Studies on the Constituents of Syringa Species. X.1) Five New Iridoid Glycosides from the Leaves of Syringa reticulata (BLUME) HARAN)

Koichi MACHIDA, Atsuko KANEKO, Tomokazu HOSOGAI, Rie KAKUDA, Yasunori YAOITA, and Masao KIKUCHI

Tohoku Pharmaceutical University, 4–4–1 Komatsushima, Aoba-ku, Sendai, Miyagi 981–8558, Japan.
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Five new iridoid glycosides, (8Z)-ligstrose (1), (8Z)-nüzhenide (3), 6'-O-α-glucopyranosylsyringopicroside (4), 3'-O-β-glucopyranosylsyringopicroside (5) and 4'-O-β-glucopyranosylsyringopicroside (6) were isolated, together with a known one, (8E)-nüzhenide (2), from the leaves of Syringa reticulata. Their structures were established on the basis of chemical and spectral data. Compounds 1 and 3 are the first findings of a (8Z)-oleo-side-type secoiridoid. Compound 4 is the first naturally occurring iridoid di-glycoside having an isomaltose.

Key words Syringa reticulata; Oleaceae; (8Z)-ligstrose; (8Z)-nüzhenide; iridoid di-glycoside; isomaltose

In previous papers, we reported on the isolation of eleven new glycosides from the leaves of Syringa reticulata (BLUME) HARAN,2—5) In the course of further studies on the constituents of the above plant, five new iridoid glycosides (1, 3—6) along with a known one (2) have been isolated. This paper deals with the structural elucidation and identification of these compounds. The isolation procedure is described in detail in the Experimental section. Compound 2 was identified as (8E)-nüzhenide by comparison of the spectral data with those reported in the literature.6) Compound 1 was obtained as an amorphous powder, $\left[\alpha\right]_{D}^{26}$ $-81.3^\circ$ (MeOH). The molecular formula of 1, C$_{25}$H$_{32}$O$_{12}$, was confirmed by high-resolution (HR)-FAB-MS and was coincident with that of (8E)-ligstrose isolated from the same plant.7) Its 1H-NMR spectral pattern was very similar to that of (8E)-ligstrose, except for the chemical shifts owing to 1-H, 5-H, 6-H$_{2}$, 8-H, 10-H$_{3}$ and 1ơ-H, respectively.9) The 1H–1H shift correlation spectroscopy (COSY) and 1H-detected heteronuclear multiple bond correlation (HMBC, Fig. 1) experiments of 1 made up the same plane structure as (8E)-ligstrose, suggesting that 1 is the 8Z-isomer of (8E)-ligstrose. The stereochemistry of 1 was defined by the 1H–1H COSY and nuclear Overhauser enhancement spectroscopy (NOESY) experiments. As shown in Fig. 2, the NOE correlations and homoallylic coupling of (8E)-ligstrose indicated that both 1-H and 6-H$_{2}$ were quasi-axial with respect to the dihydropyran ring. On the other hand, the NOE correlations (1-H/10-H$_{3}$, 5-H/8-H, 6-H$_{2}$/8-H), and homoallylic (5-H/10-H$_{3}$) and allylic (3-H/5-H) couplings of 1 indicated that the geometry of the olefinic bond at C-8 is the Z-configuration, and both 1-H and 6-H$_{2}$ are quasi-equatorial with respect to the dihydropyran ring. Consequently, the structure of 1 was determined to be (8Z)-ligstrose.

Compound 3 was obtained as an amorphous powder, $\left[\alpha\right]_{D}^{26}$ $-101.1^\circ$ (MeOH). The molecular formula of 3, C$_{31}$H$_{42}$O$_{17}$, was confirmed by HR-FAB-MS and was coincident with that of 2. Its 1H-NMR spectral pattern was similar to that of 2, except for the chemical shifts owing to 1-H, 5-H, 6-H$_{2}$, 8-H, 10-H$_{3}$ and 1ơ-H, respectively.9) The 1H–1H shift correlation spectroscopy (COSY) and 1H-detected heteronuclear multiple bond correlation (HMBC, Fig. 1) experiments of 3 made up the same plane structure as 2, suggesting that 3 is the 8Z-isomer of 2. As shown in Fig. 2, the NOE correlations (1-H/10-H$_{3}$, 5-H/8-H, 6-H$_{2}$/8-H), and homoallylic (5-H/10-
H₃) and allylic (3-H/5-H) couplings of 3 indicated that the geometry of the olefinic bond at C-8 is the Z-configuration, and both 1-H and 6-H are quasi-equatorial with respect to the dihydropyran ring. Consequently, the structure of 3 was determined to be (8Z)-nüzhenide. It is likely that the conformational changes of the dihydropyran rings arise from steric hindrance between the β-D-glucopyranose attached C-1 and 10-CH₃.

Compound 4 was obtained as an amorphous powder, [α]D₂⁰ -28.6° (MeOH). The molecular formula of 4, C₃₀H₄₀O₁₆, was confirmed by HR-FAB-MS. The ¹³C-NMR spectrum of 4 was similar to that of syringopicroside isolated from the same plant except for the presence of an additional hexosyl moiety and difference in the chemical shift at C-6 position [δ 68.0 (± 5.2 ppm)]. In the ¹H-NMR spectrum of 4, the coupling constant of the anomeric proton signal of the additional hexosyl moiety was 3.7 Hz (δ 4.83). Acid hydrolysis of 4 gave only D-glucose, which was identified by gas-liquid chromatography (GLC) after conversion to the TMSi ether of thiazolidine derivative. These indicated that the additional α-D-glucopyranosyl moiety in 4 is attached to 6-OH in syringopicroside. This finding was supported by the NOE and HMBC correlations (Fig. 3). Consequently, the structure of 4 was determined to be 6-O-α-D-glucopyranosylsyringopicroside.

Compound 5 was obtained as an amorphous powder, [α]D₂⁰ -88.9° (MeOH). The molecular formula of 5, C₃₀H₄₀O₁₆, was confirmed by HR-FAB-MS and was coincident with that of 4. The ¹³C-NMR spectrum of 5 was similar to that of syringopicroside, except for the presence of an additional hexosyl moiety and difference in the chemical shift at C-3 position [δ 87.5 (± 9.4 ppm)]. In the ¹H-NMR spectrum of 5, the coupling constant of the anomeric proton signal of the additional hexosyl moiety was 8.1 Hz (δ 4.57). Acid hydrolysis proved that both of two sugars in 5 are D-glucose in the above manner. These indicated that the additional β-D-glucopyranosyl moiety in 5 is attached to 3'-OH in syringopicroside. This finding was supported by the NOE and HMBC correlations (Fig. 3). Consequently, the structure of 5 was determined to be 3'-O-β-D-glucopyranosylsyringopicroside.

Compound 6 was obtained as an amorphous powder, [α]D₂⁰ -77.2° (MeOH). The molecular formula of 6, C₃₀H₄₀O₁₆, was confirmed by HR-FAB-MS and was coincident with that of 5. The ¹H-NMR spectrum of 6 resembled that of 5 except for the shift of the signal assigned to the additional anomeric proton [δ 4.40 (d, J = 7.8 Hz)]. In the ¹³C-NMR spectrum, the C-4' signal (δ 80.6) of 6 was shifted downfield by 9.0 ppm compared with that of syringopicroside. Acid hydrolysis proved that both of two sugars in 6 are D-glucose in the above manner. These indicated that the additional β-D-glucopyranosyl moiety in 6 is attached to 4'-OH in syringopicroside. This finding was supported by the NOE and HMBC correlations (Fig. 3). Consequently, the structure of 6 was determined to be 4'-O-β-D-glucopyranosylsyringopicroside.

The iridoid glycoside which comprises an oleoside moiety as a framework is called oleoside-type secoiridoid, and this...
type occurs only in Oleaceae plants. All of them isolated so far have E-configuration of the olefinic bond at C-8. Compounds 1 and 3 are the first findings of a (8Z)-oleoside-type secoiridoid. From a biosynthetic point of view, it is interesting to note that (8Z)-oleoside-type secoiridoid was isolated from a natural source. Previous biosynthetic investigations (bold lines) of (8E)-oleoside-type secoiridoid reported by Inouye et al.\textsuperscript{13,14} and our structural studies described above presume that these type secoiridoids are biosynthesized by the route depicted in Fig. 4.

**Experimental**

**General** Optical rotation were taken with a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrometer. The $^1$H- and $^{13}$C-NMR spectra were recorded with JEOL JNM-GSX 400 (400 MHz, 100 MHz, respectively) and JEOL JNM-LA 600 (600 MHz, 150 MHz, respectively) spectrometers. Chemical shifts are given on a $\delta$ (ppm) scale with tetramethylsilane (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), Cosmosil 75C$_{18}$-OPN (Nacalai Tesque) and Sephadex LH-20 (Pharmacia Fine Chemicals). Preparative HPLC was carried out on a Tosoh HPLC system [pump, CCCPS; detector, UV-8020; column, Cosmosil SC$_{18}$-AR (10 mm i.d.$\times$25 cm, Nacalai Tesque)]. GLC was carried out on a Shimadzu GC-7A equipped with hydrogen flame ionization detector (FID).

**Material** The leaves of *S. reticulata* (3.8 kg) were extracted with MeOH at room temperature for 10 d. The MeOH extract was concentrated under reduced pressure and the residue was suspended in water. This suspension was successively extracted with CHCl$_3$, Et$_2$O, AcOEt, n-BuOH and H$_2$O. The CHCl$_3$-soluble fraction was concentrated under reduced pressure to produce a residue (88.2 g). The extract (25.0 g) was suspended in MeOH–H$_2$O (3 : 1, 60 ml), and the soluble material (18.0 g) was chromatographed on a C$_8$ open column using MeOH–H$_2$O (3 : 1) and the eluate was separated into seven fractions (frs. 1—7). Fraction 1 was rechromatographed on a silica gel column using CHCl$_3$–MeOH (9 : 1, 5 : 1, 1 : 1) and the eluate was separated into eleven fractions (frs. 1—11). Fraction 1—6 was subjected to preparative HPLC [column, Cosmosil SC$_{18}$-AR; mobile phase, MeOH–H$_2$O (1 : 1); UV detector, 224 nm; column, Cosmosil S5L; mobile phase, CHCl$_3$–MeOH–H$_2$O (10 : 10 : 1); UV detector, 230 nm; each flow rate: 1.5 ml/min] to give syringopicroside (25.0 mg) and compound 1 (10.0 mg). The n-BuOH-soluble fraction was concentrated under reduced pressure to produce a residue (107.0 g). The extract (25.0 g) was chromatographed on a silica gel column using CHCl$_3$–MeOH (10 : 3, 5 : 2, 2 : 1, 1 : 1, 2 : 3) and the eluate was separated into three fractions (frs. 1—3). Fraction 1 was rechromatographed on a silica gel column using CHCl$_3$–MeOH (5 : 1, 1 : 1) and the eluate was separated into seven fractions (frs. 1—7). Fraction 1—4 was chromatographed on a Sephadex LH-20 column using 50% MeOH and the eluate was separated into five fractions (frs. 1—4—1—4—5). Fraction 1—4—2 was subjected to preparative HPLC [column, Cosmosil SC$_{18}$-AR; mobile phase, MeOH–H$_2$O (2 : 3); UV detector, 225 nm; column, Cosmosil S5L; mobile phase, CHCl$_3$–MeOH–H$_2$O (30 : 10 : 1); UV detector, 230 nm; each flow rate: 1.5 ml/min] to give (8E)-ligstroside (370.5 mg), syringopicroside (25.0 mg) and compound 1 (10.0 mg). The n-BuOH-soluble fraction was concentrated under reduced pressure to produce a residue (107.0 g). The extract (25.0 g) was chromatographed on a silica gel column using CHCl$_3$–MeOH (10 : 3, 5 : 2, 2 : 1, 1 : 1, 2 : 3) and the eluate was separated into three fractions (frs. 1—3). Fraction 1 was rechromatographed on a silica gel column using CHCl$_3$–MeOH (5 : 1, 1 : 1) and the eluate was separated into seven fractions (frs. 1—7). Fraction 1—4 was chromatographed on a Sephadex LH-20 column using 50% MeOH and the eluate was separated into five fractions (frs. 1—4—1—4—5). Fraction 1—4—2 was subjected to preparative HPLC [column, Cosmosil SC$_{18}$-AR; mobile phase, MeOH–H$_2$O (2 : 3); UV detector, 225 nm; column, Cosmosil S5L; mobile phase, CHCl$_3$–MeOH–H$_2$O (30 : 10 : 1); UV detector, 230 nm; each flow rate: 1.5 ml/min] to give syringopicroside (30.0 mg), compounds 2 (32.5 mg), 3 (8.0 mg), 4 (7.5 mg), 5 (13.0 mg) and 6 (28.0 mg).

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**Fig. 4.** Possible Biosynthetic Pathways of Oleoside-Type Secoiridoids

\textsuperscript{E}E=Elimination. \textsuperscript{*}They are not yet identified from the leaves of *S. reticulata*. \textsuperscript{\#}It is not associated with the general concept of a stereoselective enzymatic reaction, but probably arises from the steric hindrance between glucopyranose attached C-1 and 10-CH$_3$. 

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[Diagram of possible biosynthetic pathways of oleoside-type secoiridoids]
Each of compounds 1—6 (ca. 1 mg) was refluxed with 4% HCl for 4h. The
reaction mixture was neutralized with Ag$_2$O, filtered and excess Ag$^+$ of the filtrate was removed with H$_2$S. 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.$^{13}$ 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). $\alpha$-glucose, $\tau_g$ 39.4 min (ref.: $\alpha$-glucose, $\tau_g$ 41.2 min).

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References and Notes


9) (8E)-Ligstroside: [α]$_D^{26}$ = -184.8° (c=3.7, MeOH) [lit.$^{7}$] = -110.7° (c=1.0, EtOH), [α]$_D^{26}$ = -180.0° (c=0.23, 95% EtOH).$^3$H-NMR (400 MHz, CD$_3$OD) $\delta$: 7.51 (1H, s, 3-H), 7.05 (2H, d, $J$=8.5 Hz, 2', 6'-H), 6.71 (2H, d, $J$=8.5 Hz, 3', 5'-H), 6.07 (1H, dq, $J$=7.0, 1.0 Hz, 1-H), 4.80 (1H, d, $J$=7.8 Hz, 1'-H), 4.22, 4.10 (each 1H, dt, $J$=7.0 Hz, 1'-H, 2'-H), 3.96 (1H, dd, $J$=1.5, 1.0 Hz, 19-H), 3.30—3.43 (4H, m, 2', 3', 5', 6'-H), 2.82 (2H, 2H), $J$=7.1 Hz, 1', 2'-H), 2.70 (1H, dd, $J$=14.1, 4.6 Hz, 6'-H), 2.43 (1H, dd, $J$=14.1, 9.0 Hz, 6'-H), 1.64 (3H, dd, $J$=7.1, 1.5 Hz, 10-H).


11) Syringopicroside; [α]$_D^{26}$ = -105.3° (c=0.4, MeOH).$^3$H-NMR (400 MHz, CD$_3$OD) $\delta$: 7.44 (1H, d, $J$=1.5 Hz, 3-H), 7.04 (2H, d, $J$=8.5 Hz, 2', 6'-H), 6.71 (2H, d, $J$=8.5 Hz, 3', 5'-H), 5.61 (1H, d, $J$=3.4 Hz, 1-H), 4.67 (1H, d, $J$=8.1 Hz, 1'-H), 4.25 (2H, bbt, $J$=6.8 Hz, 19-H), 3.90 (1H, dd, $J$=12.0, 2.0 Hz, 6'-H), 3.65 (1H, dd, $J$=12.0, 6.1 Hz, 6'-H), 3.14—3.37 (5H, m, 5-H, 2', 3', 4', 5'-H), 2.84 (2H, btt, $J$=6.8 Hz, β-H), 2.56 (1H, dd, $J$=19.3, 8.3 Hz, 6-H), 2.43 (1H, dd, $J$=19.3, 2.0 Hz, 6-H), 2.32 (1H, ddd, $J$=10.2, 8.3, 3.4 Hz, 9-H), 2.10 (1H, dq, $J$=8.3, 7.1 Hz, 8-H), 1.14 (3H, dd, $J$=7.1 Hz, 10-H).

