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Studies on the Glycosides of *Epimedium grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI. III[1)]

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Ten new glycosides, icarisides A_2—A_4, B_5—B_7, E_3, F_1—F_2 and G_1, were isolated from the polar fraction of the water extract of *Epimedium grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI, together with three known glycosides, phenethyl glucoside, (*Z*)-3-hexenyl glucoside and blumenol C glucoside. Their structures were established on the basis of chemical evidence and spectral data.

Keywords——*Epimedium grandiflorum* var. *thunbergianum*; 9,10-dihydrophenanthrenol glycoside; bibenzyl glycoside; ionone derivative; lignan; icariside A; icariside B; icariside E; icariside F; icariside G

In our previous papers,[1,2)] we reported the structures of some glycosides isolated from the aerial parts of *Epimedium grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI. We now wish to report the structures of ten new glycosides, icarisides A_2 (**3**), A_3 (**4**), A_4 (**5**), B_5 (**7**), B_6 (**8**), B_7 (**9**), E_3 (**10**), F_1 (**11**), F_2 (**12**) and G_1 (**13**), which were isolated together with three known glycosides, phenethyl glucoside (**1**), (*Z*)-3-hexenyl glucoside (**2**) and blumenol C glucoside (**6**), from the polar fraction of the water extract of *E. grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI. The structures of these compounds were determined on the basis of chemical evidence and spectroscopic studies.

Compound **1** (phenethyl glucoside)[3)] and **2** [(*Z*)-3-hexenyl glucoside)][4)] were identified by comparison of various data with reported values, and compound **6** (blumenol C glucoside)[5)] was identified by comparison of the spectral data of its aglycone, derived from enzymatic hydrolysis of **6**, with those of blumenol C.[6)]

Icariside A_2 (**3**), $C_{23}H_{28}O_{10}\cdot 1/2H_2O$, $[\alpha]_D$ $-49.1°$, was obtained as an amorphous powder. The ultraviolet (UV) spectrum showed absorption maxima at 256 (sh 4.04), 263 (sh 4.16), 272 (4.20) and 296 (sh 3.02) nm ($\log\varepsilon$), suggesting the presence of a 9,10-dihydrophenanthrene skeleton.[1,7)] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum exhibited three methoxyl signals at δ 3.81, 3.85 and 3.99 (each 3H, s) and three aromatic proton signals at δ 7.48 (1H, br s), 6.90 (1H, d, $J=9$ Hz) and 6.94 (1H, d, $J=9$ Hz), and a benzylic methylene proton signal at δ 2.58 (4H, br s), which is characteristic of 9,10-dihydrophenanthrene.[1,7)] Enzymatic hydrolysis afforded an aglycone acetate **3b**, mp 164—166 °C, followed by acetylation of an aglycone **3a**. In the ^{1}H-NMR spectrum of **3b**, the nuclear Overhauser effect (NOE) was observed at the proton signal at δ 6.90 (1H, d, $J=8$ Hz) (10%) on irradiation at the methoxyl signal at δ 3.85, and long-range couplings were observed between the proton signals at δ 6.75 (1H, br s); 7.09 (1H, br d, $J=8$ Hz) and the methylene proton signal at δ 2.62 (4H, br s). In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **3**, two methoxyl carbon signals were shifted downfield at δ 61.5 and 62.2, suggesting that these

methoxyl groups were di*ortho*-substituted.[8] An anomeric carbon signal revealed a normal chemical shift (δ 102.5) for a phenolic glucoside without steric hindrance. The structure of icariside A_2 was therefore concluded to be **3**.

Icariside A_3 (**4**), $C_{23}H_{28}O_{10}\cdot 1/2H_2O$, $[\alpha]_D$ +23.0°, was obtained as an amorphous powder. The UV spectrum suggested that this compound was also a 9,10-dihydrophenanthrene derivative [233 (sh 4.27), 261 (sh 4.03), 272 (sh 4.15), 280 (4.21), 300 (4.10), 312 (4.09) nm (log ε)].[1,7] The ^{1}H-NMR spectrum exhibited three methoxyl signals and three singlet-like aromatic proton signals. NOEs were observed at the proton signals at δ 6.70 (1H, br s) (13%) on irradiation at the methoxyl signal at δ 3.76, and at δ 8.82 (1H, s) (15%), which was shifted downfield by the effect of a neighboring aromatic ring, on irradiation at the methoxyl signal at δ 3.94. Furthermore, long-range couplings were observed between a methylene proton signal at δ 2.68 (4H, br s) and the aromatic proton signals at δ 6.70; 7.12. Hydrolysis with acetyl chloride–methanol (1 : 20) afforded an aglycone acetate (**4b**), mp 152.5—153.5 °C, after acetylation of the aglycone (**4a**). Compound **4b** was identical with 4,7-diacetoxy-2,3,6-trimethoxy-9,10-dihydrophenanthrene.[7] In the ^{13}C-NMR spectrum of **4**, an anomeric carbon signals was shifted downfield at δ 104.1 (Δ +1.5 ppm) compared with that of icariside A_1 (**4c**), due to steric hindrance. The structure of icariside A_3 was therefore concluded to be **4**.

Icariside A_4 (**5**), $C_{22}H_{28}O_{10}\cdot 1/2H_2O$, $[\alpha]_D$ −27.2°, was obtained as an amorphous

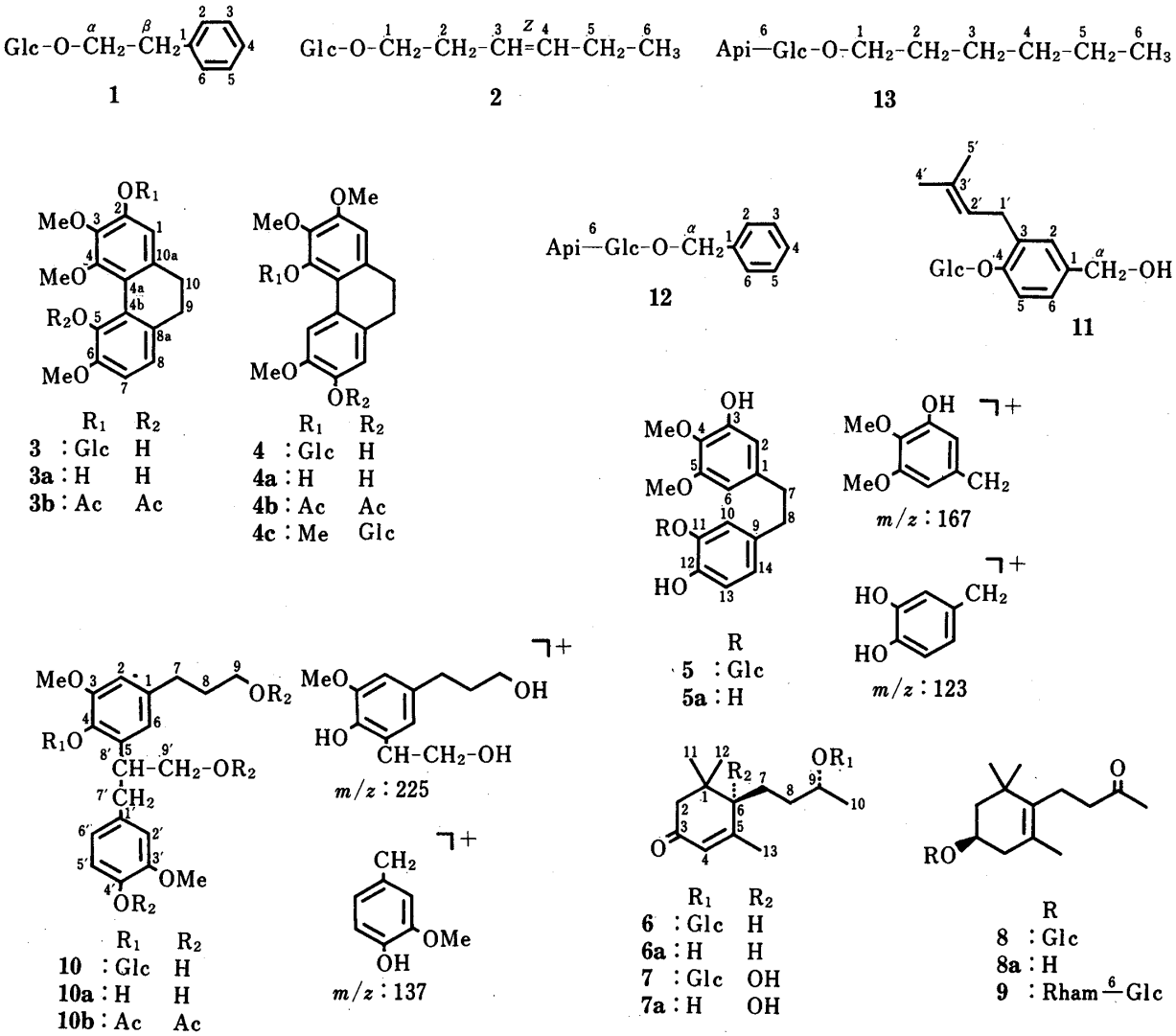
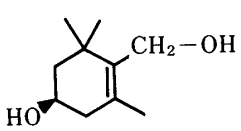
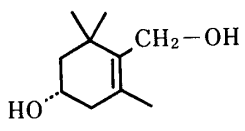


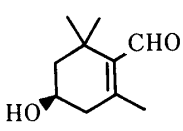
Chart 1



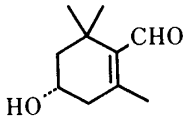
14
$[\alpha]_D^{25}$ −125.6° (dioxane)



15
$[\alpha]_D^{25}$ +125.0° (dioxane)



16
$[\alpha]_D^{25}$ −83.5° (chloroform)



17
$[\alpha]_D^{25}$ +82.1° (chloroform)

Chart 2

TABLE I. ^{1}H-NMR Chemical Shifts and Coupling Constants

Proton No.	**3**[a]	**3a**[b]	**3b**[b]	**4**[a]	**4a**[a]	**4b**[b]
1	7.48 (1H, br s)	6.76 (1H, br s)	6.75 (1H, br s)	6.70 (1H, br s)	6.52 (1H, br s)	6.73 (1H, br s)
5				8.82 (1H, s)	8.79 (1H, s)	7.59 (1H, s)
7	6.90 (1H, d, J=9 Hz)	6.79 (overlapped)	6.90 (1H, d, J=8 Hz)			
8	6.94 (1H, d, J=9 Hz)	6.79 (overlapped)	7.09 (1H, br d, J=8 Hz)	7.12 (1H, br s)	7.58 (1H, br s)	6.90 (1H, br s)
9, 10	2.58 (4H, br s)	2.63 (4H, br s)	2.62 (4H, br s)	2.68 (4H, br s)	2.78 (4H, br s)	2.73 (4H, br s)
OMe	3.81 (3H, s)	3.76 (3H, s)	3.50 (3H, s)	3.76 (3H, s)	3.82 (3H, s)	3.87 (6H, s)
	3.85 (3H, s)	3.91 (3H, s)	3.85 (3H, s)	3.94 (3H, s)	3.90 (3H, s)	3.91 (3H, s)
	3.99 (3H, s)	3.99 (3H, s)	3.92 (3H, s)	4.07 (3H, s)	3.91 (3H, s)	
OAc			2.24 (3H, s)			2.33 (3H, s)
			2.34 (3H, s)			2.35 (3H, s)
Anomeric	5.74 (1H, d, J=7 Hz)			6.39 (1H, d, J=7 Hz)		

Run at 89.55 MHz in *a*) pyridine-d_5 and *b*) $CDCl_3$ solution.

TABLE II. ^{13}C-NMR Chemical Shifts

Carbon No.	**3**	**3a**	**4**	**4a**
Aglycone moiety				
1	112.8[a]	113.6	108.5	103.9
2	149.5[b]	149.6[d]	152.2	149.4
3	142.2	140.7	134.7	131.4
4	151.4[b]	151.7[d]	147.4	147.7[g]
4a	137.1	137.5	131.7	[h]
4b	133.8	133.0	141.7	[h]
5	150.6[b]	150.6[d]	114.2[f]	113.8
6	145.2	144.9	146.6	146.3[g]
7	111.9[a]	111.6	146.6	146.7[g]
8	118.8	118.9	115.5[f]	115.9
8a	121.0[c]	121.9[e]	122.8	123.0
9, 10	31.0, 31.8	31.2, 31.6	29.7, 31.4	29.7, 31.3
10a	121.5[c]	118.3[e]	[h]	[h]
OMe	56.6, 61.5, 62.2	56.5, 61.0, 62.0	56.0, 56.4, 61.1	55.8, 56.3, 60.5
Glucose moiety				
1	102.5		104.1	
2	75.0		76.1	
3	79.1		78.5	
4	71.5		71.6	
5	78.8		78.3	
6	62.6		62.1	

Run at 22.5 MHz in pyridine-d_5 solution. *a*—*g*) Assignments may be interchanged in each column. *h*) Overlapped with solvent signals.

TABLE III. ^{1}H- and ^{13}C-NMR Data

	5[a]	5[b]	5[a]	5[b]	5a[b]	5a[b]
Aglycone moiety						
1			136.1	135.9[d]		135.8[e]
2	6.31 (1H, d, J=2 Hz)	6.23 (1H, d, J=1.5 Hz)	110.6	110.3	6.26 (1H, d, J=2 Hz)	110.1
3			152.0	152.8		151.1
4			138.5	139.2		139.4
5			154.0	154.2		154.1
6	6.87 (1H, d, J=2 Hz)	6.29 (1H, d, J=1.5 Hz)	105.3	105.6	6.33 (1H, d, J=2 Hz)	105.4
7, 8	2.88 (4H, s)	2.74 (4H, br s)	37.6, 38.5	38.3, 39.2	2.72 (4H, s)	38.4, 39.3
9			133.9	135.0[d]		134.9[e]
10	7.56 (1H, d, J=2 Hz)	6.93 (1H, br s)	120.3	119.5	6.62 (1H, d, J=2 Hz)	116.2[f]
11			147.7[c]	146.4		144.2
12			146.7[c]	146.4		146.0
13	7.22 (1H, d, J=8 Hz)	6.72 (1H, s)	117.2	116.8	6.67 (1H, d, J=8 Hz)	116.7[f]
14	6.96 (1H, dd, J=8, 2 Hz)	6.70 (1H, s)	124.7	124.8	6.50 (1H, dd, J=8, 2 Hz)	120.8
OMe	3.79 (3H, s)		56.1	56.4	3.76 (3H, s)	56.3
	3.89 (3H, s)		60.6	61.1	3.78 (3H, s)	61.0
Glucose moiety						
1	5.46 (1H, d, J=7 Hz)		104.6	104.7		
2			75.2	75.0		
3			79.0	78.3		
4			71.4	71.4		
5			78.4	77.7		
6			62.4	62.5		

Run at 89.55 and 22.5 MHz (^{1}H- and ^{13}C-NMR) in *a*) pyridine-d_5 and *b*) CD_3OD solution. *c*—*f*) Assignments may be interchanged in each column.

TABLE IV. ^{1}H-NMR Chemical Shifts and Coupling Constants

Proton No.	6[a]	6a[b]	7[a]	7a[b]
2	2.13 (1H, d, J=17 Hz)	2.03 (1H, d, J=17 Hz)	2.41 (1H, d, J=18 Hz)	2.25 (1H, dd, J=18, 1 Hz)
	2.47 (1H, d, J=17 Hz)	2.37 (1H, d, J=17 Hz)	2.83 (1H, d, J=18 Hz)	2.51 (1H, d, J=18 Hz)
4	5.92 (1H, br s)	5.82 (1H, br s)	6.00 (1H, br s)	5.84 (1H, t, J=1 Hz)
9		3.75 (1H, m, $W_{1/2}$=19 Hz)		3.82 (1H, m, $W_{1/2}$=19 Hz)
10	1.38 (3H, d, J=6 Hz)	1.20 (3H, d, J=6 Hz)	1.36 (3H, d, J=6 Hz)	1.23 (3H, d, J=6 Hz)
11	0.94 (3H, br s)	1.02 (3H, s)	1.23 (6H, br s)	1.05 (3H, br s)
12	0.98 (3H, br s)	1.07 (3H, s)		1.09 (3H, s)
13	1.87 (3H, d, J=1.3 Hz)	2.00 (3H, d, J=1.2 Hz)	2.15 (3H, br s)	2.03 (3H, d, J=1 Hz)
Anomeric	4.95 (1H, d, J=7.5 Hz)		4.93 (1H, d, J=7.5 Hz)	

Proton No.	8[a]	8a[b]	9[a]
3		3.92 (1H, m, $W_{1/2}$=23 Hz)	
10	2.14 (3H, s)	2.14 (3H, s)	2.10 (3H, s)
11, 12	0.91 (3H, br s)	1.03 (6H, s)	0.96 (3H, br s)
	0.94 (3H, br s)		1.04 (3H, br s)
13	1.52 (3H, br s)	1.59 (3H, br s)	1.48 (3H, br s)
Anomeric	5.10 (1H, d, J=8 Hz)		4.96 (1H, d, J=7.5 Hz)
			5.44 (1H, br s)
Methyl of rhamnose			1.59 (3H, d, J=6 Hz)

Run at 89.55 MHz in *a*) pyridine-d_5 and *b*) $CDCl_3$ solution.

TABLE V. ^{13}C-NMR Chemical Shifts and Coupling Constants

	6[a]	6a[b]	7[a]	7a[b]	8[a]	8a[a]	9[a]
Aglycone moiety							
1	36.6	36.2	42.6	41.7	38.0	37.9	38.0
2	47.9	47.2	50.9	50.0	47.0	49.6	47.1
3	198.9	199.2	197.9	197.8	71.9[f]	64.2	72.0[i]
4	125.4	125.1	126.5	126.0	39.6	43.3	39.7
5	165.8	165.3	168.8	167.4	125.3	125.9	125.2
6	51.4	51.1	78.3	77.8	136.9	135.8	136.9
7	25.8	26.3	34.0[c]	33.5[d]	22.4	22.3	22.3
8	36.8	38.7	32.5[c]	33.4[d]	44.4	44.3	44.4
9	76.2	68.2	76.9	68.7	208.2	207.7	208.1
10	22.2	23.6	22.3	23.8[e]	29.9[g]	29.7[h]	29.8[j]
11	27.3	27.0	24.3	23.8[e]	28.4[g]	28.4[h]	28.5[j]
12	28.9	28.8	24.7	23.9[e]	29.8[g]	29.7[h]	29.7[j]
13	24.7	24.5	21.9	20.9	19.9	19.8	19.8
Glucose moiety							
1	104.1		104.4		102.6		103.2 (154 Hz)
2	75.3		75.4		75.4		75.3
3	78.6		78.7		78.6		78.7
4	71.6		71.9		71.8[f]		72.3[i]
5	78.3		78.3		78.5		76.9
6	63.0		63.0		63.0		68.4
Rhamnose moiety							
1							102.4 (167 Hz)[10]
2							72.8[i]
3							72.7[i]
4							74.1
5							69.8
6							18.8

Run at 22.5 MHz in *a*) pyridine-d_5 and *b*) $CDCl_3$ solution. *c*—*j*) Assignments may be interchanged in each column.

powder. The UV spectrum showed absorption maxima at 278 (3.45) and 286 (sh 3.30) nm (log ε). The ^{1}H-NMR spectrum exhibited a benzylic methylene proton signal at δ 2.88 (4H, s), two methoxyl proton signals at δ 3.79 and 3.89 (each 3H, s), an anomeric proton signal at δ 5.46 (1H, d, J=7 Hz), AB-type proton signals at δ 6.31 (1H, d, J=2 Hz) and 6.87 (1H, d, J=2 Hz), and ABX-type proton signals at δ 6.96 (1H, dd, J=8, 2 Hz), 7.22 (1H, d, J=8 Hz) and 7.56 (1H, d, J=2 Hz). From these data, this compound was assumed to be a bibenzyl derivative.[9] Acid hydrolysis afforded glucose as the sugar moiety, while enzymatic hydrolysis afforded an aglycone **5a**, whose mass spectrum (MS) showed a molecular ion peak at m/z 290 in agreement with the molecular formula $C_{16}H_{18}O_5$ and ion peaks at m/z 167 and 123 due to β-cleavage. The UV spectrum of **5a** exhibited absorption maxima at 279 (3.78), 290 (sh 3.63), 305 (sh 3.34) and 313 (sh 3.31) nm (log ε) and was shifted at 286, 297 sh, 315 sh and 321 sh nm by the addition of $NaOAc+H_3BO_3$, suggesting an *ortho*-diphenol structure. The ^{13}C-NMR spectrum of **5** showed glycosylation shifts at C-10 (*ortho*) (Δ+*ca.* 3 ppm), C-11 (*C*-1) (Δ+*ca.* 2 ppm) and C-14 (*para*) (Δ+*ca.* 4 ppm) compared with those of **5a**, but little shift at C-9 (*meta*) or C-13 (*meta*), suggesting that glucose was attached to C-11.[10] The structure of icariside A_4 was therefore concluded to be **5**.

Icariside B_5 (**7**), $[\alpha]_D$ −12.9°, was obtained as an amorphous powder. The fast atom bombardment mass spectrum (FAB-MS) exhibited an ion peak at m/z 389 $(C_{19}H_{32}O_8+H)^+$. The ^{1}H-NMR spectrum revealed a singlet methyl proton signal at δ 1.23 (6H, s), a doublet methyl proton signal at δ 1.36 (3H, d, J=6 Hz), a vinyl methyl proton signal at δ 2.15 (3H,

TABLE VI. ^{1}H- and ^{13}C-NMR Data

	10[a]	**10**[b]	**10a**[c]	**10a**[b]	**10b**[c]	**10b**[b]
Aglycone moiety						
1		139.1[d]		133.0		138.9[f]
2		113.5	7.09 (1H, d, J=1 Hz)	113.8	6.96 (1H, br s)	113.9
3		152.6		149.3		g)
4		143.3		146.7[e]		g)
5		139.7[d]		133.0		140.3[f]
6		122.3	7.14 (1H, d, J=1 Hz)	g)	7.00 (1H, br s)	123.1
7	2.86 (2H, dd, J=9, 7 Hz)	33.0	2.85 (2H, t, J=7 Hz)	32.7	2.64 (2H, t, J=7 Hz)	32.5
8	2.10 (2H, m)	35.7	2.11 (2H, qui, J=7 Hz)	36.0	1.91 (2H, m)	30.7
9	3.94 (2H, t, J=6 Hz)	61.6	3.94 (2H, t, J=7 Hz)	61.5	4.14 (2H, t, J=6 Hz)	63.9
1′		132.5		130.5		135.0
2′		111.4	6.81 (1H, d, J=1 Hz)	110.3	6.83 (1H, br s)	115.0
3′		148.3		148.2		g)
4′		146.1		146.0[e]		g)
5′		116.1	7.11 (1H, d, J=8 Hz)	116.1	6.92 (1H, d, J=8 Hz)	119.9
6′		120.0	7.03 (1H, dd, J=8, 1 Hz)	121.4	7.12 (1H, dd, J=8, 1 Hz)	121.6
7′		39.2	3.37 (1H, dd, J=14, 8 Hz) 3.63 (1H, dd, J=14, 6 Hz)	37.3	3.14 (2H, m)	38.2
8′		42.4	4.3 (3H, m)	44.7	3.89 (1H, qui, J=7 Hz)	39.9
9′		67.0		65.3	4.51 (2H, d, J=7 Hz)	67.1
OMe	3.66 (3H, s)	55.9	3.68 (3H, s)	55.8	3.71 (3H, s)	55.9
	3.69 (3H, s)	56.1	3.69 (3H, s)	55.9	3.72 (3H, s)	56.0
OAc					1.96 (3H, s), 2.03 (3H, s), 2.22 (3H, s), 2.38 (3H, s)	20.5 × 2, 20.9 × 2, 168.9, 169.1, 170.8 × 2
Sugar moiety						
1	5.42 (1H, d, J=7 Hz)	105.8				
2		76.2				
3		78.5				
4		71.2				
5		78.3				
6		62.5				

a) Run at 89.55 MHz in pyridine-d_5 solution. *b*) Run at 22.5 MHz in pyridine-d_5 solution. *c*) Run at 399.65 MHz in pyridine-d_5 solution. *d*—*f*) Assignments may be interchanged in each column. *g*) Overlapped with solvent signals.

TABLE VII. ^{1}H- and ^{13}C-NMR Data

	11[a]	**11**[b]	**12**[a]	**12**[b]	**13**[a]	**13**[b]
Aglycone moiety						
1		137.0		138.9	3.67 (2H, m)	69.9
2	6.60 (1H, d, J=2 Hz)	128.5		128.7	1.57 (2H, m)	30.3
3		131.8[c]		128.7		26.2
4		155.3	7.25—7.65 (5H, m)	127.9	1.16 (6H, m)	32.0
5	6.65 (1H, d, J=9 Hz)	115.7		128.7		22.9
6	6.47 (1H, dd, J=9, 2 Hz)	125.9		128.7	0.78 (3H, t, J=7 Hz)	14.3
α	4.95 (2H, br s)	64.2	4.89 (1H, d, J=12 Hz) 5.22 (1H, d, J=12 Hz)	71.0		
1′	3.72 (2H, br d, J=7.5 Hz)	29.0				
2′	5.60 (1H, m)	123.6				
3′		131.2[c]				
4′	1.63 (6H, br s)	25.7				
5′		17.7				
Glucose moiety						
1	5.63 (1H, d, J=8 Hz)	102.9	4.93 (1H, d, J=7 Hz)	103.7	4.82 (1H, d, J=7.5 Hz)	104.7
2		74.9		75.1		75.2
3		78.6		78.6		78.6
4		71.2		72.0		72.0
5		78.6		77.3		77.2
6		62.4		69.1		69.1
Apiose moiety						
1			5.84 (1H, d, J=2.5 Hz)	111.2	5.84 (1H, d, J=2.5 Hz)	111.2
2			4.81 (1H, d, J=2.5 Hz)	78.0	4.80 (1H, d, J=2.5 Hz)	78.0
3				80.6		80.6
4			4.40 (1H, d, J=9.5 Hz) 4.61 (1H, d, J=9.5 Hz)	75.1	4.39 (1H, d, J=9.5 Hz) 4.61 (1H, d, J=9.5 Hz)	75.2
5			4.20 (2H, s)	65.7	4.19 (2H, s)	65.7

a) Run at 89.55 MHz in pyridine-d_5 solution. *b*) Run at 22.5 MHz in pyridine-d_5 solution. *c*) Assignments may be interchanged.

br s), and an olefinic proton signal at δ 6.00 (1H, br s). In the ^{13}C-NMR spectrum, nineteen carbon signals were observed, including six carbon signals due to a glucopyranosyl moiety. From a comparison of these NMR data with those for icarisides B_1—B_4, this compound was assumed to be a glucoside of an ionone derivative.[1,2] The circular dichroism (CD) spectrum displayed positive Cotton effects, $[\theta]_{218}$ +42800, $[\theta]_{324}$ +5700, and a negative Cotton effect, $[\theta]_{250}$ −30800.[11] Enzymatic hydrolysis afforded an aglycone **7a** which was identical with blumenol B.[12] In the ^{13}C-NMR spectrum of **7**, an anomeric carbon signal was shifted to δ 104.4, suggesting that glucose was attached to a secondary hydroxyl group.[10] The structure of icariside B_5 was therefore concluded to be **7**.

Icariside B_6 (**8**), $C_{19}H_{32}O_7 \cdot 1/4H_2O$, $[\alpha]_D$ −67.0°, was obtained as colorless needles, mp 143—144 °C. Icariside B_7 (**9**), $C_{25}H_{42}O_{11}$, $[\alpha]_D$ −78.3°, was also obtained as colorless needles, mp 202—203 °C. These two glycosides revealed similar ^{1}H-NMR spectra (Table IV) and afforded the same aglycone **8a**, $[\alpha]_D$ −84.4°, by enzymatic hydrolysis, while acid hydrolysis afforded glucose as the sugar moiety of **8** and rhamnose–glucose (1 : 1) as the sugar moiety of **9**. The ^{1}H-NMR spectrum of **8a** showed the presence of two quaternary methyl at δ 1.03 (6H, s), a vinyl methyl at δ 1.59 (3H, br s), an acetyl methyl group at δ 2.14 (3H, s) and a carbinyl proton at δ 3.92 (1H, m, $W_{1/2}$=23 Hz), indicating that this compound was also an ionone derivative.[1,2] In the ^{13}C-NMR spectrum of **8**, an anomeric carbon signal (δ 102.6) suggested that glucose was attached to a secondary hydroxyl group without steric hindrance.[11] From

the above data, **8a** was assumed to be 3-hydroxy-dihydro-β-ionone. The absolute stereochemistry of C-3 was decided as *R* by comparison of $[\alpha]_D$ with that of similar compounds (**14**—**17**) (Chart 2).[13] The structure of icariside B_6 was therefore concluded to be **8**, and that of icariside B_7 to be **9** by comparison of its ^{13}C-NMR spectrum with that of **8**.

Icariside E_3 (**10**), $C_{26}H_{36}O_{11} \cdot 1/2H_2O$, $[\alpha]_D$ $-61.3°$, was obtained as an amorphous powder. The UV spectrum showed an absorption maximum at 278 (3.66) nm ($\log \varepsilon$). The ^{1}H-NMR spectrum exhibited three methylene proton signals at δ 2.10 (2H, m), 2.86 (2H, dd, J= 9, 7 Hz) and 3.94 (2H, t, J=6 Hz), two methoxyl proton signals at δ 3.66 and 3.69 (each 3H, s) and the anomeric proton signal at δ 5.42 (1H, d, J=7 Hz). The MS of an aglycone **10a**, obtained by enzymatic hydrolysis, showed a molecular ion peak at *m/z* 362 and ion peaks at *m/z* 137 and 225 due to β-cleavage. In the ^{1}H-NMR spectrum of **10b**, derived by acetylation of **10a**, NOEs were observed at an aromatic proton signal at δ 6.96 (1H, br s) (15%), which was coupled with a proton signal at δ 7.00 (1H, br s), and at δ 6.83 (1H, br s) (19%), which was coupled with proton signals at δ 7.12 (1H, dd, J=8, 1 Hz) on irradiation of the methoxyl signals. Therefore, C-5 was substituted. In the ^{13}C-NMR spectrum of **10**, glycosylation shifts were observed at C-1 (*para*) (Δ+*ca.* 6 ppm), C-3 (*ortho*) (Δ+*ca.* 3 ppm), C-4 (*C*-1) (Δ−*ca.* 3 ppm) and C-5 (*ortho*) (Δ+*ca.* 6 ppm) compared with those of **10a**, and an anomeric carbon signal was shifted downfield at δ 105.8 due to steric hindrance. The structure of icariside E_3 was therefore concluded to be **10**.

Icariside F_1 (**11**), $C_{18}H_{26}O_7 \cdot 1/2H_2O$, $[\alpha]_D$ $-50.0°$, was obtained as an amorphous powder. The UV spectrum showed an absorption maximum at 266 (3.56) nm ($\log \varepsilon$). The ^{1}H-NMR spectrum suggested the presence of a γ,γ-dimethyl allyl group [δ 1.63 (6H, br s), 3.72 (2H, br d, J=7.5 Hz), 5.60 (1H, m)], a hydroxymethyl group [δ 4.95 (2H, br s)] and a 1,2,4-trisubstituted aromatic ring [δ 6.46 (1H, dd, J=9, 2 Hz), 6.60 (1H, d, J=2 Hz), 6.65 (1H, d, J=9 Hz)]. The chemical shifts of an anomeric proton and carbon (δ 5.63 and 102.9, respectively) suggested that this compound was a phenolic glucoside. Long-range couplings were observed between an aromatic proton signal at δ 6.60 and two methylene proton signals at δ 3.72 and 4.95, and between an aromatic proton signal at δ 6.47 and a methylene proton (of a hydroxymethyl group) signal at δ 4.95. These NMR data led us to conclude that the structure of icariside F_1 was **11**.

Icariside F_2 (**12**), $C_{18}H_{26}O_{10} \cdot 1/2H_2O$, $[\alpha]_D$ $-97.6°$, was obtained as an amorphous powder. The ^{1}H-NMR spectrum showed AB-type proton signals due to a benzylic methylene group at δ 4.89 and 5.22 (J=12 Hz) and a multiplet proton signal due to a phenyl group at δ 7.25—7.65 (5H). Acid hydrolysis afforded apiose and glucose as the sugar moiety. In the ^{13}C-NMR spectrum of **12**, the sp^2 carbon signals were similar to those of benzyl glucoside,[2] while C-6 of glucose was shifted downfield at δ 69.1. Apiose was thus attached to the C-6 of glucose as in the case of icariside D_1.[1] The structure of icariside F_2 was concluded to be **12**.

Icariside G_1 (**13**), $[\alpha]_D$ $-95.1°$, was obtained as a colorless viscous oil. The FAB-MS revealed an ion peak at *m/z* 419 $(C_{17}H_{32}O_{10}+Na)^+$. The ^{1}H-NMR spectrum showed a triplet methyl signal at δ 0.79 (3H, t, J=7 Hz) and a methylene proton signal due to a long chain at δ 1.16 (6H, m), suggesting that this compound had an *n*-alkyl group. Acid hydrolysis afforded apiose and glucose as the sugar moiety, while enzymatic hydrolysis afforded *n*-hexanol as an aglycone. In the ^{13}C-NMR spectrum of **13**, the carbon signals due to the sugars were very similar to those of **12**. The structure of icariside G_1 was therefore concluded to be **13**.

Although the existence of flavonol glycosides, lignans and an alkaloid in *Epimedium* spp. has been described, our reports[1,2] are the first to describe of the isolation of 9,10-dihydrophenanthrenol glycosides and terpenic glycosides.

Experimental

Melting points were taken with a Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical

rotations were determined with a JASCO DIP-140 digital polarimeter. UV spectra were run on a Shimadzu UV-360 recording spectrometer. MS and FAB-MS were measured with JEOL JMS-D100 and DX-303 mass spectrometers, respectively. CD spectra were recorded on a JEOL J-20A spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL FX-90Q (89.55 and 22.5 MHz, respectively) and JEOL GX-400 (399.65 MHz) NMR spectrometers. Chemical shifts are given on the δ scale with tetramethylsilane as the internal standard (s, singlet; d, doublet; t, triplet; qui, quintet; m, multiplet; br, broad). Gas chromatography (GC) was carried out on a Hitachi K53 gas chromatograph. High-performance liquid chromatography (HPLC) was performed with a Kyowa Seimitsu model K880 instrument.

Isolation——Aerial parts of *E. grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI (15 kg), collected in summer 1985, in Niigata Prefecture, Japan, were extracted twice with hot water. The extract was absorbed on Amberlite XAD-2 and the resin was eluted with methanol after being washed with water. Following repeated chromatography of the methanol eluate (420 g) on silica gel with a chloroform–methanol system and HPLC (column: Develosil ODS-10, Develosil Ph-10) with a water–acetonitrile or water–methanol system, thirteen glycosides were isolated.

Phenethyl Glucoside (1)[3]——Amorphous powder (350 mg), $[\alpha]_D^{21}$ −36.6° (c=1.16, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 3.03 (2H, t, J=7 Hz, H_2-β), 4.92 (1H, d, J=7.5 Hz, H-1′), 7.30 (5H, s, aromatic H). ^{13}C-NMR (pyridine-d_5) δ: 36.8 (C-β), 63.0 (C-6′), 70.7 (C-α), 71.8 (C-4′), 75.2 (C-2′), 78.6 (C-3′, C-5′), 104.8 (C-1′), 126.6 (C-4), 128.8; 129.5 (C-2, C-6/C-3, C-5), 139.5 (C-1).

(*Z*)-3-Hexenyl Glucoside (2)[4]——Amorphous powder (350 mg), $[\alpha]_D^{21}$ −35.5° (c=2.75, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 0.95 (3H, t, J=7.5 Hz, H_3-6), 1.92 (2H, m, H_2-5), 2.46 (2H, m, H_2-2), 3.72 (2H, m, H_2-1), 4.89 (1H, d, J=7.5 Hz, H-1′), 5.48 (2H, m, H-3, H-4). ^{13}C-NMR (pyridine-d_5) δ: 14.5 (C-6), 21.0 (C-5), 28.5 (C-2), 62.9 (C-6′), 69.6 (C-1), 71.8 (C-4′), 75.3 (C-2′), 78.5 (C-5′), 78.6 (C-3′), 104.7 (C-1′), 125.6 (C-3), 133.7 (C-4).

Blumenol C Glucoside (6)[5]——Amorphous powder (330 mg), $[\alpha]_D^{22}$ +46.8° (c=0.63, MeOH). *Anal.* Calcd for $C_{19}H_{32}O_7 \cdot 1/2H_2O$: C, 59.82; H, 8.72. Found: C, 60.06; H, 8.71. UV λ_{max}^{MeOH} nm (log ε): 240 (3.98). CD (c=0.0048, MeOH) [θ] (nm): +14800 (241).[11] ^{1}H- and ^{13}C-NMR: Tables IV and V.

Icariside A_2 (3)——Amorphous powder (200 mg), $[\alpha]_D^{22}$ −49.1° (c=0.55, MeOH). *Anal.* Calcd for $C_{23}H_{28}O_{10} \cdot 1/2H_2O$: C, 58.34; H, 6.17. Found: C, 58.10; H, 5.97. UV λ_{max}^{MeOH} nm (log ε): 256 (sh 4.04), 263 (sh 4.16), 272 (4.20), 296 (sh 3.02). ^{1}H- and ^{13}C-NMR: Tables I and II.

Icariside A_3 (4)——Amorphous powder (250 mg), $[\alpha]_D^{20}$ +23.0° (c=1.61, MeOH). *Anal.* Calcd for $C_{23}H_{28}O_{10} \cdot 1/2H_2O$: C, 58.34; H, 6.17. Found: C, 58.08; H, 5.97. UV λ_{max}^{MeOH} nm (log ε): 233 (sh 4.27), 261 (sh 4.03), 272 (sh 4.15), 280 (4.21), 300 (4.10), 312 (4.09). ^{1}H- and ^{13}C-NMR: Tables I and II.

Icariside A_4 (5)——Amorphous powder (40 mg), $[\alpha]_D^{20}$ −27.2° (c=1.84, MeOH). *Anal.* Calcd for $C_{22}H_{28}O_{10} \cdot 1/2H_2O$: C, 57.26; H, 6.33. Found: C, 56.96; H, 6.32. UV λ_{max}^{MeOH} nm (log ε): 227 (sh 4.20), 278 (3.45), 286 (sh 3.30). No bathochromic shift was observed on addition of NaOAc + H_3BO_3. ^{1}H- and ^{13}C-NMR: Table III.

Icariside B_5 (7)——Amorphous powder (40 mg), $[\alpha]_D^{25}$ −12.9° (c=0.62, MeOH). FAB-MS m/z: 389 $(M+1)^+$. UV λ_{max}^{MeOH} nm (log ε): 243 (3.85). CD (c=0.0034, MeOH) [θ] (nm) +42800 (218), −30800 (250), +5700 (324).[11] ^{1}H- and ^{13}C-NMR: Tables IV and V.

Icariside B_6 (8)——Colorless needles from ethyl acetate (100 mg), mp 143—144 °C, $[\alpha]_D^{22}$ −67.0° (c=0.88, MeOH). *Anal.* Calcd for $C_{19}H_{32}O_7 \cdot 1/4H_2O$: 60.54; H, 8.69. Found: C, 60.76; H, 8.59. ^{1}H- and ^{13}C-NMR: Tables IV and V.

Icariside B_7 (9)——Colorless needles from methanol (160 mg), mp 202—203 °C, $[\alpha]_D^{21}$ −78.3° (c=2.30, MeOH). *Anal.* Calcd for $C_{25}H_{42}O_{11}$: C, 57.90; H, 8.16. Found: C, 57.79; H, 8.16. ^{1}H- and ^{13}C-NMR: Tables IV and V.

Icariside E_3 (10)——Amorphous powder (280 mg), $[\alpha]_D^{22}$ −61.3° (c=0.80, MeOH). *Anal.* Calcd for $C_{26}H_{36}O_{11} \cdot 1/2H_2O$: C, 58.53; H, 6.99. Found: C, 58.81; H, 6.90. UV λ_{max}^{MeOH} nm (log ε): 278 (3.66). ^{1}H- and ^{13}C-NMR: Table VI.

Icariside F_1 (11)——Amorphous powder (315 mg), $[\alpha]_D^{23}$ −50.0° (c=1.11, MeOH). *Anal.* Calcd for $C_{18}H_{26}O_7 \cdot 1/2H_2O$: C, 59.49; H, 7.49. Found: C, 59.53; H, 7.28. UV λ_{max}^{MeOH} nm (log ε): 266 (3.59). ^{1}H- and ^{13}C-NMR: Table VII.

Icariside F_2 (12)——Amorphous powder (190 mg), $[\alpha]_D^{22}$ −97.6° (c=0.41, MeOH). *Anal.* Calcd for $C_{18}H_{26}O_{10} \cdot 1/2H_2O$: C, 52.55; H, 6.62. Found: C, 52.43; H, 6.42. ^{1}H- and ^{13}C-NMR: Table VII.

Icariside G_1 (13)——Colorless viscous oil (50 mg), $[\alpha]_D^{21}$ −95.1° (c=0.72, MeOH). FAB-MS m/z: 419 $(M+Na)^+$. ^{1}H- and ^{13}C-NMR: Table VII.

Enzymatic Hydrolysis of Icarisides A_2 (3), A_4 (5), B_5 (7), B_6 (8), B_7 (9), E_3 (10) and Blumenol C Glucoside (6)——A solution of a glycoside in water (1 ml) was treated with cellulase (Sigma type II) (about equal in weight to the glycoside) at 38 °C overnight. The reaction mixture was diluted with water and extracted with ethyl acetate 3 times. The ethyl acetate was evaporated off and the residue was purified by HPLC (Develosil ODS-10, CH_3CN–H_2O system) to give an aglycone in a yield of 30—90%. The glycosidic linkages of the glycosides were decided to be β (apiose and glucose) from the $J_{H_1-H_2}$ and (rhamnose) from the $J_{C_1-H_1}$.[11] **3a**: Amorphous powder (23 mg). MS m/z: 302 (M^+, 100), 286 (14). UV λ_{max}^{MeOH} nm (log ε): 266 (sh 4.15), 275 (4.21), 297 (sh 3.20). No bathochromic shift was observed on addition of NaOAc + H_3BO_3. ^{1}H- and ^{13}C-NMR: Tables I and II. **5a**: Colorless gum (4 mg). MS m/z: 290

(M^+, 100), 168 (65), 167 (52), 123 (93). UV λ_{max}^{MeOH} nm (log ε): 279 (3.78), 290 (sh 3.63), 305 (sh 3.34), 313 (sh 3.31). UV $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 286, 297 sh, 315 sh, 321 sh. ^{1}H- and ^{13}C-NMR: Table III. **6a**: Colorless gum (7 mg), $[\alpha]_D^{22}$ +112.5° (c=0.52, $CHCl_3$). MS m/z: 210 (M^+, 52), 195 ($M^+ - CH_3$, 9), 192 ($M^+ - H_2O$, 13), 177 ($M^+ - CH_3 - H_2O$, 37), 150 (58), 135 (87), 123 (50), 121 (37), 111 (44), 109 (65), 108 (84), 95 (75), 93 (75), 69 (73), 42 (100). ^{1}H- and ^{13}C-NMR: Tables IV and V. **7a**: Colorless gum (2.5 mg), $[\alpha]_D^{25}$ +19.7° (c=0.23, MeOH). MS m/z: 226 (M^+, 2), 208 ($M^+ - H_2O$, 4), 170 (29), 153 (37), 152 (58), 125 (25), 111 (58), 110 (100). ^{1}H- and ^{13}C-NMR: Tables IV and V. **8a**: Colorless gum (5 mg), $[\alpha]_D^{23}$ −84.4° (c=0.48, MeOH). MS m/z: 210 (M^+, 1), 192 ($M^+ - H_2O$, 10), 177 ($M^+ - H_2O - CH_3$, 4), 159 (9), 149 (5), 121 (14), 119 (100), 107 (6). ^{1}H- and ^{13}C-NMR: Tables IV and V. **10a**: Amorphous powder (3 mg), $[\alpha]_D^{22}$ −80.6° (c=0.36, MeOH). MS m/z: 362 (M^+, 56), 225 (8), 208 (100), 179 (40), 137 (94). ^{1}H- and ^{13}C-NMR: Table VI.

Acetylation of 3a——**3a** (2 mg) was dissolved in acetic anhydride and pyridine (2 drops each) and the reaction mixture was left at room temperature overnight. The reagents were evaporated off *in vacuo* and the residue was recrystallized from methanol to give a diacetate (**3b**) (1 mg) as colorless needles, mp 164—166 °C. MS m/z: 386 (M^+, 10), 344 ($M^+ - CH_2=CO$, 34), 302 ($M^+ - 2\times CH_2=CO$, 100). UV λ_{max}^{MeOH} nm (log ε): 265 (4.21), 295 (3.77). ^{1}H-NMR: Table I.

Hydrolysis of Icariside A_3 (4)——Icariside A_3 (**4**) (8 mg) was refluxed with acetyl chloride–methanol (1 : 20) (1 ml) for 80 min. The reagents were evaporated off to give a residue. After purification by HPLC [Develosil ODS-10, CH_3CN–H_2O (35 : 65)], an aglycone (**4a**) (4 mg) was obtained as an amorphous powder. $[\alpha]_D^{25}$ 0° (c=0.40, MeOH). MS m/z: 302 (M^+, 100), 287 (16), 269 (9), 254 (42). UV λ_{max}^{MeOH} nm (log ε): 270 (sh 4.11), 279 (4.21), 295 (sh 4.16), 300 (4.20), 306 (sh 4.18), 311 (sh 4.15). ^{1}H- and ^{13}C-NMR: Tables I and II.

Acetylation of 4a——**4a** (4 mg) was acetylated in the same manner as described for **3a**. A diacetate (**4b**) (3 mg) was obtained as colorless prisms, mp 152.5—153.5 °C, after recrystallization from methanol. MS m/z: 386 (M^+, 21), 344 (31), 302 (100). UV λ_{max}^{MeOH} nm (log ε): 270 (sh 4.16), 277 (4.18), 303 (sh 4.09), 312 (4.15). ^{1}H-NMR: Table I.

Acetylation of 10a——**10a** (3 mg) was acetylated in the same manner as described for **3a**. A tetraacetate (**10b**) (3 mg) was obtained as an amorphous powder. ^{1}H- and ^{13}C-NMR: Table VI.

Enzymatic Hydrolysis of Icariside G_1 (13)——Icariside G_1 (**13**) (*ca.* 0.1 mg) was treated with cellulase (2 mg) in water (0.1 ml) at 38 °C for 2 h. The reaction mixture was extracted with ether. *n*-Hexanol was detected by GC from the ether extract. Conditions: column, Spelco SPB-35 capillary column, 0.75 mm × 30 m; column temperature, 50 °C; carrier gas, N_2; t_R 5.2 min.

Acid Hydrolysis of the Glycosides——Standard gas chromatographic analysis of the sugar was carried out in the same manner as described in our previous paper.[1] The following were detected by GC: from icarisides F_2 (**12**) and G_1 (**13**), apiose and glucose; from icarisides A_2 (**3**), A_3 (**4**), A_4 (**5**), B_5 (**7**), B_6 (**8**), E_3 (**10**), F_1 (**11**), and blumenol C glucoside (**6**), glucose, and from icariside B_7 (**9**), rhamnose and glucose (1 : 1). Conditions: column, Spelco SPB-35 capillary column, 0.75 mm × 30 m; column temperature, 200 °C; carrier gas, N_2; t_R, 5.1 min (apinitol acetate), 5.2 min (rhamnitol acetate), 12.0 min (glutitol acetate).

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