[Chem. Pharm. Bull. 36(3)1158—1161(1988)]

Two New Phenylpropanoid Glycosides from *Wikstroemia sikokiana*

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(Received August 5, 1987)

Two new phenylpropanoid diglucosides named syringinoside and coniferinoside have been isolated from the water-soluble fraction of *Wikstroemia sikokiana* FRANCH. *et* SAV. (Thymelaeaceae). Their structures have been characterized on the basis of spectral and chemical evidence.

Keywords——Thymelaeaceae; *Wikstroemia sikokiana*; phenylpropanoid; syringinoside; coniferinoside; glycoside; gentiobiose

Introduction

Although the less polar constituents of plants have been extensively studied, the polar ones, especially the water-soluble constituents, have rarely been examined. Continuing our work on the constituents of *Wikstroemia sikokiana* FRANCH. *et* SAV. (Thymelaeaceae),[2,3)] we have focused on the water-soluble constituents of the plant, and have isolated two new phenylpropanoid diglycosides named syringinoside and conferinoside, as well as a known phenylpropanoid monoglycoside, syringin.[4)] In this paper, we wish to report the isolation and structural characterization of these phenylpropanoid glycosides by means of spectroscopic and chemical methods.

Results and Discussion

The high-performance liquid chromatography (HPLC) of the water-soluble fraction described in the previous paper[2)] showed that it contains mainly three compounds (Fig. 1). Each component (**1**—**3**) corresponding to the peaks **1**—**3** in Fig. 1 was separated by a combination of reversed-phase medium-pressure liquid chromatography (MPLC) and HPLC. The major component, named syringinoside (**1**), was obtained as colorless needles, mp 178—179 °C, $[\alpha]_D$ −37.6°. Its ultraviolet (UV) spectrum showed absorption maxima at 265 and 220 nm and no change on addition of sodium hydroxide. This finding indicated that a free phenolic hydroxyl group was not present in the molecule, though the infrared (IR) spectrum (KBr) showed a strong hydroxyl group absorption band (3400 cm^{-1}, br). The molecular formula was established as $C_{23}H_{34}O_{14}$ by elemental analysis and the secondary ion mass spectrum (SIMS). The SIMS of **1** showed $[M+H]^+$ at *m/z* 535 and $[M+Na]^+$ at *m/z* 557. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed the presence of a *trans*-allylic moiety [δ 6.55 (1H, dt, J=15.87, 1.46 Hz), 6.33 (1H, dt, J=15.87, 5.61 Hz), and 4.22 (2H, dd, J=5.61, 1.46 Hz)], two methoxy groups [δ 3.86 (6H, s)], two aromatic protons [δ 6.75 (2H, s)], and two anomeric protons of sugars [δ 4.87 (1H, d, J=7.57 Hz) and 4.24 (1H, d, J=7.81 Hz)]. Permethylation of **1** with dimsylsodium and methyl iodide afforded an octamethylated compound (**4**). On catalytic hydrogenation, followed by acid hydrolysis and then acetylation, **4** was converted into an acetate (**5**) which was identical with an authentic

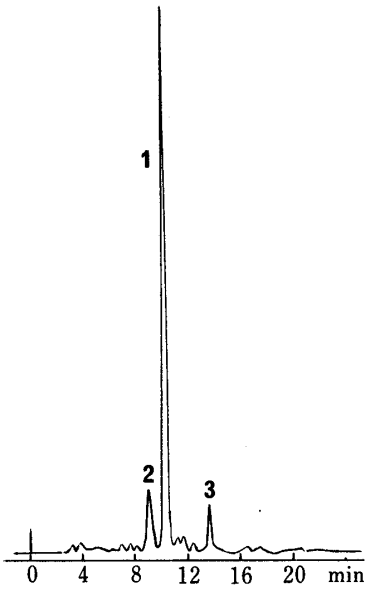
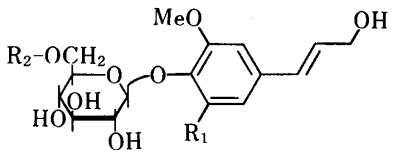


Fig. 1. HPLC of Water-Soluble Fraction

Column: Develosil C-8, 4.6 i.d. × 250 mm. Mobile phase: H_2O–MeOH (70 : 30). Flow rate: 0.5 ml/min.



1: R_1 = OMe, R_2 = β-D-glucopyranosyl
2: R_1 = H, R_2 = β-D-glucopyranosyl
3: R_1 = OMe, R_2 = H

Chart 1

sample of 4-(3-methoxypropyl)-2,6-dimethoxyphenyl acetate prepared from commercially available 3,5-dimethoxy-4-hydroxycinnamic acid (**6**) (see Experimental). Next, the octamethylated compound **4** was treated with hydrogen chloride in methanol to afford α-D-octamethylgentiobiose, whose structure was confirmed by synthesis from D-gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucose). The structure of syringinoside (**1**) has thus been established to be [4-(3-hydroxy-1-*E*-propenyl)-2,6-dimethoxyphenyl]-6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside.

The second component (**2**) named conferinoside, $[\alpha]_D$ −56.0°, SIMS *m/z* 505 $(M+H)^+$ and 527 $(M+Na)^+$, showed very similar spectral data [δ 6.55 (1H, dt, J = 15.86, 1.47 Hz), 6.28 (1H, dt, J = 15.86, 5.86 Hz), and 4.21 (2H, dd, J = 5.86, 1.47 Hz)] to those of syringinoside (**1**) except for the following point: the former has only one methoxy group [δ 3.87 (3H, s)]. The aglycone of **2** was characterized as coniferyl alcohol by comparison of the ^{1}H-NMR spectral data between **1** and **2** [δ 7.16 (1H, d, J = 8.30 Hz), 7.06 (1H, d, J = 1.96 Hz), and 6.98 (1H, dd, J = 8.30, 1.96 Hz)]. This characterization was further supported by nuclear Overhauser effect (NOE) experiments on **2**. Irradiation of the methoxy group at δ 3.87 caused a 9.8% enhancement of the signal coupled with a *meta* proton at δ 7.06 and irradiation of the anomeric proton at δ 4.89 caused an 8.5% enhancement of the signal coupled with an *ortho* proton at δ 7.16. The sugar moiety of **2** [δ 4.89 (1H, d, J = 7.32 Hz) and 4.36 (1H, d, J = 7.82 Hz)] was also identified as D-gentiobiose according to the same procedure as described above. Thus, the structure of coniferinoside should be represented by **2**.

The third component (**3**) was identified as a known compound, syringin, occurring in various plants.[4)]

Disaccharides such as syringinoside (**1**) and coniferinoside (**2**) seem to be included in the plant as protected and stored forms of coniferyl alcohol and synapyl alcohol, which are susceptible to oxidation and are regarded as precursors of lignans or lignin.

Experimental

Optical rotations were measured on a JASCO DIP-181 digital polarimeter. UV spectra were obtained on a

JASCO UVIDEC-610 spectrometer. IR spectra were taken on a JASCO IR-80 spectrometer. ^{1}H-NMR spectra were taken on a JEOL GX-400 spectrometer; chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). MS were obtained on Hitachi M-80 and M-52 spectrometers. HPLC was carried out with a JASCO BIP-1[column; Develosil C-8, 4.6 i.d. ×250 mm and 10 i.d. × 250 mm (Nomura Chemical Co. Ltd.), detector; JASCO UVIDEC 100-V, 254 nm.

Isolation——The water-soluble fraction (78 g) reported in the previous paper,[2] was charged on an Amberlite XAD-2 resin (1000 ml) column and eluted successively with H_2O (8 l), 40% EtOH (5 l) and 80% EtOH (5 l). A part (2.0 g) of the fraction (10.4 g) eluted with 40% EtOH was separated by MPLC (Lobar RP-8, 25 × 310 mm (Merck), H_2O–MeOH (70 : 30)) and HPLC (H_2O–MeOH (70 : 30)) to afford three compounds, syringinoside (**1**) (1127 mg), conferinoside (**2**) 91 mg) and syringin (**3**) (82 mg).

Syringinoside (1)——Colorless needles from aqueous MeOH, mp 178—179 °C. $[\alpha]_D$ −37.6° (c=1.0, H_2O). *Anal.* Calcd for $C_{23}H_{34}O_{14}\cdot H_2O$: C, 49.99; H, 6.57. Found: C, 49.48; H, 6.94. SIMS *m/z*: 557 $(M+Na)^+$, 535 $(M+H)^+$. ^{1}H-NMR (CD_3OD) δ: 3.86 (6H, s), 4.22 (2H, dd, J=5.61, 1.46 Hz), 4.24 (1H, d, J=7.81 Hz), 4.87 (1H, d, J=7.57 Hz), 6.33 (1H, dt, J=15.87, 5.61 Hz), 6.55 (1H, dt, J=15.87, 1.46 Hz), 6.75 (2H, s). IR ν_{max}^{KBr}: 3400 cm^{-1}.

Conferinoside (2)——Amorphous powder. $[\alpha]_D$ −56.0° (c=1.0, H_2O). *Anal.* Calcd for $C_{22}H_{32}O_{13}\cdot H_2O$: C, 50.57; H, 6.56. Found: C, 49.89; H, 7.10. SIMS *m/z*: 527 $(M+Na)^+$, 505 $(M+H)^+$. ^{1}H-NMR (CD_3OD) δ: 3.87 (3H, s), 4.21 (2H, dd, J=5.86, 1.47 Hz), 4.36 (1H, d, J=7.82 Hz), 4.89 (1H, d, J=7.32 Hz), 6.28 (1H, dt, J=15.86, 5.86 Hz), 6.55 (1H, dt, J=15.86, 1.47 Hz), 6.98 (1H, dd, J=8.30, 1.96 Hz), 7.06 (1H, d, J=1.96 Hz), 7.16 (1H, d, J=8.30 Hz). IR ν_{max}^{KBr}: 3400 cm^{-1}.

Syringin (3)——Colorless needles from aqueous MeOH, mp 193—194 °C (lit.,[4b] mp 191—192 °C). SIMS *m/z*: 395 $(M+Na)^+$, 373 $(M+H)^+$. ^{1}H-NMR (CD_3OD) δ: 3.86 (6H, s), 4.22 (2H, dd, J=5.62, 1.71 Hz), 4.87 (1H, d, J=7.57 Hz), 6.32 (1H, dt, J=15.87, 5.62 Hz), 6.54 (1H, dt, J=15.87, 1.71 Hz), 6.75 (2H, s).

Permethylation of Syringinoside (1)——A solution of **1** (21 mg) in dimethyl sulfoxide (DMSO) (1 ml) was added to a solution of dimsylsodium prepared from NaH (58 mg) and DMSO (1 ml), and the mixture was stirred at room temperature under an N_2 atmosphere. After 2 h, CH_3I (0.5 ml) was added to the solution and stirred for 3 h. The reaction mixture was poured into water (20 ml) and then extracted with AcOEt. The extract was worked up as usual and the residue was purified by preparative thin layer chromatography (TLC) (Merck 5744, $CHCl_3$–EtOH; 19 : 1) to give **4** (16 mg). Electron impact mass spectra (EIMS) *m/z*: 422 ($C_{19}H_{34}O_{10}$ corresponding to methylated gentiobiose) and 244 ($C_{12}H_{16}O_4$ corresponding to methylated phenylpropanoid). ^{1}H-NMR ($CDCl_3$) δ: 3.28 (3H, s), 3.37 (3H, s), 3.39 (3H, s), 3.49 (3H, s), 3.51 (3H, s), 3.56 (3H, s), 3.64 (3H, s), 3.68 (3H, s), 3.84 (6H, s), 4.08 (2H, dd, J=6.10, 1.46 Hz), 4.17 (1H, d, J=7.81 Hz), 4.96 (1H, d, J=7.57 Hz), 6.18 (1H, dt, J=15.87, 6.10 Hz), 6.51 (1H, dt, J=15.87, 1.46 Hz), 6.60 (2H, s).

Hydrogenation, Acid Hydrolysis and Acetylation of Permethylated Syringinoside (4)——A mixture of **4** (16 mg) and 10% Pd–C (5 mg) in MeOH (5 ml) was stirred for 1 h under an H_2 atmosphere and filtered to give the corresponding dihydro compound (14 mg). EIMS *m/z*: 648 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 3.30 (3H, s), 3.35 (3H, s), 3.37 (3H, s), 3.50 (3H, s), 3.52 (3H, s), 3.58 (3H, s), 3.64 (3H, s), 3.68 (3H, s), 3.82 (6H, s), 4.18 (1H, d, J=7.81 Hz), 4.90 (1H, d, J=7.57 Hz), 6.40 (2H, s). The dihydro compound (14 mg), without purification, was hydrolyzed with 10% HCl (0.4 ml) in dioxane (0.4 ml) at 50 °C for 5 h, acetylated with Ac_2O (0.1 ml) and pyridine (0.1 ml) and then purified by preparative TLC (Merck 5744, $CHCl_3$–EtOH; 19 : 1) to give **5** (3 mg). High resolution mass spectra (HRMS) *m/z*: 268.1310 Calcd for $C_{14}H_{20}O_5$. Found: 268.1285. IR ν_{max}^{film}: 1765, 1600 cm^{-1}. ^{1}H-NMR δ: 2.33 (3H, s), 3.36 (3H, s), 3.80 (6H, s), 6.44 (2H, s).

Methanolysis of Permethylated Syringinoside (4)——A solution of **4** (21 mg) in hydrogen chloride–methanol Reagent 10 (3 ml) (Tokyo Kasei Kogyo Co., Ltd.) was heated under reflux for 3 h. The reaction mixture was separated by preparative TLC (Merck 5744, $CHCl_3$–EtOAc; 1 : 3) to give α-D-octamethylgentiobiose (7 mg). $[\alpha]_D$ +46.1° (c=0.32, $CHCl_3$) (an authentic sample prepared from D-gentiobiose: $[\alpha]_D$ +51.6°). ^{1}H-NMR ($CDCl_3$) δ: 3.39 (3H, s), 3.40 (3H, s), 3.50 (3H, s), 3.52 (3H, s), 3.56 (3H, s), 3.60 (3H, s), 3.62 (3H, s), 3.63 (3H, s), 4.28 (1H, d, J=7.39 Hz), 4.82 (1H, d, J=3.36 Hz).

Esterification of 6 with Diazomethane——Treatment of **6** (123 mg) in ether (10 ml) with diazomethane at −60 °C gave a methyl ester (125 mg). EIMS *m/z*: 238 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 3.80 (3H, s), 3.91 (6H, s), 6.30 (1H, d, J=15.87 Hz), 6.76 (2H, s), 7.60 (1H, d, J=15.87 Hz).

Hydrogenation——The ester (120 mg) was hydrogenated over 10% Pd–C in MeOH to give a dihydro compound (120 mg). EIMS *m/z*: 240 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.61 (2H, t, J=7.81 Hz), 2.88 (2H, t, J=7.81 Hz), 3.67 (3H, s), 3.87 (6H, s), 6.42 (2H, s).

Benzylation——The dihydro compound (115 mg) in acetone (5 ml) was benzylated with benzyl bromide (100 mg) and K_2CO_3 (300 mg) under reflux for 5 h to give a benzylated compound (83 mg). HRMS *m/z*: 330.1466 Calcd for $C_{19}H_{22}O_5$. Found: 330.1427. ^{1}H-NMR δ: 2.63 (2H, t, J=7.73 Hz), 2.90 (2H, t, J=7.73 Hz), 3.68 (3H, s), 3.80 (3H, s), 3.81 (3H, s), 4.97 (2H, s), 6.41 (2H, s), 7.26—7.50 (5H, m).

Reaction with $LiAlH_4$——The benzylated compound (80 mg) in ether (6 ml) was treated with $LiAlH_4$ (50 mg) at room temperature overnight to give an alcohol (68 mg). EIMS *m/z*: 302 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.89 (2H, m), 2.65 (2H, t, J=7.57 Hz), 3.68 (2H, t, J=6.35 Hz), 3.81 (6H, s), 4.97 (2H, s), 6.41 (2H, s), 7.28—7.50 (5H, m).

Methylation——The alcohol (60 mg) in tetrahydrofuron (THF) (7 ml) was treated with NaH (6 mg) under reflux for 3 h and then with CH_3I (2 ml) under reflux for 5 h to give a methylated compound (39 mg). HRMS *m/z*: 316.1674 Calcd for $C_{19}H_{24}O_4$. Found: 316.1660. ^{1}H-NMR δ: 1.88 (2H, m), 2.63 (2H, t, J=7.39 Hz), 3.35 (3H, s), 3.39 (2H, t, J=6.38 Hz), 3.80 (6H, s), 4.97 (2H, s), 6.40 (2H, s), 7.27—7.50 (5H, m).

Hydrogenolysis——The methylated compound (31 mg) in MeOH (3 ml) was treated with 10% Pd–C (10 mg) under H_2 for 3 h to give a phenolic compound (19 mg). EIMS *m/z*: 226 (M^+). ^{1}H-NMR δ: 1.86 (2H, m), 2.62 (2H, t, J=7.39 Hz), 3.35 (3H, s), 3.39 (2H, t, J=6.39 Hz), 3.87 (6H, s), 6.42 (2H, s).

Acetylation——The phenolic compound (10 mg) was treated with Ac_2O (0.1 ml) and pyridine (0.1 ml) to give an acetate (10 mg). The acetate was identical with **5**.

Acknowledgements We wish to thank Prof. S. Yamamura (Keio University) and Dr. Y. Mori (Meijo University) for valuable comments. Thanks are also due to Dr. H. Tanino for 400 MHz NMR measurements, Mr. K. Masuda for MS measurements, Miss T. Sakai (Meijo University) for elemental analyses and Mrs. H. Kato (Kato Seishi, Ishikawa Prefecture) for collection of the plant.

References and Notes

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