Studies on the Constituents of *Syringa* Species. XII.[1] New Glycosides from the Leaves of *Syringa reticulata* (Blume) Hara

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Received February 24, 2003; accepted March 31, 2003

Three new glycosides, 6′-*O*-α-D-galactopyranosylsyringopicroside (1), secologanoside 7-methyl ester (2) and (+)-lariciresinol 4′-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (3), were isolated from the leaves of *Syringa reticulata*. Their structures were established on the basis of chemical and spectral data. Compound 1 is the first naturally occurring iridoid di-glycoside having melibiose. Comparison of the spectral data of 2 and that previously recognized as secologanoside 7-methyl ester led to the conclusion that the recognized structure should be revised to the sodium salt of secoxyloganin (2′).

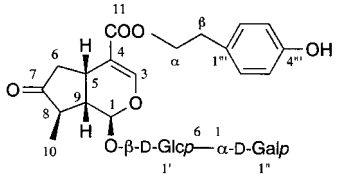
Key words *Syringa reticulata*; Oleaceae; iridoid; melibiose; secologanoside 7-methyl ester

We have recently reported the isolation of five new iridoid glycosides, including the first findings of a (8*Z*)-oleoside-type secoiridoid [(8*Z*)-ligstroside, (8*Z*)-nuzhenide] and an iridoid di-glycoside having an isomaltose, from the leaves of *Syringa reticulata* (Blume) Hara (Oleaceae).[2] In the course of further studies on the constituents of this plant, three new glycosides (**1**—**3**) have been isolated. This paper deals with the structural elucidation and identification of these compounds. The isolation procedures are described in detail in the experimental section.

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{25}$ −21.1° (MeOH). The molecular formula of **1**, $C_{30}H_{40}O_{16}$, was confirmed by high-resolution (HR)-FAB-MS. The ^{13}C-NMR spectrum of **1** was similar to that of syringopicroside[3] isolated from the same plant,[2,4] except for the presence of an additional hexosyl moiety and a difference in the chemical shifts at the C-5′ and C-6′ positions [δ_C 76.8 (C-5′, −1.7 ppm), δ_C 68.3 (C-6′, +5.5 ppm)]. In the ^{1}H-NMR of **1**, the coupling constant of the anomeric proton signal of the additional hexosyl moiety was 3.7 Hz (δ_H 4.87). Acid hydrolysis of **1** gave D-glucose and D-galactose, which were identified by gas-liquid chromatography (GLC) after conversion of the TMSi ether of thiazolidine derivative.[5] These indicated that the additional α-D-galactopyranosyl moiety of **1** is attached to 6′-OH in syringopicroside. This finding was supported by the ^{1}H-detected heteronuclear multiple bond correlation (HMBC) between H_A-6′ and C-1″. Consequently, the structure of **1** was determined to be 6′-*O*-α-D-galactopyranosylsyringopicroside. Compound **1** is the first naturally occurring iridoid di-glycoside having melibiose [α-D-galactopyranosyl-(1→6)-β-D-glucopyranose].

Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{25}$ −106.1° (MeOH). The FAB-MS showed quasi-molecular ion peaks $[M+H]^+$ and $[M+Na]^+$ at *m/z* 405 and 427, respectively. The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of secoxyloganin isolated from the stems of *Lonicera periclymenum*,[6] except for the chemical shifts due to the C-7 and C-11 carbonyl carbons. The molecular formula of **2**, $C_{17}H_{24}O_{11}$, was confirmed by HR-FAB-MS, and was coincident with that of secoxyloganin. These features suggested that **2** was a secologanoside 7-methyl ester. This deduction was supported by HMBC correlations [H-3/C-11 (δ_C 170.5) and C-1, H_2-6 and OCH_3 (δ_H 3.63)/C-7 (δ_C 174.9)]. From the above data, the structure of **2** was determined to be secologanoside 7-methyl ester.

Secologanoside 7-methyl ester has been previously isolated from the bark of *Osmanthus asiaticus*.[7] However, direct comparison of the NMR chemical shifts of **2** and the compound (**2′**)[8] previously reported as secologanoside 7-methyl ester revealed that the two glycosides are not identical. After re-examining the spectral data of **2′**, we find that the FAB-MS of **2′** exhibited two quasi-molecular ion peaks at *m/z* 427 $[M+H]^+$ and 449 $[M+Na]^+$, indicating an increase of 22 mass units in comparison with those of **2**.[8,9] Furthermore, the HMBC correlations [H-3 and OCH_3 (δ_H 3.66)/C-11 (δ_C 169.3), H_2-6/C-7 (δ_C 180.7)][9] and the significant downfield shift of the carbonyl carbon signal due to C-7 in **2′** led us to the conclusion that structure previously recognized as secologanoside 7-methyl ester from *O. asiaticus* should be re-



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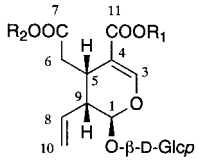
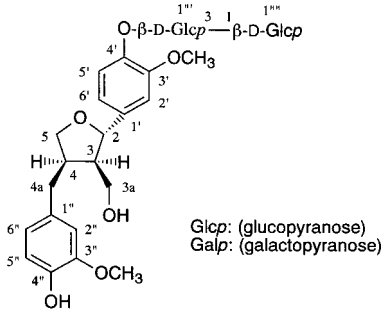


2: R_1=H, R_2=CH_3
2′: R_1=CH_3, R_2=Na



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vised to the sodium salt of secoxyloganin (**2′**).

Compound **3** was obtained as an amorphous powder, $[\alpha]_D^{25}$ −20.0° (MeOH). The molecular formula of **3**, $C_{32}H_{44}O_{16}$, was confirmed by HR-FAB-MS. The ^{13}C-NMR spectrum of **3** was very similar to that of (+)-lariciresinol 4′-*O*-*β*-D-glucopyranoside,[1,10] except for the presence of an additional hexosyl moiety. In the ^{1}H-NMR spectrum of **3**, the coupling constant of the anomeric proton signal of the additional hexosyl moiety was 7.8 Hz [δ_H 4.61 (H-1‴)]. Acid hydrolysis proved that both of two sugars in **3** are D-glucose. The location of the additional *β*-D-glucopyranosyl moiety in **3** was deduced to be at 3‴-OH of (+)-lariciresinol 4′-*O*-*β*-D-glucopyranoside, because the signal due to C-3‴ was markedly displaced downfield at δ_C 87.5 (+9.3 ppm), while the signal due to C-4‴ was shifted upfield at δ_C 69.8 (−1.6 ppm)],[2] respectively, when comparing the ^{13}C-NMR spectrum of **3** with that of (+)-lariciresinol 4′-*O*-*β*-D-glucopyranoside. This deduction was supported by the HMBC correlation between H-1‴ and C-3‴. The circular dichroism (CD) spectrum of **3** [276.5 nm ($\Delta\varepsilon$ −0.60), 229.0 nm ($\Delta\varepsilon$ −2.70)] showed two similar negative Cotton effects with those of (+)-lariciresinol 4′-*O*-*β*-D-glucopyranoside [280.0 nm ($\Delta\varepsilon$ −0.47), 228.0 nm ($\Delta\varepsilon$ −1.38)].[11—13] Consequently, the structure of **3** was determined to be (+)-lariciresinol 4′-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside.

Experimental

The instruments, materials and experimental conditions were the same as in our recent paper.[2]

Extraction and Isolation The extraction and isolation procedures were as described in our recent paper.[2] Fraction 1 obtained from the *n*-BuOH-soluble portion of the fresh leaves of *S. reticulata* was chromatographed on a silica gel column using $CHCl_3$–MeOH (5 : 1, 1 : 1), and the eluate was separated into seven fractions (frs. 1-1—1-7). Fraction 1-5 was chromatographed on a Sephadex LH-20 column using 50% MeOH, and the eluate was separated into ten fractions (frs. 1-5-1—1-5-10). Fraction 1-5-4 was subjected to preparative HPLC [column, Cosmosil 5C18-AR (10 mm i.d.×25 cm, Nacalai Tesque); mobile phase, MeOH–H_2O (1 : 2); UV detector, 206 nm: column, Cosmosil 5SL (10 mm i.d.×25 cm, Nacalai Tesque); mobile phase, CH_2Cl_2–MeOH–H_2O (30 : 10 : 1); UV detector, 225 nm; each flow rate, 1.5 ml/min] to give **1** (6.5 mg), **2** (5.5 mg) and **3** (4.0 mg).

6′-*O*-*α*-D-Galactopyranosylsyringopicroside (1) An amorphous powder, $[\alpha]_D^{25}$ −21.1° (*c*=0.389, MeOH); UV λ_{max} (MeOH) nm (log ε): 276 (3.21), 225 (4.13). FAB-MS *m/z*: 679 $[M+Na]^+$. HR-FAB-MS *m/z*: 679.2196 $[M+Na]^+$ ($C_{30}H_{40}O_{16}Na$, Calcd for 679.2214). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.44 (1H, d, *J*=1.2 Hz, H-3), 7.04 (2H, d, *J*=8.5 Hz, H-2‴, 6‴), 6.71 (2H, d, *J*=8.5 Hz, H-3‴, 5‴), 5.53 (1H, d, *J*=3.9 Hz, H-1), 4.87 (1H, d, *J*=3.7 Hz, H-1″), 4.69 (1H, d, *J*=8.1 Hz, H-1′), 4.27 (2H, m, H_2-*α*), 3.79 (1H, dd, *J*=12.0, 2.0 Hz, H-6′$_B$), 3.73 (1H, dd, *J*=10.4, 3.7 Hz, H-2″), 3.69—3.91 (6H, m, H-6′$_A$, H-3″, 4″, 5″, H_2-6″), 3.54 (1H, m, H-5′), 3.36 (2H, m, H-3′, 4′), 3.30 (1H, m, H-5), 3.21 (1H, dd, *J*=8.8, 8.1 Hz, H-2′), 2.84 (2H, t, *J*=6.8 Hz, H_2-*β*), 2.57 (1H, dd, *J*=19.3, 8.3 Hz, H-6*β*), 2.40 (1H, dd, *J*=19.3, 2.7 Hz, H-6*α*), 2.30 (1H, ddd, *J*=9.0, 7.3, 3.9 Hz, H-9), 2.14 (1H, br quartet, *J*=7.3 Hz, H-8), 1.14 (3H, d, *J*=7.3 Hz, H_3-10). ^{13}C-NMR (100 MHz, CD_3OD) δ: 220.6 (C-7), 168.4 (C-11), 157.2 (C-4‴), 153.3 (C-3), 131.0 (C-2‴, 6‴), 130.2 (C-1‴), 116.4 (C-3‴, 5‴), 111.3 (C-4), 100.8 (C-1′), 100.5 (C-1″), 96.1 (C-1), 78.1 (C-3′), 76.8 (C-5′), 74.8 (C-2′), 72.6 (C-5″), 71.8 (C-4′), 71.7 (C-3″), 71.1 (C-4″), 70.4 (C-2″), 68.3 (C-6′), 66.4 (C-*α*), 62.8 (C-6″), 46.5 (C-9), 44.8 (C-8), 43.7 (C-6), 35.4 (C-*β*), 28.6 (C-5), 14.0 (C-10).

Secologanoside 7-Methyl Ester (2) An amorphous powder, $[\alpha]_D^{25}$ −106.1° (*c*=0.337, MeOH); UV λ_{max} (MeOH) nm (log ε): 229 (3.97). FAB-MS *m/z*: 405 $[M+H]^+$, 427 $[M+Na]^+$. HR-FAB-MS *m/z*: 405.1390 $[M+H]^+$ ($C_{17}H_{25}O_{11}$, Calcd for 405.1396). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.48 (1H, d, *J*=1.7 Hz, H-3), 5.64 (1H, ddd, *J*=17.0, 10.5, 9.5 Hz, H-8), 5.47 (1H, d, *J*=4.1 Hz, H-1), 5.22 (2H, m, H_2-10), 4.66 (1H, d, *J*=7.8 Hz, H-1′), 3.89 (1H, dd, *J*=12.0, 2.0 Hz, H-6′$_B$), 3.65 (1H, m, H-6′$_A$), 3.63 (3H, s, 7-$COOCH_3$), 3.33 (3H, m, H-3′, 4′, 5′), 3.30 (1H, m, H-5), 3.21 (1H, dd, *J*=9.1, 7.8 Hz, H-2′), 2.94 (1H, dd, *J*=16.1, 5.4 Hz, H-6$_B$), 2.76 (1H, m, H-9), 2.32 (1H, dd, *J*=16.1, 8.8 Hz, H-6$_A$). ^{13}C-NMR (100 MHz, CD_3OD) δ: 174.9 (C-7), 170.5 (C-11), 153.5 (C-3), 134.8 (C-8), 120.3 (C-10), 111.2 (C-4), 100.0 (C-1′), 97.5 (C-1), 78.5 (C-5′), 78.1 (C-3′), 74.7 (C-2′), 71.6 (C-4′), 62.8 (C-6′), 52.0 (7-$COOCH_3$), 45.5 (C-9), 35.3 (C-6), 29.2 (C-5).

(+)-Lariciresinol 4′-*O*-*β*-D-Glucopyranosyl-(1→3)-*β*-D-glucopyranoside (3) An amorphous powder, $[\alpha]_D^{25}$ −20.0° (*c*=0.252, MeOH); UV λ_{max} (MeOH) nm (log ε): 278 (3.63), 225 (4.07), 205 (4.38). CD (*c*=7.35×10^{-5} M, MeOH) $\Delta\varepsilon$ (nm): −0.60 (276.5), −2.70 (229.0), −2.50 (209.5). FAB-MS *m/z*: 707 $[M+Na]^+$. HR-FAB-MS *m/z*: 707.2515 $[M+Na]^+$ ($C_{32}H_{44}O_{16}Na$, Calcd for 707.2528). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.13 (1H, d, *J*=8.3 Hz, H-5′), 6.98 (1H, d, *J*=2.0 Hz, H-2′), 6.88 (1H, dd, *J*=8.3, 2.0 Hz, H-6′), 6.79 (1H, d, *J*=2.0 Hz, H-2″), 6.71 (1H, d, *J*=8.1 Hz, H-5″), 6.64 (1H, dd, *J*=8.1, 2.0 Hz, H-6″), 4.94 (1H, d, *J*=7.3 Hz, H-1‴), *ca.* 4.80 (H-2, overlapped with solvent signal), 4.61 (1H, d, *J*=7.8 Hz, H-1⁗), 4.00 (1H, dd, *J*=8.3, 6.6 Hz, H-5$_B$), 3.85 (3H, s, H_3CO-3′), 3.83 (3H, s, H_3CO-3″), 2.91 (1H, dd, *J*=13.4, 5.0 Hz, H-4$_{a\text{-}B}$), 2.72 (1H, m, H-4), 2.51 (1H, dd, *J*=13.4, 11.2 Hz, H-4$_{a\text{-}A}$), 2.35 (1H, m, H-3). ^{13}C-NMR (100 MHz, CD_3OD) δ: 151.0 (C-3′), 149.1 (C-3″), 147.3 (C-4′), 145.9 (C-4″), 139.7 (C-1′), 133.6 (C-1″), 122.2 (C-6″), 119.6 (C-6′), 118.2 (C-5′), 116.3 (C-5″), 113.5 (C-2″), 111.6 (C-2′), 105.3 (C-1‴), 102.6 (C-1‴), 87.5 (C-3‴), 83.9 (C-2), 78.2 (C-5‴), 77.91 (C-5⁗), 77.90 (C-3⁗), 75.6 (C-2‴), 74.4 (C-2⁗), 73.7 (C-5), 71.6 (C-4⁗), 69.8 (C-4‴), 62.7 (C-6⁗), 62.5 (C-6‴), 60.6 (C-3a), 56.8, 56.4 (3′, 3″-OCH_3), 54.2 (C-3), 43.9 (C-4), 33.7 (C-4a).

Determination of Absolute Structure of Sugar Moieties in 1—3 Each of compounds **1**—**3** (*ca.* 1 mg) was refluxed with 4% HCl for 4 h. The reaction mixture was neutralized with Ag_2O, filtered, and excess Ag^+ of 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.[5] GLC conditions: column, G-column (Kagakuhin Kensa Kyokai, 1.2 mm i.d.×40 m); column temp., 260 °C; carrier gas, N_2 (28 ml/min). D-glucose, t_R 27.2 min, D-glactose, t_R 29.0 min (ref.: L-glucose, t_R 28.4 min, L-glactose, t_R 30.8 min).

Acknowledgments The authors are grateful to Mrs. S. Sato and T. Matsuki of their university for NMR and MS measurements.

References and Notes

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8) **2′** (sodium salt of secoxyloganin): $[\alpha]_D^{25}$ −115.0° (MeOH). FAB-MS *m/z*: 427 $[M+H]^+$, 449 $[M+Na]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.39 (1H, d, *J*=1.8 Hz, H-3), 5.66 (1H, ddd, *J*=17.2, 10.3, 7.0 Hz, H-8), 5.47 (1H, d, *J*=3.7 Hz, H-1), 5.25 (1H, dd, *J*=10.3, 2.2 Hz, H-10$_B$), 5.18 (1H, dd, *J*=17.2, 2.2 Hz, H-10$_A$), 4.61 (1H, d, *J*=8.1 Hz, H-1′), 3.87 (1H, br d, *J*=11.7 Hz, H-6′$_B$), 3.66 (3H, s, 11-$COOCH_3$), 3.30 (1H, m, H-5), 2.88 (1H, dd, *J*=15.6, 4.6 Hz, H-6$_B$), 2.84 (1H, m, H-9), 2.07 (1H, dd, *J*=15.6, 10.0 Hz, H-6$_A$). ^{13}C-NMR (100 MHz, CD_3OD) δ: 180.7 (C-7), 169.3 (C-11), 152.8 (C-3), 135.0 (C-8), 120.0 (C-10), 111.6 (C-4), 99.7 (C-1′), 97.6 (C-1), 78.4 (C-5′), 77.9 (C-3′), 74.7 (C-2′), 71.7 (C-4′), 62.8 (C-6′), 51.5 (11-$COOCH_3$), 38.4 (C-6), 29.6 (C-5).
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