared at a higher field (2 3 ppm) compared with that of **5**, which also supports the absence of a sugar at C-7. An enzymatic hydrolysis of **5** with β-glucosidase gave **2** Thus **2** is anhydroicaritin 3-*O*-β-xylosyl-(1 → 2)-α-L-rhamnoside and is named sagittatoside B.

Compound **3**, mp 143–144 , obtained as a yellow powder, was confirmed to possess an acetyl group by spectroscopic evidence, m/z 43 (mass), 1 95 ppm (s) (^{1}H NMR), and 20 7 and 169 0 ppm (^{13}C NMR) On hydrolysis of **3** in alkali medium, sagittatoside A was detected by HPLC The position of acetyl group was determined by comparison of the chemical shifts in the ^{13}C NMR, the signals based on C-2 and C-4 of the L-rhamnose moiety shifted by −4 2 ppm and −3 5 ppm compared with those of **1** The acetyl group was concluded to substituted at C-3 of the rhamnose by the low-fielded shift caused by acetylation [3] Consequently, **3** is anhydroicaritin 3-*O*-β-D-glucosyl-(1 − 2)-α-L-3-acetyl-rhamnoside, and is named sagittatoside C

Compounds **4** (mp 167), **5** (mp 172–174) and **6** (mp 141) were obtained as pale yellow powders. Their aglycones were the same as those of **1**–**3** by their ^{1}H NMR and mass spectra. The UV and ^{13}C NMR (Table 1) spectral data suggested that **4**–**6** were the known flavonol glycosides, epimedins A, B and C, isolated from *Epimedium koreanum* [2] All six compounds **1**–**6** could be separated and quantified by HPLC on a cosmosil 5C$_{18}$ column with gradient elution (see Experimental)

EXPERIMENTAL

Details of the apparatus used were described in our previous paper [1]

Extraction and isolation of flavonol glycosides An 35% ethanolic extract of *Epimedium sagittatum*, described in detail in our previous paper [1], was subjected to a medium pressure (2–3 atom) liquid chromatography (eluent $CHCl_3$–MeOH 5 1 by gradient on silica gel column, acetonitrile (25%) by linear gradient on octadecylsilylated silica gel column) By combination of both column chromatography and recrystallization, compound **1** (20 mg), **2** (100 mg), **3** (15 mg), **4** (60 mg), **5** (50 mg) and **6** (200 mg) were obtained

Acid hydrolysis of **1** A 3% H_2SO_4 soln (3 ml) of **1** (2 mg) was heated under reflux for 2 hr The soln was neutralized with $BaCO_3$, and the filtrate was subjected to TLC (eluent $CHCl_3$-MeOH–H_2O = 13 7 2 lower phase) D-glucose and L-rhamnose detected in the soln by spraying with 0 2% naphthoreresorcinol-ethanol (1 1) (heating 105)

Enzymatic hydrolysis A boric acid buffer soln (pH 5 0) containing epimedin B(**5**) (10 mg) and β-glucosidase (500 units) (2 mg) was incubated at 37 for 24 hr The soln was compared by TLC ($CHCl_3$–MeOH–H_2 = 13 7 2, lower phase) with sagittatoside B

Alkaline hydrolysis of **3** A 0 05 M NaOH soln (1 ml) containing **3** (2 mg) was warmed (60) for 1 hr After neutralization with 0 05 M HCl, the soln was subjected to HPLC (condition as undermentioned) to be confirmed the presence of **1**

HPLC equipment Liquid chromatograph Shimadzu LC-6A Conditions. column, cosmosil 5C$_{18}$ (Nakarai chemicals Ltd) 250 mm × 4 6 d , flow rate, 1 2 ml min solvent acetonitril gra-

dient, detection, UV 272 nm, chart speed, 2 mm/min.

Compound **1** (*sagittatoside A*) $C_{33}H_{40}O_{15}$, UV λ_{max}^{MeOH} nm. 271, 299sh, 345, +NaOMe 284, 380, +$AlCl_3$ 280, 307sh, 345, 406, +$AlCl_3$/HCl 281, 305sh, 340, 402, +AcONa 281, 314sh, 340, +AcONa/H_3BO_3 271, 372. EIMS (*m/z*) (rel. int.) 368 (aglycone, 89), 353 (aglycone−Me, 78), 313 (aglycone−C_4H_7, 100), 300 (49), 165 (22), 135 (62) ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.86 (3H, *d*, *J* = 5 9 Hz, rhamnosyl Me), 1 62, 1 81 (each 3H, *s*, $C_{14\ 15}$, Me), 2 98–4 93 (sugar protons), 3 13 (2H, *m*, H-11), 3.84 (3H, *s*, OMe), 4 09 (1H, *m*, rham, H-2), 4 24 (1H, *d*, *J* = 7 1 Hz, glc, H-1), 5 15 (1H, *br t*, *J* = 5 0 Hz, H-12), 5 53 (1H, *d*, *J* = 1 6 Hz, rham, H-1), 6 31 (1H, *s*, H-6), 7 12 (2H, *d*, *J* = 8 4 Hz, 3′, 5′), 7 87 (2H, *d*, *J* = 8 4 Hz, H-2′,6′), 10 83 (1H, *s*, C_7–OH), 12.40 (1H, *s*, C_5–OH)

Compound **2** (*sagittatoside B*). $C_{32}H_{38}O_{14}$ UV λ_{max}^{MeOH} nm· 273, 310sh, 350, +NaOMe· 282, 380, +$AlCl_3$ 282, 308sh, 344, 405, +$AlCl_3$/HCl· 282, 308sh, 344, 405, +AcONa 282, 345, +AcONa/H_3BO_3 272, 340 EIMS (*m/z*) (rel int.) 368 (aglycone, 63), 353 (aglycone − Me, 60), 313 (aglycone − C_4H_7, 38), 300 (35), 165 (13), 135 (45) ^{1}H NMR (DMSO-d_6, 300 MHz)· δ 0.88 (3H, *d*, *J* = 5 2 Hz, rhamnosyl Me), 1 63, 1 69 (each 3H, *s*, $C_{14,15}$, Me) 2 80–4 93 (sugar protons), 3 09 (2H, *m*, H-11), 3.85 (3H, *s*, OMe), 4 01 (1H, *br s*, rham, H-2), 4 16 (1H, *d*, *J* = 7 5 Hz, xyl, H-1), 5 14 (1H, *br t*, *J* = 5.2 Hz, H-12), 5 31 (1H, *d*, *J* = 1 2 Hz, rham, H-1), 6 33 (1H, *s*, H-6), 7 13 (2H, *d*, *J* = 7 7 Hz, H-3′, 5′), 7.85 (2H, *d*, *J* = 7 7 Hz, H-2′, 6′), 12.56 (1H, *s*, C_5–OH).

Compound **3** (*sagittatoside C*) $C_{35}H_{42}O_{16}$· UV λ_{max}^{MeOH} nm 270, 314 sh, 350, +NaOMe. 281, 380, +$AlCl_3$ 280, 308, 348, 410, +$AlCl_3$/HCl 280, 307, 340, 410, +AcONa: 281, 340, +AcONa/H_3BO_3. 269, 312, 350 EIMS (*m/z*) (rel int)· 368 (aglycone, 100), 353 (aglycone−Me, 78), 313 (aglycone −C_4H_7, 50), 300 (44), 165 (11), 153 (47), 43 (82). ^{1}H NMR (DMSO-d_6, 300 MHz). δ 0.84 (3H, *d*, *J* = 5 5 Hz, rhamnosyl Me), 1 63, 1.68 (each 3H, *s*, $C_{14,15}$, Me), 1.95 (3H, *s*, COMe), 2 98–4 96 (sugar protons), 3 20 (2H, *m*, H-11), 3.86 (3H, *s*, OMe), 4 23 (1H, *d*, *J* = 6 7 Hz, glc, H-1), 5 17 (1H, *br t*, *J* = 5.0 Hz, H-12), 5.39 (1H, *br s*, rham, H-1), 6.33 (1H, *s*, H-6), 7.16 (2H, *d*, *J* = 8.4 Hz, H-3′, 5′), 7 89 (2H, *d*, *J* = 8.4 Hz, H-2′, 6′), 12 45 (1H, *s*, C_5–OH)

Compound **4–6** Properties and spectra identical to those reported earlier [2] For ^{13}C NMR data, see Table 1.

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