

Studies on the Constituents of *Osmanthus* Species. X.¹⁾ Structures of Phenolic Glucosides from the Leaves of *Osmanthus asiaticus* NAKAI

Masataka SUGIYAMA and Masao KIKUCHI*

Tohoku College of Pharmacy, 4-4-1 Komatsushima Aoba-ku, Sendai-shi 981, Japan. Received July 22, 1991

Three new phenolic glucosides were isolated from the leaves of *Osmanthus asiaticus* NAKAI (Oleaceae). The structures of **1**, **2** and **3** were determined to be 2-hydroxy-5-(2-hydroxyethyl)phenyl β -D-glucopyranoside, 4-(2,3-dihydroxypropyl)-2,6-dimethoxyphenyl β -D-glucopyranoside and D-threo-guaiacylglycerol 7-O- β -D-glucopyranoside, respectively, on the basis of chemical and spectral data.

Keywords *Osmanthus asiaticus*; phenolic glucoside; NMR; NOE; heteronuclear multiple-bond correlation; optical rotation; osmanthuside F; osmanthuside G; D-threo-guaiacylglycerol 7-O- β -D-glucopyranoside

In the preceding paper,¹⁾ we have reported the isolation of phenolic glycosides from the AcOEt- and water-soluble fractions of *Osmanthus asiaticus* NAKAI (Oleaceae). We now wish to report the isolation and structure elucidation of new phenolic glucosides from the water-soluble fraction of the methanol extract.

The extraction and separation were carried out as described in the experimental section. Compound **1** was obtained as an amorphous powder. The fast atom bombardment mass spectrum (FAB-MS) of **1** showed the $[M+Na]^+$ at m/z 339. The proton nuclear magnetic resonance (¹H-NMR) spectrum of **1** showed the signals of two sets of methylene protons [δ 2.71 (2H, t, $J=6.9$ Hz, 7-H₂) and δ 3.70 (2H, t, $J=6.9$ Hz, 8-H₂)], a glucosyl-anomeric proton [δ 4.75 (1H, d, $J=7.7$ Hz, 1'-H)] and aromatic protons [δ 6.75 (1H, d, $J=8.1$ Hz, 3-H), 6.77 (1H, dd, $J=8.1, 1.8$ Hz, 4-H) and 7.07 (1H, d, $J=1.8$ Hz, 6-H)] indicative of the presence of 3,4-dihydroxyphenethyl alcohol and a glucose. The carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum of **1** (see Experimental) also showed the presence of a β -D-glucose. The position of the glucosyl linkage in **1** was investigated as follows. In a nuclear Overhauser effect (NOE) experiment, irradiation at δ 4.75 (1'-H) enhanced the intensity of the signal at δ 7.07 (6-H), suggesting that the glucosyl group is attached to 1-OH. On the basis of the above-mentioned evidence, the structure of **1** was determined to be 2-hydroxy-5-(2-hydroxyethyl)phenyl β -D-glucopyranoside.

Compound **2** was obtained as an amorphous powder. The FAB-MS of **2** showed the $[M+Na]^+$ at m/z 413. The ¹H-NMR spectrum of **2** showed the presence of two sets of methylene protons [δ 2.60 (1H, dd, $J=13.6, 8.0$ Hz, 7-H_A), 2.80 (1H, dd, $J=13.6, 5.1$ Hz, 7-H_B) and 3.48 (2H, m, 9-H₂)], a methine proton [δ 3.76 (1H, m, 8-H)], two methoxyl groups [δ 3.84 (6H, s)], a glucosyl-anomeric proton [δ 4.80 (1H, d, $J=7.7$ Hz, 1'-H)] and aromatic protons [δ 6.60 (2H, s, 3-H and 5-H)]. The ¹³C-NMR spectrum of **2** (see Experimental) also showed the presence of a β -D-glucose. The position of the glucosyl linkage in **2** was investigated as follows. The ¹H-detected heteronuclear multiple-bond correlation (HMBC)²⁾ spectrum of **2** showed the correlation of the carbon at δ 134.8 (1) with the glucosyl-anomeric proton at δ 4.80. Thus, the glucose must be attached to 1-OH. On the basis of the above-mentioned evidence, the structure of **2** was determined to be 4-(2,3-dihydroxypropyl)-2,6-dimethoxyphenyl β -D-glucopyranoside. The absolute configuration of C-8 could not be

determined, because the amount of the sample was too small.

These two compounds, **1** and **2** are the first phenolic glucosides in nature, to have been isolated from a natural source, and we named them osmanthuside F and osmanthuside G, respectively.

Compound **3** was obtained as an amorphous powder. The FAB-MS of **3** showed the $[M+H]^+$ at m/z 377. The ¹H-NMR spectrum of **3** showed the presence of methylene protons [δ 3.40 (1H, m, 9-H_A) and 3.57 (1H, m, 9-H_B)], two methine protons [δ 3.71 (1H, dd, $J=6.2, 5.1$ Hz, 8-H) and 4.86 (1H, d, $J=5.1$ Hz, 7-H)], a methoxyl group [δ 3.85 (3H, s)], a glucosyl-anomeric proton [δ 4.11 (1H, d, $J=7.3$ Hz, 1'-H)] and aromatic protons [δ 6.76 (1H, d, $J=8.1$ Hz, 5-H), 6.81 (1H, dd, $J=8.1, 1.8$ Hz, 6-H) and 7.09 (1H, d, $J=1.8$ Hz, 2-H)] indicative of the presence of a guaiacylglycerol and a glucose. The position of the glucosyl linkage in **3** was investigated as follows. In an NOE experiment, irradiation at δ 4.11 (1'-H) enhanced the intensity of the signals at δ 4.86 (7-H), 6.81 (6-H) and 7.09 (2-H). Irradiation at δ 4.86 (7-H) enhanced the intensity of the 8-H, 1'-H, 6-H and 2-H signals. Thus, the glucosyl group is attached to 7-OH. From the above results, **3** was concluded to be guaiacylglycerol 7-O- β -D-glucopyranoside. Theander reported guaiacylglycerol 7-O- β -D-glucopyranoside as a *threo*-form (**4**) from *Pinus silvestris* L.³⁾ but did not establish whether it is D- or L-form. On the other hand, the optical rotation is different from that of **3** (**4**, $[\alpha]_D +45^\circ$ (MeOH), **3**, $[\alpha]_D -80^\circ$ (MeOH)). Com-

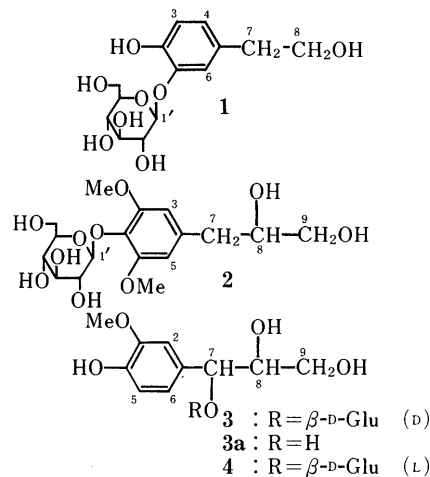


Chart 1

pounds **3** and **4** seem to be diastereomers. Hydrolysis of **3** with β -glucosidase gave an aglycon, *D*-*threo*-guaiacylglycerol, $[\alpha]_D -18.5^\circ$ (EtOH) (**3a**).⁴⁾ From the above result, **3** was concluded to be *D*-*threo*-guaiacylglycerol 7-*O*- β -*D*-glucopyranoside.

Experimental

Melting points were determined on a Yanagimoto MP-S3 micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-360 digital polarimeter. Ultraviolet (UV) spectra were recorded with a Beckman DU-64 spectrometer. ¹H- and ¹³C-NMR spectra were recorded with a JEOL JMX-GSX 400 (400 and 100 MHz, respectively) spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet). Mass spectra (MS) were recorded on a JEOL JMS-DX 300 mass spectrometer. Column chromatography was carried out on Diaion HP-20 (Mitsubishi Chemical Ind. Ltd.), Kieselgel 60 (Merck, 70–230 and 230–400 mesh) and Sephadex LH-20 (Pharmacia Fine Chemical). Preparative high-performance liquid chromatography (HPLC) was carried out on a Tosoh HPLC system (pump, CCPM prep; detector, UV-8010) using a TSK gel ODS 120A (Tosoh) column. Thin layer chromatography (TLC) was carried out with precoated Kieselgel 60 plates (Merck) and detection was achieved by spraying 50% H₂SO₄ followed by heating.

Isolation Fresh leaves of *O. asiaticus* (2.2 kg), collected in October 1988, in Sendai, Japan, were extracted with MeOH at room temperature for one month. The MeOH extract was concentrated under reduced pressure and the residue was suspended in a small excess of H₂O. This suspension was extracted with CHCl₃, Et₂O, AcOEt, *n*-BuOH and H₂O, successively. The H₂O-soluble fraction was passed through a Diaion HP-20 column. The adsorbed material was eluted with MeOH and the eluate was concentrated under reduced pressure to afford the residue (14.8 g). This residue was chromatographed on a silica gel column (CHCl₃-MeOH-H₂O) and subjected to HPLC (MeOH-H₂O) to give compounds **1** (6 mg), **2** (2 mg) and **3** (12 mg).

2-Hydroxy-5-(2-hydroxyethyl)phenyl β -*D*-Glucopyranoside (1) An amorphous powder, $[\alpha]_D -40.0^\circ$ (*c*=0.6, MeOH). FAB-MS *m/z*: 339 (M+Na)⁺. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 206 (3.96), 224 sh (3.76), 276 (3.63). ¹H-NMR (CD₃OD) δ : 2.71 (2H, t, *J*=6.9 Hz, 7-H₂), 3.70 (2H, t, *J*=6.9 Hz, 8-H₂), 4.75 (1H, d, *J*=7.7 Hz, 1'-H), 6.75 (1H, d, *J*=8.1 Hz, 3-H), 6.77 (1H, dd, *J*=8.1, 1.8 Hz, 4-H), 7.07 (1H, d, *J*=1.8 Hz, 6-H). ¹³C-NMR (CD₃OD) δ : 39.6 (7), 62.5 (6'), 64.4 (8), 71.4 (4'), 75.0 (2'), 77.7 (5'), 78.4 (3'), 104.5 (1'), 117.0 (3), 119.6 (6), 125.2 (4), 132.1 (5), 146.6 (2), 146.8 (1).

4-(2,3-Dihydroxypropyl)-2,6-dimethoxyphenyl β -*D*-Glucopyranoside (2) An amorphous powder, $[\alpha]_D -22.7^\circ$ (*c*=0.2, MeOH). FAB-MS *m/z*: 413 (M+Na)⁺. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 208 (4.17), 223 sh (3.82), 267 (2.82). ¹H-NMR (CD₃OD) δ : 2.60 (1H, dd, *J*=13.6, 8.0 Hz, 7-H_A), 2.80 (1H, dd, *J*=13.6, 5.1 Hz, 7-H_B), 3.48 (2H, m, 9-H₂), 3.76 (1H, m, 8-H), 3.84 (6H, s, 2 \times OCH₃), 4.80 (1H, d, *J*=7.7 Hz, 1'-H), 6.60 (2H, s, 3-H and 5-H). ¹³C-NMR (CD₃OD) δ : 41.2 (7), 57.0 (2 \times OCH₃), 62.6 (6'), 66.7 (9), 71.4 (4'), 74.4 (8), 75.8 (2'), 77.9 (5'), 78.4 (3'), 105.6 (1'), 108.5 (3 and 5), 134.8 (1), 137.2 (4), 154.0 (2 and 6).

***D*-*threo*-Guaiacylglycerol 7-*O*- β -*D*-Glucopyranoside (3)** An amorphous powder, $[\alpha]_D -81.2^\circ$ (*c*=1.0, MeOH). FAB-MS *m/z*: 377 (M+H)⁺, 399 (M+Na)⁺. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 208 (3.93), 229 (3.77), 278 (3.38). ¹H-NMR (CD₃OD) δ : 3.40 (1H, m, 9-H_A), 3.57 (1H, m, 9-H_B), 3.71 (1H, dd, *J*=6.2, 5.1 Hz, 8-H), 3.85 (3H, s, OCH₃), 4.11 (1H, d, *J*=7.3 Hz, 1'-H), 4.86 (1H, d, *J*=5.1 Hz, 7-H), 6.76 (1H, d, *J*=8.1 Hz, 5-H), 6.81 (1H, dd, *J*=8.1, 1.8 Hz, 6-H), 7.09 (1H, d, *J*=1.8 Hz, 2-H). ¹³C-NMR (CD₃OD) δ : 56.4 (OCH₃), 62.8 (6'), 64.0 (9), 71.9 (4'), 75.1 (2'), 76.9 (8), 77.7 (5'), 77.9 (3'), 79.4 (7), 100.6 (1'), 112.6 (2), 115.9 (5), 122.0 (6), 130.9 (1), 147.5 (4), 149.1 (3).

Enzymatic Hydrolysis of 3 A solution of **3** (5 ml) in H₂O (3 ml) was incubated with β -glucosidase (10 mg, Sigma Chemical Company) at 37°C overnight, then evaporated under reduced pressure, and the residue was extracted with MeOH. The extractive was fractionated by silica gel column chromatography (solvent; CHCl₃:MeOH:H₂O=8:2:0.2) into the aglycon (**3a**, 2 mg) and glucose (1 mg). *D*-*threo*-Guaiacylglycerol (**3a**), an amorphous powder, $[\alpha]_D -18.5^\circ$ (*c*=0.2, EtOH). ¹H-NMR (acetone-*d*₆) δ : 3.83 (3H, s, OCH₃), 4.56 (1H, d, *J*=3.7 Hz, 7-H), 6.76 (1H, d, *J*=8.1 Hz, 5-H), 6.82 (1H, dd, *J*=8.1, 1.8 Hz, 6-H), 7.01 (1H, d, *J*=1.8 Hz, 2-H). *D*-Glucose: a syrup, PC (paper, Toyo Roshi No. 51; solvent, BuOH-AcOH-H₂O (4:1:2); visualizing agent, aniline hydrogen phthalate), *R*_f=0.24 (glucose).

Acknowledgment The authors are grateful to Dr. K. Hisamichi of our college for NMR measurement.

References

- 1) Part IX: M. Sugiyama and M. Kikuchi, *Phytochemistry*, **30**, 3147 (1991).
- 2) A. Box and M. F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986); M. F. Summers, L. G. Marzilli and A. Bax, *ibid.*, **108**, 4285 (1986); A. Bax, A. Aszalos, Z. Dinya and K. Sudo, *ibid.*, **108**, 8056 (1986).
- 3) O. Theander, *Acta Chem. Scand.*, **19**, 1792 (1965).
- 4) K. Ishimaru, G. Nonaka and I. Nishioka, *Phytochemistry*, **26**, 1147 (1987).