EPIMEDINS A, B AND C, FLAVONOID GLYCOSIDES OF EPIMEDIUM KOREANUM HERBS[1]

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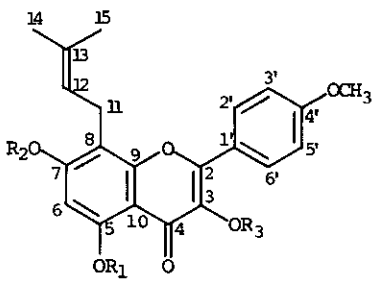
Abstract ——Three new flavonoid glycosides, epimedins A, B and C, have been isolated from Epimedium koreanum herbs, the structures of which have been elucidated on the basis of chemical and spectroscopic data as shown in formulas I, II and III, respectively.

Epimedium koreanum Nakai as well as certain other related species belonging to the genus Epimedium (Berberidaceae) are important original plants of the crude drug "inyokaku" which has been employed as a tonic, antirheumatic and aphrodisiac in Oriental medicine. The previous chemical works on this genus have been focused on flavonoids.[2,3] During the course of our investigation on medicinal plants having anticomplementary activity, the methanol extract of Epimedium koreanum herbs was found to exhibit a promising action. Accordingly, fractionation of this methanol extract was conducted to afford three new flavonoid glycosides, epimedins A, B and C.

Epimedin A (I), an amorphous yellow solid, $[\alpha]_D$ -99.8° (c 0.39, MeOH), showed IR absorption bands (KBr) at 3300 (hydroxyls) and 1635 cm^{-1} (conjugated and hydrogen-bonded carbonyl), and UV maxima (MeOH) at 271 (ε 13800), 318 (ε 8800) and 349 nm (ε 7300). Analysis of its ^{13}C NMR spectrum (125 MHz) demonstrated the presence of thirty-nine carbons as CH_3- x 3, $-CH_2$- x 1, CH_3-O x 1, $-CH_2$-O x 2, >CH-O x 12, O-$\dot{C}$H-O x 3, =CH- x 6, >C= x 4, =$\dot{C}$-O x 6, and >C=O x 1 (Table I). These spectral data, along with positive color reactions with ferric chloride and magnesium-hydrochloric acid, indicated that it is a flavonoid glycoside. In the ^1H NMR spectrum (500 MHz, pyridine-d_5), epimedin A displayed aromatic hydrogen signals at δ 7.14 (1H s), 7.15 and 8.09 (2H each d, J 9.2 Hz), where the latter two were assigned to the 3'-, 5'- and 2'-, 6'-hydrogens of the B-ring of the flavone nucleus. In addition to these, a signal due to a methoxyl group was observed at δ 3.72 (3H s), and when it was irradiated, a distinct NOE was detected in the aromatic hydrogen signal at δ 7.15. This finding fully justified the presence of a 4'-methoxyphenyl type B-ring in epimedin A. In the ^{13}C NMR spectrum of epimedin A, signals for C-2, C-3 and C-4 appeared at δ 157.1, 135.0 and 178.3, respectively, which matched well with those of 5-hydroxyflavonol-3-O-glycosides such as rutin.[4] This evidence clarified the substitution patterns at C-3 and C-5 of epimedin A, which was further substantiated by the carbonyl absorption band in the IR spectrum and the ^1H NMR signal due to the C-5 hydroxyl at δ 13.05 (1H s). The ^{13}C NMR resonance at δ 160.5, together with the fact that the UV



I: R_1=H, R_2=Glc, R_3=Rham(2→1)Glc
II: R_1=H, R_2=Glc, R_3=Rham(2→1)Xyl
III: R_1=H, R_2=Glc, R_3=Rham(2→1)Rham
IV: R_1=H, R_2=Glc, R_3=Rham
V: R_1=R_2=H, R_3=Rham(2→1)Glc
VI: R_1=H, R_2=Ac, R_3=Rham(OAc)$_2$(2→1)Glc(OAc)$_4$
VII: R_1=R_2=H, R_3=Rham(2→1)Xyl
VIII: R_1=R_2=Ac, R_3=Rham(OAc)$_2$(2→1)Xyl(OAc)$_3$
IX: R_1=R_2=H, R_3=Rham(2→1)Rham
X: R_1=R_2=Ac, R_3=Rham(OAc)$_2$(2→1)Rham(OAc)$_3$

Table I. Carbon-13 shieldings of carbons of I, II, III and V.

	I (DMSO-d_6)	I (Py-d_5)	V† (Py-d_5)	II (Py-d_5)	III (Py-d_5)
C-2	157.1	157.7	157.0	157.5	157.6
C-3	135.0	*	*	135.8	*
C-4	178.3	179.3	179.0	179.1	179.3
C-5	159.1	160.6	160.4	160.3	160.6
C-6	98.2	99.4	99.2	99.0	99.3
C-7	160.5	161.8	163.2	161.4	161.7
C-8	108.3	109.4	107.0	109.1	109.4
C-9	153.0	154.0	154.8	153.7	153.9
C-10	105.6	107.0	105.4	106.7	107.0
C-11	21.4	22.5	22.2	22.3	22.4
C-12	122.2	*	*	*	*
C-13	131.2	131.6	131.3	131.3	131.5
C-14	25.5	25.7	25.7	25.7	25.6
C-15	17.9	18.1	18.0	18.0	18.1
C-1'	122.2	*	*	123.0	*
C-2'	130.6	131.2	131.0	130.9	131.2
C-3'	114.1	114.5	114.4	114.3	114.4
C-4'	161.5	162.3	162.0	162.0	162.2
C-5'	114.1	114.5	114.4	114.3	114.4
C-6'	130.6	131.2	131.0	130.9	131.2
OMe	55.6	55.5	55.4	55.4	55.4
C-1"	100.6	102.7		102.3	102.6
C-2"	73.4	74.9		74.6	74.9
C-3"	76.3	78.4		78.0	78.6
C-4"	69.7	71.2		70.9	71.1
C-5"	77.2	79.0		78.6	78.9
C-6"	60.7	62.3		62.1	62.2
C-1"'	101.1	102.6	102.5	102.3	102.4
C-2"'	81.4	82.5	82.4	81.7	77.2
C-3"'	70.5	72.2	72.2	72.0	72.6
C-4"'	71.7	73.5	73.5	73.3	74.1
C-5"'	70.2	71.7	71.6	71.5	72.2
C-6"'	17.4	18.1	18.0	18.0	18.5
C-1""	106.2	107.6	107.5	107.5	103.4
C-2""	73.8	75.7	75.7	75.1	72.1
C-3""	76.7	78.7	78.5	78.2	72.0
C-4""	69.3	71.2	71.0	70.6	73.2
C-5""	76.6	78.5	78.3	67.2	70.3
C-6""	60.4	62.4	62.2		18.2

* Undetermined due to overlapping.
† Discrimination of two sugar moieties was made by PRFT measurements.

absorption maxima remained unaffected on addition of sodium acetate, pointed to the presence of a substituted hydroxyl group at C-7 as a glycoside.[5] Further, the presence of an isoprenyl group was indicated from the ^{1}H and ^{13}C NMR signals observed at δ 1.61 and 1.76 (3H each s), and δ 17.9 (q), 21.4 (t), 25.5 (q), 122.2 (d) and 131.2 (s), respectively. The isoprenyl group was logically placed at C-8 from the fact that the UV maximum at 271 nm of epimedin A underwent a bathochromic shift by 9 nm on addition of aluminum chloride,[6] and from the finding that the ^{13}C NMR resonances assigned to C-7 and C-9 of the flavonol suffered upfield shifts due to the presence of an alkyl substituent at C-8.[4,7] It thus became clear from the above spectral data that epimedin A was a close relative of icariin (IV) isolated from the same source.[3]

Enzymatic hydrolysis of epimedin A using β-glucosidase readily afforded D-glucose and a product (V) (FAB-MS m/z: 677 (M^++1)) which was, in turn, hydrolyzed with 1N sulfuric acid in methanol to yield D-glucose and L-rhamnose as sugar moieties. The UV spectrum of the product (V) showed a consistent bathochromic shift (271 → 280 nm) on addition of sodium acetate,[5] and the ^{13}C NMR signal for C-7 was shifted downfield by 1.40 ppm (pyridine-d_5) as compared to that of epimedin A,

suggesting that the product (V) was 7-desmonoglucopyranosylepimedin A bearing a disaccharide moiety composed of D-glucose and L-rhamnose at C-3. In order to clarify the position of the interglycosidic linkage in the disaccharide moiety, the product (V) was acetylated with acetic anhydride in pyridine to afford a heptaacetate (VI) whose ^{1}H NMR spectrum (500 MHz, $CDCl_3$), still showing a hydrogen-bonded hydroxyl signal at δ 12.28, was analyzed in detail by double resonance experiments.[8] Thus, it was found that the carbinyl hydrogen signal due to H-2 of the rhamnopyranosyl moiety at δ 4.42 (1H br s) was significantly shifted upfield in comparison to the other hydrogens on the carbons bearing acetoxyl group, revealing a 1 → 2 linkage between the outer glucopyranosyl and inner rhamnopyranosyl unit.[9]

The glycosidic linkages at C-1 of two glucopyranosyl residues were easily assigned to be both β from the coupling constants of the anomeric hydrogen signals (J 6.4 and 8.3 Hz), the chemical shifts of the ^{13}C NMR signals (δ 100.6 and 106.2)[4] and the ^{13}C-^{1}H coupling constants (159 and 159 Hz) for their anomeric centers in epimedin A.[10] The configuration at C-1 of the rhamnopyranosyl residue was also established to be α from the chemical shift (δ 101.1) and the large ^{13}C-^{1}H coupling constant (J 176 Hz) of the signal of that carbon.[4,10,11]

Epimedin B (II), an amorphous yellow solid, $[\alpha]_D$ -90.4° (c 0.44, MeOH), also showed characteristic color reactions for flavonoids (positive ferric chloride and magnesium-hydrochloric acid tests). The UV and IR absorption bands[12] perfectly matched with those of epimedin A, indicating that epimedin B has the identical oxygen substitution pattern. The ^{1}H NMR (90 MHz, pyridine-d_5) showed signals as follows δ 1.38 (3H d, J 4.3 Hz), 1.60, 1.76 (3H each s), 3.71 (3H s), 6.22 (1H br s), 7.14 (1H s), 7.15, 8.10 (2H each d, J 8.6 Hz) and 13.15 (1H s). These findings suggested that it is a congener of epimedin A, and from a comparative study of the ^{13}C NMR spectra of epimedins B and A, it was revealed that epimedin B has the same aglycone as well as glucopyranosyl and rhamnopyranosyl moieties as those of epimedin A, and in addition possesses a pentose sugar portion instead of the terminal glucopyranosyl moiety present in epimedin A (Table I). In order to confirm the sugar moiety, enzymatic hydrolysis followed by acid hydrolysis of epimedin B was carried out, to yield D-glucose, L-rhamnose and D-xylose as sugar components. The ^{1}H NMR spectrum of a heptaacetate (VIII) of an enzymatically hydrolyzed product (VII) (FAB-MS m/z: 647 (M^++1)) of epimedin B also displayed a signal ascribed to H-2 of the inner rhamnopyranosyl group at δ 4.32 (1H dd, J 3.1 and 1.7 Hz), whose chemical shift and splitting pattern were almost identical with those of the acetate (VI) prepared from epimedin A, confirming that the xylopyranosyl moiety was present as the terminal sugar at C-2 of the rhamnopyranosyl moiety [13]

The configurations of two anomeric centers of the sugar residues were determined to be β (glucopyranose) and α (rhamnopyranose) from the anomeric carbon signal (δ 102.3 and 102.3) in epimedin B, and that of the terminal xylopyranosyl residue was assigned to be β from the large coupling constant (J 6.7 Hz) for the anomeric hydrogen signal in the ^{1}H NMR spectrum of the acetate (VIII).

Epimedin C (III), an amorphous yellow solid, $[\alpha]_D$ -109.5° (c 0.19, MeOH), gave positive flavonoid color reactions. It was found to have the structural similarity to the above epimedins from its UV, IR and ^{1}H NMR spectra.[14] In the ^{13}C NMR spectrum of epimedin C, the signals attributed to the carbons of the aglycone part and the glucopyranosyl moiety at C-7 appeared at essentially the same positions as those observed in the other epimedins (Table I). Further, the ^{13}C NMR signals at δ 18.2 and 18.5 (each q) suggested the presence of two rhamnose moieties in the molecule, and this assumption was verified by the observation that acid-hydrolysis of an enzymatic hydrolysis product (IX) of epimedin C afforded only L-rhamnose. The position of the interglycosidic linkage between the two rhamnopyranosyl units was deduced to be 1 → 2 from the ^{1}H NMR spectrum of a peracetylated derivative (X) of the product (IX) in which the carbinyl hydrogen signal assigned to H-2 of the inner rhamnopyranosyl part appeared at δ 4.42 (1H br s).[15]

The ^{13}C-^{1}H coupling constants (J 159, 170 and 174 Hz) of the signals for the anomeric centers of the glucopyranosyl and two rhamnopyranosyl moieties demonstrated their configurations to be β, α and α, respectively.[10,11]

Based on the above evidence, it was concluded that the structures of epimedins A, B and C are represented by formulas I, II and III, respectively.

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NOTES AND REFERENCES

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8) ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81 (3H d, J 6.8 Hz, H-6"'), 3.27 (1H dq, J 10.2, 6.8 Hz, H-5"'), 3.64 (1H br d, J 10.2 Hz, H-5""), 4.04 (1H dd, J 12.3, 2.0 Hz, H-6""), 4.26 (1H dd, J 12.3, 3.5 Hz, H-6""), 4.42 (1H br s, H-2"'), 4.53 (1H d, J 7.7 Hz, H-1""), 4.80 (1H t, J 10.5 Hz, H-4"'), 4.98 (1H dd, J 10.2, 7.7 Hz, H-2""), 5.03 (1H t, J 10.2 Hz, H-4""), 5.16 (1H t, J 10.2 Hz, H-3""), 5.18 (1H dd, J 10.5, 3.5 Hz, H-3"'), 5.61 (1H br s, H-1"').
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12) UV λ_{max}^{MeOH} nm (ε): 271 (18300), 317 (11500), 350 (10500); IR ν_{max}^{KBr} cm^{-1}: 3400, 1645.
13) ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (3H d, J 6.7 Hz, H-6"'), 3.25 (1H dd, J 11.2, 8.1 Hz, H-5""), 3.47 (1H dq, J 9.8, 6.7 Hz, H-5"'), 3.97 (1H dd, J 11.2, 4.2 Hz, H-5""), 4.32 (1H dd, J 3.1, 1.7 Hz, H-2"'), 4.50 (1H d, J 6.7 Hz, H-1""), 4.80 (1H t, J 9.8 Hz, H-4"'), 4.80 (1H dt, J 4.2, 8.1 Hz, H-4""), 4.85 (1H dd, J 8.1, 6.7 Hz, H-2""), 5.07 (1H t, J 8.1 Hz, H-3""), 5.20 (1H dd, J 9.8, 3.1 Hz, H-3"'), 5.39 (1H d, J 1.7 Hz, H-1"').
14) UV λ_{max}^{MeOH} nm (ε) 271 (14700), 316 (9700), 348 (8100); IR ν_{max}^{KBr} cm^{-1}: 3350, 1640; ^{1}H NMR (90 MHz, pyridine-d_5): δ 1.30 (3H d, J 5.0 Hz), 1.60, 1.74, 3.70 (3H each s), 6.01, 6.10 (1H each s), 7.09 (2H d, J 8.5 Hz), 7.10 (1H s), 8.07 (2H d, J 8.5 Hz), 13.18 (1H s).
15) ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.82, 1.21 (3H each d, J 6.0 Hz, H-6"', H-6""), 3.23 (1H m, H-5"'), 3.90 (1H m, H-5""), 4.42 (1H br s, H-2"'), 4.87 (1H br s, H-1""), 4.92 (1H t, J 9.7 Hz, H-4"'), 5.04 (1H t, J 9.7 Hz, H-4""), 5.23 (1H dd, J 9.7, 4.2 Hz, H-3"'), 5.25 (1H br s, H-2""), 5.29 (1H dd, J 9.7, 4.2 Hz, H-3""), 5.56 (1H br s, H-1"').

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