A New Lignan Glycoside from the Leaves of *Sambucus sieboldiana* (Miq.) Blume ex. Graebn

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A new lignan glycoside, (−)-massoniresinol 4′-*O*-*β*-D-glucopyranoside (4), was isolated, together with six known ones (1—3, 5—7), from the leaves of *Sambucus sieboldiana*. Their structures were established on the basis of chemical and spectral data.

Key words *Sambucus sieboldiana*; Caprifoliaceae; lignan glycoside

The leaves of *Sambucus sieboldiana* (Caprifoliaceae) have been used in China as an herbal drug for anodyne and diuretic effects.[1] As a continuation of our studies of constituents from the plants in the Caprifoliaceae family,[2] we have now examined the chemical constituents of the leaves of *S. sieboldiana* in detail; this paper describes the structural elucidation of a new lignan glycoside (**4**), isolated, along with six known ones (**1**—**3**, **5**—**7**), from this plant. The isolation procedure is described in detail in the experimental section.

The known compounds were identified as (+)-syringaresinol *O*-*β*-D-glucopyranoside (**1**),[3] (−)-olivil 4″-*O*-*β*-D-glucopyranoside (**2**),[4] (−)-massoniresinol 4″-*O*-*β*-D-glucopyranoside (**3**)[5] and (2*R*,3*S*)-2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol 4′-*O*-*β*-D-glucopyranoside (**7**),[6] by direct comparison with authentic sample and/or by comparison of various spectral and chemical data with those reported in literature.

Compound **4** was obtained as an amorphous powder, $[\alpha]_D^{26}$ −63.8° (MeOH). The molecular formula of **4**, $C_{26}H_{34}O_{13}$, was confirmed by high-resolution (HR)-FAB-MS and was coincident with that of **3**. The ^{1}H- and ^{13}C-NMR spectral data of **4** were very similar to those of **3**, except for chemical shifts due to the aromatic moieties. These findings suggested that the *β*-D-glucopyranosyl group in **4** is on C-4′ of (−)-massoniresinol,[7] and not on the C-4″ hydroxyl moiety. This deduction was supported by the nuclear Overhauser enhancement spectroscopy (NOESY) and ^{1}H-detected heteronuclear multiple bond correlation (HMBC) experiments as follows: 2-H [δ 5.05 (1H, s)] showed HMBC correlation with δ_C 135.0 (s), which is also correlated to δ_H 7.12 (1H, d, J=8.3 Hz). Thus, the signals at δ_C 135.0 and δ_H 7.12 were assigned at C-1′ and 5′-H, respectively. NOE correlation was observed between the 5′-H and *β*-D-glucopyranosyl anomeric proton [δ 4.87 (1H, d, J=7.6 Hz)]. Furthermore, the NOESY spectrum of **4** showed correlations between 2-H and 3a-H_2, and between 2-H and 4a-H_2, which were also observed in the NOESY spectrum of **3**. The circular dichroism (CD) spectrum (Fig. 1) of **4** [278.5 nm ($\Delta\varepsilon$ −1.98) and 230.0 nm ($\Delta\varepsilon$ −6.71)] showed two similar negative Cotton effects, with those of **2** [$\Delta\varepsilon$ −2.68 (282.0 nm), $\Delta\varepsilon$ −7.66 (230.5 nm)], **3** [$\Delta\varepsilon$ −2.75 (281.0 nm), $\Delta\varepsilon$ −7.32 (230.5 nm)] and analogous compounds[8] [(+)-lariciresinol 4′-*O*-*β*-D-glucopyranoside; $\Delta\varepsilon$ −0.47 (280.0 nm), $\Delta\varepsilon$ −1.38 (228.0 nm).[9] (+)-lariciresinol 4″-*O*-*β*-D-glucopyranoside; $\Delta\varepsilon$ −0.85 (280.0 nm), $\Delta\varepsilon$ −1.94 (230.0 nm)[10]], suggesting that C-2, 3 and 4 have *R*, *S* and *R*-configurations, respectively.[11] From the above data, the structure of **4** was determined to be (−)-massoniresinol 4′-*O*-*β*-D-glucopyranoside. To our knowledge, the isolation of a

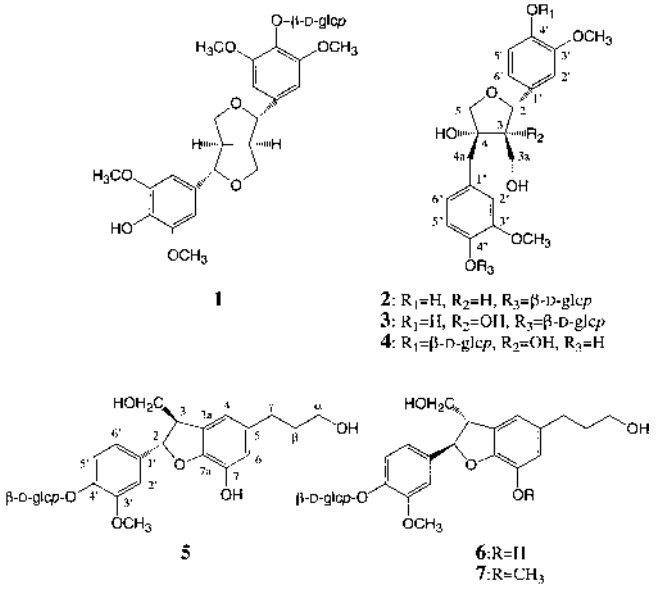

Chart 1

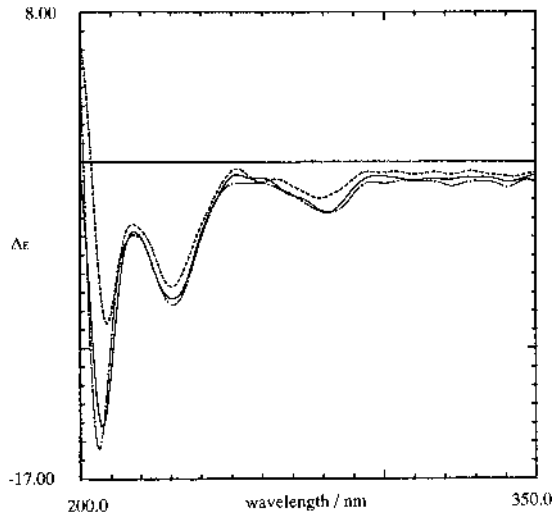

Fig. 1. The CD Spectra of **2**—**4** (MeOH)
2 (–·–·–), **3** (——), **4** (------).

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2-aryl-4-benzyltetrahydrofuran-type lignan having two hydroxyl groups at C-3 and C-4 from a natural source is rare.[5,7,12)]

Compounds **5** and **6** were isolated as an amorphous powder, $[\alpha]_D^{26}$ −35.7° and −20.0°, respectively. Interestingly, the ^{1}H- and ^{13}C-NMR spectra of **5** and **6** were quite similar. The HMBC experiments of **5** and **6** exhibited the same planar structure as 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-*β*-D-glucopyranoside, which was already isolated from *Pinus silvestris*,[13)] *P. contorta*,[14)] and *Cedrus deodara*,[15,16)] but its stereochemistry was not elucidated completely, to the best of our knowledge. Accordingly, the aglycone parts of **5** and **6** were deduced to be enantiometric structures. The relative configuration of 2-H and 3-H in each of **5** and **6** was *trans* from the NOE correlations between 2-H and one of the 3-hydroxymethyl protons [**5**; *δ* 3.74 (1H, dd, *J*=11.0, 7.6 Hz), **6**; *δ* 3.75 (1H, dd, *J*=10.9, 7.6 Hz)] and between 3-H and 2′-H. The CD data of **5** and **6** showed similar Cotton effects [**5**: *Δε* −3.40 (227.0 nm), **6**: *Δε* +2.04 (225.0 nm)] in the region corresponding to the strong UV band at *ca.* 230 nm[17,18)] with those of analogous compounds[6)] [(2*S*,3*R*)-2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol 4′-*O*-*β*-D-glucopyranoside: *Δε* −2.07 (225.0 nm),[19)] **7**: *Δε* +1.19 (223.0 nm)], respectively. From the above data, the absolute structures of **5** and **6** were determined to be (2*S*,3*R*)- and (2*R*,3*S*)-2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-*β*-D-glucopyranosides, respectively.

Experimental

General Optical rotations were measured with a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrometer. The CD spectra were obtained with a JASCO J-720 spectropolarimeter. The ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JNM-GSX 400 (400 MHz, 100 MHz, respectively) spectrometer. Chemical shifts are given on a *δ* (ppm) scale with TMS as an internal standard. FAB-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 70—230 mesh) and Sephadex LH-20 (Pharmacia Fine Chemicals). Preparative HPLC was carried out on a Tosoh HPLC system [pump, CCPM prep; detector, UV-8010; column, Cosmosil 5C_{18}-AR (10 mm i.d.×25 cm, Nacalai Tesque), Cosmosil 5SL (10 mm i.d.×25 cm, Nacalai Tesque)]. GLC was carried out on a Shimadzu GC-7A equipped with FID. Analytical TLC was performed on precoated silica gel plates (Merck, 0.25 mm thickness), and detection was achieved by spraying with 5% H_2SO_4 followed by heating.

Materials The leaves of *S. sieboldiana* were purchased from Uchida Wakanyaku Co. (Japan).

Isolation The leaves of *S. sieboldiana* (2.0 kg) were extracted with MeOH at room temp. for 10 d. Evaporation of the solvent under reduced pressure provided the MeOH extract (235.0 g), and this extract was fractionated between $CHCl_3$ and H_2O. The H_2O-soluble fraction was concentrated under reduced pressure to produce a residue (134.0 g). The residue was passed through a Mitsubishi Diaion HP-20 column, and adsorbed material was eluted with H_2O, MeOH and $CHCl_3$. The MeOH eluate fraction from the HP-20 column was concentrated, the residue (71.7 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1), and the eluate was separated into seven fractions (frs. 1—7). Fraction 2 was rechromatographed on Sephadex LH-20 (50% MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (7 : 1 : 0.5)] columns, and purified by preparative HPLC [Cosmosil 5C_{18}-AR column; MeOH–H_2O (2 : 3); 205 nm, Cosmosil 5SL column; $CHCl_3$–MeOH–H_2O (30 : 10 : 1); 230 nm, each flow rate: 1.5 ml/min] to give compounds **1** (3.5 mg) and **7** (8.0 mg). Fraction 4 was rechromatographed on Sephadex LH-20 (50% MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (30 : 10 : 1)] columns, and purified by preparative HPLC [Cosmosil 5C_{18}-AR column; MeOH–H_2O (1 : 3, 1 : 5); 205, 225 nm, Cosmosil 5SL column; CH_2Cl_2–MeOH–H_2O (30 : 10 : 1); 225 nm, each flow rate: 1.5 ml/min] to give compounds **2** (8.0 mg), **3** (7.0 mg), **4** (3.4 mg), **5** (4.3 mg) and **6** (3.0 mg).

(+)-Syringaresinol *O*-*β*-D-Glucopyranoside (**1**): An amorphous powder, $[\alpha]_D^{26}$ −23.8° (*c*=0.08, MeOH). FAB-MS *m/z*: 603 $[M+Na]^+$. The spectral data were identified with those of reported data.[3)]

(−)-Olivil 4″-*O*-*β*-D-Glucopyranoside (**2**): An amorphous powder, $[\alpha]_D^{26}$ −61.3° (*c*=0.37, MeOH). FAB-MS *m/z*: 561 $[M+Na]^+$. CD (*c*=4.07×10^{-5} M, MeOH) *Δε* (nm): −15.37 (206.0), −7.66 (230.5), −2.68 (282.0). The spectral data were identified with those of the authentic sample.[4)]

(−)-Massoniresinol 4″-*O*-*β*-D-Glucopyranoside (**3**): An amorphous powder, $[\alpha]_D^{26}$ −70.0° (*c*=0.20, MeOH). FAB-MS *m/z*: 577 $[M+Na]^+$. HR-FAB-MS *m/z*: 577.1867 $[M+Na]^+$ ($C_{26}H_{34}O_{13}Na$, Calcd for 577.1897). UV λ_{max} (MeOH) nm (log *ε*): 207 (4.38), 227 (4.19), 277 (3.73). CD (*c*=4.52×10^{-5} M, MeOH) *Δε* (nm): −14.20 (207.0), −7.32 (230.5), −2.75 (281.0). ^{1}H-NMR (400 MHz, CD_3OD) *δ*: 7.10 (1H, d, *J*=8.3 Hz, 5″-H), 7.02 (2H, br s, 2′, 2″-H), 6.86 (1H, dd, *J*=8.3, 2.0 Hz, 6″-H), 6.79 (1H, dd, *J*=8.1, 1.8 Hz, 6′-H), 6.75 (1H, d, *J*=8.1 Hz, 5′-H), 5.01 (1H, s, 2-H), 4.87 (1H, d, *J*=7.6 Hz, glc 1-H), 3.87 (3H, s, 3″-OCH_3), 3.86 (2H, m, 5-, glc 6-H_B), 3.85 (3H, s, 3′-OCH_3), 3.78 (1H, d, *J*=11.5 Hz, 3a-H_B), 3.68 (3H, m, 3a-, 5-, glc 6-H_A), 3.01, 2.95 (each 1H, d, *J*=13.5 Hz, 4a-H_2).

(−)-Massoniresinol 4′-*O*-*β*-D-Glucopyranoside (**4**): An amorphous powder, $[\alpha]_D^{26}$ −63.8° (*c*=0.19, MeOH). FAB-MS *m/z*: 577 $[M+Na]^+$. HR-FAB-MS *m/z*: 577.1867 $[M+Na]^+$ ($C_{26}H_{34}O_{13}Na$, Calcd for 577.1897). UV λ_{max} (MeOH) nm (log *ε*): 206 (4.35), 227 (4.12), 277 (3.66). CD (*c*=5.43×10^{-5} M, MeOH) *Δε* (nm): −8.66 (208.5), −6.71 (230.0), −1.98 (278.5). ^{1}H-NMR (400 MHz, CD_3OD) *δ*: 7.12 (1H, d, *J*=8.3 Hz, 5′-H), 7.10 (1H, d, *J*=2.2 Hz, 2′-H), 6.92 (2H, m, 6′, 2″-H), 6.73 (2H, m, 5″, 6″-H), 5.05 (1H, s, 2-H), 4.87 (1H, d, *J*=7.6 Hz, glc 1-H), 3.86 (3H, s, 3′-OCH_3), 3.86 (2H, m, 5-, glc 6-H_B), 3.85 (3H, s, 3″-OCH_3), 3.79 (1H, d, *J*=11.5 Hz, 3a-H_B), 3.71 (1H, d, *J*=11.5 Hz, 3a-H_A), 3.69 (1H, d, *J*=9.0 Hz, 5-H_A), 3.68 (1H, m, glc 6-H_A), 2.95, 2.90 (each 1H, d, *J*=13.9 Hz, 4a-H_2). ^{13}C-NMR (100 MHz, CD_3OD) *δ*: 86.0 (C-2), 82.4 (C-3), 64.5 (C-3a), 82.3 (C-4), 40.1 (C-4a), 75.02 (C-5), 135.0 (C-1′), 113.7 (C-2′), 150.4 (C-3′), 147.5 (C-4′), 117.4 (C-5′), 123.9 (C-6′), 130.2 (C-1″), 115.8 (C-2″), 148.6 (C-3″), 146.3 (C-4″), 115.4 (C-5″), 124.1 (C-6″), 56.4, 56.8 (3′, 3″-OCH_3), 103.0 (glc C-1), 74.98 (glc C-2), 78.0 (glc C-3), 71.4 (glc C-4), 77.9 (glc C-5), 62.6 (glc C-6).

(2*S*,3*R*)-2,3-Dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-*β*-D-Glucopyranoside (**5**): An amorphous powder, $[\alpha]_D^{26}$ −35.7° (*c*=0.14, MeOH). FAB-MS *m/z*: 531 $[M+Na]^+$. UV λ_{max} (MeOH) nm (log *ε*): 206 (4.54), 230sh (4.14), 278 (3.59). CD (*c*=5.59×10^{-5} M, MeOH) *Δε* (nm): −3.40 (227.0). ^{1}H-NMR (400 MHz, CD_3OD) *δ*: 7.13 (1H, d, *J*=8.5 Hz, 5′-H), 7.06 (1H, d, *J*=2.0 Hz, 2′-H), 6.95 (1H, dd, *J*=8.5, 2.0 Hz, 6′-H), 5.59 (1H, br s, 4-H), 6.57 (1H, br s, 6-H), 5.55 (1H, d, *J*=5.9 Hz, 2-H), *ca.* 4.87 (glc 1-H), 3.84 (2H, m, 3-C$\underline{H}_2$OH, glc 6-H_B), 3.83 (3H, s, 3′-OCH_3), 3.74 (1H, dd, *J*=11.0, 7.6 Hz, 3-C$\underline{H}_2$OH), 3.67 (1H, m, glc 6-H_A), 3.55 (2H, t, *J*=6.6 Hz, *α*-H_2), 3.43 (1H, m, 3-H), 2.56 (2H, t, *J*=7.8 Hz, *γ*-H_2), 1.78 (2H, m, *β*-H_2). ^{13}C-NMR (100 MHz, CD_3OD) *δ*: 88.2 (C-2), 56.0 (C-3), 65.3 (3-$\underline{C}H_2$OH), 129.5 (C-3a), 116.7 (C-4), 136.9 (C-5), 117.1 (C-6), 142.0 (C-7), 146.5 (C-7a), 32.8 (C-*γ*), 35.8 (C-*β*), 63.4 (C-*α*), 138.7 (C-1′), 111.2 (C-2′), 151.0 (C-3′), 147.6 (C-4′), 118.1 (C-5′), 119.4 (C-6′), 56.8 (3′-OCH_3), 103.0 (glc C-1), 74.9 (glc C-2), 78.3 (glc C-3), 71.4 (glc C-4), 77.9 (glc C-5), 62.4 (glc C-6).

(2*R*,3*S*)-2,3-Dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-*β*-D-Glucopyranoside (**6**): An amorphous powder, $[\alpha]_D^{26}$ −20.0° (*c*=0.05, MeOH). FAB-MS *m/z*: 531 $[M+Na]^+$. UV λ_{max} (MeOH) nm (log *ε*): 206 (4.56), 230sh (4.09), 278 (3.57). CD (*c*=4.92×10^{-5} M, MeOH) *Δε* (nm): +2.04 (225.0). ^{1}H-NMR (400 MHz, CD_3OD) *δ*: 7.14 (1H, d, *J*=8.3 Hz, 5′-H), 7.06 (1H, d, *J*=2.0 Hz, 2′-H), 6.95 (1H, dd, *J*=8.3, 2.0 Hz, 6′-H), 5.59 (1H, br s, 4-H), 6.57 (1H, br s, 6-H), 5.55 (1H, d, *J*=5.9 Hz, 2-H), *ca.* 4.88 (glc 1-H), 3.84 (2H, m, 3-C$\underline{H}_2$OH, glc 6-H_B), 3.83 (3H, s, 3′-OCH_3), 3.75 (1H, dd, *J*=10.9, 7.6 Hz, 3-C$\underline{H}_2$OH), 3.66 (1H, m, glc 6-H_A), 3.55 (2H, t, *J*=6.4 Hz, *α*-H_2), 3.44 (1H, m, 3-H), 2.56 (2H, t, *J*=7.5 Hz, *γ*-H_2), 1.77 (2H, m, *β*-H_2). ^{13}C-NMR (100 MHz, CD_3OD) *δ*: 88.2 (C-2), 56.0 (C-3), 65.3 (3-$\underline{C}H_2$OH), 129.5 (C-3a), 116.6 (C-4), 136.8 (C-5), 117.1 (C-6), 142.0 (C-7), 146.5 (C-7a), 32.8 (C-*γ*), 35.9 (C-*β*), 63.4 (C-*α*), 138.7 (C-1′), 111.2 (C-2′), 151.0 (C-3′), 147.6 (C-4′), 118.2 (C-5′), 119.4 (C-6′), 56.8 (3′-OCH_3), 102.9 (glc C-1), 75.0 (glc C-2), 78.3 (glc C-3), 71.4 (glc C-4), 77.9 (glc C-5), 62.4 (glc C-6).

(2*R*,3*S*)-2,3-Dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol 4′-*O*-*β*-D-Glucopyranoside (**7**): An amorphous powder, $[\alpha]_D^{26}$ −15.0° (*c*=0.20, MeOH). FAB-MS *m/z*: 545 $[M+Na]^+$. CD (*c*=3.99×10^{-5} M, MeOH) *Δε* (nm): +1.19 (223.0). The spectral data were identified with those of the authentic sample.[6)]

Determination of Absolute Structures of Glucosyl Moieties in 1—7 Each of compounds **1**—**7** (*ca.* 1 mg) was refluxed with 4% HCl for 5 h. The reaction mixture was neutralized with Ag_2O, filtered, and excess Ag^+ in the filtrate was removed with H_2S. The solution was concentrated *in vacuo* and dried to give a glycosyl residue which was subjected to preparation of the corresponding thiazolidine derivative, followed by trimethylsilylation and GLC analysis, according to the reported procedure.[20] GLC conditions: column, G-column (Kagakuhin Kensa Kyokai, 1.2 mm i.d.×40 m); column temp., 240 °C; carrier gas, N_2 (30 ml/min). D-glucose, t_R 39.4 min (ref.: L-glucose, t_R 41.2 min).

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10) Its CD spectrum was measured in MeOH ($c=5.15\times10^{-5}$ M).
11) The CD spectra of (−)-massoniresinol and (−)-olivil showed positive Cotton effects according to ref. 7, however, compounds **2**—**4** showed negative Cotton effects, which are very similar CD curves with those of (−)-olivil [$(\theta)_{282}$ −15600, $(\theta)_{231}$ −101000] and (−)-berchemol [$(\theta)_{283}$ −8450, $(\theta)_{229}$ −38400] reported by Inoue *et al*. [Sakurai N., Nagashima S., Kawai K., Inoue T., *Chem. Pharm. Bull.*, **37**, 3311—3315 (1989)].
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