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IONONE AND LIGNAN GLYCOSIDES FROM *EPIMEDIUM DIPHYLLUM*

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Key Word Index—*Epimedium diphyllum*; Berberidaceae; ionone derivative; lignan; phenylethanoid; icariside B; icariside D; icariside E.

Abstract—From the aerial parts of *Epimedium diphyllum*, a new ionone derivative glucoside, icariside B_8, two new lignan glycosides, icarisides E_4 and E_5 and a new phenylethanoid glucoside, icariside D_2, have been isolated together with 20 known compounds. The structures of four new compounds were established by spectral and chemical evidence.

INTRODUCTION

The aerial parts of *Epimedium* species have been used since ancient times as a tonic in China and Japan. The glycosides of *E. grandiflorum* Morr. var. *thunbergianum* (Miq.) Nakai were reported previously [1–3]. We have now examined the aerial parts of *E. diphyllum* Lodd. (syn. *Aceranthus diphyllus* Morr. et Decne.) and isolated a new ionone derivative glucoside, two new lignan glycosides and a new phenylethanoid glucoside together with 20 known polar compounds. The identification of the new compounds is described in this paper.

RESULTS AND DISCUSSION

Compounds **1–3**, **5**, **7–9**, **15**, **18–23** have already been isolated from *E. grandiflorum* [1–3] and were identified by direct comparison. Compound **6** was identified as the *threo* isomer of **5** by comparing its ^{13}C NMR spectrum with that of **5** [2]. Compounds **10**, **12–14**, **17** were identified by comparing their spectral data with reported data [4–8].

The spectrum of **4** showed signals for two tertiary methyl at δ0.88 and 0.97, a vinyl methyl at δ1.90, an acetyl methyl at δ2.09 and an anomeric proton signal at δ5.23 (*d*, $J=7$ Hz) in the ^{1}H NMR spectrum and the ^{13}C NMR spectrum exhibited 19 carbon signals, including six carbon signals due to a glucopyranosyl residue. These NMR data suggested that **4** had an ionone-type skeleton. Enzymatic hydrolysis afforded **4a** as an aglycone, whose ^{1}H NMR spectrum showed two carbinyl proton signals at δ4.12 (*dt*, $J=12.5$, 3 Hz) and 4.19 (*d*, $J=3$ Hz) and a methylene proton signals at δ1.73 (*dd*, $J=12.5$, 3 Hz) and 2.21 (*t*, $J=12.5$ Hz) in addition to four singlet methyl signals. The above results showed that **4a** had an α-glycol. In the ^{13}C NMR spectrum of **4a**, C-5 (δ129.4) was shifted down-field by 4.1 ppm and C-2 (δ42.5) was shifted up-field by 7.1 ppm compared with those of the aglycone of icariside B_6 (**4b**) which has one hydroxyl group at C-3 [3]. These results led us to conclude that the structure of icariside B_8 is **4**.

Icariside E_4 (**11**) showed a similar ^{1}H NMR spectrum to that of **10** [4] except for a doublet methyl at δ1.59 ($J=6$ Hz) and a singlet anomeric proton signal at δ6.07. In the ^{13}C NMR spectrum of **11**, six carbon signals due to a rhamnopyranosyl residue were observed and the glycosylation shifts were observed at C-1 (+4.0 ppm), C-3 (+1.1 ppm), C-4 (+3.2 ppm) and C-5 (+2.5 ppm) compared with those of **11a**, obtained from **11** by enzymatic hydrolysis. This aglycone was identical to the aglycone of **10** [4]. The CD spectrum of **11** showed a negative Cotton effect $[\theta]_{279}-4700$, suggesting C-7 and C-8 to be *R* and *S*, respectively [9]. From these data the structure of icariside E_4 was decided to be **11**.

Icariside E_5 (**16**) showed absorption maxima at 219 (4.56), 259 (4.22), 267 (sh 4.17), 288 (sh 3.82) and 306 (sh 3.54) nm and a similar ^{1}H NMR spectrum to that of **15** [3], except for a vinylic proton signal at δ6.58 (*dt*, $J=16$, 5 Hz). The ^{13}C NMR spectrum showed two sp^2 carbon signals at δ129.8 and 131.0 in addition to aromatic carbon signals, suggesting that **16** had a *trans*-double bond in a side chain. Catalytic hydrogenation afforded **15** in good yield. Thus, the structure of icariside E_5 was decided to be **16**.

Icariside D_2 (**24**) showed a similar ^{1}H and ^{13}C NMR spectrum compared with those of **23** [1] but an oxymethylene carbon signal (C-α) was shifted up-field by 8.0 ppm and C-1 was shifted down-field by 4.4 ppm. Thus, the structure of icariside D_2 was decided to be **24**.

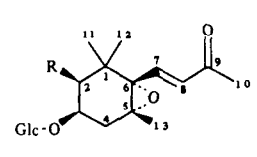
There are some morphological differences between *E. diphyllum* and *E. grandiflorum* but the chemical constituents are similar to each other.

EXPERIMENTAL

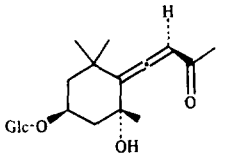
The instruments used in this study were the same as described in the previous paper [1].

Plant material. Aerial parts of *E. diphyllum* were harvested in summer of 1987 from cultivations in the botanical garden of Yomeishu Seizo Co., Ltd (Nakaminowa, Minowa-cho, Kamiina-gun, Nagano, Japan). The plant was identified by Prof. A. Ueno and a voucher specimen has been deposited in the Herbarium, School of Pharmaceutical Sciences, University of Shizuoka.

Extraction and isolation. Dried aerial parts (550 g) were extracted with hot H_2O. The H_2O extract was passed through an



	R
1	H
2	OH



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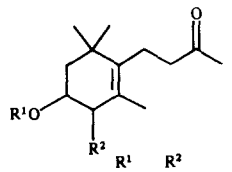
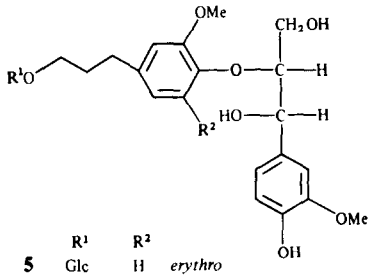
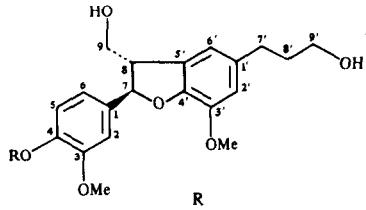


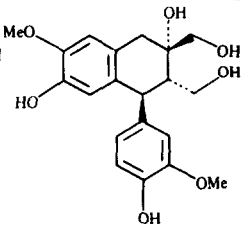
	R^1	R^2
4	Glc	OH
4a	H	OH
4b	Glc	H



	R^1	R^2	
5	Glc	H	*erythro*
6	Glc	H	*threo*
7	Rham	H	*erythro*
8	Rham	H	*threo*
9	Rham	OH	*erythro*



	R
10	Glc
11	Rham
11a	H



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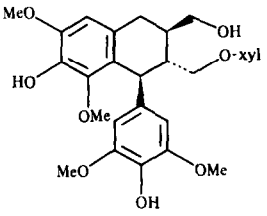

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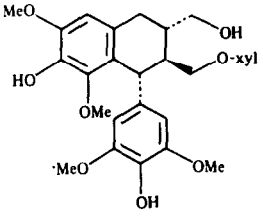

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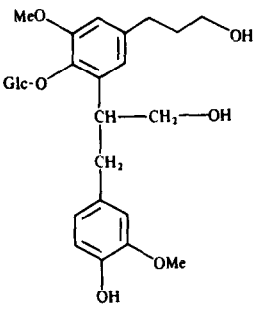

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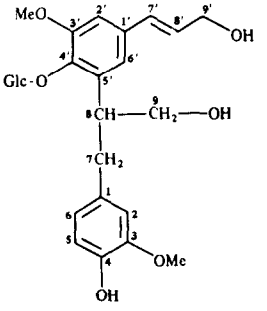

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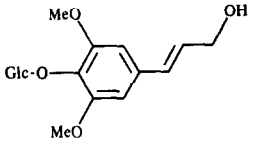

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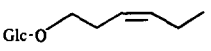

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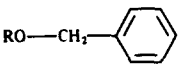
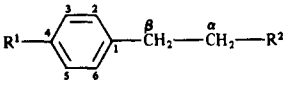


	R
19	Glc
20	Glc-6-Api



	R	R^2
21	O-Glc	NO_2
22	H	O Glc-6-Api
23	OH	O-Glc
24	O-Glc	OH

Amberlite XAD-2 column and the eluate with MeOH was concd under red. pres. The residue (50 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH (93:7–9:1) and each fr. was purified by HPLC, YMC Pack ODS-7 20 mm × 25 cm; MeCN–H_2O (23:2–3:1) to give 26 mg **1**, 4 mg **2**, 19 mg **3**, 7 mg **4**, 17 mg **5**, 2 mg **6**, 58 mg **7**, 46 mg **8**, 2 mg **9**, 13 mg **10**, 37 mg **11**, 5 mg **12**, 16 mg **13**, 13 mg **14**, 21 mg **15**, 15 mg **16**, 10 mg **17**, 56 mg **18**, 10 mg **19**, 7 mg **20**, 4 mg **21**, 19 mg **22**, 4 mg **23** and 6 mg **24**.

Icariside B_8 (**4**). $[\alpha]_D^{23}$ −60.9° (MeOH; *c* 0.64). FAB MS (MeOH + *m*-nitrobenzyl alcohol) *m/z*: 411 $[M+Na]^+$. ^{1}H NMR (pyridine-d_5): δ0.88; 0.97 (each 3H, *s*, H_3-11/H_3-12), 1.90 (3H, *s*, H_3-13), 2.09 (3H, *s*, H_3-10), 5.23 (1H, *d*, *J* = 7 Hz, anomeric H). ^{13}C NMR (pyridine-d_5): δ18.5 (C-13), 22.4 (C-7), 27.1; 29.3; 29.6 (C-10/C-11/C-12), 37.8 (C-1), 39.8 (C-2), 43.7 (C-8), 62.8 (C-6'), 68.9 (C-4), 71.7 (C-4'), 74.6 (C-3), 75.6 (C-2'), 78.8 (C-5'), 79.0 (C-3'), 101.5 (C-1'), 128.0 (C-5), 141.2 (C-6), 207.4 (C-9).

Icariside E_4 (**11**). Amorphous powder. $[\alpha]_D^{22}$ −22.0° (MeOH; *c* 1.32). (Found: C, 60.65; H, 6.94. $C_{26}H_{34}O_{10}\cdot 1/2\,H_2O$ requires: C, 60.57; H, 6.84%). UV λ_{max}^{MeOH} nm (log ε): 225 (sh 4.12), 280 (3.56). CD [θ] (nm): −4700 (279). ^{1}H NMR (pyridine-d_5): δ1.59 (3H, *d*, *J* = 6 Hz, H_3-6''), 2.08 (2H, *m*, H_2-8'), 2.87 (2H, *dd*, *J* = 8, 7 Hz, H_2-7'), 3.86 (6H, *s*, OMe × 2), 3.93 (2H, *t*, *J* = 7 Hz, H_2-9'), 6.07 (1H, *s*, H-1''), 6.10 (1H, *d*, *J* = 6 Hz, H-7), 6.95; 7.04 (each 1H, *br s*, H-2', H-6'), 7.2–7.6 (3H, *m*, H-2, H-5, H-6). ^{13}C NMR (pyridine-d_5): δ18.6 (C-6''), 32.7 (C-7'), 36.0 (C-8'), 55.2 (C-8), 56.0; 56.4 (OMe), 61.5 (C-9'), 64.5 (C-9), 71.0 (C-5''), 72.1 (C-3''), 72.7 (C-2''), 73.8 (C-4''), 88.0 (C-7), 101.6 (C-1''), 111.3 (C-2), 113.9 (C-2'), 117.6 (C-6'), 119.0 (C-5, C-6), 129.9 (C-1'), 138.0 (C-1), 136.4 (C-5'), 144.7 (C-3'), 146.4 (C-4'), 147.3 (C-3), 151.4 (C-4).

Icariside E_5 (**16**). Amorphous powder. $[\alpha]_D^{22}$ −118.8° (MeOH; *c* 1.04). (Found: C, 58.06; H, 6.50. $C_{26}H_{34}O_{11}\cdot H_2O$ requires: C, 57.77, H, 6.71%). UV λ_{max}^{MeOH} (log ε): 219 (4.56), 259 (4.22), 267 (sh 4.17), 288 (sh 3.82), 306 (sh 3.54). ^{1}H NMR (pyridine-d_5): δ3.70 (6H, *s*, OMe × 2), 4.56 (2H, *br d*, *J* = 5 Hz, H_2-9'), 6.58 (1H, *dt*, *J* = 16, 5 Hz, H-8'), 6.7–7.3 (aromatic H). ^{13}C NMR (pyridine-d_5): δ39.2 (C-7), 42.3 (C-8), 55.9; 56.1 (OMe), 62.5; 63.0 (C-6''/C-9'), 66.6 (C-9), 71.3 (C-4''), 76.2 (C-2''), 78.3 (C-5''), 78.5 (C-3''), 105.9 (C-1''), 108.9 (C-2'), 113.6 (C-2), 116.1 (C-5), 118.8 (C-6'), 129.8 (C-8'), 131.0 (C-7'), 132.3 (C-1), 134.7 (C-1'), 139.6 (C-5'), 144.7 (C-4'), 146.1 (C-4), 148.3 (C-3), 152.9 (C-3').

Icariside D_2 (**24**). Amorphous powder. $[\alpha]_D^{23}$ −61.9° (MeOH; *c* 0.11). FAB MS (MeOH + *m*-nitrobenzyl alcohol) *m/z*: 323 $[M+Na]^+$, 301 $[M+H]^+$. ^{1}H NMR (pyridine-d_5): δ2.96 (2H, *t*, *J*

= 7 Hz, H_2-β), 4.03 (2H, *t*, *J* = 7 Hz, H_2-α), 5.60 (1H, *d*, *J* = 7 Hz, H-1′), 7.29 (4H, *s*, aromatic H). ^{13}C NMR (pyridine-d_5): δ 39.6 (C-β), 62.5 (C-6′), 63.7 (C-α), 71.4 (C-4), 75.0 (C-2′), 78.6 (C-5′), 78.8 (C-3′), 102.5 (C-1′), 117.0 (C-3, C-5), 130.5 (C-2, C-6), 133.9 (C-1), 157.2 (C-4).

Enzymatic hydrolysis of **4**. A soln of **4** (5 mg) in H_2O (0.5 ml) was treated with cellulase (Sigma type II) (10 mg) at 38° for 2 hr. The reaction mixt. was extd × 3 with EtOAc. Solvent was evapd off and the residue was purified by HPLC (YMC Pack ODS-7, MeCN–H_2O (9:31) to give aglycone **4a** as a colourless syrup (2 mg). $[\alpha]_D^{23}$ −102.2° (MeOH; *c* 0.22;). EIMS *m/z* (rel. int.): 218 $[M-H_2O]^+$ (5), 168 (16), 152 (19), 135 (23), 109 (45), 42 (100). ^{1}H NMR (pyridine-d_5) (400 MHz): δ 0.99; 1.04 (each 3H, *s*, H_3-11/H_3-12), 1.73 (1H, *dd*, *J* = 12.5, 3 Hz, H-$2_{pseud-ax}$), 1.92 (3H, *s*, H_3-13), 2.09 (3H, *s*, H_3-10), 2.21 (1H, *t*, *J* = 12.5 Hz, H-$2_{pseud-eq}$), 4.12 (1H, *dt*, *J* = 12.5, 3 Hz, H-3), 4.19 (1H, *d*, *J* = 3 Hz, H-4). ^{13}C NMR (pyridine-d_5): δ 18.4 (C-13), 22.5 (C-7), 27.4; 29.4; 29.6 (C-10/C-11/C-12), 37.9 (C-1), 42.5 (C-2), 43.8 (C-8), 67.1 (C-3), 72.2 (C-4), 129.4 (C-5), 140.5 (C-6), 207.5 (C-9).

Enzymatic hydrolysis of **11**. A soln of **11** (8 mg) in H_2O (1 ml) was treated with cellulase (Sigma type II) (10 mg) at 38° for 4 hr. The reaction mixt. was extracted with EtOAc as described for **4** and the residue purified by HPLC (YMC Pack ODS-7, MeOH–H_2O (11:9) to give aglycone (**11a**) as an amorphous powder (5 mg). $[\alpha]_D^{23}$ −17.7° (Me_2CO; *c* 0.48). EIMS *m/z* (rel. int.): 360 $[M]^+$ (51), 342 $[M-H_2O]^+$ (100), 330 (56), 327 (36). ^{1}H NMR (pyridine-d_5): δ 2.07 (2H, *m*, H_2-8′), 2.87 (2H, *dd*, *J* = 8, 6 Hz, H_2-7′), 3.63; 3.83 (each 3H, *s*, OMe), 3.90 (2H, *t*, *J* = 6 Hz, H_2-9′), 6.04 (1H, *d*, *J* = 7 Hz, H-7), 6.90; 7.03 (each 1H, *br s*, H-2′/H-6′), 7.20 (2H, *br s*, H-5, H-6), 7.30 (1H, *br s*, H-2). ^{13}C NMR (pyridine-d_5): δ 32.7 (C-7′), 36.0 (C-8′), 55.1 (C-8), 55.9; 56.3 (OMe), 61.5 (C-9′), 64.5 (C-9), 88.4 (C-7), 110.9 (C-2), 113.8 (C-2′), 116.5 (C-5), 117.6 (C-6′), 119.8 (C-6), 130.3 (C-1′), 134.0 (C-1), 136.2 (C-5′), 144.7 (C-3′), 147.4 (C-4′), 148.2 (C-3, C-4).

Catalytic hydrogenation of **16**. A soln of **16** (5 mg) in MeOH (1 ml) was treated with H_2 for 5 min. using 5% Pd-C (5 mg) as catalyst at room temp. After removal of catalyst by filtration, MeOH was evapd off and the residue purified by HPLC [YMC Pack ODS-7, MeCN–H_2O (3:17)] to give **15** as an amorphous powder (4 mg), which was identified as icariside E_3 by direct comparison with an authentic sample.

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