Studies on the Constituents of *Osmanthus* Species. VI.[1] Structures of Phenylpropanoid Glycosides from the Leaves of *Osmanthus asiaticus* NAKAI

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Three new phenylpropanoid glycosides, named osmanthuside B_6 (I), osmanthuside D (II) and osmanthuside E (III), were isolated from the leaves of *Osmanthus asiaticus* NAKAI (Oleaceae). The structures of I, II and III were determined to be β-(*p*-hydroxyphenyl) ethyl *O*-α-L-rhamnopyranosyl-(1→3)-6-*O*-*trans*-*p*-coumaroyl-β-D-glucopyranoside, β-(*p*-hydroxyphenyl) ethyl *O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-*cis*-*p*-coumaroyl-β-D-glucopyranoside and β-(*p*-hydroxyphenyl) ethyl 6-*O*-*trans*-feruloyl-β-D-glucopyranoside, respectively, on the basis of chemical and spectral data.

Keywords *Osmanthus asiaticus*; phenylpropanoid glycoside; osmanthuside B_6; osmanthuside D; osmanthuside E; ^1H–^{13}C long-range COSY

We have already reported the isolation of phenylpropanoid glycosides from *Osmanthus fragrans* LOUR. var. *aurantiacus* MAKINO, *O. fortunei* CARR. and *O. ilicifolius* MOUILL. (Oleaceae).[1–5] As a continuation of our investigation on the constituents of phenylpropanoid glycosides, we now wish to report the isolation and structure elucidation of three new phenylpropanoid glycosides, named osmanthusides B_6, D and E, as well as the isolation of one known phenylpropanoid glycoside, osmanthuside B.[3]

The fresh leaves of *O. asiaticus* NAKAI. (Japanese name; ginmokusei)[6] were extracted with MeOH and the MeOH extract was suspended in a small excess of water. This suspension was extracted with $CHCl_3$, Et_2O, AcOEt and *n*-BuOH, successively. The AcOEt-soluble fraction was chromatographed on silica gel to give six fractions (fr. 1–6). After repeated chromatography (silica gel, Sephadex LH-20 and high-performance liquid chromatography (HPLC)) of these fractions, four phenylpropanoid glycosides, osmanthusides B_6 (I), D (II), E (III), and B (IV), were isolated.

Osmanthuside B_6 (I) was isolated as an amorphous powder, $[\alpha]_D^{22}$ −45.8° (c=0.24, MeOH). The infrared (IR) spectrum suggested the presence of hydroxyl groups (3600—3000 cm^{-1}), a conjugated ester (1680 cm^{-1}), a double bond (1620 cm^{-1}) and aromatic rings (1600, 1505 cm^{-1}). On fast atom bombardment mass spectrometry (FAB-MS), fragments of m/z 593 $(M+H)^+$ and 615 $(M+Na)^+$ were observed, and from the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, the molecular formula of I was determined to be $C_{29}H_{36}O_{13}$. The ultraviolet (UV) spectrum showed absorption maxima at 222 and 280 nm. The proton nuclear magnetic resonance (^1H-NMR) spectrum of I showed signals of a methyl group of rhamnose [δ 1.24 (3H, d, J=6.4 Hz)], a glucosyl-anomeric proton [δ 4.33 (1H, d, J=7.8 Hz)], a rhamnosyl-anomeric proton [δ 5.18 (1H, s)], two *trans* olefinic protons [δ 6.35, 7.62 (1H each, d, J=16.1 Hz)] and two *p*-hydroxyphenyl group A_2B_2-type signals [δ 6.84 (4H, q, A_2B_2 type, J=8.3 Hz, $\Delta\delta$=0.38 ppm, arom. protons) and δ 7.10 (4H, q, A_2B_2 type, J=8.3 Hz, $\Delta\delta$=0.62 ppm, arom. protons)]. The ^{13}C-NMR spectrum of I suggested the presence of *p*-hydroxyphenethyl, *p*-coumaroyl, glucosyl and rhamnosyl groups. The chemical shifts of I were compared with those of osmanthuside B and acteoside,[7] especially in the sugar carbon region (Table I). From these spectral data, rhamnose was attached to the glucosyl 3′-OH of *p*-hydroxyphenethyl β-D-glucoside, and the *p*-coumaroyl group was determined to be at the 6′-OH position of the glucose moiety.

On the basis of the above-mentioned evidence, the structure of osmanthuside B_6 was determined to be β-(*p*-hydroxyphenyl) ethyl *O*-α-L-rhamnopyranosyl-(1→3)-6-*O*-*trans*-*p*-coumaroyl-β-D-glucopyranoside (I).

The structure of osmanthuside D (II) was investigated as the heptaacetate (IIa), which was isolated after acetylation of the mixture of *cis*/*trans* isomers. The mixture itself could be separated into two peaks by use of HPLC with an H_2O–CH_3CN system or an H_2O–MeOH system as the mobile phase (Fig. 1). Each eluate was concentrated under

TABLE I. ^{13}C-NMR Chemical Shifts (100 MHz, CD_3OD)

Carbon		I	Osmanthuside B (IV)	Acteoside
Glc	1	104.5	104.1	104.2
	2	75.7	75.9	76.0
	3	84.0	81.6	81.7
	4	70.1	70.4	70.4
	5	75.4	76.1	76.2
	6	64.7	62.3	62.4
Rha	1	102.8	103.0	103.1
	2	72.5	72.3	72.3
	3	72.4	72.2	72.1
	4	74.0	73.8	73.8
	5	70.5	70.6	70.6
	6	17.9	18.4	18.5

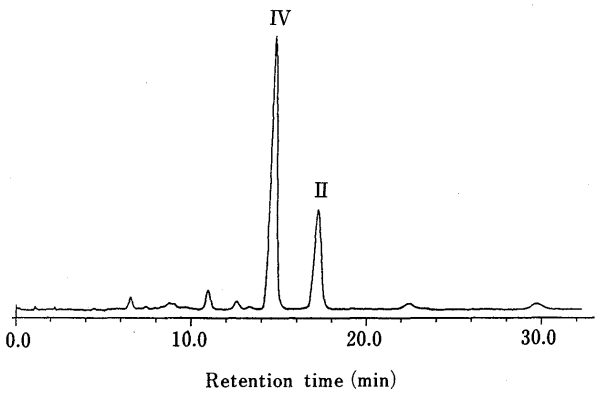
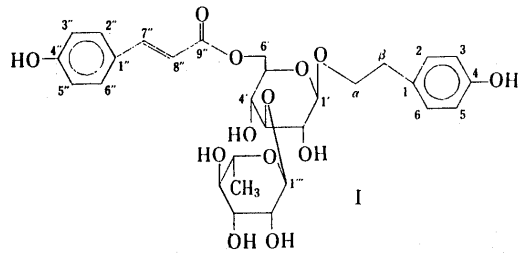
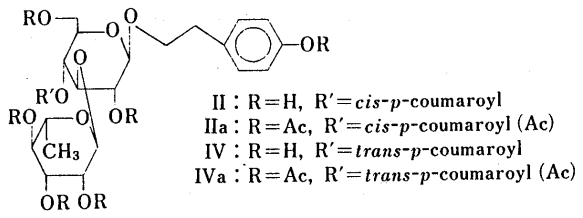


Fig. 1. High-Performance Liquid Chromatogram of Fraction 3-1

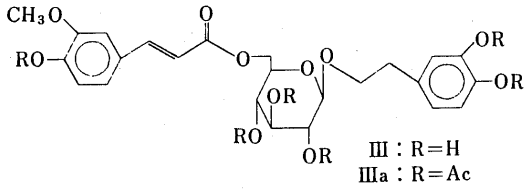
Column, TSKgel ODS-120T; column size, 4.6 mm i.d. × 15 cm; mobile phase, H_2O–CH_3CN (8:2); flow rate, 1.0 ml/min; UV detector, 313 nm (0.05 AUFS). II, osmanthuside D; IV, osmanthuside B.



I



II : R=H, R'=*cis*-*p*-coumaroyl
IIa : R=Ac, R'=*cis*-*p*-coumaroyl (Ac)
IV : R=H, R'=*trans*-*p*-coumaroyl
IVa : R=Ac, R'=*trans*-*p*-coumaroyl (Ac)



III : R=H
IIIa : R=Ac

Chart 1

reduced pressure. However, it appeared to be impossible to separate II and IV in this way, because of mutual interconversion. Therefore, the mixture was acetylated in the usual way, and IIa and IVa were isolated. Compound IVa was identified as osmanthuside B heptaacetate by spectral comparison with an authentic sample. The IR spectrum of IIa suggested the presence of a conjugated ester (1745, 1700 cm^{-1}) and aromatic rings (1600, 1500 cm^{-1}). The ^{1}H-NMR spectrum of IIa showed signals of a methyl group of rhamnose [δ 1.04 (3H, d, J=6.2 Hz)], five alcoholic acetoxyl groups [δ 1.84, 1.95, 1.99, 2.06 and 2.12 (3H each, s)], two phenolic acetoxyl groups [δ 2.28 and 2.30 (3H each, s)], methylene protons [δ 2.86 (2H, m)], a glucosyl-anomeric proton [δ 4.36 (1H, d, J=8.1 Hz)], a rhamnosyl-anomeric proton [δ 4.81 (1H, d, J=1.8 Hz)], two *cis* olefinic protons [δ 5.91 (1H, d, J=12.5 Hz) and 7.01 (1H, d, J=12.5 Hz)] and *p*-acetoxyphenyl group A_2B_2 type signals [δ 7.09 (4H, q, A_2B_2 type, J=8.4 Hz, $\Delta\delta$=0.21 ppm, arom. protons) and δ 7.38 (4H, q, A_2B_2 type, J=8.4 Hz, $\Delta\delta$=0.57 ppm, arom. protons)]. The ^{13}C-NMR spectrum of IIa suggested the presence of *p*-acetoxyphenyl, glucosyl, *cis*-*p*-coumaroyl and rhamnosyl groups. From the ^{13}C-NMR signals of the glucosyl moiety, the location of rhamnose was determined to be the C-3 position of the glucosyl moiety. The ^{1}H–^{13}C long-range shift correlation spectrum (COSY) of IIa showed the correlation of the carbonyl carbon at δ 163.9 (9″) with the glucosyl 4′-H proton at δ 5.11. Thus, the *cis*-*p*-coumaroyl group must be attached to the glucosyl 4′-OH.

On the basis of the above-mentioned evidence, the structure of osmanthuside D was determined to be β-(*p*-hydroxyphenyl) ethyl *O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-*cis*-*p*-coumaroyl-β-D-glucopyranoside (II).

Osmanthuside E (III) was purified as the hexaacetate (IIIa), a colorless powder. The IR spectrum suggested the presence of a conjugated ester (1710 cm^{-1}), a double bond (1635 cm^{-1}) and aromatic rings (1600, 1505 cm^{-1}). The ^{1}H-NMR spectrum of IIIa showed the signals of three alcoholic acetoxyl groups [δ 1.93, 1.99, 2.03 (3H each)], three phenolic acetoxyl groups [δ 2.26 (6H) and 2.32 (3H)], methylene protons [δ 2.86 (2H, m)], a methoxyl group [δ 3.88 (3H, s)], a glucosyl-anomeric proton [δ 4.50 (1H, d, J=7.8 Hz)], two *trans* olefinic protons [δ 6.41, 7.66 (1H each, d, J=16.1 Hz)] and aromatic protons [δ 6.94—7.13 (6H, m)]. The ^{13}C-NMR spectrum of IIIa suggested the presence of a 3,4-dihydroxyphenethyl β-D-glucoside moiety and a feruloyl group. The ^{1}H–^{13}C long-range COSY of IIIa showed the correlation of the carbonyl carbon at δ 166.3 (9″) with the glucosyl C-6 proton at δ 4.33. Thus, the feruloyl group must be attached to the glucosyl 6′-OH.

On the basis of the above-mentioned evidence, the structure of osmanthuside E was determined to be β-(*p*-hydroxyphenyl) ethyl 6-*O*-*trans*-feruloyl-β-D-glucopyranoside (III).

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. IR spectra were recorded with a Shimadzu IR-430 infrared spectrophotometer and UV spectra with a Beckman DU-64 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a JEOL JNM-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). MS were recorded on a JEOL JMS-DX 300 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 70—230 and 230—400 mesh) and Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) was carried out with precoated Kieselgel 60 plates (Merck) and detection was achieved by spraying 50% H_2SO_4 followed by heating. Preparative HPLC was carried out on a Tosoh HPLC system (pump, CCPM prep; detector, UV-8010) using a TSK gel ODS-80TM (Tosoh) column.

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 water. This suspension was extracted with $CHCl_3$, Et_2O, AcOEt and *n*-BuOH, successively. The AcOEt-soluble fraction was concentrated under reduced pressure to afford the residue (17.4 g). This residue was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and the eluate was separated into six fractions (fr. 1—6). Fraction 3 was rechromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1) repeatedly to give I (35 mg) and fraction 3-1 (50 mg). Fraction 3-1 was separated into two peaks at t_R (min) 17.2 (II) and 14.6 (IV) by HPLC (Fig. 1). Fraction 2 was rechromatographed on a silica gel column using $CHCl_3$–MeOH (4 : 1) repeatedly to give a crude powder containing III (25 mg). The ^{1}H-NMR spectrum of the crude powder showed the absence of acetyl groups. The crude powder was acetylated with acetic anhydride in pyridine, and the crude acetate was purified by chromatography on silica gel with *n*-hexane–acetone (3 : 2) to give the hexaacetate (IIIa) (15 mg). Fraction 3-1; amorphous powder. FAB-MS *m/z*: 593 $(M+H)^+$, 615 $(M+Na)^+$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 1.03 (3H, d, J=5.9 Hz, 6‴-H_3), 1.17 (3H, d, J=6.4 Hz, 6‴-H_3), 4.35 (1H, d, J=7.8 Hz, 1′-H), 4.38 (1H, d, J=7.8 Hz, 1′-H), 5.17 (1H, d, J=2.0 Hz, 1‴-H), 5.20 (1H, d, J=1.5 Hz, 1‴-H), 5.79 (1H, d, J=13.2 Hz, *cis*-H), 6.34 (1H, d, J=16.1 Hz, *trans*-H), 6.70—7.80 (6H, arom. protons and olefinic protons). Acetylation of 3-1; 3-1 (30 mg) was acetylated with acetic anhydride in pyridine. The crude acetate was subjected to silica gel chromatography with benzene–AcOEt (2 : 1) as a developer. The fractions showing TLC spots at *Rf* 0.35 (IIa) and *Rf* 0.25 (IIIa) were each collected, yielding IIa (10 mg) and IIIa (13 mg).

Osmanthuside B_6 (I) An amorphous powder. mp 135—138 °C. $[\alpha]_D^{22}$ −45.8° (c=0.24, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3600—3000, 1680, 1620, 1600, 1505. UV λ_{max}^{MeOH} nm (log ε): 222 (4.28), 280 (4.27). FAB-MS *m/z*: 593 $(M+H)^+$, 615 $(M+Na)^+$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 1.24 (3H, d,

$J=6.4$ Hz, 6‴-H_3), 2.83 (2H, t, $J=7.3$ Hz, β-H_2), 4.33 (1H, d, $J=7.8$ Hz, 1′-H), 4.33 (1H, dd, $J=12.2$, 6.8 Hz, 6′-H_A), 4.50 (1H, dd, $J=12.2$, 2.4 Hz, 6′-H_B), 5.18 (1H, s, 1‴-H), 6.35 (1H, d, $J=16.1$ Hz, C″$_8$-H), 6.84 (4H, q, A_2B_2 type, $J=8.3$ Hz, $\Delta\delta=0.38$ ppm, arom. protons), 7.10 (4H, q, A_2B_2 type, $J=8.3$ Hz, $\Delta\delta=0.62$ ppm, arom. protons), 7.62 (1H, d, $J=16.1$ Hz, C″$_7$-H). ^{13}C-NMR (100 MHz, CD_3OD) δ: 17.9 (q, C(6‴)), 36.6 (t, C(β)), 64.7 (t, C(6′)), 70.1 (d, C(4′)), 70.5 (d, C(5‴)), 72.3 (t, C(α)), 72.4 (d, C(3‴)), 72.5 (d, C(2‴)), 74.0 (d, C(4‴)), 75.4 (d, C(5′)), 75.7 (d, C(2′)), 84.0 (d, C(3′)), 102.8 (d, C(1‴)), 104.5 (d, C(1′)), 115.0 (d, C(8″)), 116.2 (d, C(2) and C(6)), 116.9 (d, C(3″) and C(5″)), 127.2 (s, C(1″)), 130.6 (s, C(1)), 131.0 (d, C(2″) and C(6″)), 131.3 (d, C(3) and C(5)), 146.9 (s, C(7″)), 156.8 (s, C(4)), 161.4 (s, C(4″)), 169.1 (s, C(9″)).

Osmanthuside D Heptaacetate (IIa) An amorphous powder. mp 80—83 °C. $[\alpha]_D^{25}$ −16.4° ($c=0.8$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745, 1700, 1600, 1500. FAB-MS m/z: 1036 (M+H+TEA[8])$^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.04 (3H, d, $J=6.4$ Hz, 6‴-H_3), 1.84, 1.95, 1.99, 2.06, 2.12 (each 3H, s, CH_3COO), 2.28, 2.30 (each 3H, s, CH_3COO), 2.86 (2H, m, β-H_2), 4.36 (1H, d, $J=8.1$ Hz, 1′-H), 4.81 (1H, d, $J=1.8$ Hz, 1‴-H), 5.91 (1H, d, $J=12.5$ Hz, C″$_8$-H), 7.01 (1H, d, $J=12.5$ Hz, C″$_7$-H), 7.09 (4H, q, A_2B_2 type, $J=8.4$ Hz, $\Delta\delta=0.21$ ppm, arom. protons), 7.38 (4H, q, A_2B_2 type, $J=8.4$ Hz, $\Delta\delta=0.57$ ppm, arom. protons). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.4 (q, C(6‴)), 20.6, 20.64, 20.7, 20.8, 20.9, 21.1, 21.2 (each q, $\underline{C}H_3COO$), 35.3 (t, C(β)), 62.3 (t, C(6′)), 67.3 (d, C(5‴)), 68.5 (d, C(4′)), 69.2 (d, C(2‴)), 70.1 (d, C(3‴)), 70.2 (t, C(α)), 70.7 (d, C(2′)), 71.9 (d, C(4‴)), 72.1 (d, C(5′)), 80.7 (d, C(3′)), 99.2 (d, C(1‴)), 100.0 (d, C(1′)), 118.5 (d, C(8″)), 121.35 (d, C(3) and C(5)), 121.4 (d, C(3″) and C(5″)), 130.0 (d, C(2″) and C(6″)), 131.5 (d, C(2) and C(6)), 132.0 (s, C(1″)), 136.2 (s, C(1)), 145.1 (s, C(7″)), 149.2 (s, C(4)), 151.4 (s, C(4″)), 163.9 (s, C(9″)), 169.2, 169.4, 169.5, 169.6, 170.0, 170.1, 170.8 (each s, $CH_3\underline{C}OO$).

Osmanthuside E Hexaacetate (IIIa) An amorphous powder. $[\alpha]_D^{23}$ −7.0° ($c=2.3$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745, 1710, 1635, 1600, 1505. UV λ_{max}^{MeOH} nm (log ε): 281 (4.20). FAB-MS m/z: 894 (M+H+TEA[8])$^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.93, 1.99, 2.03 (each 3H, s, CH_3COO), 2.26 (6H, s, 2×CH_3COO), 2.32 (3H, s, CH_3COO), 2.86 (2H, m, β-H_2), 3.88 (3H, s, OCH_3), 4.33 (2H, m, 6′-H_2), 4.50 (1H, d, $J=7.8$ Hz, 1′-H), 6.41 (1H, d, $J=16.1$ Hz, C″$_8$-H), 6.94—7.13 (6H, m, arom. protons), 7.66 (1H, d, $J=16.1$ Hz, C″$_7$-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.5, 20.6, 21.1 (each q, $\underline{C}H_3COO$), 35.3 (t, C(β)), 56.0 (q, OCH_3), 62.1 (t, C(6′)), 68.6 (d, C(4′)), 70.0 (t, C(α)), 71.1 (d, C(2′)), 71.9 (d, C(5′)), 72.8 (d, C(3′)), 100.7 (d, C(1′)), 111.3 (d, C(2″)), 117.5 (d, C(8″)), 121.5 (d, C(6″)), 123.1 (d, C(2)), 123.3 (d, C(5″)), 123.8 (d, C(5)), 127.2 (d, C(6)), 133.2 (s, C(1″)), 137.4 (s, C(1)), 140.6 (s, C(4)), 141.6 (s, C(4″)), 141.8 (s, C(3)), 144.9 (d, C(7″)), 151.4 (s, C(3″)), 166.3 (s, C(9″)), 168.28, 168.3 168.7, 169.4 (×2), 170.3 (each s, $CH_3\underline{C}OO$).

Osmanthuside B Heptaacetate (IVa) An amorphous powder. mp 80—85 °C. $[\alpha]_D^{23}$ −47.4° ($c=1.4$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1635, 1600, 1500. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.03 (3H, d, $J=6.2$ Hz, 6‴-H_3), 1.85, 1.94, 1.98 (each 3H, s, CH_3COO), 2.08 (6H, s, CH_3COO), 2.27, 2.29 (each 3H, s, CH_3COO), 2.88 (2H, t, $J=6.6$ Hz, β-H_2), 6.35 (1H, d, $J=16.0$ Hz, C″$_8$-H), 7.09 (4H, q, A_2B_2 type, $J=8.6$ Hz, $\Delta\delta=0.25$ ppm, arom. protons), 7.32 (4H, q, A_2B_2 type, $J=8.8$ Hz, $\Delta\delta=0.86$ ppm, arom. protons), 7.69 (1H, d, $J=16.0$ Hz, C″$_7$-H).

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