Two New Glycosides from the Whole Plants of *Glechoma hederacea* L.

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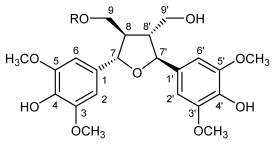
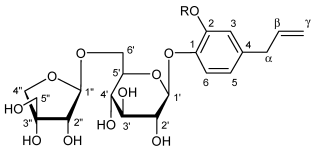
Two new glycosides, 7*S*,7′*S*,8*R*,8′*R*-icariol A_2-9-*O*-*β*-D-glucopyranoside (1) and 4-allyl-2-hydroxyphenyl 1-*O*-*β*-D-apiosyl-(1→6)-*β*-D-glucopyranoside (2), were isolated from the dried whole plants of *Glechoma hederacea* L. (Labiatae) together with four known compounds, cistanoside E (3), dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside (4), apigenin 7-*O*-*β*-D-glucuronopyranoside (5) and luteolin 7-*O*-*β*-D-glucopyranoside (6). The structures of the new compounds were elucidated on the basis of chemical and spectral analysis.

Key words *Glechoma hederacea*; Labiatae; glycoside

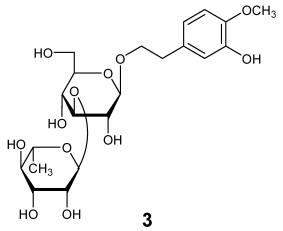
Glechoma hederacea L. (*kakidooshi* in Japanese, Labiatae) has been used in the treatment of various ailments, *e.g.* asthma, bronchitis, cold, diabetes and inflammation as a folk medicine for centuries.[1)] The constituents of *G. hederacea* have been previously investigated and shown to contain alkaloids,[1)] sesquiterpenoids,[2)] triterpenoids[3)] and rosmarinic acid.[4)] Here, we report the isolation and structural elucidation of two new glycosides, 7*S*,7′*S*,8*R*,8′*R*-icariol A_2-9-*O*-*β*-D-glucopyranoside (**1**) and 4-allyl-2-hydroxyphenyl 1-*O*-*β*-D-apiosyl-(1→6)-*β*-D-glucopyranoside (**2**), together with four known compounds from the dried whole plants of *G. hederacea*. The known compounds were identified as cistanoside E (**3**),[5)] dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside (**4**),[6)] apigenin 7-*O*-*β*-D-glucuronopyranoside (**5**)[7)] and luteolin 7-*O*-*β*-D-glucopyranoside (**6**),[8)] respectively, by comparison of their spectroscopic data with those previously described in the literature.



1 R = β-D-Glc*p*
1a R = H



2 R = H
7 R = CH_3



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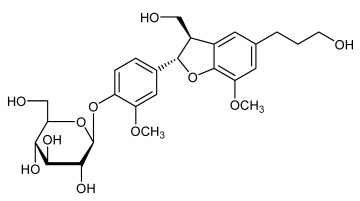

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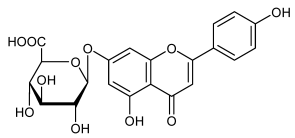

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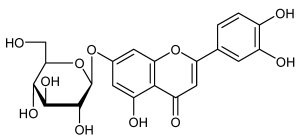

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Chart 1

Compound **1**, $[\alpha]_D$ +2.6° (MeOH), was isolated as an amorphous powder. The molecular formula was determined to be $C_{28}H_{38}O_{14}$ by high-resolution (HR)-FAB-MS. The ^{1}H-NMR spectrum showed signals due to two methines [δ_H 2.38 (1H, m, H-8′), 2.50 (1H, m, H-8)], two oxymethylenes [δ_H 3.66 (1H, dd, J=11.4, 5.4 Hz, H-9′$_A$), 3.68 (1H, dd, J=10.0, 5.4 Hz, H-9$_A$), 3.79 (1H, dd, J=11.4, 4.4 Hz, H-9′$_B$), 4.10 (1H, dd, J=10.0, 4.9 Hz, H-9$_B$)], four methoxyl groups [δ_H 3.87 (12H, s, CH_3O-3, CH_3O-5, CH_3O-3′, CH_3O-5′)], an anomeric proton [δ_H 4.28 (1H, d, J=7.6 Hz, H-1″)], two oxymethines [δ_H 4.99 (1H, d, J=8.5 Hz, H-7′), 5.07 (1H, d, J=8.3 Hz, H-7)] and four aromatic protons [δ_H 6.75 (2H, s, H-2′, H-6′), 6.78 (2H, s, H-2, H-6)]. The ^{13}C-NMR spectrum showed signals due to a glucopyranosyl group [δ_C 61.2 (C-6″), 71.6 (C-4″), 75.2 (C-2″), 78.0 (C-3″), 78.2 (C-5″), 104.8 (C-1″)]. The relatively large $^3J_{1''-2''}$ value of the anomeric proton of this glucopyranosyl group (7.6 Hz) indicated a *β* anomeric orientation. On enzymatic hydrolysis with cellulase and *β*-glucosidase (*ca.* 3 : 1), **1** gave an aglycone (**1a**) and D-glucose, which was identified by its retention time and optical rotation using chiral detection by HPLC analysis. Compound **1a** was identified as icariol A_2 by the comparison of its optical rotation value, ^{1}H-NMR spectrum and electron ionization (EI)-MS.[9)] However, the absolute configuration of **1a** was undefined. The absolute configuration of **1a** was elucidated by the circular dichroism (CD) exciton chirality method.[10)] The CD spectrum of **1a** showed a negative Cotton effect at 246.3 nm ($\Delta\varepsilon$ −3.0), indicating that two 3,5-dimethoxy-4-hydroxyphenyl groups at C-7 and C-7′ are left-handed and counter-clockwise (Fig. 1).[11,12)] Thus the absolute configurations at C-7 and C-7′ of **1a** were assigned as

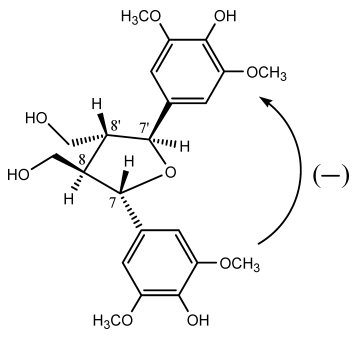

Fig. 1. Cotton Effect of **1a**

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S and *S*, respectively. Accordingly, compound **1a** was concluded to be 7*S*,7′*S*,8*R*,8′*R*-icariol A_2. The nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum determined the position of the β-D-glucopyranosyl group to be at C-9, by showing correlations between H-1″ and H-9_A, and H-1″ and H-9_B. On the basis of this evidence, the structure of **1** was determined to be 7*S*,7′*S*,8*R*,8′*R*-icariol A_2-9-*O*-β-D-glucopyranoside.

Compound **2**, $[\alpha]_D$ −43.4° (MeOH), was isolated as an amorphous powder. The molecular formula was determined to be $C_{20}H_{28}O_{11}$ by HR-FAB-MS. Acid hydrolysis of **2** gave D-apiose and D-gulcose in the above manner. The ^{1}H- and ^{13}C-NMR spectra of **2** were closely related to those of 4-allyl-2-methoxyphenyl 1-*O*-β-D-apiosyl-(1→6)-β-D-glucopyranoside (**7**),[13] except that the signal of 2-methoxyl group of **7** disappeared in **2**. The ^{13}C-NMR chemical shift at C-2 (δ_C 148.3) in **2** was shifted upfield by −2.4 ppm compared with that of **7**, suggesting that the methoxyl group at C-2 of **7** replaced the hydroxyl group in **2**.[14] Therefore, compound **2** was determined to be 4-allyl-2-hydroxyphenyl 1-*O*-β-D-apiosyl-(1→6)-β-D-glucopyranoside.

Experimental

General Procedures Optical rotations were determined using a JASCO DIP-360 digital polarimeter. CD spectra were measured on a JASCO J-720 spectropolarimeter. UV spectra were recorded with a Beckman DU-64 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JNM-LA 600 (600 and 150 MHz, respectively) and JEOL JNM-LA 400 (400 and 100 MHz, respectively) spectrometers. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane as internal standard. The EI- and HR-FAB-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on a Kieselgel 60 (230—400 mesh, Merck). HPLC was performed by using a system comprised of CCPS pump (Tosoh), a UV-8020 detector (Tosoh), and a JASCO OR-2090 plus chiral detector.

Plant Material The dried whole plants of *Glechoma hederacea* were purchased from Uchida Wakanyaku Co., Ltd., Tokyo, Japan.

Extraction and Isolation The dried whole plants of *G. hederacea* (2.0 kg) were extracted with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure. The MeOH extract (228 g) was suspended in water, and this suspension was extracted with $CHCl_3$, AcOEt, *n*-BuOH and H_2O. The *n*-BuOH extract (12 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1) to afford 106 fractions. From fraction 9, rosmarinic acid was obtained (20.0 mg). Fraction 12 was purified by preparative HPLC [column, TSKgel Amide-80 (7.8 mm i.d.×30 cm, Tosoh), column temperature, 40 °C; mobile phase, CH_3CN–H_2O (3 : 1); flow rate, 1.5 ml/min] to give **1** (1.1 mg), **2** (0.3 mg), **3** (1.0 mg) and **4** (1.1 mg). Fraction 14 was purified by preparative HPLC [column, TSKgel Amide-80 (7.8 mm i.d.×30 cm, Tosoh), column temperature, 40 °C; mobile phase, CH_3CN–H_2O (3 : 1); flow rate, 1.5 ml/min] to give **6** (2.0 mg). Fraction 80 was purified by preparative HPLC [column, TSKgel Amide-80 (7.8 mm i.d.×30 cm, Tosoh), column temperature, 40 °C; mobile phase, CH_3CN–H_2O (3 : 1); flow rate, 1.5 ml/min] to give **5** (1.0 mg).

7*S*,7′*S*,8*R*,8′*R*-Icariol A_2-9-*O*-β-D-glucopyranoside (**1**): Amorphous powder. $[\alpha]_D^{27}$ +2.6° (*c*=0.112, MeOH). UV λ_{max} (MeOH) nm (log ε): 214 (4.3), 237 (4.1), 270 (3.5). CD (*c*=5.47×10^{-5} M, MeOH) $\Delta\varepsilon$ (nm): −4.4 (246.3), +8.5 (207.6). HR-FAB-MS *m*/*z*: 621.2166 ([M+Na]$^+$, Calcd for $C_{28}H_{38}O_{14}Na$: 621.2159). ^{1}H-NMR (400 MHz, CD_3OD) δ: 2.38 (1H, m, H-8′), 2.50 (1H, m, H-8), 3.66 (1H, dd, *J*=11.4, 5.4 Hz, H-$9'_A$), 3.68 (1H, dd, *J*=10.0, 5.4 Hz, H-9_A), 3.79 (1H, dd, *J*=11.4, 4.4 Hz, H-$9'_B$), 3.87 (12H, s, CH_3O-3, CH_3O-5, CH_3O-3′, CH_3O-5′), 4.10 (1H, dd, *J*=10.0, 4.9 Hz, H-9_B), 4.28 (1H, d, *J*=7.6 Hz, H-1″), 4.99 (1H, d, *J*=8.5 Hz, H-7′), 5.07 (1H, d, *J*=8.3 Hz, H-7), 6.75 (2H, s, H-2′, H-6′), 6.78 (2H, s, H-2, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ: 51.9 (C-8′), 54.7 (C-8), 56.9 (OCH_3), 61.2 (C-6″), 62.7 (C-9′), 70.1 (C-9), 71.6 (C-4″), 75.2 (C-2″), 78.0 (C-3″), 78.2 (C-5″), 84.4 (C-7′), 84.6 (C-7), 104.8 (C-1″), 104.9 (C-2, C-6, C-2′, C-6′), 134.2, 134.3 (C-1, C-1′), 136.2 (C-4, C-4′), 149.3 (C-3, C-5, C-3′, C-5′).

4-Allyl-2-hydroxyphenyl 1-*O*-β-D-Apiosyl-(1→6)-β-D-glucopyranoside (**2**): Amorphous powder. $[\alpha]_D^{26}$ −43.4° (*c*=0.0303, MeOH). UV λ_{max} (MeOH) nm (log ε): 220 sh (4.0), 274 (3.6). HR-FAB-MS *m*/*z*: 467.1532 ([M+Na]$^+$, Calcd for $C_{20}H_{28}O_{11}Na$: 467.1529). ^{1}H-NMR (600 MHz, CD_3OD) δ: 3.33 (2H, m, H_2-α), 3.44 (1H, t, *J*=9.0 Hz, H-2′), 3.46 (1H, dd, *J*=7.6, 6.4 Hz, H-3′), 3.54 (1H, ddd, *J*=8.3, 6.6, 2.0 Hz, H-5′), 3.59 (1H, s, H-5″), 3.64 (1H, dd, *J*=11.2, 6.6 Hz, H-$6'_A$), 3.76 (1H, d, *J*=9.8 Hz, H-$4''_A$), 3.92 (1H, d, *J*=2.4 Hz, H-2″), 3.97 (1H, d, *J*=9.8 Hz, H-$4''_B$), 4.02 (1H, dd, *J*=11.2, 2.0 Hz, H-$6'_B$), 4.66 (1H, d, *J*=7.6 Hz, H-1′), 5.00 (1H, d, *J*=2.4 Hz, H-1″), 5.01 (1H, dd, *J*=10.3, 1.4 Hz, H-γ_A), 5.05 (1H, dd, *J*=16.8, 1.4 Hz, H-γ_B), 5.93 (1H, ddt, *J*=16.8, 10.3, 6.8 Hz, H-β), 6.62 (1H, dd, *J*=8.3, 2.2 Hz, H-5), 6.69 (1H, d, *J*=2.2 Hz, H-3), 7.10 (1H, d, *J*=8.3 Hz, H-6). ^{13}C-NMR (150 MHz, CD_3OD) δ: 40.6 (C-α), 65.6 (C-5″), 68.4 (C-6′), 71.6 (C-4′), 74.9 (C-4″), 75.0 (C-2′), 77.1 (C-5′), 77.6 (C-2″), 78.0 (C-3′), 80.5 (C-3″), 102.0 (C-1′), 110.2 (C-6), 110.9 (C-1″), 115.7 (C-γ), 119.2 (C-3), 121.1 (C-5), 136.7 (C-4), 139.0 (C-β), 146.3 (C-1), 148.3 (C-2).

Enzymatic Hydrolysis of 1 Compound **1** (1.1 mg), was treated with cellulase (from *Aspergillus niger*, 0.39 units/mg, Sigma Chemical Co., 1.5 mg) and β-glucosidase (from almond, 5.5 units/mg, Sigma Chemical Co., 0.5 mg) in an AcOH–AcONa buffer solution (0.02 mol/l, pH 4.6, 2.5 ml). The mixture was stirred at 37 °C for 3 d, then extracted with an equal amount of AcOEt (×3), and the AcOEt layer was evaporated under reduced pressure. The residue was dried to give the aglycone (**1a**). The sugar fraction, obtained by concentration of the water layer, was analyzed by HPLC under the following conditions: column, TSKgel Amide-80 (7.8 mm i.d.×30 cm, Tosoh; column temperature, 45 °C; mobile phase, CH_3CN–H_2O (4 : 1); flow rate, 1.0 ml/min; chiral detection. Identification of D-glucose present in the sugar fraction was carried out by the comparison of its retention time and optical rotation with that of authentic sample; t_R (min) 39.0 (D-glucose, positive optical rotation).

7*S*,7′*S*,8*R*,8′*R*-Icariol A_2 (**1a**): Amorphous powder. $[\alpha]_D^{26}$ −28.8° (*c*=0.0637, $CHCl_3$), $[\alpha]_D^{24}$ −19.0° (*c*=0.0637, EtOH) [lit.,[9]) $[\alpha]_D^{23}$ −22.0° (*c*=0.59, MeOH)]. UV λ_{max} (MeOH) nm (log ε): 235sh (3.9), 274 (3.4). CD (*c*=4.25×10^{-5} M, MeOH) $\Delta\varepsilon$ (nm): −3.0 (246.3), +7.5 (207.4). EI-MS *m*/*z*: 436 [M]$^+$, 418 [M–H_2O]$^+$, 400 [M–2H_2O]$^+$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 2.30 (2H, m, H-8, H-8′), 3.61 (2H, dd, *J*=11.4, 5.5 Hz, H-9_A, H-$9'_A$), 3.69 (1H, dd, *J*=11.4, 3.7 Hz, H-9_B, H-$9'_B$), 3.86 (12H, s, OCH_3), 4.95 (2H, d, *J*=8.7 Hz, H-7, H-7′), 6.73 (4H, s, H-2, H-6, H-2′, H-6′).

Acid Hydrolysis of 2 Compound **2** (0.3 mg) was refluxed with 5% HCl for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The solution was concentrated *in vacuo* and dried to give a sugar fraction. The sugar fraction was analyzed by HPLC under the following conditions: column, TSKgel Amide-80 (7.8 mm i.d.×30 cm, Tosoh; column temperature, 45 °C; mobile phase, CH_3CN–H_2O (4 : 1); flow rate, 1.0 ml/min; chiral detection. Identification of D-apiose and D-glucose present in the sugar fraction was carried out by the comparison of these retention times and optical rotations with those of authentic samples; t_R (min) 21.0 (D-apiose, positive optical rotation), 39.0 (D-glucose, positive optical rotation).

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