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Studies on the Glycosides of *Epimedium grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI. I

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A new phenolic glycoside, icariside A_1 (IV), and six new terpenic glycosides, icariside B_1 (V), B_2 (VI), C_1 (VII), C_2 (VIII), C_3 (IX), and C_4 (X), have been isolated from *Epimedium grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI, together with three known glycosides, salidroside (I), thalictoside (II) and benzyl glucoside (III). The structures of IV—X were established on the basis of chemical evidence and spectral data.

Keywords——*Epimedium grandiflorum* var. *thunbergianum*; 9,10-dihydrophenanthrenol glycoside; ionone derivative; sesquiterpene glycoside; icariside A; icariside B; icariside C

The aerial parts of *Epimedium grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI have been used since ancient times as a tonic in China and Japan. The constituents of this plant were investigated by Takemoto *et al.* (flavonoids and lignans)[1] and Tomita and Ishii (alkaloid).[2]

Our interest has been directed to the reinvestigation of the constituents of the aerial parts, with the aim of isolating some biologically active substances.[3] In this paper, we wish to describe the isolation of seven new glycosides, icariside A_1 (IV), B_1 (V), B_2 (VI), C_1 (VII), C_2 (VIII), C_3 (IX), and C_4 (X), along with three known glycosides, salidroside (I), thalictoside (II), and benzyl glucoside (III). The structures of these compounds were determined on the basis of chemical evidence and spectroscopic studies.

Salidroside (I) was identified by direct comparison [thin layer chromatography, infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR), and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra] with an authentic sample.[4]

Thalictoside (II), $C_{14}H_{19}NO_8$, mp 138—139 °C was identified by comparison of various data (mp, IR, ^{1}H-NMR) with reported values.[5]

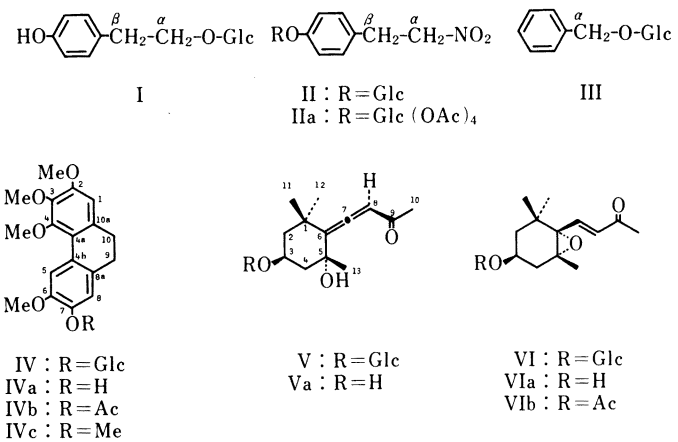
Benzyl glucoside (III), $C_{13}H_{18}O_6 \cdot 1/4H_2O$, $[\alpha]_D -59.2°$, was obtained as colorless needles, mp 123—124 °C. The ^{1}H-NMR spectrum exhibited AB-type signals due to a benzylic methylene at δ4.85 (1H, J=12 Hz) and 5.17 (1H, J=12 Hz), a doublet signal due to an anomeric proton at δ5.01 (1H, J=7 Hz) and multiplet signals due to aromatic protons at δ7.25—7.65 (5H). These data led us to conclude the structure of this compound to be III, previously synthesized by Bonner *et al.* (lit. mp 121 °C).[6] This is the first isolation of III from this plant.

Icariside A_1 (IV), $C_{24}H_{30}O_{10}$, $[\alpha]_D -22.9°$, was obtained as colorless needles, mp 220—222 °C. The ultraviolet (UV) spectrum showed absorption maxima at 280 (4.27), 302 (4.17) and 312 (4.19) nm (log ε). The ^{1}H-NMR spectrum exhibited a broad singlet signal due to benzylic methylene protons at δ2.68 (4H), four singlet signals due to methoxyl protons at δ3.84, 3.87, 3.91 and 4.11 (each 3H), a doublet signal due to an anomeric proton at δ5.75

(1H, $J=7$ Hz) and three singlet signals due to aromatic protons at δ 6.92, 7.43 and 8.31 (each 1H). From these data, IV was assumed to be a 9,10-dihydrophenanthrene derivative having four methoxyl groups and a glucosyl residue.[7] The ^{13}C-NMR spectrum exhibited four methoxyl carbon signals at δ 56.1, 56.5, 60.8 and 61.5, the latter two signals might be due to *ortho*-disubstituted methoxyl groups because of the downfield shifts.[8] Acid hydrolysis afforded glucose as the sugar moiety and enzymatic hydrolysis afforded an aglycone IVa. Acetylation of IVa afforded a monoacetate IVb and methylation of IVa afforded a methyl ether IVc. In the ^{1}H-NMR spectrum of IVa, two aromatic proton signals (δ 6.66 and 6.76) were long-range-coupled with a benzylic methylene proton signal at δ 2.71 (4H, br s) and another one was deshielded at δ 7.97.[7] Nuclear Overhauser effects (NOE) were observed at the proton signals at δ 6.76 (21%) and 7.97 (24%) on irradiation at the methoxyl signals. From these data, IVa was assumed to be 7-hydroxy-2,3,4,6-tetramethoxy-9,10-dihydrophenanthrene, previously isolated from *Combretum psidioides*.[9] The identities of IVa, IVb and IVc were established by comparison of the physical and spectral data (mp, UV, ^{1}H-NMR) with reported data. Therefore, the structure of icariside A_1 was concluded to be IV.

Icariside B_1 (V), $C_{19}H_{30}O_8 \cdot 1/2H_2O$, $[\alpha]_D -73.5°$, was obtained as an amorphous powder. The UV spectrum showed an absorption maximum at 232 (4.16) nm (log ε) and the IR spectrum showed the presence of hydroxyl groups (3450 cm^{-1}), an allenic structure (1945 cm^{-1}) and a conjugated ketone group (1670 cm^{-1}). The ^{1}H-NMR spectrum exhibited four singlet methyl signals at δ 1.09 (3H), 1.51 (6H), 2.21 (3H), the last one being due to a methyl ketone, a carbinol proton signal at δ 4.95 (1H, m), an anomeric proton signal at δ 5.12 (1H, d, $J=7$ Hz) and an olefinic proton signal at δ 5.92 (1H, s). Acid hydrolysis afforded glucose as the sugar moiety and enzymatic hydrolysis afforded an aglycone Va. In the ^{1}H-NMR spectrum, a carbinol proton signal was observed at δ 4.32 (m, $W_{1/2}=17.5$ Hz). From these data, Va was assumed to be grasshopper ketone, previously isolated from *Romalea microptera*.[10] The identity of Va was established by comparison of the physical and spectral data (mp, UV, IR, ^{1}H-NMR) with reported data. In the ^{13}C-NMR spectrum of Va, two carbinol carbon signals were observed at δ 63.8 (d) and 72.3 (s). The former was shifted downfield by 8.2 ppm in the ^{13}C-NMR spectrum of V, but the latter was shifted downfield by only 1.0 ppm. Therefore, the glucosidation position was decided to be at C-3. These results led us to conclude the structure of icariside B_1 to be V.

Icariside B_2 (VI), $C_{19}H_{30}O_8 \cdot 1/2H_2O$, $[\alpha]_D -102.1°$, was obtained as colorless needles, mp 172.5—174.0 °C. The UV spectrum showed an absorption maximum at 230 (4.06) nm (log ε)



I

II : R=Glc
IIa : R=Glc(OAc)$_4$

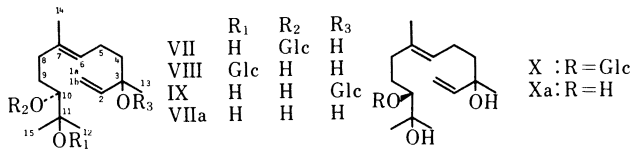
III

IV : R=Glc
IVa : R=H
IVb : R=Ac
IVc : R=Me

V : R=Glc
Va : R=H

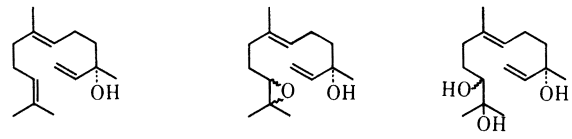
VI : R=Glc
VIa : R=H
VIb : R=Ac

Chart 1



	R_1	R_2	R_3
VII	H	Glc	H
VIII	Glc	H	H
IX	H	H	Glc
VIIa	H	H	H

X : R=Glc
Xa: R=H



XI XII XIII

Chart 2

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

Proton No.	V[a]	Va[b]	VI[a]	VI[c]
3	4.95 (1H, m)	4.32 (1H, m, $W_{1/2}$=17.5 Hz)	7.21 (1H, d, J=15 Hz)	4.84 (1H, m, $W_{1/2}$=18 Hz)
7			6.51 (1H, d, J=15 Hz)	6.92 (1H, d, J=15 Hz)
8	5.92 (1H, s)	5.87 (1H, s)		6.25 (1H, d, J=15 Hz)
10	2.21 (3H, s)	2.19 (3H, s)	2.29 (3H, s)	2.22 (3H, s)
11, 12, 13	1.09 (3H, s), 1.51 (6H, s)	1.16 (3H, s), 1.39 (3H, s), 1.43 (3H, s)	0.96 (3H, s), 1.13 (6H, s)	0.99 (3H, s), 1.19 (3H, s), 1.22 (3H, s)
Anomeric	5.12 (1H, d, J=7 Hz)		4.95 (1H, d, J=7 Hz)	
OAc				1.97 (3H, s)

Run at 89.55 MHz in *a*) pyridine-d_5 *b*) $CDCl_3$ *c*) CCl_4 solution.

and the IR spectrum showed the presence of hydroxyl groups (3500, 3400 cm^{-1}) and a conjugated ketone group (1685 cm^{-1}). The ^{1}H-NMR spectrum exhibited four singlet methyl signals at δ 0.96 (3H), 1.13 (6H), 2.29 (3H), the last one being due to a methyl ketone, an anomeric proton signal at δ 4.95 (1H, d, J=7 Hz) and a pair of *trans* olefinic proton signals at δ 6.51 (1H, d, J=15 Hz) and 7.21 (1H, d, J=15 Hz). In the ^{13}C-NMR spectrum, three oxygen-bearing carbon signals were observed at δ 67.1 (s), 69.9 (s), 71.5 (d), and the former two signals were assigned to epoxy carbons. Acid hydrolysis afforded glucose as the sugar moiety and enzymatic hydrolysis afforded an aglycone VIa, which was acetylated immediately, to give an acetate VIb. The ^{1}H-NMR spectrum of VIb exhibited an acetyl methyl signal at δ 1.97 (3H, s) and a carbinol proton signal at δ 4.84 (1H, m, $W_{1/2}$=18 Hz) suggesting that VIb has an equatorial acetoxyl group. From these data, VIb was assumed to be 3β-acetoxy-5α,6α-epoxy-β-ionone, previously synthesized from a constituent of *Nicotiana tabacum* L.[11] The identity of VIb was established by comparison of the physical and spectral data [mp, $[\alpha]_D$, UV, IR, ^{1}H-NMR, circular dichroism (CD)] with reported data. These results led us to conclude the structure of icariside B_2 to be VI.

Icariside C_1 (VII), $C_{21}H_{38}O_8$, $[\alpha]_D$ −22.5°, was obtained as an amorphous powder. The IR spectrum showed the presence of hydroxyl groups (3450 cm^{-1}) and double bonds (1645 cm^{-1}). The ^{1}H-NMR spectrum exhibited three singlet methyl signals at δ 1.36, 1.46, 1.49, a vinyl methyl signal at δ 1.66 (brs), an anomeric proton signal at δ 5.16 (1H, d, J=8 Hz), an olefinic proton signal at δ 5.51 (1H, brt, J=7 Hz) and three olefinic proton signals at δ 5.16 (1H, dd, J=11, 2 Hz), 5.52 (1H, dd, J=18, 2 Hz), 6.17 (1H, dd, J=18, 11 Hz)

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

Carbon No.	V[a]	Va[b]	VI[a]
Aglycone moiety			
1	36.3	36.1	35.1
2	47.1[c]	48.8[c]	44.8
3	72.0	63.8	71.5[c]
4	48.1[c]	49.0[c]	37.8
5	71.3	72.3	67.1
6	119.8	118.8	69.9
7	197.8	198.2	143.0
8	100.6	100.8	133.3
9	209.6	209.6	197.1
10	26.5	26.4	27.7[d]
11	29.3[d]	29.1[d]	29.0[d]
12	31.1[d]	30.9[d]	22.5[d]
13	32.0[d]	31.8[d]	20.0
Sugar moiety			
1	103.1		103.2
2	75.4		75.3
3	78.6[e]		78.7[e]
4	71.7		71.8[e]
5	78.3[e]		78.4[e]
6	62.8		62.8

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

which were due to a vinyl group. In the ^{13}C-NMR spectrum, twenty-one carbon signals were observed, including six signals due to a glucopyranosyl moiety. Acid hydrolysis afforded glucose as the sugar moiety and enzymatic hydrolysis afforded an aglycone VIIa, colorless oil, $[\alpha]_D - 13.4°$. The ^{1}H-NMR spectrum of VIIa exhibited a carbinol proton signal at δ 3.76 (1H, dd, $J=10$, 2 Hz), while the ^{13}C-NMR spectrum of VIIa exhibited fifteen carbon signals including three carbinol carbon signals at δ 72.4 (s), 72.7 (s), 78.5 (d). From a comparison of these spectral data with those of nerolidol,[12] VIIa was assumed to be 3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol. The identity of VIIa was established by chemical synthesis of XIII from (+)-nerolidol (XI).[13] Compound XIII was obviously a mixture of 10*S* and 10*R* from the synthetic process, but the two isomers were not distinguishable in the ^{1}H- and ^{13}C-NMR spectra. Thus, in order to decide the stereochemistry at C-10, the Cotton effect of the α-glycol in the presence of a shift reagent Eu(fod)$_3$ was examined. The CD spectrum of VIIa showed a positive Cotton effect, $[\theta]_{305} + 41322$, and a negative Cotton effect, $[\theta]_{285} - 27716$, suggesting C-10 to be *S*.[14] In the ^{13}C-NMR spectrum of VIIa, three carbinol carbon signals were observed at δ 72.4 (s), 72.7 (s), 78.5 (d). The last one was shifted downfield at δ 90.7 (d) in the ^{13}C-NMR spectrum of VII, so the glucosidation position was decided to be C-10. These results led us to conclude the structure of icariside C_1 to be VII.

Icariside C_2 (VIII), $C_{21}H_{38}O_8 \cdot 1/2H_2O$, $[\alpha]_D - 19.3°$, was obtained as an amorphous powder. The IR and ^{1}H-NMR spectra were very similar to those of VII. Acid hydrolysis afforded glucose as the sugar moiety and enzymatic hydrolysis afforded an aglycone VIIa. On comparison of the ^{13}C-NMR spectra of VIII and VIIa, the signal of C-10 (δ 76.9) of VIII was shifted upfield by 1.6 ppm and that of C-11 (δ 80.9) was shifted downfield by 8.2 ppm. Therefore, the structure of icariside C_2 was concluded to be VIII, with a glucosyl residue at C-11.

Icariside C_3 (IX), $C_{21}H_{38}O_8 \cdot 1/2H_2O$, $[\alpha]_D - 34.7°$, was obtained as an amorphous

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

Proton No.	VII	VIII	IX	X
1a	5.52 (1H, dd, J=18, 2 Hz)	5.54 (1H, dd, J=17, 2 Hz)	5.39 (1H, dd, J=18, 1.5 Hz)	5.58 (1H, dd, J=17, 2 Hz)
1b	5.16 (1H, dd, J=11, 2 Hz)	5.17 (1H, dd, J=10, 2 Hz)	5.23 (1H, dd, J=11, 1.5 Hz)	5.18 (1H, dd, J=10, 2 Hz)
2	6.17 (1H, dd, J=18, 11 Hz)	6.17 (1H, dd, J=17, 10 Hz)	6.28 (1H, dd, J=18, 11 Hz)	6.18 (1H, dd, J=17, 10 Hz)
6	5.51 (1H, br t, J=7 Hz)	*a*)	*a*)	*a*)
10				
12	1.36 (3H, s)	1.49 (6H, s)	1.48 (3H, s)	1.35 (3H, s)
13	1.46 (3H, s)	1.51 (3H, s)	1.52 (3H, s)	1.43 (3H, s)
15	1.49 (3H, s)		1.58 (3H, s)	1.48 (3H, s)
14	1.66 (3H, br s)	1.67 (3H, br s)	1.65 (3H, br s)	1.67 (3H, br s)
Anomeric	5.16 (1H, d, J=8 Hz)	5.23 (1H, d, J=7 Hz)	4.95 (1H, d, J=8 Hz)	5.01 (1H, d, J=8 Hz)

Proton No.	VIIa	XIII	Xa
1a	5.56 (1H, dd, J=18, 2 Hz)	5.53 (1H, dd, J=17, 2 Hz)	5.56 (1H, dd, J=17, 2 Hz)
1b	5.17 (1H, dd, J=11, 2 Hz)	5.16 (1H, dd, J=11, 2 Hz)	*a*)
2	6.17 (1H, dd, J=18, 11 Hz)	6.17 (1H, dd, J=17, 11 Hz)	6.17 (1H, dd, J=17, 11 Hz)
6	5.45 (1H, br t, J=7 Hz)	5.43 (1H, br t, J=7 Hz)	*a*)
10	3.76 (1H, dd, J=10, 2 Hz)	3.74 (1H, dd, J=10, 2 Hz)	3.77 (1H, dd, J=10, 2 Hz)
12	1.48 (3H, s)	1.48 (6H, s)	1.49 (3H, s)
13	1.49 (3H, s)	1.51 (3H, s)	1.50 (3H, s)
15	1.53 (3H, s)		1.54 (3H, s)
14	1.71 (3H, br s)	1.69 (3H, br s)	1.70 (3H, br s)

Run at 89.55 MHz in pyridine-d_5 solution. *a*) Overlapped with H_2O.

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

Carbon No.	VII	VIII	IX	X	VIIa	XIII
Aglycone moiety						
1	111.3	111.4	114.8	111.2	111.2	111.9
2	147.1	147.1	144.6	147.2	147.2	147.1
3	72.5[a]	72.5	80.1	72.4[a]	72.4[a]	73.1[a]
4	43.5	43.4	42.3	43.4	43.4	43.4
5	23.5	23.4	23.0	23.4	23.4	23.7
6	128.5	125.8	125.1	125.8	125.3	125.6
7	135.2	135.3	135.5	135.2	135.5	135.7
8	30.7	30.6	30.8	31.2	30.9	31.0
9	36.7	37.6	37.6	36.5	37.7	37.9
10	90.7	76.9	78.9[a]	90.3	78.5	78.7
11	73.6[a]	80.9	72.7	72.0[a]	72.7[a]	73.4[a]
12	24.3[b]	21.6[a]	26.0[b]	25.2[b]	26.0[b]	26.1[b]
13	28.6	28.6	23.6	28.4	28.5	28.7
14	16.4	16.4	16.3	16.1	16.3	16.8
15	26.8[b]	24.2[a]	26.1[b]	27.0[b]	26.1[b]	26.6[b]
Sugar moiety						
1	106.8	98.8	99.8	106.1		
2	76.2	75.4	75.4	75.5		
3	78.8[c]	78.8[b]	78.5[a]	78.6[c]		
4	71.8	71.8	72.0	71.8		
5	78.3[c]	78.2[b]	78.1[a]	78.5[c]		
6	62.9	62.8	63.1	62.7		

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

powder. The IR and ^{1}H-NMR spectra were similar to those of VII and VIII. Acid hydrolysis afforded glucose as the sugar moiety and enzymatic hydrolysis afforded an aglycone VIIa. In the ^{13}C-NMR spectrum of IX, the signal of C-3 (δ 80.1) was shifted downfield by 7.7 ppm, while those of C-2 (δ 144.6), C-4 (δ 42.3) and C-13 (δ 23.6) were shifted upfield by 2.6, 1.1 and 4.9 ppm, respectively, compared with those of VIIa. Therefore, the structure of icariside C_3 was concluded to be IX, with a glucosyl residue at C-3.

Icariside C_4 (X), $C_{21}H_{38}O_8 \cdot 1/2H_2O$, $[\alpha]_D + 3.4°$, was obtained as an amorphous powder. The ^{1}H- and ^{13}C-NMR spectra were very similar to those of VII, though C-11 and C-12 showed small differences in the chemical shifts. Therefore, X was assumed to be an epimer of VII at C-10. The aglycone Xa obtained by enzymatic hydrolysis of X gave the same IR and ^{1}H-NMR spectra as a synthetic product, XIII. The CD spectrum of Xa in the presence of Eu(fod)$_3$ showed a negative Cotton effect, $[\theta]_{305} - 32768$, and a positive one, $[\theta]_{284} + 20480$, opposite to those in the case of VIIa. Thus, the stereochemistry at C-10 was decided to be *R* and the structure of icariside C_4 to be X.

This is the first report of the isolation of a dihydrophenanthrene derivative and terpenic glycosides from *Epimedium* species. Studies on the structures of other minor glycosides (polar) are in progress.

Experimental

Melting points were taken on a Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-140 digital polarimeter. IR spectra were run on a JASCO A-202 IR spectrometer and UV spectra on a Shimadzu UV-360 recording spectrometer. Mass spectra (MS) were measured on a JEOL JMS-100 mass spectrometer. CD spectra were recorded on a JASCO J-20A spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX-90Q NMR spectrometer (89.55 and 22.5 MHz, respectively). Chemical

shifts are given on the δ scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Gas chromatography (GC) was done on a Hitachi K53 gas chromatograph. High-performance liquid chromatography (HPLC) was done on 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. After repeated chromatography of the methanol eluate (420 g) on silica gel with a chloroform–methanol system and HPLC (column; Develosil ODS-10, 20 × 250 mm) with a water–acetonitrile system, ten glycosides were isolated.

Salidroside (I): Amorphous powder (50 mg). IR ν_{max}^{KBr} cm^{-1}: 3420, 1670, 1620, 1600, 1525, 1445, 1380, 1260, 1250, 1165, 1130, 1075, 910. ^{1}H-NMR (pyridine-d_5) δ: 3.02 (2H, t, J=7 Hz, H_2-β), 4.94 (1H, d, J=7 Hz, H-1′), 7.17 (4H, br s, H-2, H-3, H-5, H-6). ^{13}C-NMR (pyridine-d_5) δ: 36.0 (C-β), 62.8 (C-6′), 71.1 (C-4′), 71.7 (C-α), 75.2 (C-2′), 78.5, 78.6 (C-3′/C-5′), 104.7 (C-1′), 116.2 (C-3, C-5), 129.5 (C-1), 130.5 (C-2, C-6), 157.3 (C-4).

Thalictoside (II): Colorless needles from methanol–ethyl acetate (330 mg), mp 138—139 °C. *Anal.* Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25. Found: C, 50.78; H, 5.65; N, 4.07. IR ν_{max}^{KBr} cm^{-1}: 3520, 1620, 1550, 1520, 1385, 1240, 1105, 1075, 1050, 1015. ^{1}H-NMR (pyridine-d_5) δ: 3.23 (2H, t, J=7 Hz, H_2-β), 4.84 (2H, t, J=7 Hz, H_2-α), 5.60 (1H, d, J=7 Hz, H-1′), 7.22 (2H, d, J=9 Hz, H-3, H-5), 7.31 (2H, d, J=9 Hz, H-2, H-6). ^{13}C-NMR (pyridine-d_5) δ: 32.7 (C-β), 62.4 (C-6′), 71.3 (C-4′), 74.9 (C-2′), 76.8 (C-α), 78.5, 78.8 (C-3′/C-5′), 102.2 (C-1′), 117.3 (C-3, C-5), 130.1 (C-1, C-2, C-6), 157.8 (C-4).

Benzyl Glucoside (III): Colorless needles from methanol–ethyl acetate (390 mg), mp 123—124 °C, $[\alpha]_D^{25}$ −59.2° (c=0.67, methanol). *Anal.* Calcd for $C_{13}H_{18}O_6 \cdot 1/4H_2O$: C, 56.82; H, 6.79. Found: C, 56.66; H, 6.53. IR ν_{max}^{KBr} cm^{-1}: 3450, 1640, 1505, 1460, 1420, 1375, 1160, 1110, 1085, 1055, 1030. ^{1}H-NMR (pyridine-d_5) δ: 4.85 (1H, d, J=12 Hz, H-α), 5.01 (1H, d, J=7 Hz, H-1′), 5.17 (1H, d, J=12 Hz, H-α′), 7.25—7.65 (5H, m, H-2, H-3, H-4, H-5, H-6). ^{13}C-NMR (pyridine-d_5) δ: 62.7 (C-6′), 70.8 (C-α), 71.6 (C-4′), 75.0 (C-2′), 78.3 (C-3′, C-5′), 103.8 (C-1′), 127.7 (C-4), 128.1 (C-2, C-6), 128.5 (C-3, C-5), 138.8 (C-1).

Icariside A_1 (IV): Colorless needles from methanol (1.4 g), mp 220—222 °C, $[\alpha]_D^{25}$ −22.9° (c=0.59, methanol). *Anal.* Calcd for $C_{24}H_{30}O_{10}$: C, 60.24; H, 6.32. Found: C, 60.14; H, 6.29. UV λ_{max}^{MeOH} nm (log ε): 216 (4.53), 233 (sh, 4.36), 272 (sh, 4.21), 280 (4.27), 302 (4.17), 312 (4.19). IR ν_{max}^{KBr} cm^{-1}: 3520, 3420, 1615, 1595, 1525, 1460, 1405, 1265, 1220, 1115, 1080, 1050, 1045. ^{1}H-NMR (pyridine-d_5) δ: 2.68 (4H, br s, H_2-9, H_2-10), 3.84, 3.87, 3.91, 4.11 (each 3H, s, OMe), 5.75 (1H, d, J=7 Hz, H-1′), 6.92 (1H, s, H-1), 7.43 (1H, s, H-8), 8.31 (1H, s, H-5). ^{13}C-NMR (pyridine-d_5) δ: 29.6, 30.9 (C-9/C-10), 56.1, 56.5 (methoxyl at C-2/C-6), 60.8, 61.5 (methoxyl at C-3/C-4), 62.5 (C-6′), 71.4 (C-4′), 75.0 (C-2′), 78.7, 79.0 (C-3′/C-5′), 102.6 (C-1′), 112.4, 112.7, 112.9 (C-1/C-5/C-8), 122.6, 125.8 (C-8a/C-10a), 131.6 (C-4a), 134.7 (C-3), 143.1 (C-4b), 148.4, 148.7 (C-6/C-7), 150.8, 152.1 (C-2/C-4).

Icariside B_1 (V): Amorphous powder (525 mg), $[\alpha]_D^{25}$ −73.5° (c=1.00, methanol). *Anal.* Calcd for $C_{19}H_{30}O_8 \cdot 1/2H_2O$: C, 57.71; H, 7.90. Found: C, 57.56; H, 7.76. UV λ_{max}^{MeOH} nm (log ε): 232 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3450, 1945, 1670, 1460, 1370, 1245, 1170, 1160, 1080, 1030, 955. ^{1}H- and ^{13}C-NMR: Tables I and II.

Icariside B_2 (VI): Colorless needles from methanol-ethyl acetate (510 mg), mp 172.5—174.0 °C, $[\alpha]_D^{25}$ −102.1° (c=0.97, methanol). *Anal.* Calcd for $C_{19}H_{30}O_8 \cdot 1/2H_2O$: C, 57.71; H, 7.90. Found: C, 57.92; H, 7.66. UV λ_{max}^{MeOH} nm (log ε): 230 (4.06). IR ν_{max}^{KBr} cm^{-1}: 3500, 3400, 1685, 1390, 1365, 1250, 1170, 1125, 1085, 1045, 1025, 990, 905. ^{1}H- and ^{13}C-NMR: Tables I and II.

Icariside C_1 (VII): Amorphous powder (1.12 g), $[\alpha]_D^{25}$ −22.5° (c=1.00, methanol). *Anal.* Calcd for $C_{21}H_{38}O_8$: C, 60.27; H, 9.15. Found: C, 60.27; H, 9.14. IR ν_{max}^{KBr} cm^{-1}: 3450, 1645, 1470, 1455, 1415, 1385, 1370, 1170, 1150, 1075, 1030, 965, 925, 900. ^{1}H- and ^{13}C-NMR: Tables III and IV.

Icariside C_2 (VIII): Amorphous powder (420 mg), $[\alpha]_D^{25}$ −19.3° (c=0.96, methanol). *Anal.* Calcd for $C_{21}H_{38}O_8 \cdot H_2O$: C, 57.78; H, 9.24. Found: C, 57.81; H, 8.97. IR ν_{max}^{KBr} cm^{-1}: 3450, 1645, 1470, 1455, 1415, 1390, 1375, 1160, 1080, 1040, 1020, 925. ^{1}H- and ^{13}C-NMR: Tables III and IV.

Icariside C_3 (IX): Amorphous powder (315 mg), $[\alpha]_D^{25}$ −34.7° (c=0.88, methanol). *Anal.* Calcd for $C_{21}H_{38}O_8 \cdot 1/2H_2O$: C, 59.00; H, 9.19. Found: C, 59.26; H, 9.09. IR ν_{max}^{KBr} cm^{-1}: 3450, 1640, 1455, 1415, 1390, 1375, 1160, 1075, 1040, 1030, 925. ^{1}H- and ^{13}C-NMR: Tables III and IV.

Icariside C_4 (X): Amorphous powder (35 mg), $[\alpha]_D^{25}$ +3.4° (c=0.87, methanol). *Anal.* Calcd for $C_{21}H_{38}O_8 \cdot 1/2H_2O$: C, 59.00; H, 9.19. Found: C, 59.16; H, 9.12. IR ν_{max}^{KBr} cm^{-1}: 3350, 1645, 1390, 1370, 1315, 1270, 1230, 1180, 1160, 1140, 1105, 1010, 990, 920, 870. ^{1}H- and ^{13}C-NMR: Tables III and IV.

Acetylation of Thalictoside (II)——Thalictoside (II, 10 mg) was dissolved in pyridine and acetic anhydride (each 0.3 ml), and the reaction mixture was left at room temperature. The reagents were evaporated off *in vacuo* and the residue was recrystallized from methanol to give a tetraacetate (IIa, 8 mg) as colorless crystals, mp 165—166 °C. IR ν_{max}^{KBr} cm^{-1}: 1755, 1620, 1560, 1525, 1440, 1380, 1230, 1100, 1070, 1050, 910. ^{1}H-NMR ($CDCl_3$) δ: 2.05, 2.06, 2.07, 2.09 (each 3H, s, OAc), 3.28 (2H, t, J=7 Hz, H_2-β), 4.60 (2H, t, J=7 Hz, H_2-α), 6.98 (2H, d, J=9 Hz, H-3, H-5), 7.16 (2H, d, J=9 Hz, H-2, H-6).

Enzymatic Hydrolysis of Icariside A_1 (IV)——A solution of icariside A_1 (IV, 13 mg) in water (2 ml) was treated with β-glucosidase (50 mg) at 37 °C for a day. The reaction mixture was diluted with water and extracted with ethyl acetate 3 times. Ethyl acetate was evaporated off and the residue was recrystallized from methanol to give colorless

needles (IVa, 6 mg), mp 179—180 °C. $[\alpha]_D^{25}$ 0° ($c=1.56$, chloroform). UV λ_{max}^{MeOH} nm (log ε): 215 (4.61), 233 (sh, 4.59), 273 (sh, 4.19), 281 (4.26), 301 (4.13), 313 (4.13). IR ν_{max}^{KBr} cm^{-1}: 3430, 1610, 1580, 1520, 1450, 1415, 1405, 1345, 1330, 1265, 1210, 1180, 1150, 1065, 1040, 850. MS *m/z*: 316 (M^+, 100), 301 (M^+-CH_3, 34), 286 ($M^+-2\times CH_3$, 6), 270 (16), 151 (17). ^{1}H-NMR ($CDCl_3$) δ: 2.71 (4H, br s, H_2-9, H_2-10), 3.77, 3.93, 3.95, 4.00 (each 3H, s, OMe), 5.73 (1H, s, OH), 6.66 (1H, br s, H-8), 6.76 (1H, br s, H-1), 7.97 (1H, s, H-5). ^{13}C-NMR ($CDCl_3$) δ: 29.3, 30.3 (C-9/C-10), 55.8, 56.1 (methoxyl at C-2/C-6), 60.1, 61.2 (methoxyl at C-3/C-4), 110.3, 111.0, 111.2 (C-1/C-5/C-8), 120.2 (C-8a), 125.1 (C-10a), 130.5 (C-4a), 135.0 (C-3), 139.0 (C-4b), 147.2, 147.3, 147.6 (C-4/C-6/C-7), 150.5 (C-2).

Acetylation of IVa——IVa (8 mg) was acetylated in the same way as II. A monoacetate IVb (6 mg) was obtained as colorless needles, mp 143—144 °C after recrystallization from methanol. UV λ_{max}^{MeOH} nm (log ε): 218 (4.56), 233 (sh, 4.37), 273 (sh, 4.19), 280 (4.24), 294 (sh, 4.12), 310 (4.17). IR ν_{max}^{KBr} cm^{-1}: 1785, 1615, 1590, 1525, 1470, 1455, 1415, 1405, 1370, 1340, 1290, 1265, 1250, 1220, 1200, 1110, 1065, 1040, 950, 910, 890. ^{1}H-NMR ($CDCl_3$) δ: 2.36 (3H, s, OAc), 2.73 (4H, br s, H_2-9, H_2-10), 3.77 (3H, s, OMe), 3.94 (9H, s, OMe × 3), 6.75, 6.77 (each 1H, br s, H-1/H-8), 8.03 (1H, s, H-5). ^{13}C-NMR ($CDCl_3$) δ: 20.8 (OAc), 29.1, 30.0 (C-9/C-10), 55.9, 56.2 (methoxyl at C-2/C-6), 60.4, 61.0 (methoxyl at C-3/C-4), 110.9 (C-1), 111.8 (C-5), 117.5 (C-8), 124.6, 126.4 (C-8a/C-10a), 131.3 (C-4a), 134.2 (C-3), 142.1 (C-8b), 144.3 (C-7), 147.3, 148.1 (C-6/C-4), 151.4 (C-2), 169.3 (C=O).

Methylation of IVa——A mixture of IVa (5 mg), dimethyl sulfate (0.2 ml) and anhydrous potassium carbonate (100 mg) in dry acetone (2 ml) was refluxed for 3 h with stirring. After removal of the precipitate by filtration, the filtrate was concentrated to a syrup, which was chromatographed on a thin layer plate (Kiesel gel GF_{254}; benzene–acetone (95 : 5)) to yield a methyl ether (IVc, 3 mg) as colorless needles (methanol), mp 108—109 °C. UV λ_{max}^{MeOH} nm (log ε): 216 (4.58), 233 (sh, 4.20), 273 (sh, 4.03), 281 (4.11), 301 (4.01), 312 (4.00). IR ν_{max}^{KBr} cm^{-1}: 1610, 1520, 1495, 1465, 1415, 1400, 1265, 1245, 1220, 1190, 1130, 1085, 1055, 1005. ^{1}H-NMR ($CDCl_3$) δ: 2.74 (4H, br s, H_2-9, H_2-10), 3.79, 3.90, 3.94 (each 3H, s, OMe), 3.95 (6H, s, OMe × 2), 6.62, 6.72 (each 1H, br s, H-1/H-8), 8.02 (1H, s, H-5). ^{13}C-NMR ($CDCl_3$) δ: 29.4, 30.6 (C-9/C-10), 55.9, 56.0 ((1C) and (2C), methoxyl at C-2/C-6/C-7), 60.5, 61.1 (methoxyl at C-3/C-4), 107.7 (C-8), 111.0, 111.1 (C-1/C-5), 120.9 (C-8a), 125.2 (C-10a), 130.4 (C-4a), 134.2 (C-3), 141.6 (C-4b), 147.3, 147.4 (C-4/C-6), 151.5, 151.7 (C-2/C-7).

Enzymatic Hydrolysis of Icariside B_1 (V)——A solution of icariside B_1 (V, 27 mg) in water (2 ml) was treated with cellulase (30 mg) at 37 °C for 5 h. After being diluted with water the reaction mixture was passed through an Amberlite XAD-2 column, which was washed with water. The methanol eluate was purified by HPLC (Develosil ODS-10, 20 × 250 mm; H_2O–CH_3CN (77 : 23)) to give an aglycone (Va, 11.5 mg) as colorless needles (acetone–benzene), mp 134—136 °C, $[\alpha]_D^{25}-63.0°$ ($c=1.15$, methanol). UV λ_{max}^{MeOH} nm (log ε): 232 (4.15). IR ν_{max}^{KBr} cm^{-1}: 3350, 1945, 1680, 1595, 1465, 1370, 1240, 1190, 1165, 1150, 1070, 1045, 990, 955, 860, 820. ^{1}H- and ^{13}C-NMR: Tables I and II.

Enzymatic Hydrolysis of Icariside B_2 (VI)——A solution of icariside B_2 (VI, 9 mg) in water (0.5 ml) was treated with β-glucosidase (10 mg) at 37 °C for 13 h. The reaction mixture was worked up in the same way as described for IV to give an aglycone (VIa, 5 mg) as an amorphous powder. This was acetylated in the usual way with pyridine and acetic anhydride (each 3 drops) to give VIb (4 mg) as colorless needles (ether–hexane), mp 129—130 °C, $[\alpha]_D^{25}-104.7°$ ($c=0.25$, chloroform). CD ($c=0.039$, methanol) [θ] (nm): −37326 (232). UV λ_{max}^{MeOH} nm (log ε): 230 (4.09). IR ν_{max}^{KBr} cm^{-1}: 1740, 1685, 1390, 1375, 1270, 1255, 1170, 1050, 1045, 1000, 915. MS *m/z*: 266 (M^+, trace), 191 (7), 175 (4), 163 (5), 149 (8), 135 (10), 124 (24), 123 (100). ^{1}H-NMR: Table I.

Enzymatic Hydrolysis of Icarisides C_1 (VII), C_2 (VIII) and C_3 (IX)——A solution of icariside C_1 (VI, 34 mg) in water (1 ml) was treated with cellulase (30 mg) at 37 °C overnight. The reaction mixture was worked up in the same manner as described for VI. The methanol eluate was purified by HPLC (Develosil ODS-10, 20 × 250 mm; H_2O–CH_3CN (63 : 37)) to give an aglycone (VIIa, 11 mg) as a colorless oil. $[\alpha]_D^{25}-13.4°$ ($c=1.08$, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1610, 1460, 1380, 1165, 1080, 1000, 930. CD ($c=0.010$, carbon tetrachloride with equimolar Eu(fod)$_3$): +41322 (305), −27716 (285). From VIII (14 mg), VIIa (5 mg) was obtained in the same manner. $[\alpha]_D^{25}-12.2°$ ($c=0.49$, methanol). CD ($c=0.010$, carbon tetrachloride with equimolar Eu(fod)$_3$): +35840 (305), −23040 (284). From IX (14 mg), VIIa (2.8 mg) was obtained in the same manner. $[\alpha]_D^{25}-13.4°$ ($c=0.19$, methanol). CD ($c=0.010$, carbon tetrachloride with equimolar Eu(fod)$_3$): +31530 (305), −22220 (284). ^{1}H-NMR: Table III.

Enzymatic Hydrolysis of Icariside C_4 (X)——A solution of icariside C_4 (X, 13 mg) was treated in the same manner as described for VII to give an aglycone (Xa, 7 mg) as a colorless oil. $[\alpha]_D^{25}+44.5°$ ($c=0.64$, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1610, 1460, 1380, 1160, 1080. CD ($c=0.010$, carbon tetrachloride with equimolar Eu(fod)$_3$): −32768 (305), +20480 (284). ^{1}H-NMR: Table III.

Acid Hydrolysis of Glycosides IV, V, VI, VII, VIII, IX and X——A solution of a glycoside (*ca.* 0.1 mg) in 10% sulfuric acid (2 drops) was heated in a boiling water bath for 30 min. The solution was passed through an Amberlite IRA-45 column and concentrated to give a residue, which was reduced with sodium borohydride (*ca.* 1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and the eluate was concentrated to dryness. Boric acid was removed by co-distillation with methanol and the residue was acetylated with acetic anhydride and pyridine (1 drop each) at room temperature overnight. The reagents were evaporated off *in vacuo*. From each glycoside, glucitol acetate was detected by GC. Conditions: column, 1.5% OV-17, 3 mm × 1 m; column temperature, 200 °C; carrier gas, N_2; t_R 4.8 min.

Synthesis of 3,7,11-Trimethyl-1,6-dodecadien-3,10,11-triol (XIII)——*m*-Chloroperbenzoic acid (2.5 g) was added to a stirred solution of (+)-nerolidol (XI, 2.8 g) in dichloromethane (25 ml) and saturated aqueous sodium hydrogen carbonate (25 ml). The mixture was stirred for 17 h at room temperature, then the dichloromethane layer was washed with saturated aqueous sodium chloride and dried over sodium sulfate. Removal of the solvent afforded a crude product, which was chromatographed on silica gel using benzene–acetone (9 : 1) as the eluent to give 10,11-epoxynerolidol (XII, 2.6 g) as a colorless oil. A solution of XII (850 mg) in 0.1 N sulfuric acid (33 ml) and tetrahydrofuran (33 ml) was stirred for 5.5 h at room temperature. The reaction mixture was diluted with water and passed through an Amberlite XAD-2 column. After washing of the column with water, the methanol eluate was purified by HPLC (Develosil ODS-10, 20 × 250 mm; H_2O–CH_3CN (70 : 30)) to give 3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol (XIII, 150 mg) as a colorless oil. *Anal.* Calcd for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01. Found: C, 70.24; H, 11.03. IR ν_{max}^{KBr} cm^{-1}: 3450, 1610, 1460, 1380, 1165, 1080, 1000, 930. ^1H- and ^{13}C-NMR: Tables III and IV.

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